

**Power output and tissue oxygenation of women and girls  
during repeated Wingate tests and recovery**

by

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B.Sc., University of Ottawa, 2008

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## **Supervisory Committee**

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## ABSTRACT

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The purpose of this study was to compare the exercise and recovery muscle oxygenation response of Women and Girls during two 30s Wingate anaerobic tests separated by two minutes of active cycling recovery (resistance  $\approx$  2.5% body weight, 60-80rpm). Oxygenated hemoglobin (HbO<sub>2</sub>), deoxygenated hemoglobin (HHb), total hemoglobin (tHb), and tissue saturation index (TSI) were monitored at the right vastus lateralis muscle using near infrared spectroscopy (NIRS) throughout exercise, recovery, and a post-exercise femoral artery occlusion to TSI plateau. Pressure was preset at 250mmHg for Women and 210mmHg for Girls, achieved by rapid inflation in 0.3 seconds, and maintained until a 2 minute TSI plateau occurred or 10 minutes had passed. Twenty Women (23.8[2.12] years) and 13 Girls (9[1] years, combined Tanner stage <4) completed all tasks excepting 1 girl who did not complete occlusion.

Significant group, time, and group by time interaction effects were observed for peak and mean power (Watts·kgFFM<sup>-1</sup>). Women had significantly greater power output compared to Girls for both Wingates. While both groups had reduced power output in Wingate 2, the reduction was significantly greater in Women compared to Girls. No significant group differences were found for resting TSI, recovery TSI, minimum TSI during either Wingate test, or for minimum TSI during occlusion, however a time main effect for Women was observed with minimum TSI being significantly lower in Wingate 1 compared to Wingate 2. Girls had similar minimum TSI

for both Wingate tests. Women also demonstrated a significantly greater difference between Wingate minimum TSI and occlusion minimum TSI in Wingate 2 compared to Wingate 1. During Wingate 1, HHb increase was greater in Girls compared to Women and remained elevated during recovery compared to women. Changes in HbO<sub>2</sub>, HHb, and tHb were reduced in Wingate 2 for both groups, more so in Women for tHb and in Girls for HHb. Recovery was not different between groups with the exception of a faster TSI time constant of recovery in Women ( $\tau = 20.25 [13.01]$ s) compared to Girls ( $\tau = 36.77 [13.38]$ s) which is attributed to a faster HHb time constant in Women ( $\tau = 13.6 [0.44]$ s) compared to Girls ( $\tau = 30.77 [19.47]$ s).

Both groups demonstrated similar power output results and TSI response across the two Wingate tests but Girls were better able to repeat the anaerobic performance with a consistent TSI minimum between the two tests despite a faster recovery of HHb and TSI in women. These findings, in the context of observed Hb variable differences between groups, provide evidence of greater oxidative metabolism in Girls during a high intensity exercise.

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**List of Abbreviations**

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BM	Body mass
Cr	Creatine
PCr	Creatine Phosphate
Ca <sup>2+</sup>	Calcium ion
CO <sub>2</sub>	Carbon dioxide
CSEP	Canadian Society for Exercise Physiology
DEXA	Dual energy x-ray absorptiometry
FFA	Free fatty acid
FFM	Fat free mass
FSH	Follicle stimulating hormone
g·kg <sup>-1</sup>	grams/kilogram
g·kgBM <sup>-1</sup>	Grams per kilogram of body mass
H <sup>+</sup>	Hydrogen ion
H <sub>2</sub> O	Water
Hb	Hemoglobin
HbΔ	Hemoglobin difference, the difference of oxy and deoxy hemoglobin
HbO <sub>2</sub>	Oxyhemoglobin
HHb	Deoxyhemoglobin
HIE	High intensity exercise
HIIE	High intensity interval exercise
La <sup>-</sup>	Lactate ion

LH	Luteinizing hormone
$\mu\text{M}$	Micromolar, measure of hemoglobin variable concentration
mmHg	millimeters of mercury, measure of pressure
$\text{NAD}^+$	Oxidized nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NIRS	Near infrared spectroscopy
$\text{O}_2$	Oxygen
OCC	Occlusion
OCC WG $\Delta$	Difference between Occlusion TSI and Wingate TSI minimums
OCC WG1	Difference between Occlusion TSI and Wingate 1 TSI minimum
OCC WG2	Difference between Occlusion TSI and Wingate 2 TSI minimum
Pi	Inorganic phosphate
pH	Potential hydrogen, measure of acidity
PMRS	Phosphorous magnetic resonance spectroscopy
R	Recovery
R1	Recovery 1, first recovery period
R2	Recovery 2, second recovery period
RER	Respiratory exchange ratio
SD	Standard deviation
$\tau$	Tau, the time constant of recovery, measured in seconds
tHb	Total hemoglobin
TSI	Tissue Saturation Index
$\text{Watts}\cdot\text{kgFFM}^{-1}$	Watts per kilogram of fat free mass, measure of power output
WG	The Wingate anaerobic test
WG1 or Wingate 1	The first Wingate test

WG2 or Wingate 2	The second Wingate test
XFER	Transfer (from one cycle ergometer to the other)
XFER1	Transfer 1 (after Wingate 1, from Wingate bike to Recovery bike)
XFER 2	Transfer 2 (before Wingate 2, from Recovery bike to Wingate bike)
% $\Delta$ TSI	Percent change TSI

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## CHAPTER 1 INTRODUCTION

### 1.1 Overview

High intensity interval exercise (HIIE) is a central component to many sports; successful athletic performance depends on power output and recovery. Power output is important for athletes of all ages competing in sport however maturation-related differences exist in the production of power as well as recovery from high intensity performances which impact the ability to complete repeated high intensity intervals. During high intensity interval exercise, adults are able to produce greater power relative to body size, mass, and muscle mass, while children demonstrate superior fatigue resistance and recovery (Gaul, Docherty, & Cicchini, 1995; Hebestreit, Mimura, & Bar-Or, 1993; Ratel, Williams, Oliver, & Armstrong, 2004; Zafeiridis et al., 2005).

The Wingate test is a maximal cycling test commonly used to measure power output. Studies comparing children and adults have found that peak and mean power are greater in adults, even when adjusted for mass, fat free mass, and lower leg volume (Beneke, Hütler, & Leithäuser, 2007). On the other hand, it has been found that children are better able to repeat power performance with shorter recovery times compared to adults (Chia, 2001; Hebestreit et al., 1993).

To understand anaerobic power performance and post-HIIE recovery differences between children and adults, previous research has used a variety of methodologies to examine changing metabolic characteristics during and after exercise. Cardiorespiratory measures provide evidence of central support for metabolism while muscle biopsies and blood samples more closely reflect metabolic activity of the working muscles. Imaging technologies such as phosphorous magnetic

resonance spectroscopy (PMRS) and near infrared spectroscopy (NIRS) provide the opportunity to examine muscular metabolism noninvasively, and often in real-time (Neary, 2004). Through these various methodologies, the literature points to greater oxidative metabolism in children during both high intensity interval exercise (Ratel, Duché, & Williams, 2006) and recovery (Falk & Dotan, 2006) compared to adults.

While resting levels of phosphocreatine (PCr) are similar between children and adults, evidence of PCr changes during HIIE are conflicting; overall, Pi/PCr ratio appears to increase more in adults compared to children during high intensity exercise (Kappenstein et al., 2013; Kuno et al., 1995; Zanconato, Buchthal, Barstow, & Cooper, 1993). These findings suggest a greater reliance on anaerobic metabolism in adults during HIIE. On the other hand the recovery of PCr concentrations post-exercise is faster in children (Ratel, Tonson, Le Fur, Cozzone, & Bendahan, 2008; Taylor, Kemp, Thompson, & Radda, 1997; Tonson et al., 2010), suggesting greater aerobic support for rephosphorylation of Cr to PCr post-exercise. Children have also been observed to have lower resting concentrations (relative to protein content) of the anaerobic enzyme lactate dehydrogenase (Kaczor, Ziolkowski, Popinigis, & Tarnopolsky, 2005). Lactate concentrations are greater in adults after high intensity exercise compared to children (Beneke, Hu, Jung, Leitha, & Renate, 2005; B. O. Eriksson, Karlsson, & Saltin, 1971; Ratel et al., 2004; Zafeiridis et al., 2005), however this variable reflects the balance between lactate production and clearance and therefore may not be a good indicator of anaerobic glycolysis. Petersen, Gaul, Stanton, & Hanstock (1999), using  $^{31}\text{P}$ -MRS- demonstrated that prepubescent girls' skeletal muscle pH dropped at least as low as pubescent girls' during high intensity work, suggesting similar glycolytic activities during exercise. Blood flow to muscles during maximal exercise has been reported to be greater in children compared to adults (Koch, 1974), potentially facilitating

oxygen delivery to muscles and more rapid clearance of lactate, acids and other metabolites related to exercise. Aerobic metabolism not only drives the recovery of these variables but also contributes some energy to these maximal efforts; monitoring tissue oxygenation during HIIE and post-exercise recovery may help to explain the observed maturational differences.

Compared to adults, children demonstrate faster oxygen kinetics at the onset of exercise as well as greater oxygen uptake and oxygen cost during high intensity exercise (Armon, Cooper, Flores, Zanconato, & Barstow, 1991; Beneke et al., 2007; Tonson et al., 2010). These findings suggest that during exercise a greater proportion of ATP is produced aerobically in children compared to adults. Children also demonstrate faster recovery of respiratory variables, such as minute ventilation, oxygen uptake, and CO<sub>2</sub> production, as well as heart rate following high intensity exercise (Hebestreit et al., 1993; Zafeiridis et al., 2005; Zanconato, Cooper, & Armon, 1991). These results could be viewed as evidence of greater aerobic activity facilitating recovery in children, alternatively they could reflect less recovery required in children following high intensity exercise.

Near infrared spectroscopy (NIRS) is a relatively new technology that allows for the real-time examination of peripheral metabolism and offers a reliable, non-invasive opportunity to explore maturational differences in peripheral metabolism during exercise. Unlike MRS, NIRS is portable, inexpensive and accessible. It monitors changes in local tissue oxyhemoglobin (HbO<sub>2</sub>) and deoxyhemoglobin (HHb) concentrations, which together equal total hemoglobin (tHb). A faster rate of change and shorter response time for HHb has been consistently observed in children compared to adults during constant work rate exercise (Fulford, Welford, Welsman, Armstrong, and Barker, 2008; Leclair et al., 2013; Willcocks, Williams, Barker, Fulford, & Armstrong, 2010). Although this technology has the potential to provide real time, accurate

measurement of peripheral oxygenation of working muscle, to date there has been limited NIRS research examining maturational differences during HIIE, particularly in females, and no NIRS research found of the post-exercise recovery response comparing children and adults.

To determine whether maturational differences in repeat anaerobic power performance are related to differences in the metabolic response to exercise and/or the physiology of post-exercise recovery processes, research examining peripheral metabolism during high intensity interval exercise is required.

## **1.2 Purpose**

The purpose of this study was to improve the understanding of maturational differences in muscle physiology during anaerobic exercise and recovery by examining local tissue oxygenation response in girls and women during two repeated Wingate tests separated by active recovery.

## **1.3 Research questions**

In healthy active females:

- 1) Are there maturational differences in peak and mean power output during two repeated Wingate tests separated by active recovery?
- 2) Are there maturational differences in oxygenation response at the vastus lateralis muscle during two repeated Wingate tests separated by active recovery?
  - a) For Tissue Saturation Index: range, minimum TSI, percent change TSI, comparing to occlusion minimum TSI
  - b) For the hemoglobin chromophores of HbO<sub>2</sub>, HHb, and tHb: range

- 3) Are there maturational differences in the oxygenation response at the vastus lateralis muscle during active recovery following a Wingate test?
- a) For Tissue Saturation Index: range, maximum, steady state, percent change, time constant of recovery ( $\tau$ )
  - b) For the hemoglobin chromophores of HbO<sub>2</sub>, HHb, tHb: range, time constant of recovery ( $\tau$ )

#### 1.4 Hypotheses

- 1) H<sub>1</sub>: Women will produce greater peak and mean power relative to fat free mass during both Wingate tests compared to girls.  
H<sub>1</sub>: Girls will be better able to repeat power performance on the second Wingate test compared to women.
- 2) H<sub>0</sub>: No maturational difference in tissue oxygenation response during the two Wingate tests will be found for
  - a. Any TSI measurement
  - b. Any of the HbO<sub>2</sub>, HHb, and tHb measures
- 3) H<sub>0</sub>: No maturational difference in tissue oxygenation response during the active recovery period following the first Wingate tests will be found for.
  - a. Any TSI measurement
  - b. Any of the HbO<sub>2</sub>, HHb, and tHb measures

## 1.5 Operational definitions

- High intensity interval exercise (HIIE): Repeated maximal cycling effort lasting 30 seconds with two minutes of low intensity active cycling recovery.
- Anaerobic power: power output during the Wingate tests, measured in watts, expressed relative to fat free mass.
  - Peak power: maximum power output during 1 second
  - Mean power: average power over 30 seconds
- Muscle or tissue oxygenation: changes in oxygenated hemoglobin, deoxygenated hemoglobin, total hemoglobin, and TSI measured using NIRS from the vastus lateralis muscle.
- Range: the magnitude of change of a variable, calculated as the difference between a determined start and end value.

## 1.6 Assumptions, delimitations, limitations

### *1.6.1 Assumptions*

- All Participants performed maximally during both Wingate tests
- High intensity exercise and recovery performance and metabolism are not significantly affected by birth control use and stage of menstrual cycle in women

### NIRS related assumptions

The following assumptions are stated in the Artinis Portalite Manual (Artinis Medical Systems BV, 2011, p.21):

- Slope estimations are based on the assumption that the source-detector separation is much larger than the source size and the scattering mean free pathlength

- The algorithm assumes homogeneous and infinite tissue
- The light enters the tissue perpendicular and without any air-tissue transition
- A constant scattering coefficient is assumed, and it is estimated to follow a linear relation to the wavelength

### ***1.6.2 Delimitations***

- 1) Women were aged 19-30 years, non-pregnant, apparently healthy, with no injury or disease
- 2) Girls were prepubertal (combined Tanner score <4), apparently healthy, with no injury or disease.
- 3) All participants met the Canadian Society for Exercise Physiology (CSEP) physical activity guidelines (Tremblay et al., 2011):
  - a) Adults: 150 minutes of moderate to vigorous physical activity a week
  - b) Children: 60 minutes of moderate to vigorous physical activity a day

### ***1.6.3 Limitations***

- 1) Pre-testing nutritional status, activity, and level of fatigue were not controlled for
- 2) Menstrual cycle phase of women was not be measured or controlled for
- 3) The applicability of results to non-cycling activities may not be appropriate.

## CHAPTER 2 LITERATURE REVIEW

Maturational differences during high intensity interval exercise and recovery have been researched extensively. Performance differences between children and adults are well documented and have been related to metabolic differences observed during exercise and recovery. To understand the physiological basis of maturational differences in exercise metabolism, the underlying metabolic mechanisms must be reviewed and applied to the results of studies comparing children and adults during exercise and recovery.

### **2.1 Exercise metabolism**

Much of what is known about exercise metabolism is based on research conducted with adults. These results cannot necessarily be applied to children as they are not “miniature adults”. Research comparing child and adult physiology and exercise metabolism is important to inform training programs and fitness standards for different levels of maturity.

#### ***2.1.1 Energy production***

At exercise onset, muscles begin contracting and as demand for greater force develops there is a recruitment of more muscle fibers, muscles, and muscle groups. This increases energy demand which must be matched by energy supply to continue exercise. ATP, the primary energy currency in skeletal muscle, can be produced through anaerobic and aerobic metabolism. During activities of low and moderate intensities, ATP is preferentially produced using aerobic metabolism (Brooks, Fahey, & Baldwin, 2005). With increasing exercise intensity, the contribution of energy produced aerobically decreases while anaerobic metabolism increases to meet energy demands. Aerobic metabolism can produce more ATP compared to anaerobic metabolism; however anaerobic metabolism can produce ATP more quickly, fitter individuals will have a higher

anaerobic threshold meaning they are able to produce ATP aerobically at higher relative exercise intensities, sparing their anaerobic energy stores. ATP produced through anaerobic metabolism comes from intramuscular stores of ATP, creatine phosphate (PCr) and glycogen (Brooks et al., 2005). During high intensity exercise ATP production will be primarily anaerobic for up to 2 minutes after which exercise intensity will drop as a result of muscular fatigue (Gastin, 2001). Depletion of energy stores and metabolic byproduct buildup contribute to fatigue, inhibiting anaerobic metabolism and continued effort at maximal intensity.

