The Assessment of Muscle Oxygen Saturation in Students During Maximal VO_2 Exercise and High Intensity Intervals

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Abstract

The advancement of human performance benefits from state-of-the-art technology to improve the ability to monitor and evaluate physiological adaptations in athletes. Previously validated as a reliable tool for measuring muscle oxygenation, near-infrared spectroscopy (NIRS) has evolved into a more portable platform capable of being used in a variety of settings. One such example of a portable NIRS device advertised to athletes and the military is the MOXY monitor.

Purpose: To our knowledge, no studies exist that have examined the output produced by the MOXY monitor compared to other more established variables during high intensity intervals. Therefore, the purpose of this experiment was to evaluate the output provided by the MOXY monitors (SmO2) by comparing SmO2 to more established exercise variables including VO2, heart rate and blood lactate (BL) to determine if the values agree with physiological expectations. We predicted that the SmO2 would demonstrate an inverse relationship to other measured variables including HR, VO2 and BL. Eg. As SmO2 decreased, HR, VO2 and BL would increase and peak HR, VO2,BL values would coincide with SMO2 troughs. **Methods**: Six endurance-trained East Carolina University students, aged 21±2 years completed a VO_2 max test followed by 6 high-intensity 30-second sprint intervals at 125% of VO2 max wattage on a cycle ergometer. SmO2 was measured using MOXY monitors (Fortiori Design LLC., MN, USA) placed on the quadriceps

muscles as well as the left deltoid. HR was monitored using a Garmin FR70 watch (Garmin Ltd., Switzerland) and VO2 obtained using a Parvomedic TrueOne 2400 metabolic cart (Parvo Medics, UT, USA). BL was collected within 5 seconds of the completion of each of the 6 high intensity intervals using a Lactate Plus Meter (Sports Research Group Inc.). SmO2 and VO2 and SmO2 and HR were plotted over time to present a graphical illustration demonstrating the relationships among changes in oxygen saturation and the change with VO2 and HR. BL and SmO2 were presented using tables to display changes in BL and SmO2 as the intervals progressed. **Results**: All participants had a VO2 max >30ml/kg/min and no musculoskeletal injuries in the previous 3 months were reported. During each of the six intervals, SmO2 was shown to decrease as both VO2 and HR increased. Troughs in SmO2 did not consistently align with peaks in VO2 and HR, as SmO2 tended to reach troughs about 10 seconds after the completion of many intervals. VO2 demonstrated peaks at 20 and 40-seconds following interval completion. Patterns in BL were unable to be evaluated due to inadequate BL collection. Three participants demonstrated a pattern of increasing BL with intervals 1-4 followed by a plateau or decline in the final intervals.

Conclusion: The findings from this study support the hypothesis that SmO2 would demonstrate an inverse relationship compared to HR and VO2 during high intensity intervals. e.g. As SmO2 decreased during the intervals, both HR and VO2 increased. Peak HR and VO2 values did not coincide with SmO2 troughs. A delay of about 10 seconds was observed in SmO2 troughs and a 20 to 40-second delay was seen in peak VO2 and HR values. The results obtained from this study suggests the MOXY monitors may provide reliable output as the inverse relationships among SmO2 and HR and SmO2 and VO2 agree with physiological expectations, despite peaks and troughs not coinciding. Further research will be needed to explore the relationships among these variables and to further assess the MOXY monitor as a tool for exercise training.

The Assessment of Muscle Oxygen Saturation in Students During Maximal VO_2 Exercise and High Intensity Intervals

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List of Abbreviations

SmO_2: muscle oxygen saturation; expressed as a percentage; produced by MOXY

NIRS- near-infrared spectroscopy

THb: Total hemoglobin: expressed as; produced by MOXY

VO_2: volume of oxygen consumed; expressed as ml/kg/min; produced by metabolic cart

BL: blood lactate; expressed as mmol/L; obtained using Lactate Plus Meter

HR: heart rate; expressed as beats per minute

BPM: beats per minute

VO_2 Max: maximum volume of oxygen consumed; expressed as ml/kg/min

NIR- near-infrared light

L- left quadricep MOXY monitor

R- right quadricep MOXY monitor

D- deltoid MOXY monitor

Quad- quadriceps

RPM- rotations per minute

W- wattage

Chapter 1: Introduction

The field of human performance relies on technological advancements in exercise monitoring to guide training regimens in order to push the capabilities of the human body to perform at the highest level. There are many technologies available to monitor training adaptations in athletes of all levels with the goal of increasing performance, longevity and recovery. Two established exercise tools include the heart rate (HR) monitor and the metabolic cart, used to measure heart rate and oxygen consumption (VO_2), respectively.

