Exploring the Potential to Non-Invasively Assess Lactate Threshold Using Near-Infrared Spectroscopy (NIRS)

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April 2024

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An Honours Thesis Submitted to the Department of Human Kinetics in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in Human Kinetics with Honours

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ACKNOWLEDGEMENTS

I would like to acknowledge and express my heartfelt gratitude to my supervisor, Dr. Daniel Kane, for his support throughout the duration of this project. Thank you for your time, guidance, and patience; your belief in me has truly been inspiring. Thank you to my second reader, Dr. Jennifer Jamieson, for your support and encouragement. I would also like to acknowledge the Department of Human Kinetics at St. Francis Xavier University for providing me with the opportunity to pursue an honours degree.

ABSTRACT

Lactate is a versatile molecule that plays a pivotal role in whole-body energy metabolism — a process wherein fuel sources are burned down and converted into energy. Our understanding of lactate has changed considerably over time, notably, low oxygen (O_2) was once considered to be the primary driver of lactate accumulation in human cells, however, lactate accumulation has been found to occur in the absence or presence of O_2; and yet, a relationship between tissue O_2 availability exists. The relationship between skeletal muscle oxygenation (SmO_2) and thresholds of blood lactate concentration (BLC) constitutes the basis of previous attempts to use changes in SmO_2 via near-infrared spectroscopy (NIRS) as a proxy for BLC. The objective of this study was to 1) confirm the relationship between changes in muscle oxygenation (SmO_2) using NIRS and the lactate threshold (LT) determined via traditional blood sampling; and 2) further explore the relationship between SmO_2 kinetics and exercise thresholds of respiratory gas exchange. Sixteen recreationally active runners (n = 16; age 25.4 ± 8.3yrs; body fat 14.9 ± 5.5%) performed an incremental, progressive treadmill running protocol consisting of 4-min running stages, separated by 1-min rest intervals. Heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, total hemoglobin, and SmO_2 were continuously monitored; blood lactate concentration and rating of perceived exertion were obtained during rest intervals and following each exercise stage, respectively. The SmO_2 control ratio revealed a distinct breakpoint near, but not necessarily coincident with, the lactate and gas exchange thresholds. Bland-Altman analysis resulted in a mean bias of 2.3 bpm HR and 0.1 L/min $\dot{V}O_2$ between the blood lactate-determined LT and NIRS-determined LT, suggesting an acceptable level of agreement between methods. These findings suggest that NIRS may be a valid non-invasive and practical alternative to estimate lactate threshold intensity in endurance athletes.

Keywords: lactate threshold; muscle oxygenation; NIRS; non-invasive; SmO_2 control ratio

INTRODUCTION

The citric acid cycle (CAC) and the electron transport chain (ETC) constitute the key metabolic pathways through which mitochondria generate adenosine triphosphate (ATP). Glycolysis is a highly conserved metabolic pathway involving the breakdown of glucose (6-carbon carbohydrate) into two, 3-carbon pyruvate molecules through a series of some 10 enzyme-catalyzed steps in the cytosol (Bertram et al., 2006). Glycolysis yields a quantitatively unimpressive net 2 ATP, the biochemical "currency" of the cellular "economy," but does so relatively quickly in the absence of oxygen (O_2), per se. Oxidative phosphorylation (OXPHOS), on the other hand, yields about an order of magnitude greater ATP than glycolysis (on a molar flux basis), but requires O_2 and is comparatively slower than glycolysis. Historically, the fermentive reduction of pyruvate to lactate (**Eq. 1**), catalyzed by lactate dehydrogenase (LDH), was considered to be a result of limited O_2. However, while low O_2 can certainly lead to lactate accumulation, in human cells, lactate accumulation can occur in the absence or presence of O_2. In fact, given its high-activity, and the near-equilibrium nature of LDH, lactate predominates over pyruvate in the cell, and has therefore been considered the end-product of glycolysis (Rogatzki et al., 2015).

Equation 1: $Pyruvate^- + NADH + H^+ \leftrightharpoons Lactate^- + NAD^+$ (Bertram et al., 2006).

Previous understandings of lactate production during exercise were based on an insufficient supply of O_2, however, recent research suggests that with increasing exercise intensity, the body's reliance on metabolic pathways simultaneously increases independent of oxygen availability (Ferguson et al., 2018). Consequently, glycolytic rates increase to meet heightened energy demands, resulting in a concurrent increase in the conversion of pyruvate into lactate (Ferguson et al., 2018). The body's ability to clear lactate from the blood is highly dependent on physiological

factors related to increased muscular work rate and high-intensity contractions; consequently, blood lactate concentrations rise exponentially (Heuberger et al., 2018). Moreover, the maximal lactate steady state (MLSS) can be described as the point at which the rate of lactate appearance is equal to the rate of lactate disappearance (**Fig. 1**). Before the MLSS, the rate of lactate appearance is less than the rate of disappearance. However, above the MLSS is when the rate of lactate appearance exceeds the rate of disappearance, representing the point at which continual lactate accumulation occurs (**Fig. 1**). Lactate is a versatile molecule that plays a pivotal role in whole-body energy metabolism — a process wherein fuel sources are burned down and converted into energy (Ferguson et al., 2018). The lactate threshold describes a critical metabolic level during "whole body" exercise involving the major muscle groups (e.g., running, swimming, cycling), above which the concentration of lactate in the blood begins to rise (Poole et al., 2016).

The ‘aerobic-anaerobic transition’ framework, while still used to some degree for performance assessment, is rarely used in current research due to the inherent ambiguity that exists between thresholds (Faude et al., 2009). The “aerobic” threshold describes the capacity to sustain prolonged exercise while relying primarily on aerobic metabolism for efficient energy production during exercise (Faude et al., 2009). The “aerobic” threshold is said to be the first lactate threshold (LT_1) and denotes the point at which blood lactate concentrations begin to rise once this threshold is surpassed. The term “anaerobic” threshold, used interchangeably with lactate threshold or maximal lactate steady state (MLSS), is said to be the second lactate threshold (LT_2). The “anaerobic threshold” occurs when the body's energy demand increases to the point at which it can no longer rely on aerobic metabolism and must rely on anaerobic metabolism (Faude et al., 2009). It’s important to note that aerobic metabolism necessitates O_2 for energy production, whereas anaerobic metabolism can occur in the absence of O_2.

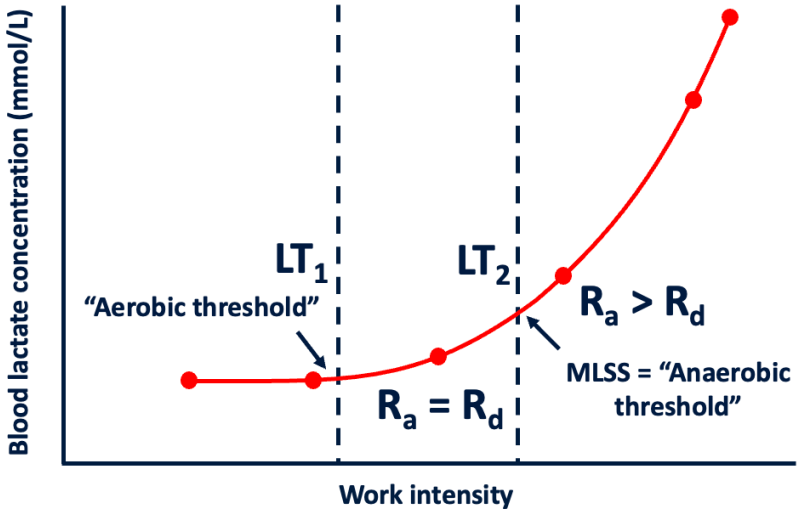


Figure 1. Lactate-intensity plot showing the first (LT_1) and second lactate threshold (LT_2), sometimes erroneously referred to as the "aerobic" and "anaerobic" thresholds, respectively. Below the maximal lactate steady-state (MLSS), the rates of lactate appearance (R_a) and disappearance (R_d) from circulation are equivalent; above the MLSS, the R_a exceeds the R_d (Adapted from Faude et al., 2009).

The lactate threshold can be defined as the level of exercise intensity at which the production of lactate exceeds the body's ability to metabolize and remove it. This results in a sudden increase in blood lactate concentration. In practical terms, the lactate threshold represents an upper limit of exercise intensity that can be maintained over extended periods of endurance exercise (e.g., race pace for a distance runner). Lactate thresholds, traditionally measured via capillary blood sampling, are a far better predictor of endurance performance compared to ventilatory thresholds (e.g., maximal oxygen uptake) (Faude et al., 2009). The invasive nature and practical challenges associated with intermitted blood sampling raise the question of whether near-infrared spectroscopy (NIRS) could offer a potentially more efficient and non-invasive alternative.

Near-infrared spectroscopy (NIRS) relies on the relative absorption of near-infrared light that readily penetrates tissue and skeletal muscle; emitting signals that differentiate between

oxygenated and deoxygenated forms of hemoglobin and myoglobin (Fadel et al., 2004). NIRS can accurately measure real-time muscle oxygenation (%SmO_2) and total hemoglobin (tHb) levels. An increase in sympathetic activity during exercise and a reduction of blood flow can be observed in response to vasoconstriction within skeletal muscle (Fadel et al., 2004). NIRS technology has been effectively used to measure sympathetically mediated responses to exercise; a decrease in tissue oxygenation and blood flow caused by sympathetic activation can be readily detected by NIRS (Fadel et al., 2004). Sympathetic activation and increased cardiac output to meet oxygen demands are possible explanations for the decline in tissue oxygenation and blood flow during exercise (Fadel et al., 2004).