Aerobic metabolism is dominant during low and moderate intensity exercise, but is unable to meet the ATP demand during maximal exercise due to slower ATP production compared to anaerobic pathways. Another factor that could limit aerobic metabolism is reduced blood flow/oxygen delivery to the muscle. Another limiting factor of aerobic metabolism is reduced blood flow to and from muscles. This may be exacerbated during isometric exercises or in situations where arteries and veins are occluded by muscular contractions.

#### **a) Anaerobic metabolism**

There are two anaerobic pathways that produce ATP, the adenosine triphosphate- creatine phosphate (ATP-PCr) system and Anaerobic Glycolysis.

##### *i) The ATP-PCr or anaerobic lactic system*

ATP and PCr are stored in the muscle. ATP is used as immediate energy and PCr is used to rapidly replenish ATP.

The ATP-PCr system produces ATP quickly but only for 0-3 seconds, after which stores are depleted and must be replenished using oxygen. The creatine phosphate shuttle restores PCr to pre exercise levels (see 2.1 b) ii) Recovery).

*ii) Anaerobic glycolysis or anaerobic lactic system*

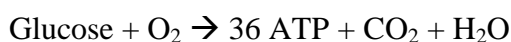
Muscles contain stores of glycogen which can supply ATP for 4-50 seconds.

Intramuscular glycogen stores are less likely to be depleted during short duration maximal exercise, rather, exercise intensity would be limited by the changing cell environment. Without adequate oxygen, lactate concentration increases, increasing hydrogen ion concentration. pH decreases, reducing glycolytic enzyme activity and inhibiting calcium release from the sarcoplasmic reticulum binding to expose the myosin binding site for ATP (Gastin, 2001). Limited binding sites for myosin means less cross bridging can occur, ultimately inhibiting muscular contractions. Recovery depends on clearance of metabolic byproducts via the aerobically powered lactate shuttle (see 2.1 b) ii) Recovery).

**b) Aerobic metabolism**

*i) ATP production*

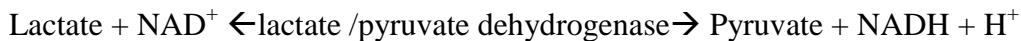
Aerobic metabolism produces ATP in the mitochondria of muscle cells using glucose or free fatty acids (FFA) as substrates. With the same amount of substrate, more ATP is produced through aerobic metabolism compared to anaerobic metabolism, however the process takes much longer. Simply put:



These processes take Acetyl Co-enzyme A from either fatty acid or glucose metabolism into the Krebs (aka Citric Acid or TCA) cycle and the electron transport chain.

### Glycolysis

With adequate oxygen, the lactate pyruvate balance is maintained via the lactate shuttle:



In the mitochondria, pyruvate dehydrogenase transforms pyruvate into acetyl CoA which can enter the Krebs cycle and produce ATP aerobically. With increasing exercise intensity, oxygen delivery cannot meet demand and the lactate/pyruvate balance will shift to a buildup of lactate, associated with an increase in hydrogen ion concentration, changing the cell environment and contributing to muscular fatigue.

### *ii) Recovery*

Aerobic metabolism is not only important for producing ATP but also in the recovery from high intensity exercise. Once exercise intensity is reduced to a level that oxygen delivery can once again meet the energy demands of the working muscle and also functions to restore the cell environment catch up to demand, the lactate shuttle will return the lactate/pyruvate balance to normal. Creatine phosphate is also resynthesized in the mitochondria with the enzyme creatine kinase by the creatine phosphate shuttle:



Both the lactate and creatine phosphate shuttles highlight the strong link between the aerobic and anaerobic metabolism for high intensity interval exercise.

### ***2.1.2 Measurement of the physiological response to exercise***

Central and peripheral physiological variables are measured to understand metabolism during exercise of different modes, intensities, durations, and compare different populations. Central variables refer to the cardiovascular and respiratory response to exercise. Common measures are heart rate, oxygen (O<sub>2</sub>) consumption, cardiac output, blood pressure, minute ventilation and respiratory exchange ratio. Peripheral variables represent those processes occurring in the muscles and blood vessels around the muscles. Metabolic byproducts (H<sup>+</sup>, ADP, Pi), enzyme activity, and O<sub>2</sub> saturation at the muscle and can be measured invasively by tissue biopsy, blood samples, or non-invasively by phosphorous magnetic resonance imaging (<sup>31</sup>P MRI), or near infrared spectroscopy (NIRS).

With increasing exercise intensity, respiratory variables (tidal volume and ventilation rate), cardiac output (heart rate and stroke volume), and blood flow to muscles increase to support increased energy demands (Brooks et al., 2005). Heart rate and blood pressure are most easily measured while stroke volume, cardiac output, and oxygen consumption require more sophisticated equipment to measure directly. As energy demand increases the partial pressure of CO<sub>2</sub> increases and the arteriovenous oxygen difference becomes greater facilitating oxygen uptake at the muscle. As more O<sub>2</sub> is consumed and CO<sub>2</sub> produced at the level of the muscle, the percentage of O<sub>2</sub> in expired air decreases while the percentage of CO<sub>2</sub> increases. Indirect calorimetry, the collection of expired gasses, allows for measurement of the respiratory exchange ratio, another reflection of metabolism.

As anaerobic and aerobic metabolism both increase ATP production to meet increasing demand from the muscles, enzyme activities increase. Accumulation of metabolic byproducts such as hydrogen ions and inorganic phosphate, as well as changing cellular pH reflects

increased anaerobic metabolism and higher intensity exercise. Lactate concentrations and rates of appearance/disappearance have been used to represent anaerobic metabolism (Eriksson, Karlsson, & Saltin, 1971), however care must be taken when interpreting results: Measures of muscle and especially blood lactate are influenced by production and clearance and therefore a greater observed concentration does not represent superior glycolytic capacity. Muscle biopsies and blood samples have also been used to examine both anaerobic and aerobic enzyme activities during exercise and recovery however these measures are invasive and no longer ethical in pediatric populations. Researchers must now rely on noninvasive methods to examine muscle metabolism in children. Newer imaging technologies such as  $^{31}\text{P}$ -MRI and NIRS offer this opportunity.

#### **a) Near Infrared Spectroscopy**

NIRS technology functions by emitting near infrared light into tissue which is absorbed by oxygenated hemoglobin ( $\text{HbO}_2$ ) and refracted by deoxygenated hemoglobin (HHb). Continuous wave photo detectors measure concentration changes (micromolar) of  $\text{HbO}_2$  and HHb based on changes in the emitted light. It should be noted that NIRS cannot differentiate between hemoglobin and myoglobin and therefore all  $\text{HbO}_2$  and HHb changes are actually reflecting all oxygen changes in hemoglobin and myoglobin in the tissue (Ferrari, Mottola, & Quaresima, 2004). Total hemoglobin (tHb), the sum of  $\text{HbO}_2$  and HHb, and hemoglobin difference ( $\text{Hb}\Delta$ ), the difference between absolute  $\text{HbO}_2$  and HHb change, are other measures monitored by NIRS.

The ratio of  $\text{HbO}_2$  to tHb is also monitored by NIRS and reported as the tissue saturation index (TSI) (Ferrari et al., 2004). TSI of muscle reflects the balance between oxygen delivery from blood flow and oxygen consumption from aerobic metabolism.  $\text{HbO}_2$  increases with

increased delivery and/or decreased aerobic metabolism and vice versa. HHb increases with increased oxygen consumption and/or decreased blood flow (HHb clearance), and decreases with decreased oxygen consumption and/or increased blood flow (HHb clearance). tHb increases when HbO<sub>2</sub> and HHb both increase, such as during exercise when oxygen delivery and oxygen consumption increase. Oxyhemoglobin, HbO<sub>2</sub>, increases in working muscles during lower intensity exercise blood flow and oxygen delivery increase, but exercise intensities above the anaerobic threshold will result in decreased oxygen saturation when blood flow and oxygen delivery can no longer meet the ATP demand (Gastin, 2001).

The application of NIRS in exercise physiology has provided opportunity to examine local tissue aerobic metabolism during various types and modes of exercise at different intensities, and during recovery in adult and child populations (Bhambhani et al., 2010; Cettolo, Ferrari, Biasini, & Quaresima, 2007; Moalla et al., 2006; Spencer, Murias, Lamb, Kowalchuk, & Paterson, 2011; Willcocks, Williams, Barker, Fulford, & Armstrong, 2010). Figure 1 demonstrates a representative tissue oxygenation response at the vastus lateralis muscle in adults during rest, warm up, during three different cycling protocols, and recovery.

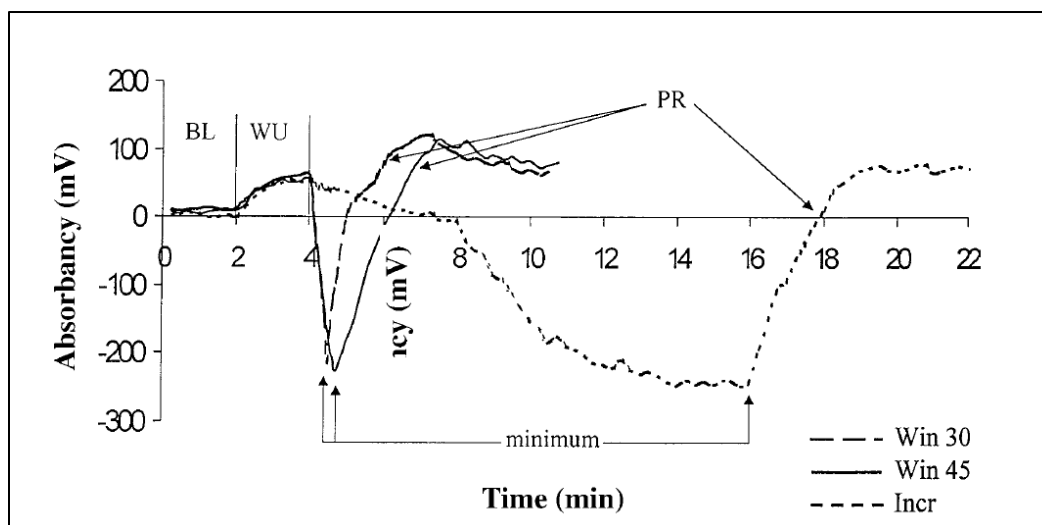


Figure 1. Muscle oxygenation trends at the vastus lateralis during a 30s and 45s Wingate and an incremental cycling test (from Bhambhani, Maikala, & Esmail, 2001).

BL= 2 minutes baseline, WU= 2 minute warm up, PR= start of 4 minutes passive recovery following 2 minutes of active recovery (active recovery was immediately after exercise, likely close to where minimums occurred).

NIRS has been validated in skeletal muscle during exercise (Sako, Hamaoka, Kurosawa, & Katsumura, 2001) and found to be a reliable measure at various exercise intensities across multiple days (van Beekvelt, van Engelen, Wevers, & Colier, 2002). Interindividual differences in tissue oxygenation response are influenced by adipose tissue thickness at the site of measurement, potentially affecting results (Niwayama, Suzuki, Yamashita, & Yasuda, 2012). NIRS exercise research focusing on skeletal muscles, most commonly measures the tissue oxygenation response at the calf, forearm, and quadriceps. For cycle exercise, the NIRS device is commonly placed at the vastus lateralis muscle. This quadriceps muscle is easily accessible for instrumentation and is active during cycling, contracting to push the pedal down (Chia, 2001). NIRS allows for noninvasive examination of peripheral tissue oxygenation. NIRS is more portable compared to PMRS, another non-invasive imaging technology. NIRS measurements can

also be monitored in real-time during whole body dynamic exercise, increasing the applicability to sports science.

To improve interpretation of the tissue oxygenation response beyond the relationship between blood flow and metabolism, results can be considered in context of blood flow measures. As well, occlusion techniques are commonly employed to isolate measures of muscular metabolism.

### **Occlusion protocols**

Occlusion protocols are often employed in NIRS research to provide reference values as well as isolate more specific blood flow and metabolism measures. Arterial occlusion limits blood flow to and from the tissue while venous occlusion limits only outflow (Hamaoka, McCully, Quaresima, Yamamoto, & Chance, 2007). During venous occlusion (pressure at approximately 50-60mmHg), HHb appearance reflects oxidative tissue metabolism while HbO<sub>2</sub> appearance represents blood flow (De Blasi, Cope, Elwell, Safoue, & Ferrari, 1993). Arterial occlusion pressure must be greater than blood pressure in the main artery delivering blood to the muscle of interest and is typically set at or above 250 mmHg. This protocol can be used to measure muscle oxygen consumption as the rate of HbO<sub>2</sub> decrease during rest and exercise. To measure muscle oxygen consumption during exercise, occlusion is applied directly after exercise and it is assumed that the tissue oxygenation response reflects metabolism during exercise (McCully & Hamaoka, 2000).

Another type of arterial occlusion is to “plateau” to determine an individual’s physiological range. NIRS data throughout exercise is then normalized to this range to account for interindividual differences (Southern, Ryan, Reynolds, & McCully, 2014). While this method

may be useful in comparing some NIRS measures between individuals and groups, not all NIRS studies practice this data “normalization”. The rationale for this method is that sensitivity of the NIRS signal will be different between individuals however when comparing change in NIRS variables between individuals or groups, normalization becomes less important. Another issue with this method is that no criteria exist for occlusion “plateau”; it appears that this plateau is determined visually by researchers. It is also unclear from the literature which NIRS variable or variables researchers are observing for “plateau”. In addition to the “plateau” endpoint for occlusion, 10 minutes is a common set end point for occlusion protocols.

## **2.2 High intensity interval exercise**

Many sports, such as soccer and tennis, depend on the ability to repeat high intensity or maximal work. This can be quantified as anaerobic power output and recovery. High intensity interval exercise performance depends on both the anaerobic system to generate power by producing lots of energy quickly, and aerobic system to recover by replenishing energy stores and removing muscle byproducts (Brooks et al., 2005). The 30 second Wingate anaerobic test was developed to quantify maximal effort in a short period of time as power output. Recovery can be quantified by comparing performance on repeated exercise tasks to the original exercise bout and active recovery is most realistic to a sport environment.

### ***2.2.1 Measuring power output and recovery performance***

High intensity interval exercise performance consists of power output and recovery. Power is produced using primarily anaerobic metabolism (Brooks et al., 2005). Anaerobic power tests are designed to elicit maximal effort in a short period of time. Anaerobic power also depends on

motivation, biomechanics, neural factors, and muscle properties such as fiber type, substrate availability, and enzyme concentration and activity (Dotan et al., 2013).

### **a) The Wingate Test**

Anaerobic powers tests involving dynamic maximal exercise, such as running or cycling, are most applicable to sport. Comparing women and girls, running economy is greater in women (Allor, Pivarnik, Sam, & Perkins, 2000) while mechanical efficiency was found to be comparable during cycling exercise in children and adults (Rowland, 1990).

The Wingate test is a cycling test used to measure anaerobic power. It has been demonstrated to be valid and reliable in children and adults (Bar-Or, Dotan, & Inbar, 1977). The test consists of 30 seconds of cycling as fast as possible against a resistance of  $75 \text{ g} \cdot \text{kg}^{-1}$  body weight. The Wingate test provides measures of peak power (maximum power achieved in 1 second, occurring within the first 10 seconds) and mean power (power over the 30 second test) based on pedal frequency and resistance. Power can be expressed in absolute terms and relative to mass, but fat free mass has been found to be a stronger predictor of power output compared to body mass (Armstrong, Welsman, & Chia, 2001; Maud & Shultz, 1986) and therefore it is recommended to express power relative to fat free mass.

### **Determination of Fat Free Mass**

Fat free mass can be estimated by using equations that consider participant characteristics and anthropometric measures such as age, height, weight, sex, and sums of skinfolds. Because body composition changes with maturity (Rogol, Roemmich, & Clark, 2002) different equations are required for children, adolescents, and adults. Using skinfolds, it is necessary to make the assumption that skinfold thickness is positively correlated with total body fat (Wells, 2005).

These equations will give an estimate of body fat percent which can be used to estimate fat free mass with the following equation:

$$\text{FFM} = M \times \text{BF}\%$$

Where FFM is fat free mass, M is body mass in kilograms, and BF% is body fat percent determined from the equations.