A lesser used technology continuing to be explored for its potential as an exercise training tool is near-infrared spectroscopy (NIRS). NIRS has been validated for its ability to accurately measure changes in oxygenation both in the brain as well as within skeletal muscle for quite some time (Mancini et al., 1994). NIRS uses near-infrared light, between 600-1,000 nanometers to penetrate the capillaries within muscle tissues. It was discovered that when near-infrared light was shown into body tissues, differences were observed in the absorbency of oxygenated and deoxygenated hemoglobin and myoglobin (Bellardinelli et al., 1995). NIRS measures the amount of near-infrared light that is reflected to a detector after being shown into the muscle and partially absorbed based on the amount of hemoglobin and myoglobin that is oxygenated. Calculations are then used by the various monitors to estimate a percentage of hemoglobin and myoglobin that is oxygenated, termed muscle oxygenation. Initially used in the clinical setting to measure oxygenation within the brain, NIRS technology was quickly adopted into the field of exercise science as a potential route for monitoring changes in skeletal muscle oxygenation during exercise.

The ability for NIRS to measure changes in muscle oxygenation has been validated, as demonstrated in studies by Grassi et al. (1999), Shibuya et al. (2003), and Tachi (2004) and summarized in a NIRS review by Bhambhani et al. (2004). Previous research has examined muscle

oxygen saturation during incremental exercise as well at higher intensities. During incremental exercise, Bellardinelli et al. (1995) found skeletal muscle oxygen decreased progressively throughout testing and was most desaturated at around 80% of VO2 max. Bellardinelli concluded that NIRS is a noninvasive method of monitoring skeletal muscle changes in oxygenation during incremental exercise. Additionally, Bhambhani's 2004 review supports the effectiveness for NIRS technologies to measure muscle oxygen trends during both incremental aerobic exercise as well as anaerobic exercise.

As NIRS has become more widely explored for its applications in exercise, more portable NIRS devices have become a focus among the commercial market. A portable and wireless NIRS monitor, and the one chosen for this study, is the MOXY monitor. It is small and economically priced for the everyday consumer, unlike many NIRS instruments that are bulky and expensive. MOXY uses four light emitting diodes that shine near-infrared light into the muscle tissues which is then reflected to two detectors, distinguishing it from other devices with a single detector. A series of calculations and algorithms are then used by the monitor to estimate a percentage of hemoglobin and myoglobin that is oxygenated, coined skeletal muscle oxygen saturation (SmO2). Little research has been published examining the SmO2 output provided by the MOXY monitor. Some available studies using MOXY monitors have examined the reoxygenation of forearm muscles in rock climbers and lower extremity oxygenation after low-intensity training in older adults (Bau et al., 2015 & Balas et al., 2015).

Similar to the MOXY monitor is another wireless NIRS device known as the PortaMon. (Artinis Medical Systems, Netherlands). Jones et al. (2015) examined muscle oxygen using the PortaMon in Olympic caliber field hockey athletes during a 6-week sprint interval cycling protocol. It was found that NIRS was able detect positive peripheral muscle oxygenation changes. Additional

studies by Miura et al. (2000) and Grassi et al. (1999) found correlations between muscle oxygenation and VO2 and blood lactate. Grassi found that as blood lactate accelerated, muscle oxygenation decreased. Examining muscle deoxygenation during sprints on a cycle ergometer, Racinais et al. (2007) also demonstrated a decrease in oxygenation coinciding with an increase in VO2 following 6-second sprints and 30-second rests.

To our knowledge, no published research has been conducted using the MOXY monitors to measure SmO2, VO2, heart rate and blood lactate during high intensity intervals. The purpose of this experiment was to evaluate the output provided by the MOXY monitors (SmO2) by comparing SmO2 to more established exercise variables including VO2, heart rate and blood lactate (BL) to determine if the values agree with physiological expectations.