Training intensity distribution (TID) among world-class athletes highlights the importance of finding a balance between moderate, heavy, and severe exercise domains; it was however determined that further research is needed to investigate sport-specific training intensities (Sperlich et al., 2023). Notable research examining the training characteristics of Norwegian elite cross-country skiers and biathletes found that aerobic endurance training accounted for ninety-four percent of training (Tønnessen et al., 2014). Of that ninety-four percent, approximately ninety percent was performed at a low intensity, denoting below the first lactate threshold; the remaining ten percent of training was performed at a high intensity, considered to be above the first lactate threshold (Tønnessen et al., 2014). Findings suggest that intensity distribution, training frequency, and training volume are key factors in the development of endurance performance among Olympic and World Champion endurance athletes (Seiler, 2010; Tønnessen et al., 2014). Moreover, previous literature recommends a tapering strategy leading up to peak performance, involving a reduction in training volume of approximately forty-one to sixty percent (Bosquet et al., 2007).

However, Tønnessen et al. (2014) did not observe this recommended decrease in training volume among Norwegian elite cross-country skiers and biathletes.

NIRS provides a solid foundation for the quantification of lactate thresholds and has the potential to replace current invasive methods, thereby offering a more efficient measurement tool, especially where intermittent blood sampling may not be feasible or practical. The purpose of this study was to examine the validity of NIRS in measuring muscle oxygenation and its correlation to lactate-based exercise thresholds. The primary aim of this study was to confirm the relationship between the NIRS trace breakpoints ($\%SmO_2$) and LT_1 and/or LT_2 using traditional blood sampling methods. The secondary aim of this study was to explore if a relationship exists between changes in the NIRS signal ($\%SmO_2$ or tHb) and the first lactate threshold (LT_1).

LITERATURE REVIEW

Traditional Approaches to Quantifying Lactate Threshold

Several traditional approaches to assessing lactate threshold are used in sport and exercise science for the optimization of training and workload intensities. Faude et al. (2009) examined the validity of traditional lactate threshold concepts, emphasizing that the lactate threshold is a key physiological parameter, with several practical applications in the diagnosis and assessment of endurance performance. Traditional methods of quantifying lactate thresholds are assessed through invasive and repetitive capillary blood sampling (e.g., finger or earlobe prick), making these approaches inconvenient to sustain for longer periods. Examining an individual's blood lactate response to exercise is key for identifying lactate thresholds and assessing an individual's aerobic and anaerobic fitness (Beneke et al., 2011; Urhausen et al., 1993). Studies by Heuberger et al. (2018), Urhausen et al. (1993), and Poole et al. (2016) investigated the anaerobic threshold and maximal lactate steady state (MLSS), concluding their validity and reliability in assessing and monitoring blood lactate concentrations during progressive exercise. Given the capacity for muscles to produce lactate during exercise, blood lactate concentrations rise based on physiological factors (Heuberger et al., 2018). Graded exercise tests are commonly used to assess training intensities in endurance sports; however, lower blood lactate concentrations are observed in glycogen-depleted individuals, therefore, incremental exercise tests are said to be a poor overall estimate of lactate concentrations (Maassen & Busse, 1989).

Midgley et al. (2007) examined the physiological determinants of time to exhaustion by assessing lactate threshold velocity during incremental exercise tests. Lactate threshold velocity can be defined as the point at which blood lactate begins to increase significantly above baseline during exercise (Midgley et al., 2007). Predicted time to exhaustion revealed lactate threshold

velocity as a key predictor of endurance performance, implying precision and accuracy in assessing lactate thresholds (Midgley et al., 2007). Blood lactate concentrations play a central role in differentiating between aerobic and anaerobic energy systems during whole-body exercise (Faude et al., 2009). The extensive applications of lactate threshold concepts come as no surprise given that blood lactate concentrations are said to provide valuable information about metabolic demands and work intensities (Beneke et al., 2011; Faude et al., 2009). Meta-analyses have shown a positive correlation between endurance training intensity and improvements in both lactate and ventilatory thresholds (Londeree, 1997). The complex relationship between increased exercise intensity and delayed lactate onset emphasizes that lactate thresholds are sensitive to changes in endurance training (Londeree, 1997; Beneke et al., 2011).

Blood lactate concentrations are typically measured using portable, hand-held blood lactate analyzers that use enzymatic amperometric detection, referring to the ability to measure electrical signals produced when lactate in blood interacts with the enzyme lactate oxidase (Bonaventura et al., 2015). A comparative research study by Bonaventura et al. (2015) assessed the reliability and accuracy of several hand-held blood lactate analyzers; findings suggest that the EDGE and Lactate Pro2 were among the most accurate and precise for measuring blood lactate concentrations (Bonaventura et al., 2015).

Muscle Oxygen Kinetics

The balance between oxygen supply and demand in muscles during exercise is associated with metabolic rate and time to exhaustion, revealing a critical threshold at which muscle oxygenation begins to decline (Brett et al., 2021; Kirby et al., 2021). Previous research focused on muscle oxygen kinetics demonstrates that there exists a critical point when oxygen availability becomes limited during exercise (Brett et al., 2021; Bangsbo et al., 2000). At the onset of exercise,

energy liberation and oxygen transport within skeletal muscle instantaneously increase, while oxygen availability reflects the biochemical capacity of the muscle (Bangsbo et al., 2000). A comprehensive overview of blood flow regulation in skeletal muscle during exercise shows a relationship between blood pressure, heart rate, and oxygen demand (Saltin et al., 1998). Research examining the capacity of near-infrared spectroscopy to measure muscle oxygen kinetics in the scope of exercise performance reiterates the dynamic changes of hemoglobin and myoglobin in active muscles (Nemoto, 2021; Spires et al., 2011). Oxygen availability can be explained in part by the increase in work rate, which subsequently causes an increase in adenosine diphosphate (ADP) and inorganic phosphate (P_i; **Eq. 2**) (Ferguson et al., 2018). As a result of the increase in ADP and P_i, the rate of glycolysis increases and thus increases lactate concentrations (Ferguson et al., 2018).

Equation 2. $[ADP] \bullet [P_i]/[ATP]$

Batterson et al. (2023) investigated the relationship between skeletal muscle oxygen saturation rates and lactate-based exercise thresholds. Key findings suggest that muscle oxygenation levels could indicate a maximal metabolic steady state during exercise. Moreover, muscle oxygenation ($\%SmO_2$) and blood lactate were found to be highly correlated across graded exercise intensity (Batterson et al., 2023). Research by Fadel et al. (2004) used NIRS to investigate the role of the sympathetic nervous system in resting and exercising skeletal muscle. Sympathetically mediated vasoconstriction in skeletal muscle during exercise can be measured non-invasively using NIRS (Fadel et al., 2004). A gradual decline in muscle oxygenation levels is found in skeletal muscle during exercise, highlighting the dynamic relationship between oxygen supply and demand (Belardinelli et al., 1995). Several interrelated factors influence metabolic rate and oxygen uptake in muscles during exercise (Xu & Rhodes, 1999).

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) technology has recently become an attractive alternative for measuring real-time muscle oxygenation (%SmO_2) and hemoglobin index (tHb) information. NIRS is a non-invasive approach that assesses differences in the transmittance of hemoglobin between oxygenated and deoxygenated states. Traditional methods of measuring blood lactate concentrations from capillary blood sampling present practical challenges, such as repeatedly drawing blood, making NIRS a far more efficient and less invasive tool to investigate lactate thresholds. Near-infrared spectroscopy (NIRS) technology has been widely used in several areas of clinical and non-clinical research, including sport and exercise science. NIRS has emerged as an attractive alternative to current methods given its capability to non-invasively measure real-time muscle oxygenation levels in various practical settings (i.e., during exercise).

The capacity of NIRS to measure primary heme compounds, including hemoglobin and myoglobin, in skeletal muscle has become increasingly sought after because of its non-invasive nature (Barstow, 2019). Several key research studies investigating the validity and reliability of NIRS technology have contributed to advancements in the scope of sport and exercise science. Barstow (2019) provided an exhaustive overview of NIRS usage, highlighting a lack of standardization in protocols and an overly broad range of terminology as potential limitations. NIRS has been widely used to measure muscle oxygenation, oxidative metabolism, and vascular dynamics because of its continuous nature and practical applications (Ferrari et al., 2011; Koirala et al., 2021).

Furthermore, NIRS is frequently used to measure the dynamic balance between oxygen supply and demand in localized skeletal muscle (Ferrari et al., 2004). The sport-specific context of NIRS, discussed by Kozlová (2018), investigated future applications of NIRS to include

performance monitoring and training optimization. Several researchers have explored the usage of NIRS for measuring muscle oxygenation and hemodynamics during graded exercise tests (Neary, 2004; Van Beekvelt et al., 2001). The relative absorption of near-infrared (NIR) light readily penetrates superficial tissue layers and emits signals that distinguish between oxygenated and deoxygenated forms of hemoglobin and myoglobin (**Fig. 2)** (Fadel et al., 2004). NIR light is absorbed by chromophores in skeletal muscle, allowing for NIRS data to be quantifiable based on light absorbency properties (Ferrari et al., 2004).