To correct power output for fat free mass, absolute peak and mean power can be divided by fat free mass in kilograms as per the following equation:

$$P_R = P/\text{FFM}$$

Where  $P_R$  is relative power in Watts per kilogram (W/kg), P is absolute power in Watts (W), and FFM is fat free mass in kilograms (kg).

## **b) Recovery**

Recovery of anaerobic power is determined by the amount of time required to repeat power output performance. It depends on the work to rest ratio, the total amount of work performed up to that point, and recovery mode (active, passive). Active recovery is inferior to passive recovery during high intensity interval exercise (see 2.2.2 Metabolism) but is most similar to the demands of high intensity interval sports.

### **2.2.2 Metabolism**

High intensity interval exercise depends on mostly anaerobic metabolism for energy production and the aerobic metabolism for recovery. The aerobic system also contributes to ATP production but much less so than the anaerobic energy system during maximal efforts, especially at the start of maximal exercise. From a review of energy metabolism during high intensity

interval exercise, 15-30% of ATP production has been attributed to aerobic metabolism during a Wingate test (Gastin, 2001). Examining repeated maximal efforts, aerobic contribution to ATP production appears to increase disproportionately to the observed decrease in power output (Gastin, 2001). This supports the contribution of aerobic ATP production to maximal exercise tasks. During a Wingate test, TSI rapidly decreases at the onset of exercise, reaching minimum around 15 seconds (Bhambhani et al., 2001). This is related to the rapid drop in HbO<sub>2</sub> and increase in HHb observed during the maximal exercise interpreted as being due to reduced blood flow to and from the muscle from intrinsic muscular occlusion, and increased aerobic metabolism. Tissue reoxygenation following a maximal effort is rapid as blood flow to muscles is restored quickly, however full recovery of TSI and underlying Hb variables depends on exercise mode, intensity, and time of recovery. Being able to repeat anaerobic, high intensity power performances requires physiological processes such as heart rate, substrate stores, pH, and oxygen saturation returning to or close to pre-exercise levels.

Active recovery leads to faster clearance of metabolic by-products, replenishment of fuels, and better repeat performance compared to passive recovery for longer duration recovery periods (Dotan, Falk, & Raz, 2000; Koizumi et al., 2011; Miladi, Temfemo, Mandengué, & Ahmaidi, 2011). It appears that passive recovery leads to better repeat power performance compared to active recovery for recovery durations less than 4 minutes (Buchheit et al., 2009; Grégory Dupont, Moalla, Guinhouya, Ahmaidi, & Berthoin, 2004; Gregory Dupont, Moalla, Matran, & Berthoin, 2007; Lopez, Smoliga, & Zavorsky, 2014). This could be because recovery of PCr, heart rate, and oxygen consumption are attenuated during active recovery (Buchheit et al., 2009; Spencer, Dawson, Goodman, Dascombe, & Bishop, 2008). During repeated 30s cycle sprints

with 2 minutes of passive recovery between, power output and tissue deoxygenation decreased over 6 sprints (Buchheit, Abbiss, Peiffer, & Laursen, 2012).

The understanding of high intensity interval exercise metabolism is mostly based on research with male adult participants and as such, the understanding of metabolism during HIIE is limited in children. Research comparing child and adult metabolism during HIIE has been conducted and will be discussed in the section 2.3.3.

## **2.3 Maturational differences during high intensity interval exercise**

High intensity interval exercise performance differences exist between children and adults. Adults demonstrate greater anaerobic power output relative to size, mass, fat free mass, and leg volume while children recover anaerobic power more quickly (Ratel et al., 2006). One proposed explanation for observed performance differences is different metabolic characteristics between children and adults (Ratel et al., 2006).

### ***2.3.1 Maturational differences in high intensity exercise performance***

High intensity exercise performance differences between children and adults have been explored extensively with consistent findings: children generate less relative anaerobic power but are more able to resist fatigue and repeat performance compared to adults (Chia, 2001; Hebestreit et al., 1993; Ratel et al., 2006).

Considering maximal effort cycling, anaerobic power output relative to body mass was greater in men compared to boys during short term (10s), intermediate (30s), and long term (90s) anaerobic maximal cycling at 4.5% and 6.5% body mass resistance for boys and men respectively (Gaul et al., 1995). Peak, mean, and minimum power output relative to body mass were greater in men than boys during a Wingate test (Beneke et al., 2007). For repeated maximal

cycling sprints, it is evident that although power output may be inferior, children are better able to repeat performance: During 10 x 10s cycle sprints at 5% body mass resistance with 15s recovery between each sprint, peak power relative to lower limb volume was greater in men compared to boys (aged 11.7 [0.5] years) for sprints 1-6 only, and mean power was higher only for sprints 1-3. Boys power decrement over the 10 sprints was not as great as that seen in the men (Ratel et al., 2004). As well, Hebestreit et al. (1993) demonstrated that prepubertal boys (Tanner stage 1) were better able to repeat power output during a second Wingate test compared to men. Boy's peak power was consistent between Wingate tests with 2 minutes, 3 minutes or 10 minutes of active recovery but men's peak power was lower in the second Wingate following 2 and 3 minutes of recovery.

Less research exists with female participants, but similar differences to males have been observed. Women (25.1 +/- 2.7 years) were found to have greater peak anaerobic power output (relative to lower limb mass) during three repeated 15 second Wingate tests with 45 seconds of recovery while girls (13.6 +/- 1 year) had better power recovery (Chia, 2001). Also, during three repeated all out cycling sprints (with brake forces of 25 g·kg<sup>-1</sup>, 50 g·kg<sup>-1</sup> and 75 g·kg<sup>-1</sup>) separated by four minutes of recovery, women demonstrated greater peak power compared to girls (Doré, Bedu, França, & Van Praagh, 2001).

### ***2.3.2 Proposed explanations for maturational differences***

Explanations for anaerobic performance differences between children and adults include, biomechanics, neuromotor variables, metabolic characteristics, and motivation (Dotan et al., 2013).

Biomechanical differences between children and adults, such as relative limb length and optimal joint angles for force production, do exist. These are controlled for as much as possible by adjusting testing equipment and correcting results for anthropometric measures, but they still may have some influence on results (Ratel et al., 2006). Examining neuromotor differences between children and adults is a promising research direction for explaining differences in high intensity interval exercise performance. Conduction velocity has been found to be similar in children and adults but greater Type II fiber recruitment in adults compared to children has been suggested to explain greater relative power output (Ratel et al., 2006). Maturity related differences in muscle characteristics have been difficult to determine. It is generally accepted that fiber type proportions are not different between children and adults although some research has shown a greater proportion of Type II fibers in adults compared to children (Ratel et al., 2006). Greater proportions of Type II fibers are associated with superior anaerobic power output (Brooks et al., 2005). A greater proportion or faster recruitment of Type II fibers in adults could therefore explain anaerobic power output differences between children and adults. Type I fibers are highly reliant on oxidative metabolism and therefore if children relied more on Type I fibers, this could explain faster recovery.

### ***2.3.3 Maturational differences in exercise metabolism***

High intensity interval exercise performance differences between children and adults have been established, but research examining potential underlying metabolic differences is less conclusive (Ratel et al., 2006). Two arguments exist: the first is that children rely less on anaerobic metabolism during high intensity exercise and therefore have less to recover from, allowing children to recover more quickly and be better able to repeat power performance. The second argument is that anaerobic metabolic contribution to high intensity exercise is the same in

children and adults but children are better able to recover due to superior or facilitated aerobic metabolism (increased vascularization, mitochondrial density, and enzyme activity (Ratel et al., 2006).

Resting levels of PCr and ATP, the primary fuels used in anaerobic power tests, were found to be similar between boys and men (Eriksson & Saltin, 1974). After high intensity intermittent calf exercise, adults had a greater PCr depletion compared to children (Kappenstein et al., 2013) indicating greater reliance on anaerobic metabolism. In a study by Petersen et al.'s (1999) using  $^{31}\text{P}$ -MRS, the mean P/PCr ratio increased significantly more in the pubertal girls than prepubertal girls for 30 seconds (minute 1:00-1:30) of a two minute supramaximal exercise but the authors interpreted the results as demonstrating no overall difference in glycolysis between puberty groups. A faster rate of PCr breakdown has been observed in adults compared to children during an incremental calf muscle exercise as well (Zanconato et al., 1993). These results all point towards a greater reliance on anaerobic metabolism for adults during high intensity exercise. In contrast, during high intensity constant work rate exercise, Willcocks et al. (2010) found no differences in the rate of PCr breakdown and end exercise PCr concentration at the vastus lateralis muscle between children and adults. The disagreement in these results may be explained by the different exercise tasks, the muscles being examined, and the maturity level of the groups (Willcocks' children were 13 years, Beneke's older group were 16 years old). Recovery of PCr concentration was found to be faster in children (Ratel et al., 2008; Taylor et al., 1997; Tonson et al., 2010). Since PCr recovery relies on aerobic metabolism, a faster rate of PCr recovery could represent a faster rate of aerobic metabolism.

Children have been found to have lower resting concentrations of the anaerobic enzyme lactate dehydrogenase (relative to protein content) (Kaczor et al., 2005). Based on greater post

exercise (VO<sub>2</sub> max test) blood and muscle lactates in adults compared to children, it was inferred that children have reduced glycolytic capacity (Eriksson & Saltin, 1974). Factors facilitating lactate clearance, such as increased vascularization, mitochondrial density, and enzyme activity, could explain this difference however (Ratel et al., 2006). Blood flow to muscles during maximal exercise has been determined to be greater in children compared to adults, potentially facilitating oxygen delivery to muscles (Koch, 1974) and potentially reducing the reliance on anaerobic energy production.

Blood lactate was not different between women and girls following 3 intermittent 15s Wingate tests (Chia, 2001). Beneke et al. (2007) found no differences in end exercise lactate or in end exercise PCr concentrations between boys and male adolescents after a Wingate test. Comparing prepubertal and pubertal females, no differences were found in pH during a supramaximal calf exercise (Petersen et al., 1999).

Regardless of whether or not children are less able to rely on anaerobic metabolism, it is evident that children rely on oxidative metabolism more during high intensity exercise compared to adults. Armon, Cooper, Flores, Zanconato, & Barstow, (1991) measured greater oxygen cost in boys compared to men during high intensity cycling. Hebestreit et al. (1998) did not find any significant differences in overall oxygen debt between boys and men after cycling at various intensities but boys demonstrated faster oxygen uptake at the onset of heavy intensity exercise. In a later study, Hebestreit et al. (1993) observed a lower RER and faster recovery of heart rate and ventilation rate in boys compared to men during a 30 second Wingate test. Beneke et al. (2007) used measures of O<sub>2</sub> consumption and CO<sub>2</sub> production (indirect calorimetry) to estimate that the contribution of energy from aerobic metabolism was greater in boys compared to male adolescents during a Wingate test.

Tissue oxygenation of the muscle during high intensity cycling exercise has been examined in adults (Bhambhani, 2004). NIRS research on skeletal muscles during whole body exercise in children is limited to constant work rate cycling: Leclair et al. (2013) found a faster HHb response time in prepubescent boys compared to men. During single joint constant work rate exercise, Fulford, Welford, Welsman, Armstrong, and Barker (2008) observed shorter HHb delay time, faster HHb time constant, and a faster HHb mean response time in children (9-10 years old) compared to adults. Willcocks et al. (2010) compared HHb changes during high intensity isometric exercise in male and female children and adults and found that children had a faster rate of deoxygenation compared to adults but that overall degree of deoxygenation was similar. It should be noted that “child” participants were on average 13 years old and therefore not necessarily prepubescent. Overall, tissue oxygenation kinetics are observed to be faster in children compared to adults during exercise. No studies to date have compared tissue oxygenation during high intensity cycling exercise and recovery in prepubescent children and adults.

## **2.4 Female physiology**

Physiological research in women is complicated by the menstrual cycle, divided into the follicular and luteal phases. Throughout the cycle, the hormones estrogen, progesterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) fluctuate, affecting body temperature, respiratory variables, and metabolism (Oosthuyse & Bosch, 2010). From a review of the topic, Oosthuyse and Bosch (2010) conclude that anaerobic power output is not significantly different between phases. Evidence of improved oxidative metabolism during exercise in the luteal phase in women with regular menstrual cycles (McCracken, Ainsworth, & Hackney, 1994) has implications for improved recovery during high intensity interval exercise.

There are no apparent differences in exercise performance between eumenorrheic and amenorrheic women (Dawson & Reilly, 2009), however very little research on the topic examined high intensity exercise. Physiological differences between the groups seem to be hormonal and anthropometric (Dawson & Reilly, 2009), however metabolic responses in eumenorrheic and amenorrheic women have not been thoroughly examined.

## **2.5 Conclusions**

While adults produce greater relative power compared to children during maximal all out efforts, children are better able to repeat power performance with less recovery time. In attempting to better understand these performance differences between children and adults, research can be conducted from several different perspectives. In terms of potential physiological differences, it appears that adults rely more on immediate anaerobic metabolism (ATP-PCr) during high intensity exercise but it is still unclear whether or not anaerobic glycolysis is limited in children or not during high intensity exercise. Children recover more quickly in terms of physiological markers of metabolism. There is limited research on peripheral measures of aerobic metabolism during high intensity exercise and recovery, and therefore comparing the peripheral tissue oxygenation response of children and adults with NIRS technology during high intensity exercise and recovery may help to improve the understanding of performance differences.

## CHAPTER 3 METHODS

### 3.1 Design

The design of this study was quasi-experimental with two non-equivalent groups recruited through purposeful sampling. Twenty-one Women and twenty-one Girls volunteered and performed two Wingate tests separated by two minutes of active Recovery on a cycle ergometer. They then underwent an arterial occlusion at the thigh to determine physiological minimum tissue oxygenation. Power output was determined from the Wingate test ergometer and tissue oxygenation was measured using near infrared spectroscopy (NIRS) throughout exercise, recovery, and during the occlusion test. Each participant was required to come to the lab only once for approximately 1.5 hours. This research study was approved by the Human Research Ethics board at the University of Victoria.

### 3.2 Participants

Girls were required to be prepubescent or in the very early stages of puberty (combined Tanner score of 2 or 3 as estimated by parents and confirmed by Girls in a self-assessment) and physically active a minimum of 60 minutes a day at a moderate to vigorous intensity. The Women were required to be 19-30 years old, eumenorrheic (or assumed so in the absence of birth control that inhibits menstruation), and active 150 minutes a week at a moderate to vigorous intensity. Physical activity criteria was based on the Canadian Society for Exercise Physiology physical activity guidelines (Tremblay et al., 2011). Participants were recruited by contacting sport organizations and activity clubs, by email, placing posters at the University of Victoria, and Recreation Centers throughout Victoria, BC, as well as through word of mouth. Of the twenty-one Girls who participated, the data from 8 Girls were excluded from analyses based on having a maturity status greater than combined Tanner stage 3 or being unable to complete the Wingate

test protocols appropriately. This left 13 data sets for analysis. One of these 13 Girls was unable to complete the occlusion protocol resulting in 12 data sets for analysis of occlusion data. Of the twenty-one young adult Women, one was excluded due to errors in the NIRS data, resulting in an adult participant group of 20 Women.

### **3.3 Experimental protocol**

#### ***3.3.1 Preparation and questionnaire***

Once recruited, participants were confirmed to have met the age and activity inclusion criteria (Appendix 1) and understood the pre-testing directions (Appendix 2), they were scheduled to come to the lab for testing. Participants were instructed to consume nothing but water within 2 hours of the lab session, have no caffeine within 6 hours of the lab session, and refrain from vigorous exercise within 12 hours of the lab session.

All testing took place at the University of Victoria in the Exercise Physiology Lab. The experimental procedures were explained to the participant by the researcher, followed by the participant completing a Par-Q+ questionnaire (Appendix 3) and signing a letter of informed consent (Appendix 4) (parent or guardian consent was obtained for Girl participants). Age and level of physical activity (hours of moderate to vigorous physical activity per week) were recorded on the data collection form (Appendix 5).