Research Hypotheses

1) SmO2 measured by the MOXY monitors during high intensity intervals will show an inverse relationship with heart rate. Eg. SmO2 will decrease during the intervals while HR rises. Troughs in SmO2 will correspond to peaks in HR.
2) SmO2 measured by the MOXY monitors during high intensity intervals will illustrate an inverse relationship with VO2. Eg. SmO2 will decrease during the intervals while VO2 increases. SmO2 troughs will correspond to peaks in VO2.
3) SmO2 measured by the MOXY monitors during high intensity intervals will decrease with each interval as the intervals progress while BL will increase with each interval.

Delimitations

Age- Age of subjects ranged from 18-23 years

Sample Size- This was a pilot study evaluating individual participants

Intensity of Cycling During Intervals- The intensity for intervals was set at 125% of max power wattage and rest periods were set at 20%

Duration of Interval- 30-second intervals were chosen based on the recommendation of Chuck Tanner (Professor at ECU in the Dept. of Kinesiology)

Duration of Rest Between Intervals- 90-second rest periods were chosen based on the recommendation of Chuck Tanner (Professor at ECU in the Dept. of Kinesiology)

Limitations

Desired Cycling Wattage- The cycling ergometer consistently took between 10-15 seconds to achieve desired wattage for the high intensities for the participants with the highest power wattages. Therefore, cycling time under desired wattage was limited

Blood Lactate Collection- BL readings were limited by sweat and calloused hands

Loss of signal from MOXY Monitors- Output provided by the MOXY monitors were unavailable for some time periods

Unusually high SmO2- Output provided by the MOXY monitors showed alarmingly high values during the high-intensity intervals for participant 006

Chapter 2: Review of Literature

Exercise Tools

Advancement in exercise technology is central to the optimization of training in athletes. In the last 100 years, many advances have been made in exercise monitoring that have broadened the scope of available biological data. Examples include the measuring of heart rate using wireless monitors, volume of oxygen consumed (VO2) using metabolic carts and blood lactate using a variety of lactate meters. Today, there are many exercise technologies available to all levels of athletes, with the ability to monitor steps, heart rate, calories burned, and distance traveled. These have been made readily available by companies such as Fitbit, Garmin and Jawbone. With the level of sports performance continually increasing, a high demand has been created for technology companies to produce even greater advancements in wearable exercise tools to optimize training.

It wasn't until the 1980's that wireless heart rate monitors became available for everyday consumers, creating a surge in popularity (Achten et al. 2003). The ability for heart rate monitors to measure heart rate has long been validated. Today, they are a staple among researchers and exercisers to monitor and optimize training and improve performance. Heart rate can directly be used as an indicator of exercise intensity and is often manipulated for training in certain 'heart rate zones' to elicit a desired training adaptation. The American Council of Sports Medicine has even used heart rate to develop general classifications of physical activity using percentages of maximum heart rate (Pollock et al., 1998). Recently, heart rate variability (HRV) has gained popularity as it may be a better indicator than sole heart rate for overall training status and health. A study by Tsuji et al. (1994) found that high HRV was associated with higher VO2 max while lower HRV was associated with increased mortality. To explain the impact an exercise tool may

have on advancing the world of sports performance requires looking no further than the heart rate monitor.

A newer exercise tool growing in popularity since transitioning into a more portable platform is near infrared spectroscopy (NIRS), a noninvasive method developed to assess changes in oxygenation within skeletal muscle. The development of NIRS as an exercise tool is credited to Glenn Allan Millikan who was the first to use a dual wavelength oximeter to assess oxygen within muscle (Mozina et al., 2011). It was Frans Jobsis who in 1977, made the observation that near-infrared light acted in a transparent manner when shown into body tissue and noted that the absorbency differed depending on the oxygenation of hemoglobin and myoglobin (Hamaoka et al., 2007). It is this difference in absorbency that allows NIRS technologies to distinguish between oxygenated/deoxygenated hemoglobin and myoglobin within muscle tissues. NIRS works by shining near-infrared light between 600-1,000 nanometers into muscle tissue. The reflected light is then returned to a detector(s) where a series of algorithms and calculations produce a useable estimation of muscle oxygen saturation. The Beer-Lambert law provides the mathematical basis for NIRS, which states "that light passing through a solution of colored compound is absorbed by the compound, resulting in reduction in intensity of emerging light" (Mozina et al., 2011). This is an integral component for calculations in all NIRS instruments as many devices have altered this equation to claim greater measurement precision. Additional calculations are added to account for various issues such as the scattering of light caused by adipose tissue. The number of light sources as well as the number of detectors may differ slightly among NIRS instruments. Early models of NIRS used a single-distance continuous wave light source while more modern models may use multiple sources and detectors.