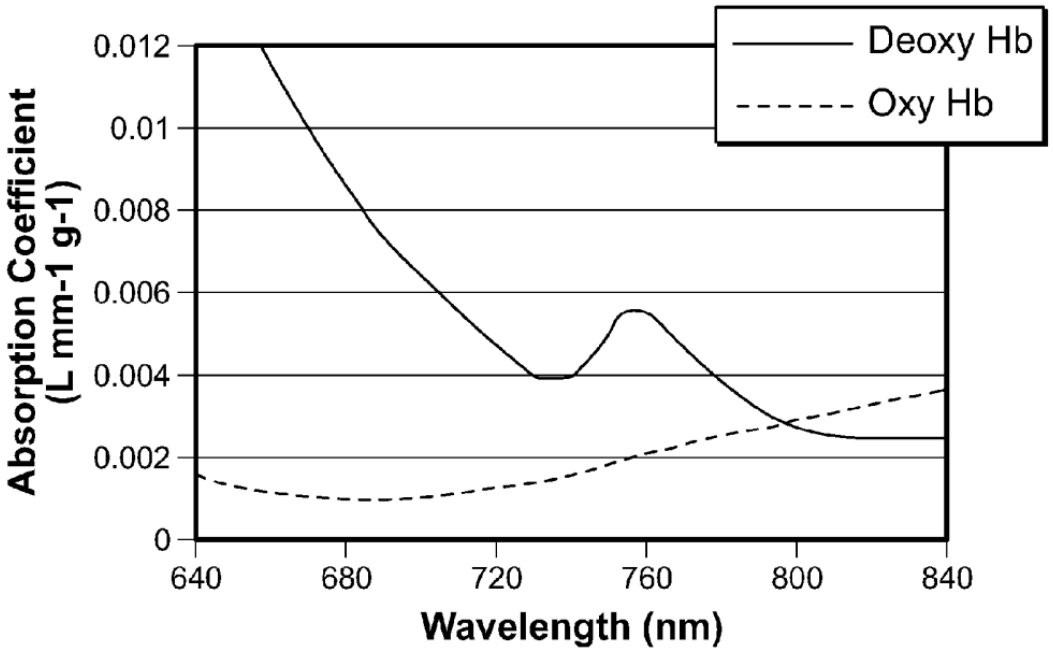


Figure 2. Absorption coefficient compared to near-infrared light wavelength for deoxyhemoglobin (Deoxy Hb) and oxygenated hemoglobin (Oxy Hb) retrieved from Moxy United States Patent No. 8,941,830 B2 (2015).

Koirala et al. (2021) presented a computational analysis of the effects that muscle oxygenation and hemodynamics have on NIRS signals. Findings indicate diverse applications of NIRS and investigate variables that influence signal interpretation. Researchers noted that changes

in whole blood volume impact NIRS signals, regardless of normal or reduced oxygen delivery during exercise (Koirala et al., 2021; Kozlová, 2018). Deoxygenated blood was found to be insensitive to changes in total blood volume, allowing for a better NIRS signal when compared to oxygenated blood (Koirala et al., 2021). NIRS has become a particularly useful tool for exercise testing because of its practicality, allowing for potential advancements in the scope of sport and exercise science. However, NIRS reportedly has a large variability between subjects (Gerz et al., 2013). Salas-Montoro et al. (2022) found no significant difference between NIRS-detected muscle oxygenation breakpoints (Humon Hex, Dynometrics Inc., Boston, MA, USA) and the second lactate threshold measured via capillary blood sampling in elite cyclists (n = 90). This research suggests that near-infrared spectroscopy could potentially replace invasive or expensive methods of estimating lactate thresholds (Salas-Montoro et al., 2022). In addition, there were no observable differences between the muscle oxygenation breakpoint and the second lactate threshold for male (n = 32) and female (n = 58) cyclists when studied independently (Salas-Montoro et al., 2022).

The novel use of near-infrared spectroscopy as a non-invasive measurement tool and its application to hemodynamics is key in exercise physiology. Research investigating muscle oxygenation at the onset of exercise highlights the value of non-invasive, real-time monitoring. NIRS allows for remote continuous monitoring of primary heme compounds, in addition to reduced safety risks when compared to capillary blood sampling. Further research aimed at investigating the relationship between the blood lactate-determined LT and the NIRS-determined LT could be advantageous to investigate the underlying physiological mechanisms of muscle oxygen dynamics in exercising skeletal muscle. The dynamic balance between oxygen supply and demand, metabolic rate, and blood flow are key mechanisms in skeletal muscle during exercise. Future research focused on broadening the scope of NIRS applications could establish a solid

foundation for endurance sports across a wide range of disciplines, eliminating the reliance on laboratory environments. Research of this nature would be extremely valuable and could positively impact exercise performance and training optimization for endurance athletes.

The Moxy Monitor (Fortiori Design LLC, Minnesota, USA) has been found to be both reliable and valid for monitoring muscle oxygenation (%SmO_2) and total hemoglobin (tHb) using near-infrared spectroscopy (Feldmann et al., 2019; Crum et al., 2017). However, the Moxy Monitor has been found to have greater variation at higher exercise intensities when compared to low and moderate intensities (Crum et al., 2017). The Moxy Monitor has also been compared to other NIRS devices, such as the NIRS PortaMon (Artinis, Elst, Netherlands), showing comparable results and producing acceptable tissue oxygen saturation index measures at rest and during exercise (McManus et al., 2018). The Moxy Monitor uses near-infrared spectroscopy and mathematical algorithms to predict muscle oxygenation (%SmO_2). Unlike visible light, near-infrared light emitted from a sensor penetrates the skin's surface and muscle tissue. Researchers have also investigated the reliability of threshold determination using NIRS, compared to lactate and ventilatory thresholds (Sendra-Pérez et al., 2023). Findings suggest that NIRS is accurate in determining the second ventilatory and lactate thresholds, however, further research is needed to detect and quantify the first threshold (Sendra-Pérez et al., 2023). It is important to note that the ability of NIRS to assess muscle oxidative capacity is indicative of aerobic fitness and is independent of biological sex (Beever et al., 2020).

METHODS

Ethical Approval

Experimental procedures and protocols were submitted to the Department of Human Kinetics Research Ethics Review Committee at St. Francis Xavier University. The application was then approved by the University Research Ethics Board (REB) for approval in accordance with the Tri-Council Policy Statement Guidelines. There was no financial incentive or compensation to participate in the research study. Participants were informed of all procedures and protocols, as well as any potential benefits and risks before providing informed written consent to demonstrate their willingness to voluntarily participate in the research study.

Participants

Recreationally active runners (at a minimum) between the ages of 18 and 59 who had competed at least once in the previous 12 calendar months were eligible to participate in the study. Exclusion criteria sought to maintain the integrity of the study outcome and ensure ethical research practices. As such, individuals with a history of anemia or neurological disorders affecting the nervous system that limited their ability to exercise normally were excluded from participating. Individuals following strict diets or regimens that limit carbohydrate or glycogen levels were not eligible to participate. Participants taking any prescription or over-the-counter medications, excluding forms of hormonal contraceptives, that significantly interfered with their ability to exercise were not eligible to participate. Additionally, participants with a previous medical history or health conditions that limited their ability to exercise were also not eligible. Eligibility was restricted to subjects who were non-smokers and otherwise in good overall health.

Participants were recruited from the general population, the student population, and the varsity student-athlete population at St. Francis Xavier University. Participant recruitment was

done by word of mouth and convenience sampling. All participants were provided with an Invitation to Participate and were required to provide informed consent.

Table 1. General characteristics of participants included in the study.*

Participant ID	***Gender***	***Age (yrs)***	***Height (cm)***	***Weight (kg)***	***BFP (%)***
1	M	43	173	76.9	24.8
2	M	21	188	90.71	18.3
3	F	21	163	57.2	21.8
4	M	19	180	66.67	7.1
6	M	20	180	68	9.8
7	M	19	172	67.5	13.5
8	F	36	161	61.9	20.4
9	F	33	163	57.4	20.0
10	M	18	178	61.3	10.2
12	M	36	172	69	13.0
13	M	21	170	68.5	12.0
14	F	33	165	63.3	20.2
15	M	23	177	71.3	10.6
16	M	18	177	72.5	11.1
17	M	21	174	65.3	10.5
Mean ± SD		Mean: 25.47, SD: 8.25	Mean: 172.87, SD: 7.56	Mean: 67.83, SD: 8.32	Mean: 14.89, SD: 5.46

*Values are reported from current year of study.

Measurement Tools

Experimental trials were performed in a laboratory setting where temperature (°C), humidity (%), and time of day were recorded at the beginning of data collection. Trials were conducted using a Trackmaster (TMX 425) motorized treadmill (**Fig.** 3) set at the lowest possible grade, determined to be 1.75%. A 1% treadmill gradient is considered the most accurate representation of the energetic cost of outdoor running (Jones & Doust, 1996). A rating of perceived exertion (RPE) on a modified 10-point modified Borg Scale was collected from

participants during the last 30 seconds of each stage. A Moxy Monitor (Fortiori Design LLC, Minnesota, USA) was used to measure light absorbency differences and collect real-time muscle oxygenation (%SmO_2) and total hemoglobin (tHb) data (**Fig. 4**).

Figure 3. The schematic of the measurement on a motorized treadmill (Trackmaster). The drawing shows the location of the Moxy Monitor (Fortiori Design LLC, Minnesota, USA) and V_{max} metabolic headset that channelled inspired and expired air.