Stage of sexual maturity for the Girl participants was determined by asking each to self-assess their level of physical maturity from anatomical drawings of the five stages of breast and pubic hair development from the Tanner Scale (Marshall & Tanner, 1969; Appendix 6). This was conducted in a confidential manner and involved clear verbal explanation of each drawing. The sum of chosen values from each scale (1-5) was used to determine a participant's combined Tanner Score (2-10). This methodological strategy for determining maturational stage is reliable

(Rabbani et al., 2013). Further strong correlations have been found between both parent and Girls' Tanner stage assessments and those of a trained health professional (Dorn, Susman, Nottelmann, Inoff-germain, & Chrousos, 1990). To confirm the self-assessment scores, a respective parent or guardian was also asked to score their daughter based on the same Tanner Scale drawings.

### ***3.3.2 Anthropometric measures***

Participant height and weight were measured while wearing light clothing and no shoes using a stadiometer (Tanita, USA) and a scale (Health-o-meter, Continental Scale Corporation, USA). Skinfolds were measured with calipers (John Bull Indicators, England) at one site (triceps) for Girls and four sites (triceps, biceps, subscapular, iliac crest) for Women. Measurements were performed following the International Standards for Anthropometric Assessment (ISAK, 2001). To estimate fat free mass, the following skinfold equations were used:

Girls: Fat Free Mass= Total Mass (kg) - Fat Mass (kg)

$$\text{Fat Mass} = (0.332 * \text{weight}) + (0.263 * \text{triceps}) + (0.760 * \text{gender}) - (0.704 * \text{ethnicity}) - 8.004$$

(Dezenberg, Nagy, Gower, Johnson, & Goran, 1999)

where triceps is the triceps skinfold thickness, gender is 2 for female, and ethnicity is 1 for Caucasian. This equation has been cross validated with DEXA (for Girls  $R^2 = 0.92$ ) (Dezenberg et al., 1999).

Women: Body Density =  $1.1599 - 0.0678 \times \log(\sum SF)$  (Durnin & Womersley, 1974)

where  $\sum SF$  is the sum of measures at the triceps, biceps, subscapular, and iliac crest skinfold sites. This equation was validated with body density determined from underwater weighing (Durnin & Womersley, 1974). To estimate body fat percent and determine fat free mass, the Siri equation (1956) was used:

$$\text{Body Fat \%} = ((4.95/\text{BD}) - 4.5) * 100$$

where BD is body density in kilograms determined from the equations above. This result was used to determine fat free mass by the following equations:

$$\text{Fat Mass} = \text{Total Mass (kg)} * \text{Body Fat \%}$$

$$\text{Fat Free Mass} = \text{Total mass (kg)} - \text{Fat mass (kg)}$$

A skinfold measurement was also taken at the site of the NIRS device placement on the thigh of all participants (at right vastus lateralis muscle, see section 3.3.3) to measure adiposity at the site of measurement.

After skinfold measures were taken, a Polar heart rate monitor was secured at the level of the sternum to monitor heart rate for safety purposes. No heart rate data were recorded or used in this study.

### ***3.3.3 Tissue oxygenation measurement***

Throughout the exercise test, including the Active Recovery period, and during the post exercise occlusion test, a continuous wave NIRS monitor (Portalite, Artinis Medical Systems BV, The Netherlands) interfaced with the Oxysoft 2.1.6 computer program was used to measure changes in total hemoglobin concentration (tHb), oxyhemoglobin concentration (HbO<sub>2</sub>), and

deoxyhemoglobin concentration (HHb) in  $\mu\text{M}$ . The ratio of  $\text{HbO}_2$  to tHb was expressed as the Tissue Saturation Index (TSI). Data were collected at 10 Hz with a differential pathlength factor (DPF) of 4 and wavelengths set at 761nm and 848nm.

The NIRS sensor was secured over the belly of the right vastus lateralis muscle following the protocols reported by others (Bhambhani et al., 2001; Moalla et al., 2006), 10-14cm from the anterior border of the patella for adults and 10-12cm for children. This placement was marked using indelible ink while the participant was in a seated position with the right knee fully extended and the left foot firmly on the ground. The NIRS sensor was tightly covered with plastic wrap (Saran<sup>TM</sup>) to protect it from sweat and positioned parallel to the direction of the muscle fibers. To block out all light, and hold it in place, the sensor was taped to the thigh with athletic tape, covered with a black cloth leg band, taped again, and then covered with a black nylon sleeve. The NIRS receiver was secured in an armband positioned on the right upper arm throughout testing (Figure 2, Appendix 7).

Table 1 provides an explanation for each of the events (explained in section 3.3.5) that were manually marked and labeled in the NIRS data throughout data collection.

*Table 1. Experimental Protocol Events Marked In NIRS Data With Label Used, Details, And Bike Information.*

<b>Event</b>	<b>Label</b>	<b>Details</b>	<b>Bike</b>
A	WU start	Warm up for 5 minutes	1
B	WU end		1→2
C	REST start	Rest period for 5 minutes	2
D	REST end	5 minutes done, participant instructed to start pedaling as fast as possible	2
E	WG1 start	Weight basket drops, Wingate test starts (30 seconds)	2
F	WG1 end	Signals the start of bike transition	2→1
G	R1 start	Active Recovery period for 2 minutes	1
H	R1 end	Signals bike transfer, once seated participant was instructed to go	1→2
I	WG2 start	Weight basket drops, Wingate test starts (30 seconds)	2
J	WG2 end	Bike transfer	2→1
K	R2 start	Start of active Recovery period for 2 minutes	1
L	R2 end	2 minute mark, participant continues to pedal to ensure full Recovery before occlusion	1
M	3min	End of extra minute of Recovery for participants	1
N	Occlusion	Rapid inflation of cuff until plateau or 10 minutes	n/a
O	Release	Cuff pressure released, participant stays lying down for 2 minutes	n/a
P	Sit	Participant sits for 3 minutes	n/a
Q	Stand	Participant stands to ensure TSI% has achieved maximum	n/a
R	End test	Max TSI response confirmed, test complete	n/a

Note: Although a second Recovery period is detailed in this Table 1 (events K, L, M), it is not relevant to the research questions and was not considered for data analyses.

### 3.3.4 *Wingate tests and recovery*

Figure 3 provides a graphic representation of the chronological order of the experimental protocol events. Two cycle ergometers were used for this test: the warm up and Recovery cycle ergometer (Bike 1, Monark ergomedic 818E, Sweden) and the Wingate cycle ergometer (Bike 2, Monark, ergomedic 894Ea, Sweden). Both ergometers were positioned side by side in the laboratory setting. The seats were adjusted to a comfortable height for participants with slight knee flexion when the pedal was at the lowest point. On Bike 1, participants completed a 5 minute warm up at 60-80 rpm with resistance of 2.5% of body weight ( $25 \text{ g kg}^{-1}$ ), adjusted depending on participant preference (Table 1, Events A  $\rightarrow$  B). A 2-3 second sprint was completed the end of minutes 1 and 4. This protocol is similar to warm-up protocols employed by Armstrong, Welsman, & Chia (2001) and Beneke, Hütler, & Leithäuser (2007). After warm up, participants moved to Bike 2 for 5 minutes of rest (Table 1, Events C  $\rightarrow$  D). At the end of 5 minutes, participants were instructed to start pedaling as fast as possible. When pedaling frequency exceeded 100 rpm, a resistance of  $75 \text{ g kg}^{-1}$  of body weight was applied automatically by the ergometer system and the Wingate test began (Table 1, Event E). After 30 seconds (Table 1, Event F), the resistance was automatically removed and participants returned to Bike 1 as quickly as possible to begin the active Recovery session (Table 1, Event G), pedaling at a rate of 60-80 rpm for two minutes at resistance consistent with that used during the individual participant's warm up. At the end of two minutes (Table 1, Event H), participants quickly returned to Bike 2 and were instructed to begin a second Wingate (Table 1, Event I $\rightarrow$ J). Throughout the two Wingate tests, constant verbal encouragement was given to participants as they were maximally exerting themselves. Participants returned to Bike 1 after the second



were averaged for determination of an occlusion plateau (the last two minutes were within 2 % TSI for both).

The Girls were offered an iPod on which they could play games quietly during the occlusion process. If a Girl reported being unmanageably uncomfortable, the cuff pressure was reduced by 10mmHg at a time to a minimum of 190mmHg. Cuff pressure was rapidly released at TSI plateau or maximum time (Table 1, Event O). Participants remained in a supine position for 2 additional minutes, then sat on the mat with legs extended for 3 minutes (Table 1, Event P). Finally they were asked to stand (Table 1, Event Q). NIRS data were collected until it was confirmed that TSI had peaked and was dropping again to baseline (reperfusion maximum achieved; Table 1, Event R).

### **3.4 Data processing and analysis**

#### **3.4.1 Data processing**

Data for 20 Women and 13 Girls were analyzed for all variables except those involving occlusion comparisons in which case data for 20 Women and 12 Girls were analyzed.

##### a) Power output

Power output was normalized to fat free mass determined from the body composition measures and equations (section 3.3.2) and expressed as Watts per kilogram of fat free mass ( $\text{Watts} \cdot \text{kgFFM}^{-1}$ ). For each Wingate test, peak power, mean power, minimum power, and power drop, where power drop was calculated as the difference between peak and minimum power over one Wingate test.

## b) NIRS data analyses

Data were averaged over each second and divided into blocks representing the first Wingate test, the first Recovery period, the second Wingate test, occlusion, and reperfusion. Figure 3 provides a visual representation of this timeline.

The time it took to transfer between bikes was labelled XFER1 (Table 1, post WG1, bike 2→1) and XFER2 (Table 1, post Recovery, bike 1→2). The first transfer (XFER1) was included in the total Recovery period. For the purposes of this thesis, the second transfer (XFER2), occurring before the second Wingate test, was not included in the current results.

Magnitude of change, denoted as range, in TSI, HbO<sub>2</sub>, HHb, and tHB during the Wingate tests and Recovery period was determined as the difference between a given start and end value in each data block. For example, Wingate 1 Range was calculated as:

$$\text{WG1 Range} = \text{Resting steady state TSI} - \text{Minimum TSI during Wingate 1}$$

Percent change TSI (% $\Delta$ TSI) was calculated as:

$$\% \Delta \text{TSI} = ((\text{TSI start value} - \text{TSI end value}) / \text{TSI start value}) * 100$$

The starting TSI value for the Wingate tests was the average TSI of the last 120 seconds of the rest period before the first Wingate test. The end TSI value was the minimum TSI during each Wingate. To calculate percent change for the Recovery period, the starting value was the TSI at the start of Recovery, and end value was the average TSI the last 60 seconds of Recovery. This 60s period was visually determined as a plateau for TSI and therefore defined as a steady state.

The time constant of Recovery tau ( $\tau$ ) was the time in seconds it took for TSI, HbO<sub>2</sub>, HHB, and tHb to return to 63% of the 60s steady state during the Recovery.

$$\tau = ((60sSS - \text{min}) * 0.63) + \text{min}$$

### 3.4.2 *Statistical analyses*

A multivariate two-way repeated measures analysis was performed to test for the main effect of time (WG1 vs WG2), main effect of group (Women vs Girls), and interaction between time and group for the following variables:

1. Wingate performances: Peak power, mean power, minimum power and power drop
2. TSI: range, maximum, minimum, percent change, difference between occlusion minimum and WG minimum
3. HbO<sub>2</sub>, HHB, tHb, Hb $\Delta$ : range

Independent t tests were performed post hoc on differences in these variables between Girls and Women (group effect). Paired sample t tests were performed separately for Girls and Women to determine differences in these variables between Wingate1 and Wingate2 (time effect).

Independent t tests were performed to test for differences in the following Recovery variables:

1. TSI range, maximum, minimum, percent change, and time constant of Recovery ( $\tau$ )
2. HbO<sub>2</sub>, HHB, tHb, and Hb $\Delta$  range; HbO<sub>2</sub>, HHB, tHb time constant of recovery ( $\tau$ )

Taking group number differences into account, equal variance was assumed when the F statistic was not significant. Means and standard deviations (SD) are reported for all variables. SPSS

(IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp) was used for all statistical analyses. Alpha level was set at 0.05 (95% confidence interval).

## CHAPTER 4 RESULTS

### 4.1 Participant physical characteristics

Table 2 provides the physical characteristics of both groups of participants. All Girls self-assessed their level of maturity with a combined Tanner stage score of either 2 (n=9) or 3 (n=4). All Women were deemed fully mature based on attainment of menses. Height and weight were significantly greater in Women compared to Girls ( $p < 0.01$ ). There were no significant group differences in percent body fat percent although Women had significantly greater skinfold thickness at the site of NIRS device placement on the vastus lateralis muscle ( $p < 0.001$ ).

*Table 2. Physical Characteristics of Women and Girls*

	Women (n=20)		Girls (n=13)	
	Mean	SD	Mean	SD
<b>Height (cm)</b>	165.81	7.14	136.91~	6.40
<b>Weight (kg)</b>	62.17	8.24	30.52~	4.73
<b>Body fat percent (%)</b>	23.70	3.54	23.12	3.16
<b>Vastus lateralis skinfold (mm)</b>	19.14	5.07	13.17~	4.34

~ Significant group difference

### 4.2 Wingate power results

A significant group by time interaction existed for the Wingate test power results. As shown in Table 3, all measures of cycling power were significantly greater during the first Wingate test (Wingate 1) compared to the second Wingate test (Wingate 2) for both groups and greater in Women compared to Girls during both tests. Power drop was not significantly different between Wingate 1 and 2 for Women or Girls. Percent power Recovery ((Wingate 2 power/Wingate 1 power) \* 100) was 88% for Girls and 80% for Women but this difference was not significant ( $p > 0.05$ ).

Table 3. Mean (SD) Peak, Average, and Minimum Power, and Power Drop ( $\text{Watts} \cdot \text{kgFFM}^{-1}$ ) of Women and Girls during Wingate 1 and Wingate 2

	Wingate 1				Wingate 2			
	Peak	Mean	Min	Power drop	Peak	Mean	Min	Power drop
<b>Women</b>								
Mean	13.16	9.64	6.55	6.62	10.50*	7.55*	4.95*	5.55
SD	1.72	0.86	0.90	2.15	1.50	0.90	1.24	2.24
<b>Girls</b>								
Mean	10.43~	7.35~	3.86~	6.58	8.94*~^	6.30*~^	3.97*~^	4.97
SD	2.31	1.41	1.92	2.62	2.12	1.28	1.08	1.51

\* Significant test difference

~ Significant group differences

^ Significant interaction

## 4.3 NIRS Results

### 4.3.1 Repeated Wingate tests

At the start of the Wingate tests, Women were able to increase pedaling frequency to 100 rpm causing the weight basket to drop and the test to begin within a few seconds of being instructed to begin (“go”). Girls took significantly longer to reach the required pedaling rate of 100rpm to initiate the weight drop and start the Wingate test.

TSI dropped significantly for both groups in the time it took to increase pedaling frequency to the requisite 100rpm. Girls’ Wingate tests took longer in seconds than Women due to the “pre-Wingate” period that started at the command to “go” and ended with the weight basket dropping. Because of this, the TSI data were analyzed in two manners: 1) as 30 second Wingate tests with data collected once the 100 rpm set the resistance, and 2) as “total Wingate tests” which included the seconds before the official start of the test where TSI was dropping at least 1 percent per second for at least 2 seconds. Including data from the pre-Wingate period was more reflective of

the demand of the exercise task and therefore the “total Wingate” period is the primary measure considered in the following results.

### a) Tissue Saturation Index (TSI)

For both groups, the TSI response was the same throughout the exercise and Recovery bouts. TSI decreased rapidly from the command “go” achieving a minimum before the end of the Wingate test. TSI recovered in a logarithmic shape to a steady state lower than rest, and then decreased rapidly again during Wingate 2, arriving at minimum approximately halfway through the test ( $\approx 15$ s). Figure 5 shows TSI from a representative participant throughout the Wingate tests and Recovery.