Using the EDGE handheld lactate analyzer, blood lactate concentrations ($mmol \cdot L^{-1}$) were measured in a small sample of capillary blood taken directly from subjects at predetermined time points. The blood lactate meter requires a very small capillary blood sample (≈3µl) extracted from a subject's finger to analyze blood lactate concentrations. The EDGE lactate analyzer takes roughly 30-45 seconds to produce results and represents a standard of comparison for traditional methods. Blood lactate concentrations were collected at rest, and subsequently following each stage of the incremental exercise test. The EDGE has been found to be one of the most precise and accurate hand-held blood lactate analyzers (Bonaventura et al., 2015). In addition, a OneTouch

VerioIQ glucose meter was used to measure the concentration of glucose (mM) in a small sample (≈0.4µl) of fingertip capillary blood using a sterile, one-time-use disposable lancet puncture on alcohol-sterilized skin. Blood glucose concentrations were only collected once at rest to ensure participants' glucose levels were within normal range. Blood glucose concentrations within normal range provide the most accurate representation and prevent complications due to hyperglycemia or hypoglycemia (Güemes et al., 2015).

A chest strap heart rate monitor (Garmin Forerunner 910XT) was used to continuously monitor heart rate (bpm) during incremental exercise. Expired air analysis (EAA) was used during the progressive incremental exercise test to measure rates of oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$), as well as minute ventilation ($\dot{V}_E$; **Fig. 3**). Subjects were equipped with a mouthpiece, headset attachment, and nose clip, coupled with an expired air analysis system (Vmax ENCORE 29C; CareFusion Yorba Linda, CA, USA). This measurement examined relative concentration differences of O_2 and CO_2 in collected inspired and expired air.

The NIRS signal is highly sensitive to adipose tissue thickness, impacting the accuracy of the Moxy Monitor (Feldmann et al., 2019). To account for this, bioelectrical impedance analysis (BIA) was used to measure body fat percentage, muscle mass, and total body water using the Inbody 270 (Inbody Canada). This approach uses a handheld device that measures variations in electrical current flow, which is contingent on body composition. The Inbody BIA divides the body into five compartments: right arm, left arm, trunk, right leg, and left leg.

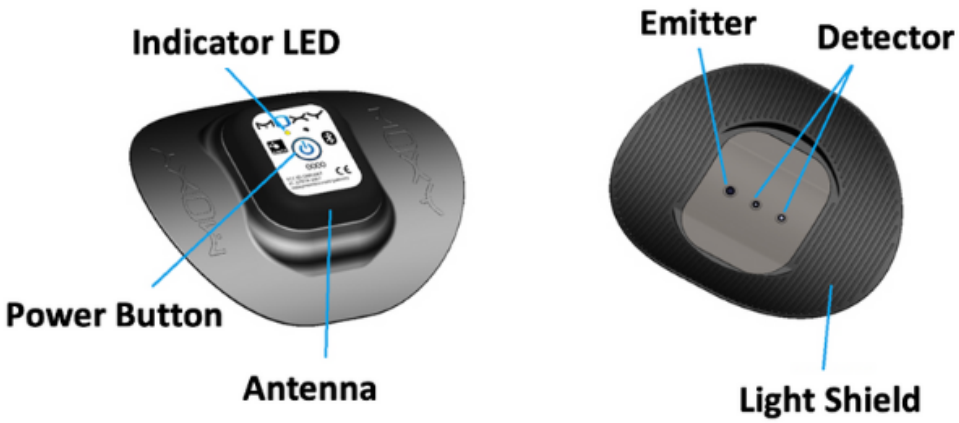


Figure 4. The distribution of the source emitter and detector on the Moxy Monitor (Fortiori Design LLC, Minnesota, USA).

Experimental Protocol: Study Design

Pre-test

After providing informed written consent, anthropometric measurements were recorded (i.e., age, height, weight, and self-identified gender). Participants were also asked to characterize their most recent training or exercise activity, including duration and intensity. Body fat percentage was assessed in confidence using bioelectric impedance analysis. Heart rate, blood lactate concentration, and blood glucose concentration were recorded at rest. Fingertip capillary blood samples were obtained via a small sterile, one-time-use disposable lancet puncture on alcohol-sterilized fingertip skin. The Moxy Monitor was affixed to the participant's right thigh using a light shield and self-adhesive wrap to prevent potential intrusion of ambient light. The Moxy Monitor was positioned directly above the *vastus lateralis* muscle, 2/3 on the line from the anterior *spina iliaca* superior to the lateral side of the patella (**Fig. 3**).

Warm-up

The participant began by walking comfortably on the Trackmaster treadmill (1.75% incline), the treadmill speed was then increased gradually over the next 15 minutes but never exceeded a slow jog. Participant's rating of perceived exertion did not exceed a 1 on a 10-point modified Borg Scale (i.e., the warm-up was gentle). Depending on the participant's resting blood lactate concentration, a slower and/or longer warm-up may have been necessary. The goal of the warm-up was for the participant to begin the incremental exercise test at a blood lactate concentration at or below 1.0 $mmol \cdot L^{-1}$. Fingertip blood sampling at the 15-minute mark during the warm-up indicated whether the participant was ready to begin the test. In the event that a participant's blood lactate concentration was above 1.0 $mmol \cdot L^{-1}$ at the 15-minute mark during warm-up, the treadmill speed was slowed, and blood lactate was assessed again at the 30-minute mark. Immediately following the warm-up, the participant was fitted with a mouthpiece, headset attachment, and nose clip for expired air analysis.

Incremental Exercise Test

Approximately one minute after the warm-up, the participant began the progressive exercise test at the slowest comfortable jogging pace above the walk-run transition, to the nearest kilometre per hour (typically 7.0 km per hour). After 4 minutes at this pace, the participant stepped off the treadmill and their BLC was measured. One minute later, the participant stepped back on the treadmill, this time with the speed increased by 1.0 km per hour. Four minutes later, the participant stepped off again and had their blood lactate measured. The test increased progressively in this manner (1.0 km per hour increments; 4-min stages, separated by a 1 min rest period) until a blood lactate concentration of at least 4.0 $mmol \cdot L^{-1}$ had been reached, at which point the exercise test was terminated (**Fig. 5**). A blood lactate concentration of approximately 4.0 $mmol \cdot L^{-1}$ typically

denotes the upper boundary for moderate intensities of exercise in most individuals and is commonly used to evaluate and monitor endurance training (Weltman, 1995).

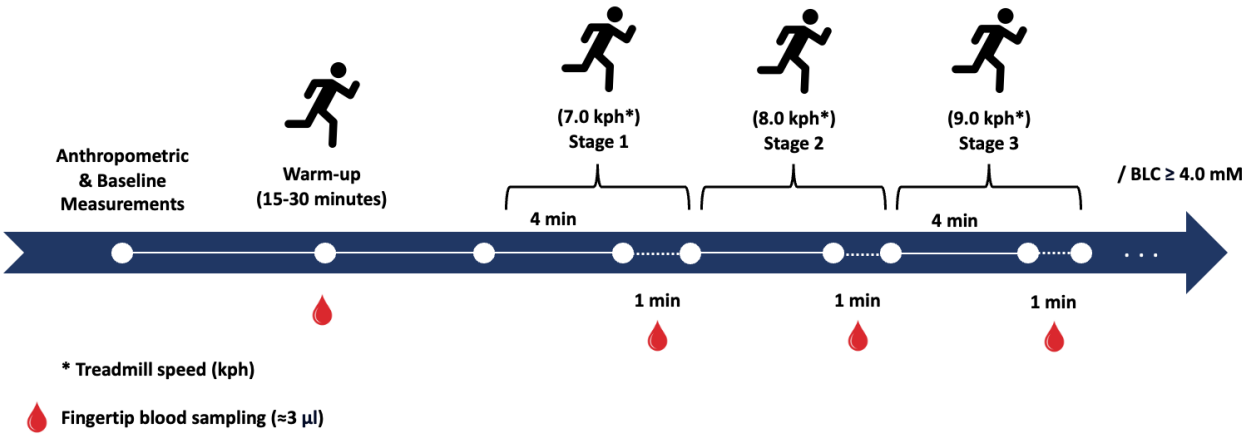


Figure 5. Protocol timeline of research study outlining anthropometric and baseline measurements, warm-up, exercise test stages, and fingertip blood sampling.

Data Analysis

The lactate threshold (LT) was considered to be the first increase of at least 0.5 mmol·L^{-1} above baseline measurement that did not decrease in the subsequent stages. The Moxy Monitor uses an algorithm based on the Monte Carlo modelling technique: a mathematical mode that detects how light propagates through different mediums (e.g., adipose tissue and muscle). The Monte Carlo modelling technique considers variations in optical properties which limit potentially confounding factors, including melanin, blood concentration, and subcutaneous adipose tissue thickness (Moxy, Fortiori Design LLC). The method chosen to quantify the SmO_2 data was based on the kinetics of the muscle oxygenation breakpoints and the boundaries of the SmO_2 trace. Bland-Altman statistical analysis was used to assess agreement between the two methods of measurement. Data analysis was done in GraphPad Prism (GraphPad Software, Boston, MA).

RESULTS

Muscle Oxygenation and Lactate Kinetics

The concurrent depiction of muscle oxygen saturation (SmO_2), blood lactate concentration (BLC), and treadmill speed (km/hr) provide insight into the physiological and metabolic responses during progressively increasing exercise intensity (**Fig. 6**). The onset kinetics of muscle oxygenation (SmO_2) reveal deoxygenation occurs almost immediately at the onset of increasing workload. Based on the premise of oxygen supply and demand, an increase in oxygen delivery occurs in excess of demand, giving a positive net SmO_2. The upward trend in SmO_2 trace following the onset of exercise was apparent in early stages at low to moderate intensities. Moreover, as intensity began to increase, the onset kinetics revealed a much different story. Following a sudden deoxygenation, the SmO_2 trace once again began to increase, however, only to the point at which it began to plateau and find a balance; the complex kinetics of these stages could represent a delayed delivery of oxygen to skeletal muscle (**Fig. 6**). Later stages of the exercise test show drastically different onset kinetics following the initial deoxygenation. Oxygen uptake is greater than oxygen delivery, resulting in a negative net SmO_2 and a downward trace. The kinetics of muscle oxygenation reflect spatial and temporal balance, as well as a balance of oxygen supply and demand. The onset kinetics of SmO_2 allow us to infer intensity domains in which physiological responses are different, however, the transition from one intensity domain to another is difficult to visually pinpoint and often requires subjective interpretation.

In addition to onset kinetics, the SmO_2 trace shows unique characteristics during reoxygenation, occurring at the point of exercise stimulus removal, where the oxygen demand simultaneously decreases. The maximum SmO_2 recovery values, represented by peaks in the SmO_2 trace, are reflective of the ability to adapt to an exercise stimulus. Generally speaking, as exercise intensity increased, the maximum SmO_2 recovery value subsequently decreased, and a negative

trend was observed. Additionally, the amplitude of change that occurred immediately following the exercise stimulus generally increased as exercise intensity progressively increased. A larger amplitude of change was observed in later stages compared to a relatively small amplitude of change in early stages. From a mechanical perspective, a stimulus is created simply by changes in pressure, whereas from a metabolic perspective, vasodilation is highly dependent on exercise intensity.

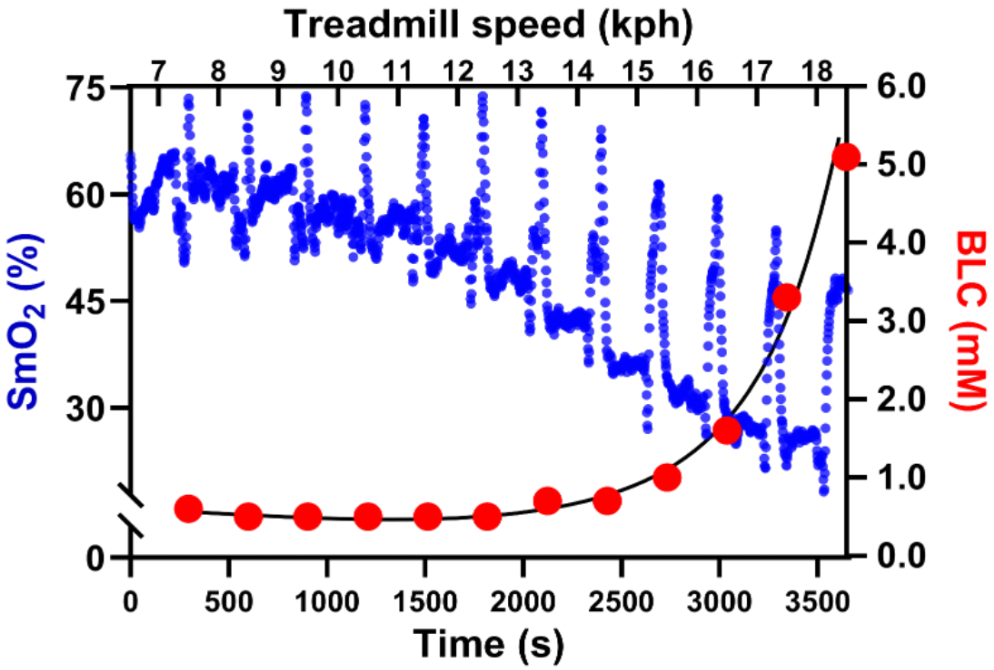
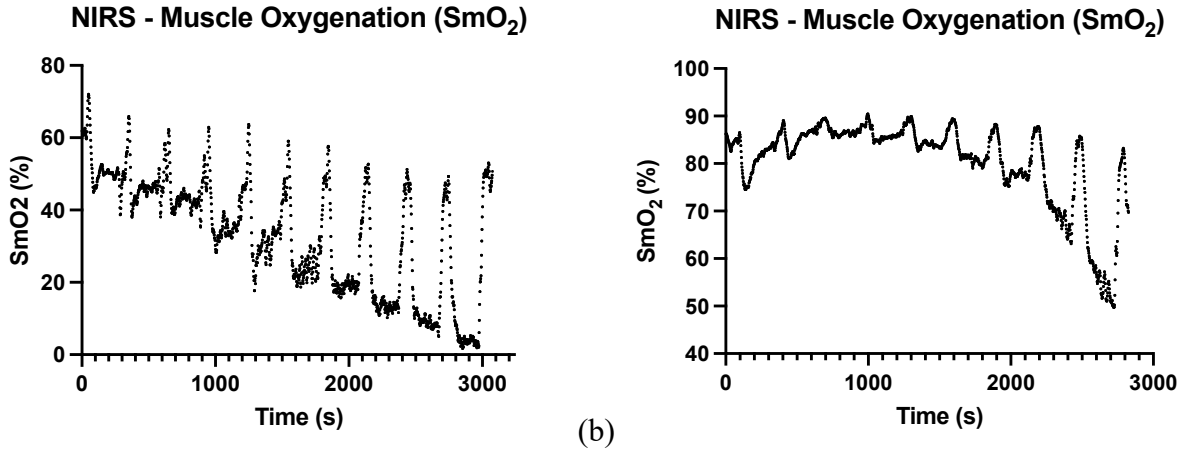


Figure 6. Muscle Oxygenation (SmO_2) and blood lactate concentration (BLC) during progressively increasing exercise as a function of treadmill speed.

Delving further into the interpretation of SmO_2 data, distinct differences were apparent between the NIRS-determined SmO_2 of self-identified men and woman participants, as shown in Figure 7. Findings show a net downward trend in muscle oxygenation in self-identified men, conversely, a curvilinear trend was observed in self-identified women. The maximum SmO_2 recovery values were relatively similar; however, the amplitude of change was drastically

different. This indicates that while both self-identified genders may reach similar maximum SmO_2 recovery values, the degree of fluctuation in oxygen saturation levels varied on an individual basis. Findings support previous research indicating the change in SmO_2 direction is inversely related to exercise intensity. Discrepancies in NIRS-determined SmO_2 trace remain largely unknown, with possible explanations pointed at subcutaneous adipose tissue and NIRS placement. Examining the influence of factors like hydration, muscle fibre type, and genetic predisposition on muscle oxygenation patterns could provide additional insights into the observed differences between self-identified men and women.



(a) (b)

Figure 7. (a) Representative NIRS-determined muscle oxygenation (SmO_2) for self-identified man. (b) Representative NIRS-determined muscle oxygenation (SmO_2) for self-identified woman.

The lactate threshold was considered to be the first 0.5 $mmol \cdot L^{-1}$ increase above baseline that did not subsequently accompany a decrease. Blood lactate concentrations, highlighted in Figure 6., followed an exponential curve given the dynamic balance between rates of lactate appearance and disappearance during progressively increasing exercise. The blood lactate-determined LT was found to occur at an average blood lactate concentration of 0.98 ± 0.4 $mmol \cdot L^{-1}$ and an average heart rate of 144.9 ± 17.0 bpm. The blood lactate-determined LT occurred at an average speed of 11.1 ± 2.4 km/hr. Previous research has investigated the slope of the NIRS trace,

conversely, we were interested in examining the kinetics of the SmO_2 curve concerning the upper and lower boundaries of the trace. As such, the 'SmO_2 Control Ratio' was used to interpret the SmO_2 kinetics, accounting for individual differences in physiology and endurance. The SmO_2 Control Ratio was then used to compare two methods of determining lactate threshold: blood lactate-determined LT and NIRS-determined LT. Similar to blood lactate concentrations, the SmO_2 Control Ratio generally followed an exponential curve, suggesting a threshold effect (**Fig. 8**).

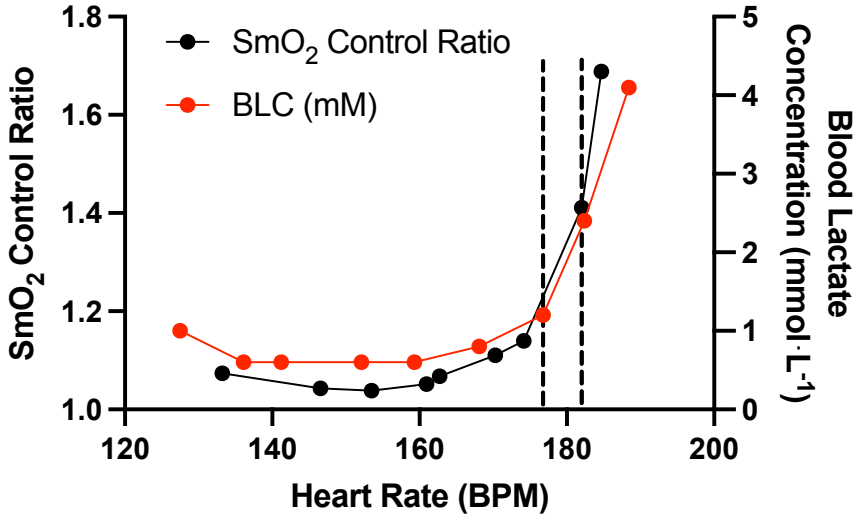


Figure 8. SmO_2 Control Ratio plotted against blood lactate concentration ($mmol \cdot L^{-1}$) as a function of heart rate (bpm).