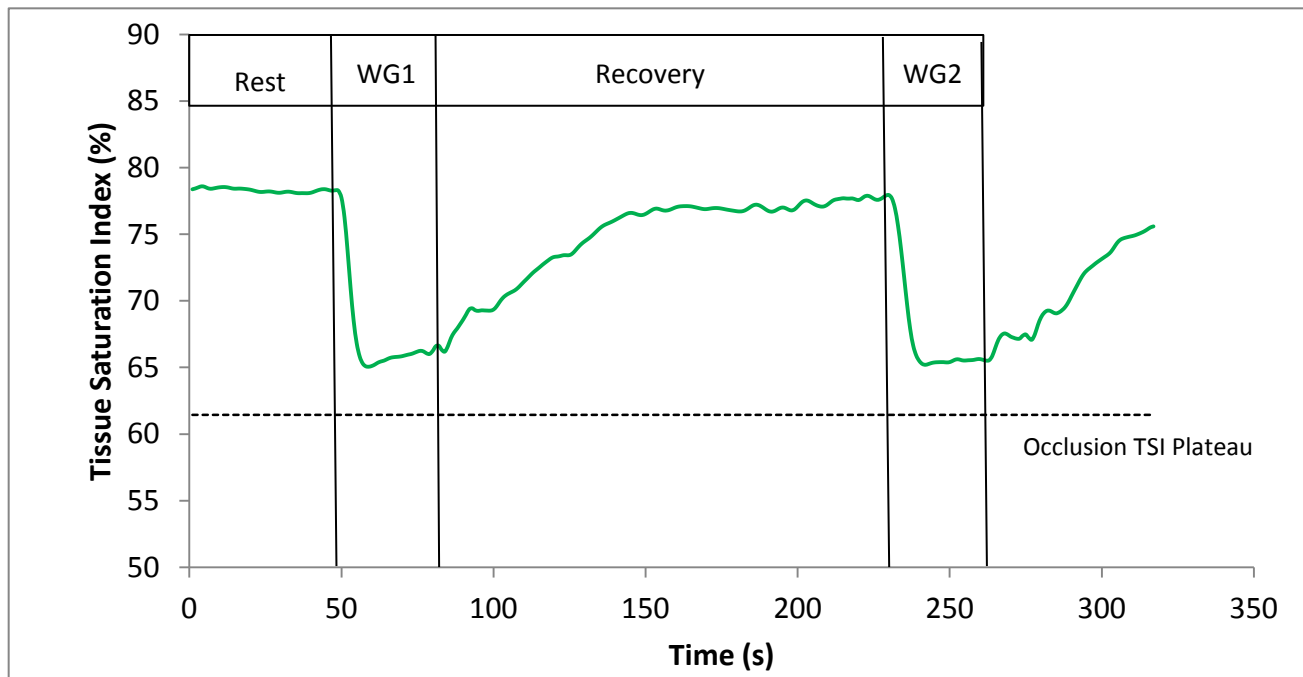


Figure 5. TSI of a representative participant during rest, Wingate 1 (WG1), Recovery, and Wingate 2 (WG2) with occlusion TSI plateau line included for reference.

Resting steady state TSI before Wingate was not significantly different between Women and Girls. Table 4 shows that there were no maturity group differences in TSI Range for either Wingate test but TSI Range was greater in Wingate 1 compared to Wingate 2 for both Women and Girls. Wingate 2 range was 51% and 52% of Wingate 1 range for Women and Girls respectively.

The starting TSI value for Wingate 2 (Recovery steady state) was significantly lower than the starting value TSI from Wingate 1 (resting steady state), but not different between Women and Girls. The lower starting point could account for the smaller observed range in Wingate 2, however Wingate 1 range was still significantly greater than Wingate 2 range even if the starting TSI difference was taken into account.

Table 4 shows TSI percent change was significantly greater in Wingate 1 than Wingate 2 with no group main effect found. Women's Wingate 1 minimum TSI was smaller than in Wingate 2 while minimum TSI was consistent for the Girls for both tests. No interactions existed for any variable.

*Table 4. Mean (SD) TSI results for Women and Girls during Wingate 1 and Wingate 2.*

	Wingate 1		Wingate 2	
	Women	Girls	Women	Girls
<b>Test Time (s)</b>	31.8 (1.15)	35.08 (3.30)~	30.90 (1.21)	35.77 (4.96)~
<b>TSI min (%)</b>	61.43 (6.02)	57.83 (4.80)	62.35 (5.25)*	58.60 (4.34)
<b>Range (%)</b>	17.60 (5.78)	20.38 (3.57)	8.91 (4.23)*	10.50 (5.50)*
<b>%Change</b>	21.33 (6.70)	25.10 (4.24)	20.23 (5.42)	24.19 (4.84)

Minimum TSI=TSI min, Time to minimum TSI=Time to min, TSI Range=Range, %Change=TSI Percent change

\* Significant test differences

~ Significant group differences

## b) Hemoglobin variables

The results provided in Table 5 demonstrate that during the Wingate tests, HbO<sub>2</sub> and tHb decreased while HHb increased with greater changes occurring in Wingate 1 compared to Wingate 2 for both groups.

Table 5. Mean (SD) NIRS Hemoglobin Range for Women and Girls during Wingate 1 and Wingate 2.

	Wingate 1		Wingate 2	
	Women	Girls	Women	Girls
<b>HbO<sub>2</sub></b>	12.06 (4.04)#	13.20 (3.8)	6.38 (2.70)*	8.58 (4.89)* #
<b>HHb</b>	7.16 (3.84)	12.20 (4.18)~	4.65 (4.12)*	6.10 (4.13)*^
<b>tHb</b>	6.42 (4.77)	3.42 (1.48)	3.14 (2.56)*	3.26 (1.66)^
<b>HbΔ</b>	4.90 (1.02)	1.00 (1.72)	1.73 (4.65)*	2.48 (1.94)*^

\* Significant test effect

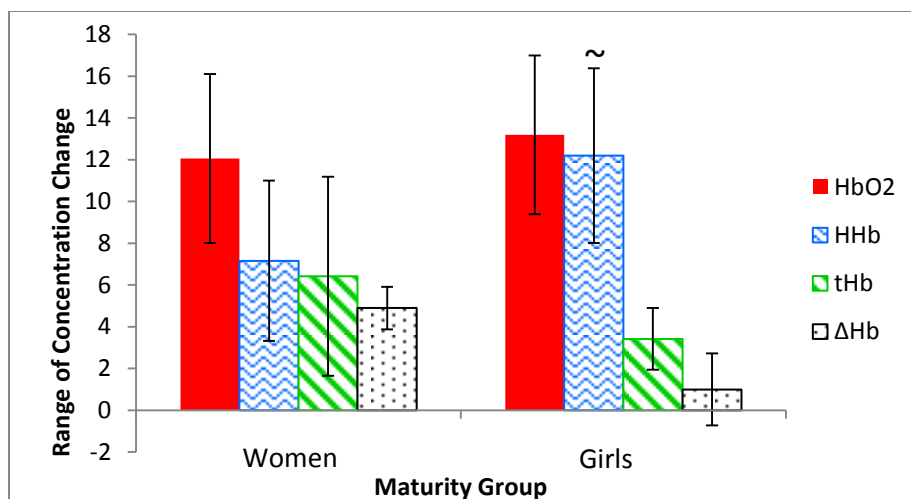
~ Significant group differences within same test

^ Significant interaction

# Significant difference between HbO<sub>2</sub> and HHb within group and test

Starting values of HbO<sub>2</sub> and tHb were higher in Wingate 1 compared to Wingate 2 in both maturity groups. Women and Girls had opposite changes in starting value for HHb from Wingate 1 to Wingate 2: For Women, HHb was lower before Wingate 2 but for Girls, HHb was lower before Wingate 1.

Figure 6 demonstrates the Hb responses for Wingate 1. No group differences in HbO<sub>2</sub> and tHb range existed for Wingate 1 or Wingate 2, but Girls demonstrated greater range in HHb in Wingate 1 compared to Women.



~ Significant group difference

*Figure 6.* Mean range of concentration change (SD) of HbO<sub>2</sub> HHb, tHb, and Hb difference during Wingate 1 in Women and Girls.

No group by test interaction existed for HbO<sub>2</sub> but Girls HHb range decreased more from Wingate 1 to Wingate 2 than in Women. Group by test interactions also existed for a) tHb: Women's tHb changed less and Girls tHb changed more in Wingate 2 compared to Wingate 1, and b) Hb difference: Hb difference was greater in Wingate 1 compared to Wingate 2 for Women while it was greater in Wingate 2 compared to Wingate 1 for Girls.

#### ***4.3.2 Active recovery post Wingate 1***

For analysis of Recovery, only data from the first transfer and the 2 minutes of Recovery pedaling between the two Wingate tests were considered. Recovery time was significantly longer (7.05 seconds) in Women compared to Girls but neither portion of Recovery (transfer and pedaling) was significantly different between groups. Average transfer time was 15.35 (8.2)s in Women and 10.85(5.44)s in Girls.

### a) Tissue Saturation Index

Recovery was characterized by the following variables: TSI range, TSI percent change, and the time constant of recovery ( $\tau$ ). While the time constant of recovery ( $\tau$ ) was significantly longer in Girls compared to Women, no other group differences were found for TSI in Recovery (Table 6).

*Table 6. Mean (SD) TSI Range, Percent change, and Time Constant of Recovery (Tau) during Recovery from Wingate 1 in Women and Girls*

	Women	Girls
<b>TSI Range (%)</b>	10.40 (4.30)	12.02 (4.84)
<b>Tau (s)</b>	20.35 (11.97)	37.15 (13.15)~
<b>TSI Percent Change</b>	-12.53 (9.68)	-17.3 (8.76)

~ Significant group difference (Women and Girls)

Based on the time constant of recovery ( $\tau$ ), two different profiles of Recovery were observed and are represented in Figure 7. Profile A is characterized by a faster  $\tau$ , occurring within the time it took to transfer to the Recovery bike. In all instances where this occurred,  $\tau$  was less than 15s. Profile B is characterized by a  $\tau$  greater than 15s, occurring once pedaling Recovery had begun. All Girls were classified as Profile B while the Women were divided: 7 Women had the characteristics of Profile A and 13 Women were Profile B.  $\tau$  was significantly greater in women with profile B compared to A, but women with profile B still had significantly shorter  $\tau$  compared to Girls (who all had the B profile). Follow up analysis comparing Women with Profile A and B, revealed that women with a shorter recovery time (Profile A) had significantly lower mean power output and a higher fatigue index in Wingate 2 compared to Profile B women who had a slower recovery time.

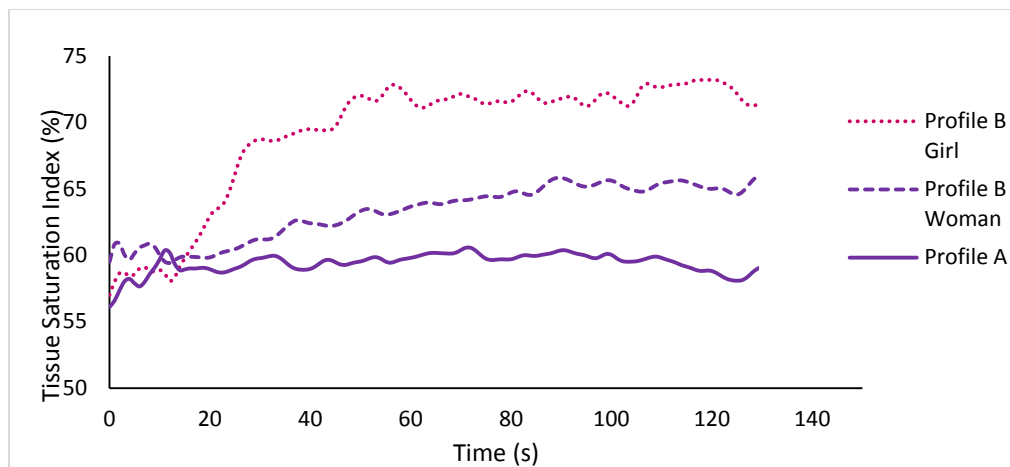
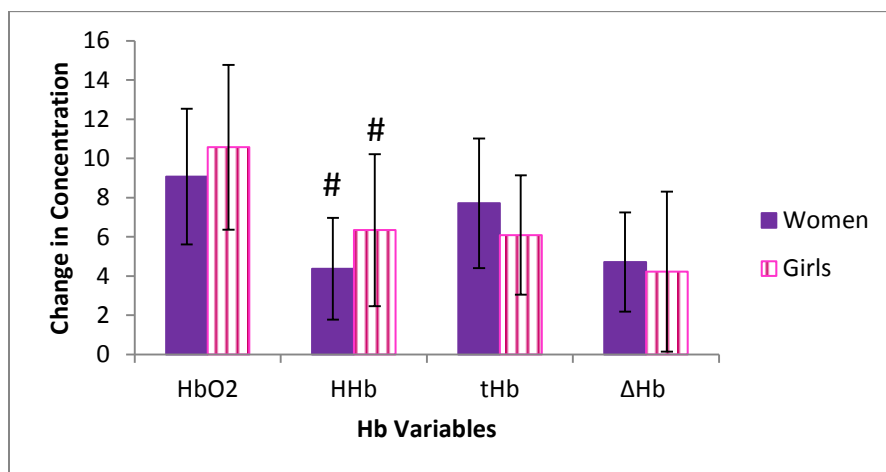


Figure 7. TSI Recovery Profiles A (Woman) and B (Woman and Girl example) from representative participants

### b) Hemoglobin variables

Figure 8 shows that during Recovery,  $\text{HbO}_2$  and tHb increased while HHb decreased. No group differences existed for Recovery range of any one hemoglobin variable, but  $\text{HbO}_2$  increased significantly more than HHb decreased in Women and Girls.



# Significant difference between  $\text{HbO}_2$  and HHb within group

Figure 8. Mean range of concentration change (SD) of hemoglobin variables during recovery in Women and Girls.

Table 7 shows that while HHb recovered significantly more slowly in Girls compared to Women, no group differences for the time constant of recovery ( $\tau$ ) were observed for HbO<sub>2</sub> and tHb.

*Table 7. Group Mean (SD) for the Time Constant of Recovery of HbO<sub>2</sub>, HHb, and tHb in Women and Girls*

	Tau	
	Women	Girls
<b>HbO<sub>2</sub></b>	25.75 (19.34)	36.92 (14.53)
<b>HHb</b>	13.6 (10.44)	30.77 (19.47)~
<b>tHb</b>	24.25 (26.00)	17.69 (16.64)

~ Significant group differences

### **4.3.3 Occlusion TSI**

To determine the degree TSI decreased during exercise relative to a resting state with no blood flow, TSI values from the occlusion protocol were compared to the minimum TSI recorded from each Wingate test. The occlusion TSI value chosen for this comparison was the average TSI of the observed TSI plateau. Other occlusion TSI values that could be considered for this comparison were a) the real-time plateau determined visually in the lab with a stopwatch, b) the minimum TSI value of the plateau period, and c) the minimum value from the entire occlusion period. When all TSI values during occlusion were compared to the minimum TSI values from the Wingate tests no differences were found. Therefore the average TSI during occlusion plateau was chosen to represent occlusion plateau TSI.

As represented in Table 8, Wingate minimum TSI values in both groups were significantly greater than those observed during the occlusion protocol but there was no group main effect. Women demonstrated a smaller difference between Occlusion TSI and Wingate 1 minimum TSI compared to Wingate 2, while for girls the difference between the Occlusion TSI and Wingate minimum TSI were consistent across both Wingate tests.

*Table 8. Mean (SD) TSI Minimums during Occlusion (OCC), and Each Wingate Test (WG1min and WG2min) and Difference between OCC and WG Minimums (OCC WG  $\Delta$ )*

	<b>Women (n=20)</b>	<b>Girls (n=12)</b>
<b>OCC TSI (%)</b>	56.14 (5.09)	54.02 (4.03)
<b>WG1 min TSI (%)</b>	61.43 (6.02)^	57.83 (4.80)^
<b>WG2 min TSI (%)</b>	62.35 (5.25)*^	58.60 (4.34)^
<b>OCC WG1 <math>\Delta</math></b>	5.30 (3.00)	3.98 (2.93)
<b>OCC WG2 <math>\Delta</math></b>	6.22 (2.53)*	4.65 (2.03)

^ Significantly different from OCC TSI

\* Significant test effect

## CHAPTER 5 DISCUSSION

This study examined maturational differences in tissue oxygenation during high intensity interval exercise, providing new evidence to consider in the discussion of maturational differences in energy metabolism and contributing to the growing body of research using NIRS technology. The differences in tissue oxygenation responses observed between Women and Girls' support a greater aerobic metabolic contribution to high intensity exercise in Girls relative to Women.