The most widely accepted method, or "gold standard", for determining the lactate threshold is considered to be a graded exercise test paired with invasive blood lactate sampling. The SmO_2 Control Ratio was found to coincide with both lactate-based and ventilatory-based thresholds, as determined through Bland-Altman analysis. Bland-Altman analysis comparing the blood lactate-determined LT and the NIRS-determined LT with HR suggests a good level of agreement, represented with a bias of 2.253 using a 95% confidence interval (**Fig. 9a**). Results indicate that on average, the NIRS-determined LT tends to occur before the blood lactate-determined LT in

terms of heart rate. Additionally, Bland-Altman analysis comparing the blood lactate-determined LT and the NIRS-determined LT with VO_2 suggests a high level of agreement, represented with a bias of 0.09927 (**Fig. 9b**). This suggests the NIRS-determined LT occurs ever so slightly before the blood lactate-determined LT in terms of VO_2. Findings suggest a clear agreement between the blood lactate-determined LT and the NIRS-determined LT, regardless of whether HR or VO_2 was used as a reference. This strongly indicates that NIRS is a valid predictor of lactate threshold and suggests an effective non-invasive alternative to traditional blood sampling.

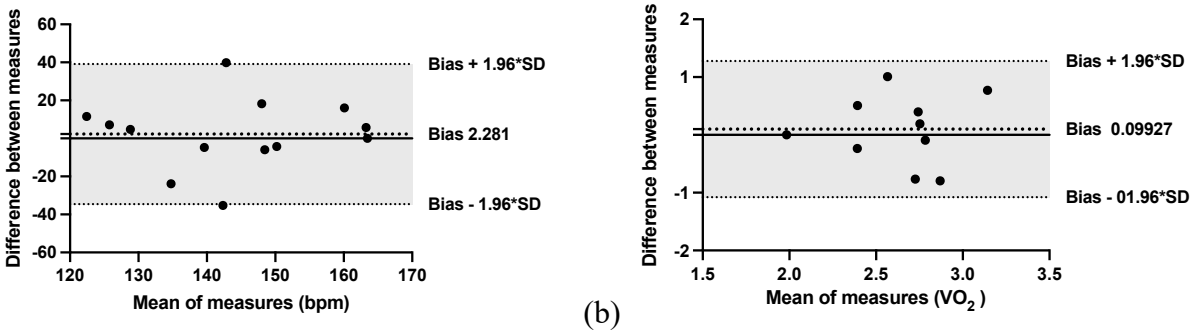


Figure 9. (a) Bland-Altman plot of heart rate (bpm) at blood lactate-determined LT compared to NIRS-determined LT. (b) Bland-Altman plot of VO_2 (L/min) at blood lactate-determined LT compared to NIRS-determined LT.

Bland-Altman analysis was used to assess the agreement between the gas exchange LT and both blood lactate-determined LT and NIRS-determined LT (**Fig. 10**). Bland-Altman analysis revealed a high level of agreement between the two methods using HR, with a bias of -0.2222 for blood lactate-determined LT (**Fig. 10a**), and -3.913 for NIRS-determined LT (**Fig. 10b**), as indicated by the narrow spread of data points around the mean difference line within the 95% confidence interval. With respect to VO_2, a mean bias of -0.01657 for blood lactate-determined LT (**Fig. 10c**) and -0.1158 for NIRS-determined LT (**Fig. 10d**) suggests a high level of agreement. Previous research has indicated that changes in muscle oxygenation (SmO_2) coincide with

metabolic demands, oxygen availability, and ventilatory responses during exercise. It is therefore reasonable to suggest NIRS could serve as a valid method to estimate ventilatory thresholds in this context.

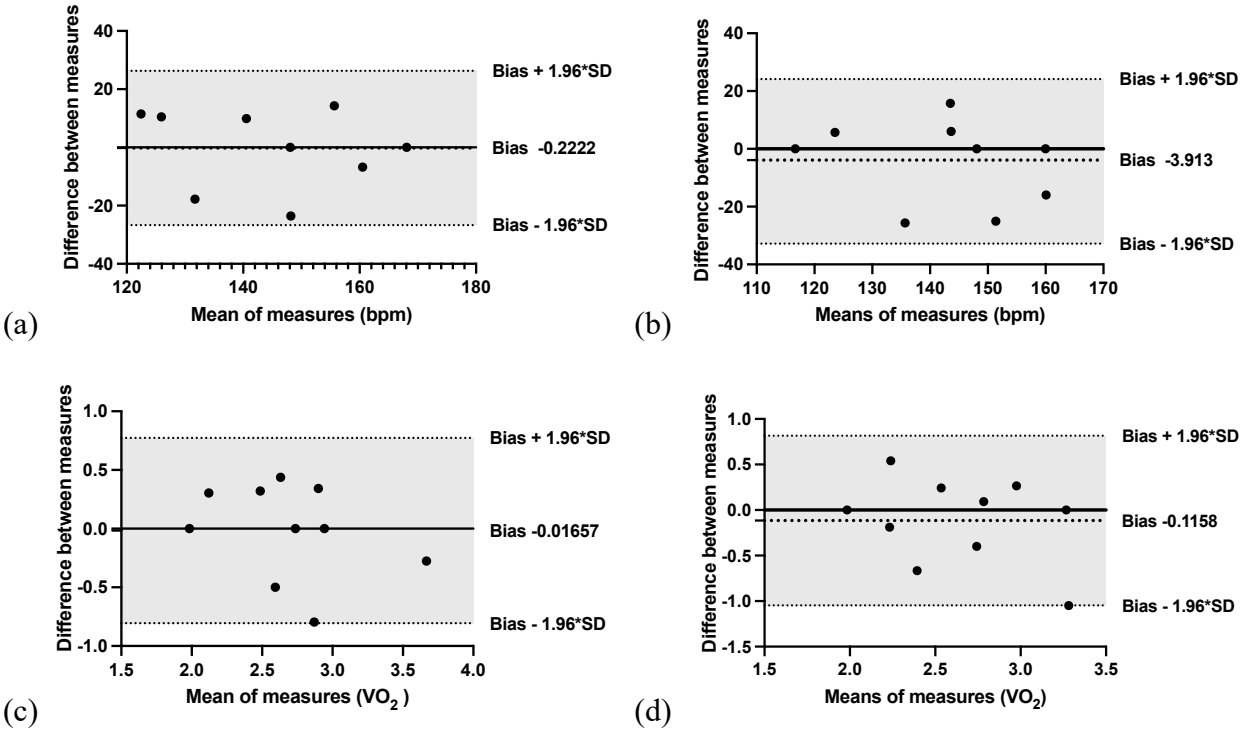


Figure 9. (a) Bland-Altman plot of heart rate (bpm) at blood lactate-determined LT compared to gas exchange LT. (b) Heart rate (bpm) at NIRS-determined LT compared to gas exchange LT. (c) VO_2 (L/min) at blood lactate-determined LT compared to gas exchange LT. (d) VO_2 (L/min) at NIRS-determined LT compared to gas exchange LT.

Fat and Carbohydrate Oxidation Rates

The dynamic balance between carbohydrate and fat metabolism during exercise is key for sustaining energy production in skeletal muscle. Research has explored the complex regulatory network governing these fuel sources, influenced by both substrate availability and exercise intensity (Spriet, 2014). The regulation of fat metabolism during exercise is highly dependent on fatty acid transport, intracellular triacylglycerol dynamics, and mitochondrial processes (Spriet,

2014). Drastic changes in intramuscular triacylglycerol content, achieved through dietary interventions, highly influence fuel selection processes during exercise, as found with both low-carbohydrate and high-fat diets (Spriet, 2014). Understanding the relative contributions of fat and carbohydrate oxidation during exercise, in addition to the mechanisms governing fuel selection in skeletal muscle, is important from a metabolic perspective. The body relies heavily on fat oxidation during low to moderate-intensity exercise, however, as exercise intensity increases, a greater reliance on carbohydrate oxidation is needed to meet energy demands (Spriet, 2014). Moreover, this shift in substrate usage is unsurprising considering carbohydrates are readily converted into ATP. Rates of total fat (FATox) and carbohydrate oxidation (CHOox) were calculated from measurements of inspired O_2 and expired CO_2 using stoichiometric equations, described by Frayn (1983).

Equation 3: FATox ($g \cdot min^{-1}$) = $1.67VO_2$ ($L \cdot min^{-1}$) - $1.67\ VCO_2$ ($L \cdot min^{-1}$)

Equation 4: CHOox ($g \cdot min^{-1}$) = $4.55VO_2$ ($L \cdot min^{-1}$) - $3.21VO_2$ ($L \cdot min^{-1}$)

Fat and carbohydrate oxidation (FATox/CHOox) constitute the rates at which fats and carbohydrates are metabolized, respectively, during exercise. These metabolic processes govern the use of energy substrates and play a pivotal role in meeting the energetic demands of exercise. FATox occurred at an average speed of 9.9 ± 2.2 km/hr and an average heart rate of 134 ± 16.9 bpm, whereas CHOox occurred at an average speed of 14.3 ± 1.7 km/hr and an average heart rate of 171 ± 10.2 bpm. It was found, however, that both metabolic processes were used simultaneously to varying degrees across different exercise intensities. Higher exercise intensities tend to be associated with carbohydrate oxidation, generally speaking, as found with CHOox rates measured through inspired O_2 and expired CO_2. Low to moderate-intensity exercise tends to rely more on fat oxidation, evident in FATox rates that occurred at both a lower average speed and lower heart

rate. In general, CHOox rates were highest in later stages of the exercise test wherein exercise intensity was greatest (**Fig. 10**).