### 5.1 Maturational differences in anaerobic power output

As hypothesized, the Women produced greater peak and mean power during both Wingate tests, but Girls were better able to maintain their power output in the second Wingate test following two minutes of active recovery. These results were expected based on previous research by Hebestreit, Mimura, & Bar-Or (1993) who found that power output was higher in men but repeat power performance was superior in prepubescent boys during two 30 second Wingate tests separated a similar recovery protocol. Chia (2001) used found that peak power output was greater in Women during 3x 15s Wingate tests separated by 45 seconds of active recovery compared to Girls who had better power recovery (the ratio of power output from the second and third Wingate tests to power output from the first Wingate test).

Because children have less muscle mass relative to lean body mass compared to adults (Tonson, Ratel, Fur, Cozzone, & Bendahan, 2008), there is some criticism of using the same ergometer brake force based on body mass for the Wingate test comparing maturational groups. Doré, Bedu, França & Van Praagh, (2001) compared different brake forces during all out cycle sprints to maximum velocity and found that Women demonstrated greater power output than

prepubescent Girls at 2.5%, 5%, and 7.5% body weight resistance. The authors state that 7.5% resistance is too great to achieve peak power in Girls however the power output results of the present study are comparable to the highest power output results of the study by Doré, Bedu, França, & Van Praagh, (2001) at the suggested 5.0% body weight resistance. This provides supporting evidence that the Wingate resistance chosen in this current study was appropriate to elicit maximum power output in Girls as well as Women. Further support comes from a study by Carlson and Naughton (1994) who compared Wingate performance of children with brake forces ranging from 40 to 80 grams per kilogram of body mass ( $\text{g}\cdot\text{kgBM}^{-1}$ ), and found that higher resistances yielded better power results and these results were not different between 65, 75, and  $85\text{g}\cdot\text{kgBM}^{-1}$  of brake force.

## **5.2 Maturation differences in tissue oxygenation**

Due to limited research in this area there is no strong evidence-based position on maturational differences in tissue oxygenation during exercise and recovery, especially in females. As a result, it was hypothesized that there would be no differences in the tissue oxygenation response between Girls and Women during the anaerobic HIIE protocol used. However, this hypothesis was not supported by the results as significant differences in the tissue oxygenation response were found to exist between Women and Girls during both exercise and recovery. To describe the tissue oxygenation response to exercise, research with NIRS commonly reports the measures of TSI and HHb. TSI reflects the balance of oxygen delivery and oxygen consumption while HHb reflects aerobic metabolic activity (true measurement of oxidative metabolism is indirect and can only occur in an occluded state) (Ferrari et al., 2004).

Previous NIRS-based research comparing maturational differences during exercise has compared males and females (Fulford et al., 2008; Willcocks et al., 2010) and males only

(Leclair et al., 2013). Participants in Fulford's and Leclair's studies were of similar age and maturity status as those in the current study (Tanner stage  $\leq 3$ , 9-11 years), but participants in Willcock's study were 13 +/- 1 years and the authors suggest that they were unlikely prepubertal. These studies have consistently found faster HHb kinetics during exercise in children compared with adults. A faster mean HHb response time was reported in children compared to adults during single joint heavy intensity (Willcocks et al., 2010), single joint moderate intensity (Fulford et al., 2008) and moderate intensity cycling exercise (Leclair et al., 2013). If a greater rate of HHb appearance is related to accelerated oxygen consumption in children, then these results (Willcocks et al., 2010) suggest that aerobic metabolism is more active in children during high intensity exercise. Willcocks et al. (2010) interpret the faster observed decrease in HHb at the onset of exercise as a superior matching of oxygen delivery and consumption in adults. The authors relate this to faster oxygen extraction in adults. Fulford et al. (2008) drew the same conclusions as Willcocks et al. (2010) but the exercise task intensity in their study was considered moderate intensity (80% of the Pi/PCr intracellular threshold). During such moderate intensity exercise, ATP production is mostly aerobic (Gastin, 2001) and therefore it would be unexpected for either group to demonstrate a greater overall aerobic contribution to total work. It is possible that children have a greater rate of aerobic metabolism compared to adults: during moderate intensity cycling exercise, Leclair et al. (2013) attribute the faster HHb kinetics observed in children to a faster oxygen extraction at the active muscle. It is also possible that the exercise intensity in these studies was high enough to elicit a different metabolic response in children and adults.

For the present study, tissue oxygenation kinetics (response time, time constants) were not calculated during the Wingate tests. This was because the "pre-Wingate" period resulted in a

significant decrease in TSI and this time period was different between the two maturity groups. The variables were considered in terms of the entire exercise task and not once the weight basket dropped as it would have been inaccurate to calculate rate of change without a consistent exercise task duration.

Other measures of metabolism during high intensity exercise and recovery have revealed that children exhibit greater reliance on aerobic metabolism than adults (Armon et al., 1991; Beneke et al., 2007; Hebestreit et al., 1998, 1993). The variables collected and reported in this study, including TSI, HbO<sub>2</sub>, HHb, and tHb, do not directly measure aerobic metabolism but they do represent the relationships that exist between aerobic metabolism and blood flow through the active muscle. Comparing the changes in these variables between Women and Girls in the context of what is known about maturational differences in blood flow during high intensity cycling exercise, and post exercise recovery, can provide some insight into aerobic metabolism.

### ***5.2.1 Wingate tests***

The results of this study demonstrate that the Girls relied more on aerobic metabolism compared to Women during the Wingate tests. Previously, children have been reported to have greater blood flow during maximal cycle exercise compared to adults (Koch, 1974). These differences have been related to greater capillarization and less lung to muscle transit time in children compared with adults (Falk & Dotan, 2006), facilitating oxygen delivery to muscles during exercise. In addition to facilitated oxygen delivery, greater aerobic enzyme concentrations (Berg, Kim, & Keul, 1986) and greater mitochondrial density (Bell, MacDougall, Billeter, & Howald, 1980) may contribute to children's relatively larger aerobic potential, however the relative contribution of each remains unclear (Ratel et al., 2006).

The greater leg volume in Women, compared to Girls, may also restrict vessels to a greater degree during the maximal exercise compared to children further supporting facilitated flow in children during exercise. Results of this study show a greater reduction in HbO<sub>2</sub> than HHb increase for Women during Wingate 1 supporting evidence that blood flow is more of a limiting factor for Women compared to Girls during this type of exercise. Consequently, the greater HHb appearance observed in Girls during Wingate 1 is unlikely due to a greater restriction of blood flow but due to greater oxygen consumption driving greater oxy-hemoglobin desaturation at the working muscle level in children. This was the only group difference observed from the Hb results, and provides novel evidence of greater aerobic muscle metabolism in children during a bout of maximal exercise.

Both maturity groups demonstrated an attenuated tissue oxygenation response in the second Wingate test. Considered alone, Wingate 2 tissue oxygenation response was not different between the maturity groups, however from Wingate 1 to Wingate 2, Girls demonstrated a more consistent TSI minimum compared to Women. This response is confirmed by comparing TSI minimum values during the Wingate tests to minimum TSI from the occlusion protocol. Girls' TSI minimum decreased to the same degree of Occlusion TSI for Wingate 1 and 2, while Women's TSI minimum did come as close to Occlusion TSI in Wingate 2.

Although TSI minimum was not different between groups, TSI decrease to minimum appears to be more strongly influenced by HHb in Girls in Wingate 1 compared to Women, evidenced by the smaller Hb difference. In Wingate 2, Women's Hb difference is significantly smaller than in Wingate 1, more similar to the Girls' response, providing evidence that Women were relied more on oxidative mechanisms of ATP production during Wingate 2. This is parallel to the greater

significant reduction in peak and mean power output in Women compared to Girls from Wingate 1 to Wingate 2.

Girls' Hb difference actually increased in Wingate 2 but it is likely due to the fact that HHb was more elevated during recovery and had less potential for change compared to HbO<sub>2</sub>. Since TSI minimums are not different between maturity groups, the more consistent response in Girls must be related to the process of TSI decrease and associated Hb variable changes. The results of the present study support the fact that blood flow and/or aerobic metabolism are more consistent in Girls during a second Wingate test compared to Women.

### *5.2.2 Active recovery*

Previous NIRS research has not examined maturational differences in the recovery response from high intensity exercise. Aerobic metabolism provides the necessary energy needed to support post-exercise recovery by way of restoring resting concentrations of energy substrates, supporting the lactate and creatine phosphate shuttles, and clearing exercise induced metabolic byproducts such as lactate (Brooks et al., 2005). In terms of tissue oxygenation, the recovery response observed in the current study did not appear to be influenced by maturity status with the exception of faster TSI and HHb recovery in Women, which was unexpected. Previous research examining maturational differences in recovery of males reported that boys' heart rate and ventilation rate recovered more quickly compared to men following a Wingate test (Hebestreit et al., 1993). PCr recovery time, often used to quantify oxidative capacity, has been observed to be faster in children compared to adults following dynamic moderate intensity plantar flexion exercise (Kappenstein et al., 2013), isometric incremental plantar flexion (Taylor et al., 1997), and dynamic moderate intensity finger flexion exercise (Ratel et al., 2008; Tonson et al., 2010). A faster recovery of these variables may reflect a faster consumption of available oxygen which

could explain a greater presence of HHb in children during recovery from Wingate 1. Faster HHb recovery in Women could also be related to a faster rate of HHb clearance by way of an enhanced post-exercise blood flow. A post exercise hyperemic response is directly related to the strength of muscular contraction (Arai & Endoh, 1974) and since Women demonstrated greater power output relative to fat free mass, this could explain a faster HHb recovery response.

Within the Women there appeared to be two different recovery profiles. Profile B women demonstrated a slower tau closer to what the Girls tau was although still significantly faster. As expected with a slower tau, these women had better performance in Wingate 2 compared to Profile A women who had a faster tau. These differences are potentially related to fitness level, as aerobic fitness would influence blood flow and metabolism post-exercise. Fiber type may also play a role; those with more type I fibers would theoretically have greater capillarization, enhancing recovery. This can only be speculated however, since fitness was not measured, nor was fiber type examined in this study. Applying these speculations to child/adult differences is challenging. The only study to compare vastus lateralis muscle fiber type from between children and adults observed an overall decrease in percentage of type I fibers from 0 to 20 years of age, however the percentage of type I muscle fibers was not different between children 6-10 years and adults (Oertel, 1988). Two separate reviews on the topic have concluded that while changes in muscle fiber type appear to exist with age and maturity, there is insufficient evidence to conclude that children have a greater percentage of type I fibers compared to adults (Armstrong, Barker, & McManus, 2015; Ratel et al., 2006). Another possible explanation is that children do not fully recruit type II fibers during maximal exercise (Ratel et al., 2006). A recent study by Pitt et al. (2015) found that boys' EMG threshold (indicating recruitment of type II fibers) occurred

at a higher percentage of peak power during an incremental test to exhaustion compared to men, suggesting less use of type II fibers for higher intensity exercise.

In both maturity groups, aerobic metabolism remained elevated during recovery which was expected since recovery involved active low intensity cycling. This was reflected by the lower HHb disappearance compared to HbO<sub>2</sub> appearance. Overall both groups recovered to the same degree in terms of tissue oxygenation but Women had a faster initial tissue oxygenation recovery response compared to Girls following high intensity exercise.

Because Wingate 2 performance was more significantly reduced in Women, it could be that while the groups were comparably recovered in terms of tissue oxygenation, they were not in terms of other measures such as anaerobic energy sources. Previous research found that adults have a greater reduction in PCr concentration during high intensity exercise compared to children (Kappenstein et al., 2013), which could indicate that adults need longer to recover. These findings, considered along with the results from the present study, support the argument that PCr is not as important for power production in Girls, pointing to a greater reliance on aerobic metabolism for power production.

#### **5.4 Conclusion**

The results of this study show that maturational differences exist in female participants for power output and muscle tissue oxygenation during two repeated Wingate tests separated 2 minutes of active recovery. Repeat power performance appears to be superior in Girls due to a greater aerobic contribution to power output during exercise. Presumably, the Women in this study were unable to recover the anaerobic energy stores they needed to rely on for greater power output in the second Wingate test, but this was not as much of a problem in the

prepubescent Girls: their greater reliance on aerobic metabolism for power production means they did not have as much to recover in terms of energy substrates. There was no evidence of faster or better recovery in terms of the tissue oxygenation response.

It is clear from this study that relying on TSI to represent muscle tissue oxygenation response during exercise may lead to misinterpretation of results. The importance of examining Hb variables when interpreting NIRS data should be disseminated to the research community. Future research could examine the tissue oxygenation response in children and adults during shorter duration maximal efforts which are more sport specific. In addition to NIRS variables, measures of anaerobic metabolism could be included to provide a more comprehensive understanding of maturational differences in exercise metabolism. Blood flow measures could also be included to strengthen the metabolic interpretation of peripheral Hb changes. Finally, it would be interesting to compare the results of this study with the tissue oxygenation response during high intensity exercise at the same relative power output or same total work in children and adults.

This research offers a strong starting point to further examination of maturational differences in the peripheral exercise metabolism during HIIE. More research is required to determine exact recovery processes and requirements however these results could serve to inform different training protocols for children and adults to optimize athletic performance.

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## **Appendix 1: Inclusion Criteria**

### Women

- Must be in Victoria for the lab session.
- Must be 19-30 years old
- Must not be pregnant
- Must be regularly menstruating or would be in the absence of exogenous hormone intake
- Must participate in a minimum of 2.5 hours a week of moderate to vigorous physical activity

### Girls

- Must be in Victoria for the lab session.
- Must be at least 8 years old.
- Must be prepubescent (Parents/guardians asked to estimate based on the information that prepubescent would mean no evidence of breast or pubic hair development).
- Must participate in moderate to vigorous physical activity at least 60 minutes a day.
- Must be familiar with how to ride a bike.

## **Appendix 2: Pre Testing Directions**

1. No food or drink (except water) within 2 hours of coming to the lab
2. No caffeine within 6 hours of coming to the lab
3. Refrain from hard physical activity within 12 hours of coming to the lab
4. Wear running shoes and athletic wear, preferably shorts

## Appendix 3: ParQ+ forms

CSEP approved Sept 12 2011 version

# PAR-Q+

## The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

### SECTION 1 - GENERAL HEALTH

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	<input type="checkbox"/>	<input type="checkbox"/>
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Are you currently taking prescribed medications for a chronic medical condition?	<input type="checkbox"/>	<input type="checkbox"/>
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	<input type="checkbox"/>	<input type="checkbox"/>
7.	Has your doctor ever said that you should only do medically supervised physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

If you answered NO to all of the questions above, you are cleared for physical activity.



Go to Section 3 to sign the form. You do not need to complete Section 2.

- › Start becoming much more physically active – start slowly and build up gradually.
- › Follow the Canadian Physical Activity Guidelines for your age ([www.csep.ca/guidelines](http://www.csep.ca/guidelines)).
- › You may take part in a health and fitness appraisal.
- › If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist\* (CSEP-CEP) or CSEP Certified Personal Trainer\* (CSEP-CPT).
- › If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.



If you answered YES to one or more of the questions above, please GO TO SECTION 2.



Delay becoming more active if:

- › You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- › You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- › Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

## SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?	<input type="checkbox"/> If yes, answer questions 1a-1c	<input type="checkbox"/> If no, go to question 2
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	<input type="checkbox"/>	<input type="checkbox"/>
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you have Cancer of any kind?	<input type="checkbox"/> If yes, answer questions 2a-2b	<input type="checkbox"/> If no, go to question 3
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	<input type="checkbox"/>	<input type="checkbox"/>
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm	<input type="checkbox"/> If yes, answer questions 3a-3e	<input type="checkbox"/> If no, go to question 4
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	<input type="checkbox"/>	<input type="checkbox"/>
3c.	Do you have chronic heart failure?	<input type="checkbox"/>	<input type="checkbox"/>
3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	<input type="checkbox"/>	<input type="checkbox"/>
3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	<input type="checkbox"/>	<input type="checkbox"/>
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes	<input type="checkbox"/> If yes, answer questions 4a-4c	<input type="checkbox"/> If no, go to question 5
4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	<input type="checkbox"/>	<input type="checkbox"/>
4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	<input type="checkbox"/>	<input type="checkbox"/>
4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)	<input type="checkbox"/> If yes, answer questions 5a-5b	<input type="checkbox"/> If no, go to question 6
5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
5b.	Do you also have back problems affecting nerves or muscles?	<input type="checkbox"/>	<input type="checkbox"/>

Please read the questions below carefully and answer each one honestly: check YES or NO.		YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure	<input type="checkbox"/> If yes, answer questions 6a-6d	<input type="checkbox"/> If no, go to question 7
6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	<input type="checkbox"/>	<input type="checkbox"/>
6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	<input type="checkbox"/>	<input type="checkbox"/>
6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	<input type="checkbox"/>	<input type="checkbox"/>
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia	<input type="checkbox"/> If yes, answer questions 7a-7c	<input type="checkbox"/> If no, go to question 8
7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	<input type="checkbox"/>	<input type="checkbox"/>
7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	<input type="checkbox"/>	<input type="checkbox"/>
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event	<input type="checkbox"/> If yes, answer questions 8a-c	<input type="checkbox"/> If no, go to question 9
8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	<input type="checkbox"/>	<input type="checkbox"/>
8b.	Do you have any impairment in walking or mobility?	<input type="checkbox"/>	<input type="checkbox"/>
8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	<input type="checkbox"/>	<input type="checkbox"/>
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?	<input type="checkbox"/> If yes, answer questions 9a-c	<input type="checkbox"/> If no, read the advice on page 4
9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	<input type="checkbox"/>	<input type="checkbox"/>
9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	<input type="checkbox"/>	<input type="checkbox"/>
9c.	Do you currently live with two chronic conditions?	<input type="checkbox"/>	<input type="checkbox"/>

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

## PAR-Q+



If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- › It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- › You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- › As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- › If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.



If you answered YES to one or more of the follow-up questions about your medical condition:

- › You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.



Delay becoming more active if:

- › You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- › You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- › Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

### SECTION 3 - DECLARATION

- › You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- › The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- › If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- › Please read and sign the declaration below:

*I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.*

NAME \_\_\_\_\_ DATE \_\_\_\_\_

SIGNATURE \_\_\_\_\_ WITNESS \_\_\_\_\_

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER \_\_\_\_\_

For more information, please contact:  
Canadian Society for Exercise Physiology  
[www.csep.ca](http://www.csep.ca)

#### KEY REFERENCES

1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation; background and overall process. APNM 36(51):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(51):S266-S298, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.



## Appendix 4: Letters of Informed Consent



University of Victoria | School of Exercise Science,  
Physical & Health Education

### Participant Consent Form: Women

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#### **Muscle oxygen kinetics at the vastus lateralis muscle during anaerobic cycling and recovery in women and girls**

You are invited to participate in a study entitled **Muscle oxygen kinetics at the vastus lateralis muscle during anaerobic cycling and recovery in women and girls** that is being conducted by Emily R. Medd (MSc. Kinesiology Candidate). Emily is a graduate student in the department of Exercise Science, Physical and Health Education at the University of Victoria and you may contact her if you have further questions by phone: 250-508-9489, or email: [ermedd@uvic.ca](mailto:ermedd@uvic.ca).

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

#### **Purpose and Objectives**

The purpose of this research project is to examine maturational differences in muscle metabolism during intervals of high intensity cycling exercise and recovery. Specifically, muscle tissue oxygenation during exercise and recovery will be compared in women and girls.

#### **Importance of this Research**

Research of this type is important because relatively little exercise physiology research has been conducted in females, especially comparing children and adults. This study will contribute to the evidence on maturational differences in muscle metabolism improving the understanding of human physiology which could benefit medical practices. As well, improved understanding of metabolism during high intensity exercise and recovery in women and girls could inform sport and athletic training programs making them safer and more effective.

#### **Participants Selection**

You are being asked to participate in this study because you are a healthy, non-pregnant, eumenorrheic woman aged 19-30 years old who is active at least 150 minutes a week at a moderate to vigorous intensity, but not a trained cyclist.

#### **What is involved**

This study involves the following procedures:

- Brief questionnaire (menstrual cycle stage, birth control, activity level)
- Height, weight, and body composition
- High intensity interval cycling exercise with recovery periods (8 mins)
- Leg occlusion (10 mins)

If you consent to voluntarily participate in this research, your participation will include one visit to the exercise physiology laboratory at the university of Victoria (McKinnon 171) lasting approximately 2 hours. You will be asked to refrain from vigorous physical activity for 24 hours before coming into the lab, not to smoke or drink caffeine 6 hours before coming into the lab, and not to eat 2 hours before coming into the lab. When you arrive at the lab, you will be required to answer a questionnaire on your menstrual cycle and birth control (if applicable) and your activity level. The questions regarding your menstrual cycle and birth control will give us some information on your hormonal profile at the time of data collection. Next, your height, weight, and skinfolds will be measured. Skinfold measures will be taken at 5 sites on the body by the principle investigator (who is female) in a secluded area of the lab. Skinfolds must be taken to estimate body composition which will allow for a better comparison of power output (relative to fat free mass) between women and girls.

You will then be fitted with a heart rate monitor and a near infrared spectroscopy (NIRS) device will be secured to the lower thigh with athletic tape. The NIRS measures tissue oxygenation of your quadriceps muscle. You will be familiarized with the exercise test protocol on the cycle ergometer and the occlusion protocol with the blood pressure cuff on the leg. The exercise test involves a 5 minute warm up followed by two 30 second sprints separated by 2 minutes of recovery cycling. These sprints are called "Wingate tests" during which you pedal as fast as possible against a resistance set based on body weight. There is no minimum expected work requirement beyond asking you to do your best during each Wingate. The total duration of the exercise test including rest and warm up will be less than 20 minutes. Following the exercise test, you will sit down and the blood pressure cuff will be inflated to a maximum of 250mmhg for no longer than 10 minutes, following standard occlusion protocol. You will be offered an iPod to play games to help pass the time. This procedure will be used as a means of significantly reducing blood flow to the thigh, in order to determine if any additional oxygen was available following the exercise. This procedure is necessary to allow for comparison of tissue oxygenation changes during the exercise test between women and girls. The occlusion protocol including rest and recovery will take no longer than 25 minutes.

### **Inconvenience**

Participation in this study may cause some inconvenience to you, including potential physical discomfort during the exercise test related to maximal physical effort, as well as during leg occlusion. You will be asked not to eat 2 hours before coming in to the lab and not to ingest any caffeine 6 hours before coming in. You will also be asked not to participate in any vigorous physical activity for 24 hours before coming in to the lab. You will be provided with a parking pass so you will not have to pay to come in to the lab.

**Risks**

There are some potential risks to you by participating in this research and they include physical stress and fatigue and a low risk of injury during the exercise test. You may also experience discomfort during the leg occlusion.

To prevent or to deal with these risks the following steps will be taken:

- a) You will fill out a physical activity readiness questionnaire (Par-Q+) to ensure it is safe for you to participate in the exercise test.
- b) All testing will be conducted by the principle investigator a certified exercise physiologist (Canadian Society of Exercise Physiology).
- c) You will be familiarized with the equipment and protocol to minimize potential discomfort and the risk of injury. All methods will be explained and demonstrated so you understand what will happen during data collection.

**Benefits**

The potential benefits of your participation in this research include experiencing fitness tests employed with elite and Olympic athletes. As well, your results will contribute to an improved understanding of maturational differences in exercise metabolism benefiting the sport and medical communities.

**Voluntary Participation**

Your participation in this research must be completely voluntary. If you do decide to participate, you may withdraw at any time without judgment or repercussion. If you do withdraw from the study your data will be disposed of and not used in any analysis.

**Anonymity**

Due to the nature and location of the study, your anonymity cannot be fully protected during data collection. All data collected will be saved under your participant code and analyzed under this code. 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 participant code so that your data will not be identifiable to anyone other than the principle 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 the following ways: a written thesis, oral presentations, and published in a peer-reviewed academic journal.

**Disposal of Data**

Data from this study will be disposed of within five years of study completion. Electronic data will be permanently erased and paper copies will be shredded.

**Contacts**

Individuals that may be contacted regarding this study include:

- a) Emily Medd, Principle Investigator  
Phone: 250-508-9489  
Email: [ermedd@uvic.ca](mailto:ermedd@uvic.ca)
  
- b) Dr. Kathy Gaul, Supervisor  
Phone: 250-721-8380  
Email: [kgaul@uvic.ca](mailto:kgaul@uvic.ca).

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](mailto: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.

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*Name of Participant*

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*Signature*

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*Date*

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

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**Muscle oxygen kinetics at the vastus lateralis muscle during anaerobic cycling and recovery  
in women and girls**

You are invited to participate in a study entitled **Muscle oxygen kinetics at the vastus lateralis muscle during anaerobic cycling and recovery in women and girls** that is being conducted by Emily R. Medd (MSc. Kinesiology Candidate). Emily is a graduate student in the department of Exercise Science, Physical and Health Education at the University of Victoria and you may contact her if you have further questions by phone: 250-508-9489, or email: [ermedd@uvic.ca](mailto:ermedd@uvic.ca).

As a graduate student, I must do a research study as part of the requirements for a degree in Master of Science in kinesiology. My study is being supervised by Dr. Kathy Gaul. You can contact my supervisor at 250-721-8380 (local: 8380) or [kgaul@uvic.ca](mailto:kgaul@uvic.ca).

### **Purpose and Objectives**

The purpose of this research project is to see what is happening in your muscles when you are exercising really hard and when you are resting after exercise. We want to see if what happens in your muscles is different from what happens in an adult's muscle.

### **Importance of this Research**

Research of this type is important because not much is known about females' muscles during exercise and recovery, especially comparing kids and adults. The results from my study could help inform sports training and/or medical practices.

### **Participants Selection**

You are being asked to participate in this study because you are an active, healthy girl, who has not gone through puberty.

### **What is involved**

If you agree to voluntarily participate in this research, you will come to the exercise physiology laboratory at the University of Victoria (McKinnon 171) for around 2 hours with your parent/guardian. You will be asked not to do any intense exercise for the day before coming into the lab and not to eat 2 hours before coming into the lab. When you arrive, you will be asked some questions about yourself. One of these questions is a self-assessment of maturity based on the tanner scale. These are drawings girls and women's bodies in the 5 stages of breast and pubic hair development. With your parent/guardians help, you will choose which picture you think your body is closest too. All you have to do is write the number that goes with the drawing you choose down on the piece of paper, you don't have to tell us out loud. I understand this might be a little uncomfortable but it is very important for my research to have prepubescent girls participate and so we have to include this question.

Next, we will measure your height, weight, and skinfolds. The principle investigator (who is a woman) will measure skinfolds by measuring folds of skin at 3 sites on the body (back of the arm, shoulder blade, and top of the thigh). It can be a little bit ticklish but you can try it out first to see what it is like. Skinfold measures give us an estimate of what your body is made up of. This will help us with comparing your power output during the exercise test with a woman's power output. A heart rate monitor will be secured to your chest and a near infrared spectroscopy device will be taped to your lower thigh to measure the oxygen in your muscle. We will then explain the exercise test and make sure the bike fits you properly. After this, we will explain and demonstrate the occlusion procedure. Occlusion means blocking off; we will be blocking off the blood flow to your leg using a pressure cuff filled with air. You will get to try out the leg pressure cuff to see what it feels like.

The exercise test involves a 5 minute warm up followed by two 30 second maximum sprints with 2-3 minutes of cycling recovery. The maximum intensity sprints are called "Wingate" tests which are completely safe and reliable fitness tests with children the Wingate test involves the participant pedaling as fast as they can against resistance set based on body weight. The exercise test (including the warm up) will be less than 20 minutes.

After the exercise test, you will sit down and we will inflate the leg pressure cuff and keep the pressure on for no more than 10 minutes. You will have iPod games to play to help pass the time. After the pressure is released you will stay sitting down for another 5 minutes. The reason we are putting pressure on your leg is to try and see what the minimum amount of oxygen in your leg is (during the occlusion) and the maximum amount of oxygen that can be delivered to your muscles (right after the pressure cuff is released). We need this information to help us compare girls' data with the women's data. This part of the experiment will take no longer than 25 minutes. After that we will take all the instruments off of you and you can go home!

### **Inconvenience**

Participation in this study may cause some inconvenience to you, including feeling tired from exercising (your breathing, heart, and muscles) and some discomfort in your leg during the occlusion test. You will also be asked not to eat 2 hours before coming into the Lab and not to exercise intensely the day before.

### **Risks**

There are some potential risks to you by participating in this research and they include physical stress and fatigue and a low risk of injury during the exercise test. You might also be uncomfortable during the leg occlusion.

To prevent or to deal with these risks the following steps will be taken:

- a) You will fill out a Physical Activity Readiness questionnaire (Par-Q+) to make sure it is safe for you to participate in the exercise test.
- b) All testing will be conducted by the Principle Investigator who is a Certified Exercise Physiologist (Canadian Society of Exercise Physiology).

- c) You will be familiarized with the equipment and protocol to minimize potential discomfort and the risk of injury. All methods will be explained and demonstrated so you understand what will happen during data collection.

### **Benefits**

You will have the opportunity to experience fitness tests used with elite and Olympic athletes and get to see what a research laboratory is like. Also, your results will help us understand differences in the muscles of adults and kids during exercise and recovery.

### **Voluntary Participation**

Your participation in this research must be completely voluntary. If you do decide to participate, you can choose to stop at any time and you don't have to give a reason and there won't be any consequences. If you do withdraw from the study we will be disposed of and not used for analysis.

### **Anonymity**

Because the study is taking place at UVIC and you need to be there for data collection, your anonymity cannot be fully protected. This means we will know who you are and you might run in to others you know while here. All data collected will be saved under your participant code and analyzed under this code. Electronic data will be stored in a password protected laboratory computer. All paper data will be stored in a locked filing cabinet belonging to my supervisor Dr. Gaul, in McKinnon Building, room 128.

### **Confidentiality**

Your confidentiality and the confidentiality of the data will be protected by a participant code so no one except the principle investigator will be able to link your personal information with your data. 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 the following ways: a written paper, presentations, and published in a peer-reviewed academic journal. You will receive a report of results of the study once the study has been completed.

### **Disposal of Data**

Data from this study will be disposed of within five years of study completion. Electronic data will be erased and paper copies will be shredded.

### **Contacts**

People to contact about this study include:

a) Emily Medd, Principle Investigator  
Phone: 250-508-9489  
Email: [ermedd@uvic.ca](mailto:ermedd@uvic.ca)

b) Dr. Kathy Gaul, Supervisor  
Phone: 250-721-8380 (local: 8380)  
Email: [kgaul@uvic.ca](mailto:kgaul@uvic.ca).

In addition, you can check the ethical approval of this study, ask any questions you might have, by contacting the Human Research Ethics Office at the University of Victoria (250-472-4545 or [ethics@uvic.ca](mailto: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.

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*Name of Participant*

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*Signature*

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*Date*

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

## Participant Consent Form: Parent/Guardian

### **Muscle oxygen kinetics at the vastus lateralis muscle during anaerobic cycling and recovery in women and girls**

Your daughter is invited to participate in a study entitled **Muscle oxygen kinetics at the vastus lateralis muscle during anaerobic cycling and recovery in women and girls** that is being conducted by Emily R. Medd (MSc. Kinesiology Candidate). Emily is a graduate student in the department of Exercise Science, Physical and Health Education at the University of Victoria and you may contact her if you have further questions by phone: 250-508-9489, or email: [emedd@uvic.ca](mailto:emedd@uvic.ca).

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

#### **Purpose and Objectives**

The purpose of this research project is to examine maturational differences in muscle metabolism during intervals of high intensity cycling exercise and recovery. Specifically, muscle tissue deoxygenation during exercise and reoxygenation during recovery will be compared in women and girls.

#### **Importance of this Research**

Research of this type is important because relatively little exercise physiology research has been conducted in females, especially comparing children and adults. This study will contribute to the evidence on maturational differences in muscle metabolism improving the understanding of human physiology which may serve to benefit medical practices. As well, improved understanding of metabolism during high intensity exercise and recovery in women and girls may inform sport and athletic training programs making them safer and more effective.

#### **Participants Selection**

Your daughter is being asked to participate in this study because she is an active, healthy, prepubescent girl.