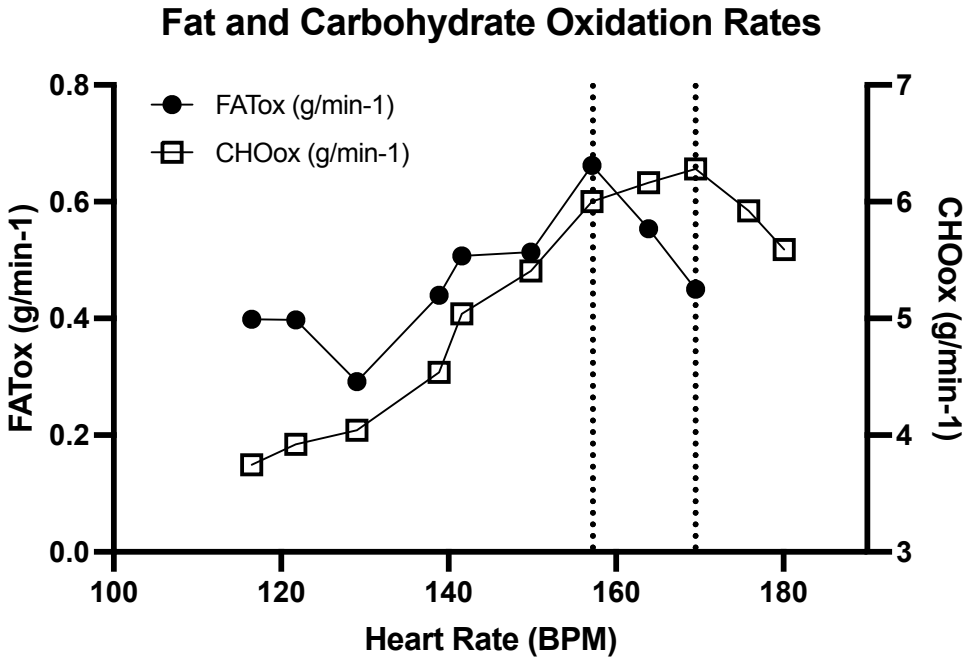


Figure 10. Fat (FATox) and carbohydrate (CHOox) oxidation rates (g/min^{-1}) calculated from inspired O_2 and expired CO_2 as a function of heart rate (bpm) using **Eq. 3 and 4** (Frayn, 1983).

FATox rates occurred near, but not necessarily coincident with the NIRS Control Ratio. A bias of -12.5 indicated that on average, FATox values were somewhat lower compared to the corresponding heart rate values of the NIRS Control Ratio using Bland-Altman analysis (**Fig. 11**). On the other hand, CHOox rates had a bias of 25.31, indicating that CHOox values were significantly higher compared to the corresponding heart rate of the NIRS Control Ratio. Findings suggest a systematic difference, indicating that NIRS may not be the best overall estimate of CHOox rates during progressively increasing exercise intensity.

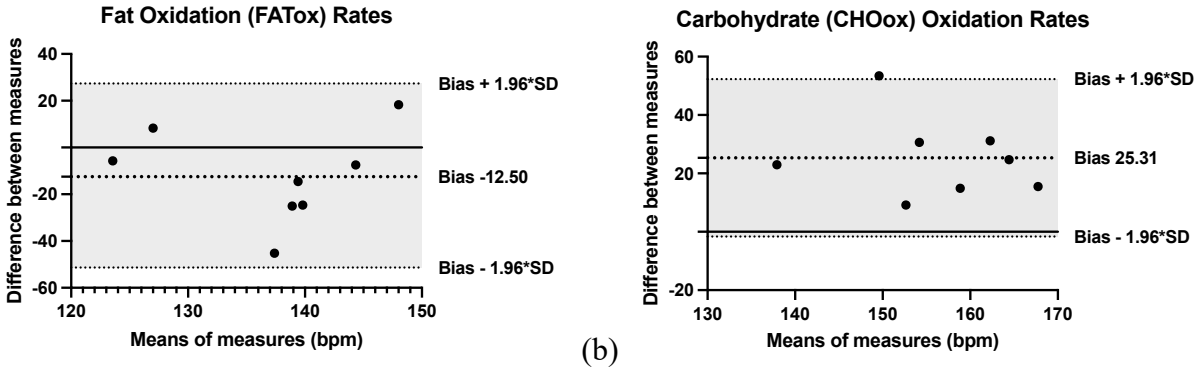


Figure 11. (a) Bland-Altman plot of HR at fat oxidation (FATox) compared to NIRS Control Ratio. (b) Bland-Altman plot of HR at carbohydrate oxidation (CHOox) compared to the NIRS Control Ratio.

DISCUSSION

This study aimed to 1) confirm the relationship between changes in muscle oxygenation (SmO_2) and the lactate threshold (LT) determined via traditional blood sampling, and 2) further explore the relationship between SmO_2 kinetics and exercise thresholds of respiratory gas exchange. The results of the study suggest that near-infrared spectroscopy (NIRS) provides valuable insights into the physiological and metabolic responses during progressively increasing exercise intensity. The discrepancies in the SmO_2 trace underscore the importance of considering individual variability in metabolic responses. The significance of this research lies in the ability to continuously monitor muscle oxygenation in real-time. Compared to traditional blood sampling, the NIRS device is a non-invasive approach and can therefore be used beyond the confines of a laboratory setting. From a health and wellness perspective, NIRS may be useful for populations where blood sampling is less practical, such as older populations or young children. Moreover, NIRS offers a non-invasive method to monitor metabolic thresholds in clinical applications, as with chronic diseases or conditions that may limit one's ability to undergo traditional exercise testing.

Future research should focus on validating these findings in larger and more diverse populations, as well as exploring the impact of training status and exercise modalities on muscle oxygenation. Additionally, investigating the relationship between muscle oxygenation and performance outcomes in endurance sports could provide valuable insights for optimizing training programs and enhancing endurance performance. The NIRS device was placed at the site of the *vastus lateralis*, it is unknown whether this relationship holds true across other muscle sites, or multiple muscle sites simultaneously. Though it would require extensive research and

development, integrating NIRS technology into wearable devices could be transformative for health monitoring and endurance performance.

Limitations

Based on current literature, the most significant limitation of the Moxy device is the effects of subcutaneous adipose tissue thickness on the NIRS signal, and consequently on SmO_2. Near-infrared light, as previously discussed, relies solely on the propagation of light through different mediums. Body fat percentages were estimated using bioelectrical impedance analysis, however, subcutaneous adipose tissue thickness on the *vastus lateralis* was not measured. All participants were active runners with an average body fat percentage of 14.89 ± 5.46 %. Nevertheless, subcutaneous adipose tissue thickness can impact the absorption spectrum detected by NIRS, future research investigating samples with uniform adipose tissue distribution may provide additional insights. It should be noted that an error in the NIRS signal occurred in one participant, this could be due to interferences or the Moxy device itself, however, this represents a relatively small percentage of the sample and doesn't meaningfully impact findings.

The treadmill used in this study (Trackmaster TMX 425) was set using a 1° (≈ 1.75°) grade at the "zero" setting; a 1° grade is considered the most accurate representation of the energetic cost of outdoor running (Jones & Doust, 1996). This relationship suggests that the energetic cost of running could slightly differ between a 1° and a 1.75% grade, therefore, it is reasonable to suggest the energetic cost of running may have exceeded that of outdoor running for select portions of the exercise test, eliciting a greater VO_2. Further research would be required to investigate whether this meaningfully limits the external validity of the results. Other limitations include the inability to precisely control temperature and humidity due to laboratory constraints, however, every effort was made to simulate consistent conditions among all participants. The testing protocol was

carried out in a laboratory setting with an average temperature of 21.65 ± 1.3°C and humidity of 23.13 ± 9.2%. Running in extreme temperature conditions, whether too hot or too cold, can impact physiological and metabolic processes. Nevertheless, given a relatively small standard deviation in temperature and humidity, it is reasonable to suggest a reasonable degree of internal validity.

CONCLUSION

The control kinetics of SmO_2 during exercise revealed a distinct breakpoint near, but not necessarily coincident with, the lactate and gas exchange thresholds in endurance athletes. Moreover, results suggest a meaningful relationship between SmO_2 kinetics and exercise thresholds of respiratory gas exchange, reporting an admissible level of agreement between methods. These findings have significant practical implications for both research and performance optimization, offering a convenient and accessible method for evaluating metabolic thresholds without the need for invasive blood sampling. Nevertheless, further investigation is warranted to better understand the precise physiological relationship between the NIRS-determined SmO_2 and exercise intensity; confirming the reliability of NIRS across various populations and exercise modalities is essential for contributing to the growing body of knowledge in the field of exercise physiology.

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APPENDICES

July 18, 2023

Miss. Melina Collins (Principal Investigator)
Dr. Daniel Kane (Supervisor)
Faculty of Science\Human Kinetics
St. Francis Xavier University

ROMEO File #: 26563
Project Title: Investigating the Validity of Near-Infrared Spectroscopy as a Non-Invasive Alternative for Assessing Lactate Threshold

Dear Melina Collins,

The Research Ethics Board (REB) has cleared the above cited proposed research project for ethics compliance with the Tri-Council Guidelines (TCPS) and St. Francis Xavier University's ethics policies. In accordance with the Tri-Council Guidelines, your project has been cleared for one year. At the end of each year, the REB will ask if your project has been completed and, if not, what changes have occurred or will occur in the next year. This will be required each year following approval until the project is reported to be completed, up to a maximum of five years.

Thank you for your careful attention to the recommended changes. One small point is in section 5.10. You are under no obligation to distribute individualized results to participants, and in fact, it may be best if you did not do so. This question asks about how you will distribute the overall *summary* results to participants. For example, will you email participants an executive summary once the data has been collected and analyzed?

Renewal Due-2024/07/18

You are reminded of your obligation to advise the REB of any adverse event(s) that occur during this one-year period. An adverse event includes, but is not limited to, a complaint, a change or unexpected event that alters the level of risk for the researcher or participants or situation that requires a substantial change in approach to a participant(s).

You are also reminded that all changes that might affect human participants must be cleared by the REB. For example, you must report changes in study procedures or implementations of new aspects in the study procedures. These changes must be sent to the undersigned prior to implementation.

On behalf of the Research Ethics Board, I wish you continued success in your research.

Sincerely

Dr. Christine Lomore
Professor and Chair
STFX Research Ethics Board

INVITATION TO PARTICIPATE

Title of Research: Using Near-Infrared Spectroscopy (NIRS) to Assess Lactate Thresholds During Running.

Romeo No: 26563

Name of Researcher(s):

Principal Investigator: Melina Collins
Email: x2020avj@stfx.ca

Supervisor: Dr. Daniel Kane
Associate Professor at St. Francis Xavier University, Department of Human Kinetics
Email: dkane@stfx.ca

This research study is being conducted as part of the requirements for an undergraduate honours' thesis in the Department of Human Kinetics at St. Francis Xavier University, in Antigonish, N.S.

I am writing to invite you to participate in a research study that seeks to validate the use of near-infrared spectroscopy (NIRS) to assess lactate threshold during exercise.

What is the Study About?

This study aims to investigate the validity of near-infrared spectroscopy (NIRS) to measure endurance performance and predict lactate thresholds during incremental exercise. The following research study seeks to examine near-infrared spectroscopy (NIRS) technology in the scope of exercise science as a non-invasive alternative to current methodologies. Near-infrared spectroscopy (NIRS) will be used to accurately measure real-time muscle oxygenation (%SmO_2) and total hemoglobin (tHb) levels. Near-infrared spectroscopy benefits from the relative absorption of near-infrared (NIR) light that readily penetrates tissue and skeletal muscle; emitting signals that differentiate between oxygenated and deoxygenated forms of hemoglobin and myoglobin (Fadel et al., 2004).

Prospective participants must meet the inclusion and exclusion criteria to be considered for this research study. Eligibility is restricted to recreationally active runners (at a minimum) between the ages of 18 and 59 who have competed at least once at any level in the previous 18 calendar months. Alternatively, prospective participants may be endurance athletes competing in any endurance sport who train at a minimum frequency of 3 times per week.

Exclusion criteria specific to this research study will seek to maintain the integrity of the study outcome and ensure ethical research practices. Participants will be excluded from participation if they have a history of anemia or neurological disorders affecting the nervous system that would limit one's ability to exercise normally. Individuals following strict diets or regimens that limit carbohydrate or glycogen levels will not be eligible to participate. Participants must not have a previous medical history or health conditions that would limit their ability to exercise. Participants must be non-smokers and otherwise in good overall health. Prospective participants should not answer "yes" to any of the items on The Physical Activity Readiness Questionnaire for Everyone PAR-Q+.

What Will I be Expected to Do?

If you choose to participate in the study, you will be asked to complete an incremental exercise test performed on a motorized treadmill. The incremental exercise test consists of a warm-up period of 15-30 minutes, followed by several 4-minute stages of running that increase in speed over time with a 1-minute rest period following each stage for blood sampling. The time commitment for participating in this study is approximately 1 hour in total carried out in one session. Blood testing is involved in the study, a very small capillary blood sample (less than 20 µl) will be sampled from a participant's finger at rest, during warm-up, and after each stage of the exercise test. In addition to blood sampling, data will be collected for variables including, but not limited to, heart rate, muscle oxygenation, total hemoglobin, blood glucose, and expired air.

Participants will be asked to refrain from caffeine and exercise at least 4 hours before the start of the exercise test. Participants should be properly nourished and hydrated at the time of participation. Participants will be required to provide demographic information for research purposes, including age, weight, height, and gender.

There is no financial incentive or compensation to participate in this study.

Confidentiality

The investigators and possibly other individuals of the research team will have access to study data and information, including the identities of those who choose to participate, but participants are guaranteed that every effort will be made to ensure that no one else becomes aware.

This research study is conducted with the intent to publish results. No identifying information will be included in any document resulting from this study.

What Happens if I Change My Mind and Wish to Withdraw?

Your participation in this research study is completely voluntary, and at any time you will be able to withdraw from the study without facing any negative consequences. Should you choose to withdraw, simply contact researchers, and express your intentions to withdraw. Prospective research participants will be able to withdraw at any point before their involvement, as well as during the exercise test, at which point participants can simply stop exercising/running at any point and signal their intention to withdraw from the study. Withdrawal of your participation does not necessarily include withdrawal of any data compiled up to that point. By consenting to be involved in this research you have not waived your right to legal recourse in the event of any research-related harm.

What are the Potential Benefits and Harms Associated with Participation in the Study?

Potential benefits of this research include a deeper understanding of the potential usages of near-infrared spectroscopy (NIRS) and its applications to lactate-based exercise thresholds. For participants, this study will provide personalized exercise test results, which may be of personal benefit or interest. In the scope of clinical exercise science, validation of a non-invasive measurement tool could replace current invasive methods and improve research practices.

As for potential harm, it is possible that your data may be intercepted by a third party, however, the risk to your privacy is very low. There are currently no identifiable conflicts of interest nor possibility of commercialization of the research. There are no known physical harms associated with your participation in this research. However, there may be harms that we don't yet know about. Exercise testing in clinical populations is considered a safe procedure, however, complications may arise unexpectedly. Risks associated with physical activity and exercise include fatigue and muscle soreness. Participants will perform a warm-up to avoid potential injuries related to exercise, and handrails are available on the treadmill to avoid potential injuries or falls. Operation of a motorized treadmill can result in injuries and must be used in accordance with the manufacturer's guidelines to avoid inherent dangers. Blood sampling involved in this study could result in infection or bruising, however, every precaution will be made to avoid complications. It is possible that a small number of participants may experience lightheadedness or fainting, in such cases, participants will be offered juice and will be asked to remain under observation in the lab until they feel well. An AED (automated external defibrillator) is in proximity to where the participant will be performing the exercise test (Room 103). In addition, the below emergency contacts are available.

Emergency Services (Public Safety Answering Point) - 911

St. Martha's Regional Hospital, Antigonish - (902) 867-4500

Where and How Will My Data Be Stored?

All information will be stored in password-protected computers, and any information collected on paper will be stored in a locked filing cabinet. Data collected, including demographic information, heart rate, blood lactate concentration, blood glucose concentration, muscle oxygenation levels, expired air data, and rating of perceived exertion, will be placed in a data repository for future research purposes. No biological materials will be stored in a data repository. The data repository used will be an electronic file folder designated specifically for research data related to this study. The nature and types of future research that may be conducted are not specified at this time. All data collected will be stored, and human biological materials, including blood, will be discarded appropriately, and will not be stored. Consent to the collection of data is voluntary, however, it may not be feasible to withdraw data after the completion of the study. The benefits of storage include the potential to revisit data and conduct future research, as well as potential correlational research. Potential risks to data storage do not exceed that of normal cyber security threats. Participants may opt to be contacted for additional future research should they choose to do so.

Where Do I Get Questions Answered?

If you have any questions or concerns, please do not hesitate to contact us at dkane@stfx.ca or x2020avj@stfx.ca.

Supervisor: Dr. Daniel Kane
Email: dkane@stfx.ca
Phone: (902) 867-2251
Room 103C
42 West Street

Please direct all questions or concerns regarding ethics issues to:

Dr. Christine Lomore
Chair of the StFX Research Ethics Board
clomore@stfx.ca
902-867-5387

Thank you for considering this request.

Melina Collins & Dr. Daniel Kane

CONSENT FORM

I have received a copy of the Invitation to Participate in the research project titled "Using Near-Infrared Spectroscopy (NIRS) to Assess Lactate Threshold During Exercise", have had an opportunity to read the information provided or it has been explained to me, and have had all questions that I may have had answered.

I agree to participate in this research project, understanding that I am doing so voluntarily, that confidentiality will be maintained, and that I have the right to withdraw from the study at any point using the means outlined in the Invitation to Participate.

Signature: _____

Date (dd/mm/yyyy): _____

I agree to permit my data to be deposited in a data repository for future unspecified research. I understand that I am doing so voluntarily. I understand that my data will be stored, excluding identifiable information such as name, and that I have the right to withdraw my data at any point using the means outlined in the Invitation to Participate.

Signature: _____

Date (dd/mm/yyyy): _____

Contact Information

Supervisor: Dr. Daniel Kane
Associate Professor at St. Francis Xavier University
Department of Human Kinetics

Email: dkane@stfx.ca
Phone: (902) 867-2251
Room 103C
42 West Street