#### **What is involved**

This study involves the following procedures:

- Self-assessment of sexual maturity
- Height, weight, and body composition
- High intensity interval cycling exercise with recovery periods (8 mins)
- Leg occlusion (10 mins)

If your daughter consents to voluntarily participate in this research, you will come with her to the Exercise Physiology Laboratory at the University of Victoria (Mckinnon 171) for approximately 2 hours. Your daughter will be asked not to do any intense exercise for the day before coming into the lab and not to eat 2 hours before coming into the lab. When you arrive, your daughter will be asked some questions about herself. One of these questions is a self-assessment of maturity based on the Tanner scale. These are anatomical drawings of the 5 stages of breast and pubic hair development. With your help, your daughter will be asked to choose which picture she thinks her body is most similar to. Because of the screening questions already asked, I expect this will not be difficult and that all girls will most closely resemble the first set of drawings (prepubescent). I understand this might be a little uncomfortable, however it is very important for my research to have prepubescent girls participate and therefore it is essential to include this question. Once I explain the scale, I will leave you and your daughter to choose an answer by yourselves. If you are already aware of any breast or pubic hair development of your daughter, unfortunately she will not be able to participate in this study.

Next, we will measure your daughter's height, weight, and skinfolds. The principle investigator (who is a woman) will measure skinfolds by measuring folds of skin at 3 sites on the body. Your daughter will get to see how the skinfold measuring tool works beforehand and even try it out on the researcher if she likes. Skinfold measures give us an estimate of body composition which will help us with comparing power output during the exercise test between women and girls. After this, a heart rate monitor will be secured at the chest and a Near Infrared Spectroscopy device will be taped to the lower thigh to measure the oxygen in the muscle. We will then explain the exercise test and make sure the bike fits your daughter properly. After familiarizing her with the bike, we will explain and demonstrate the occlusion procedure and your daughter will get to try out the leg pressure cuff to see what it feels like.

The exercise test involves a 5 minute warm up followed by two 30 second maximum sprints with 2 minutes of cycling recovery. The maximum intensity sprints are called "Wingate" tests which are completely safe and reliable fitness tests with children. The Wingate test involves the participant pedaling as fast as they can against resistance set based on body weight. Including the warm up and the rest period, the exercise test will be no longer than 20 minutes.

After the exercise test, your daughter will sit down and the leg pressure cuff will be placed around her thigh. The pressure will be inflated to the same pressure she experienced in the familiarization (maximum of 250 mmHg) and maintained for a maximum of 10 minutes following standard occlusion protocol. She will be offered an iPod to play games to help pass the time. This procedure will be used as a means of significantly reducing blood flow to the thigh, in order to determine if any additional oxygen was available following the exercise. This procedure is necessary to allow for comparison of tissue oxygenation changes during the exercise test between women and girls. After the pressure is released your daughter will remain sitting for another 5 minutes. The occlusion protocol including rest and recovery will take no longer than 25 minutes. After the occlusion we will remove all measurement instruments and you may go home!

**Inconvenience**

Participation in this study may cause some inconvenience to your daughter, including feeling tired from exercising (breathing, heart, and muscles) and some discomfort in your leg during the occlusion test. She will also be asked not to eat 2 hours before coming into the Lab and not to exercise intensely the day before. You will be provided with a parking pass so you will not have to pay to come in to the lab.

**Risks**

There are some potential risks to you by participating in this research and they include physical stress and fatigue and a low risk of injury during the exercise test. She might also be uncomfortable during the leg occlusion.

To prevent or to deal with these risks the following steps will be taken:

- d) Your daughter will fill out a Physical Activity Readiness questionnaire (Par-Q+) to make sure it is safe for her to participate in the exercise test.
- e) All testing will be conducted by the Principle Investigator who is a Certified Exercise Physiologist (Canadian Society of Exercise Physiology) and a woman.
- f) Your daughter will be familiarized with the equipment and protocol to minimize potential discomfort and the risk of injury. All methods will be explained and demonstrated so she understands what will happen during data collection.

**Benefits**

The potential benefits of your daughter's participation in this research include experiencing fitness tests used with elite and Olympic athletes. As well, her results will contribute to an improved understanding of maturational differences in exercise metabolism benefiting the sport and medical communities.

**Voluntary Participation**

Your daughter's participation in this research must be completely voluntary. If she does decide to participate, she can choose to stop at any time without a reason and there will not be any consequences. If your daughter does withdraw from the study we dispose of her data and it will not be used in our analysis. You will be still be provided with a parking pass regardless of whether or not she completes the study.

**Anonymity**

Due to the nature and location of the study, your daughter's anonymity cannot be fully protected during data collection. All data collected will be saved under a participant code and analyzed under this code. Electronic data will be stored in a password protected laboratory computer. All paper data will be stored in a locked filing cabinet belonging to my supervisor Dr. Gaul, in McKinnon Building, room 128.

### Confidentiality

Your daughter's confidentiality and the confidentiality of the data will be protected by a participant code so that your data will not be identifiable to anyone other than the principle 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 the following ways: a written thesis, oral presentations, and published in a peer-reviewed academic journal. You will receive a report of the study results once the study has been completed.

### Disposal of Data

Data from this study will be disposed of within five years of study completion. Electronic data will be permanently erased and paper copies will be shredded.

### Contacts

Individuals that may be contacted regarding this study include:

c) Emily Medd, Principle Investigator  
Phone: 250-508-9489  
Email: [ermedd@uvic.ca](mailto:ermedd@uvic.ca)

d) Dr. Kathy Gaul, Supervisor  
Phone: 250-721-8380 (local: 8380)  
Email: [kgaul@uvic.ca](mailto:kgaul@uvic.ca).

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](mailto: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.

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*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 5: Data Collection Form

### Muscle oxygen kinetics at the vastus lateralis muscle during anaerobic cycling and recovery in women and girls

Participant ID: \_\_\_\_\_

Date: \_\_\_\_\_

**Participant Characteristics:**

Age (years)	
Height (cm)	
Weight (kg)	

**Women only**

Birth Control	Y N	Type:
Menstrual Cycle Phase	Luteal vs. Follicular	

Hours of moderate/vigorous physical activity per week: \_\_\_\_\_

**Girls only**

Tanner Stages		
A. Breast	B. Pubic Hair	Total

**Body Composition/Skinfolds:***For women measure 1,2,3,4,5**For girls measure 2,5*

Measure	Time 1	Time 2	Time 3	Final
1. Biceps SF				
2. Triceps SF				
3. Subscapular SF				
4. Iliac Crest SF				
5. Vastus Lateralis SF				

**Anaerobic Power:**

Measure	Wingate 1	Wingate 2
Peak Power (W) 1 second		
Peak Power (W/kg) 1 second		
Mean Power (W) 30 seconds		
Mean Power (W/kg) 30 seconds		
Fatigue Index (%)		

Plateau (TSI change &lt;1% in 2 minutes) time: \_\_\_\_\_ Event letter range: \_\_\_\_\_

Notes: \_\_\_\_\_

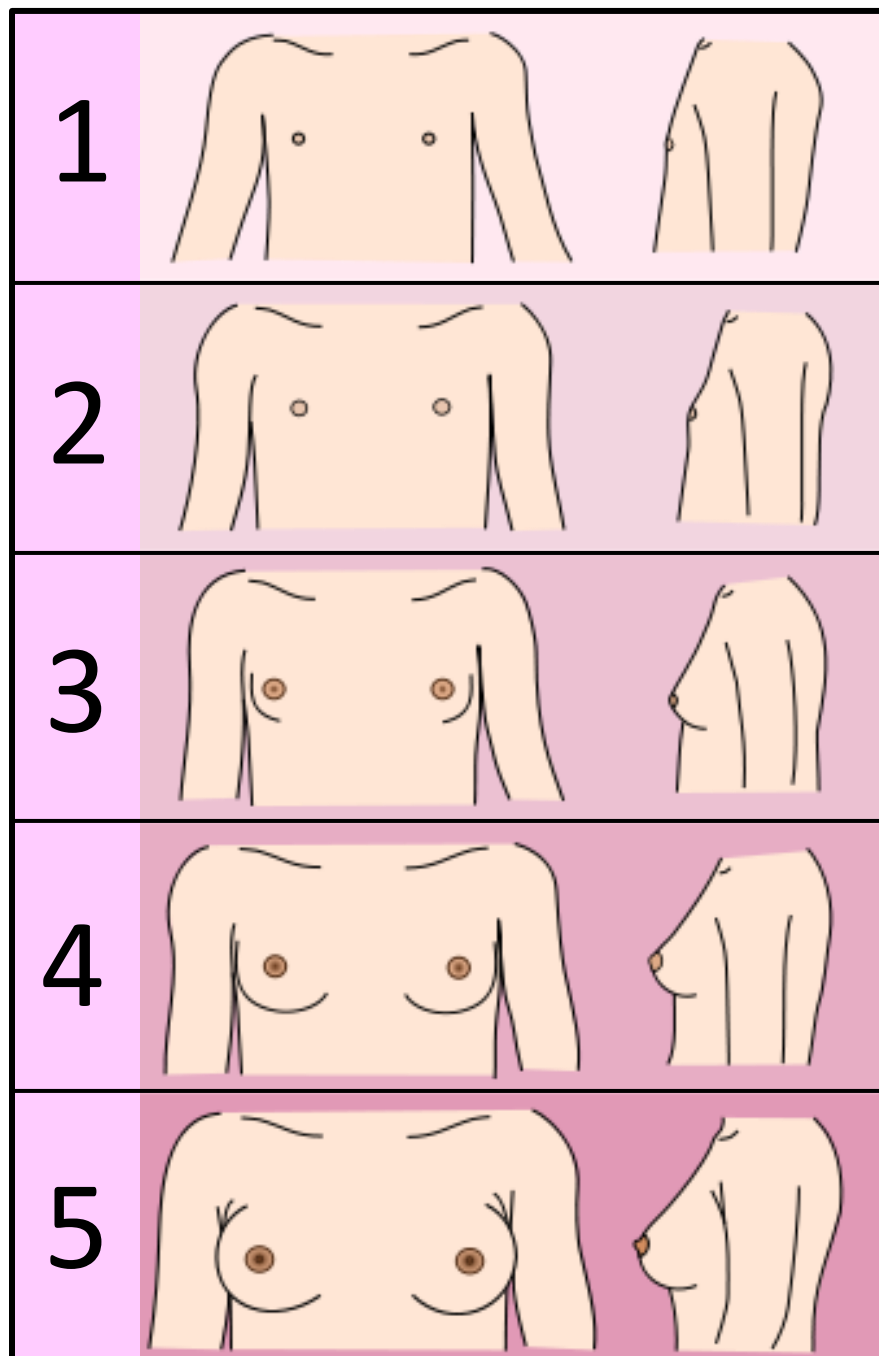
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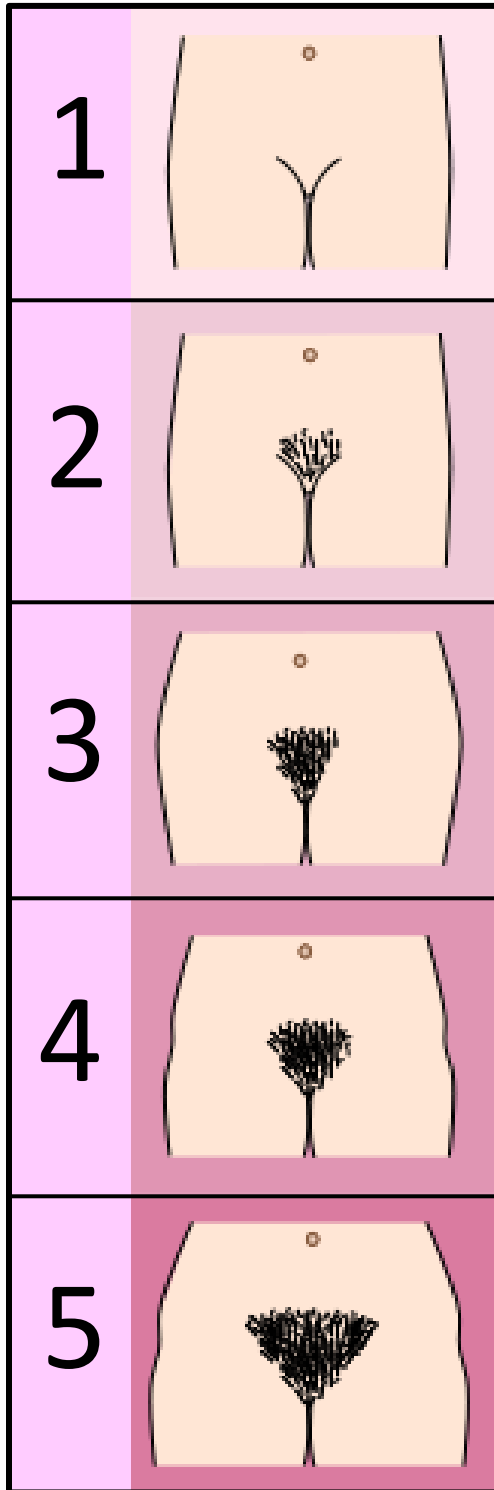
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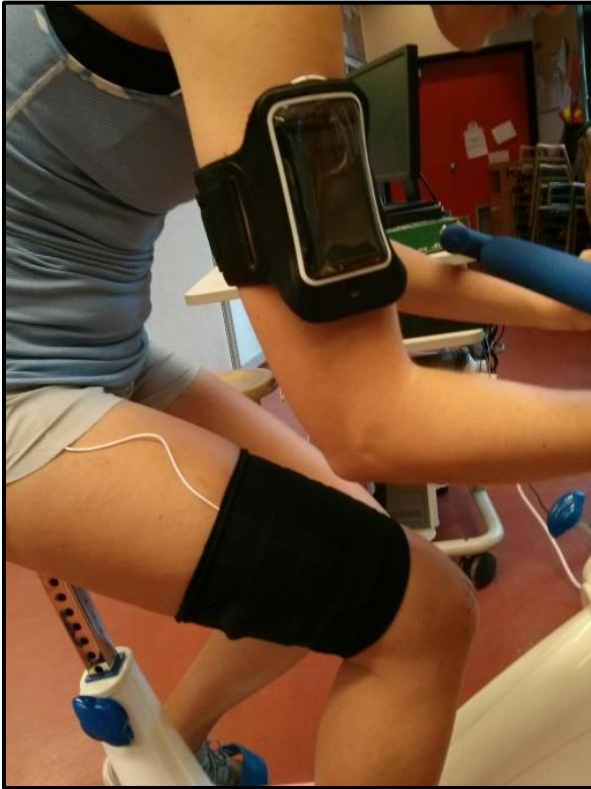
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## Appendix 6: Tanner Scale Drawings

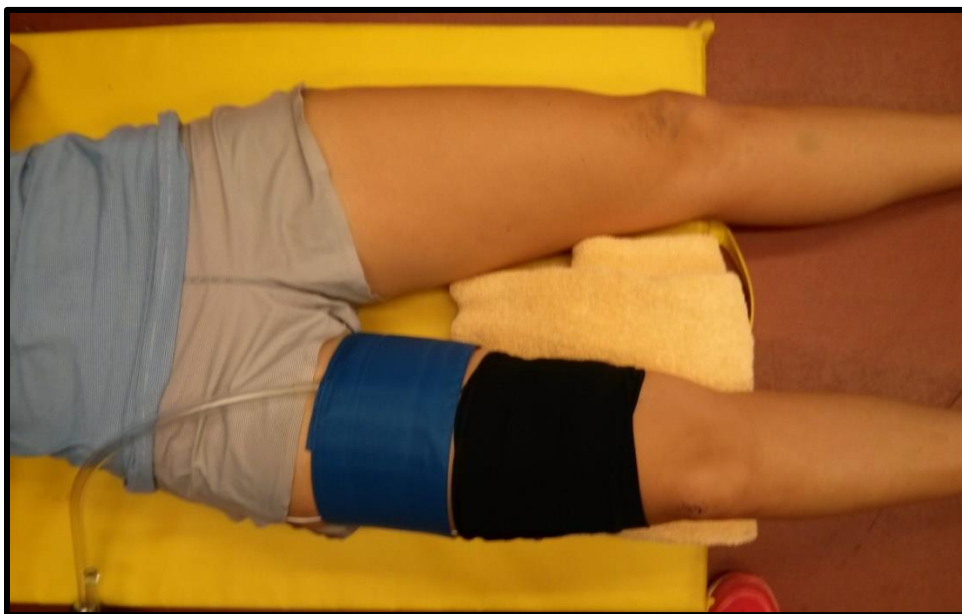
## A) BREAST DEVELOPMENT



**B) PUBIC HAIR DEVELOPMENT**

**Appendix 7: Figures**

*Figure 2.* Portalite setup on participants thigh with Portamon secured in armband



*Figure 4.* Occlusion cuff placement proximal to Portalite