Original NIRS instruments were used to assess oxygenation within the brain and were quite large, expensive and immobile. More portable models were later created for more dynamic use but still required wires and a control box, restricting its use to the laboratory (Hamaoka et al., 2011). In recent years, wireless NIRS monitors have been developed to offer the user free rein to be used in a variety of settings. Two such monitors are the PortaMon (Artinis Medical Systems, Netherlands) and the MOXY monitor (Fortiori Design, LLC, Minnesota) and allow researchers to openly explore muscle oxygen saturation during an array of exercises including running, cycling, hiking, swimming as well as during sport. The PortaMon and MOXY are similar in that they are both portable, wireless monitors that are attached directly to the skin of the testing muscle and transmit muscle oxygen estimations via Bluetooth directly to a software database provided with purchase. These monitors are marketed to a wide population including researchers, recreational exercisers, as well as elite athletes. Like all NIRS instruments, the two devices differ in the number of lights and detectors as well as mathematical estimation of muscle oxygenation. MOXY uses 4 light emitting diodes as well as two detectors, distinguishing it from other single light diodes with one detector. Like most NIRS instruments, both the PortaMon and MOXY use the Beer-Lambert law in their calculations; however, many companies choose to modify the equation, attempting to correct for possible scattered light caused by adipose tissue between the skin and muscle. With this new-found portability, NIRS has emerged as a potential frontrunner in exercise monitoring.

NIRS in the Literature

Since its adoption as an exercise tool, the ability for NIRS to observe changes in muscle oxygenation has been well examined. Few studies have been published using MOXY monitors. Recently, two noteworthy studies investigated forearm muscle oxygen recovery in rock climbers

and the effects of low-intensity exercise training on muscle strength and oxygenation in older adults. Bau et al. (2015) found that a 6-week training program in older adults in community dwellings counterbalanced the muscular function decline seen in the control group. Balas et al. (2015) assessed the effects of different recovery techniques during rock climbing on forearm reoxygenation It was found using MOXY monitors that shaking the forearm beside the body allowed for greater reoxygenation and recovery between climbs. These studies demonstrate the potential applicational use MOXY monitors may have in exercise monitoring.

In addition to its use as an exercise tool, NIRS has often been used in the clinical setting. A study by Wilson et al. (1989) found that the range of 760-800 nanometers proved to correlate closely with venous hemoglobin oxygen saturation. To assess the ability for NIRS to detect flow-related changes in hemoglobin and myoglobin, this same range of near-infrared light was used to assess oxygen saturation in the vastus lateralis muscle during progressive cycling exercise in both normal subjects as well as those with heart failure. This study found a lower oxygenation among the heart failure patients and suggested NIRS as a method to detect impaired oxygen delivery in patients with heart failure. In estimating muscle oxygen during incremental exercise, Bellardinelli et al. (1995) found that oxygen saturation decreased with incremental exercise. During progressive workloads on a cycle ergometer, a slow decrease in oxygen saturation was observed followed by a more rapid decrease around the lactate threshold and a plateau of minimum saturation point around 80% VO2 max. Works by Bellardinelli et al. (1995) and Bhambhani et al. (1997) demonstrated a four-phase oxygen saturation trend consistently observed during incremental exercise. At the onset of exercise, an initial increase in muscle oxygen saturation is seen, followed by a decrease below resting baseline with increased work rate, followed by a leveling off as fatigue or VO2 max is attained and concluding with a rapid

increase in saturation during the 1-2 minutes following exercise. Additionally, Neary et al. (2001) found that longer duration exercise elicited greater deoxygenation of hemoglobin and myoglobin.

NIRS technology has also been utilized during high intensity exercise. Racinais et al. (2007) assessed muscle oxygen patterns during ten 6-second sprints with 30-second rests using a cycle ergometer. Significant muscle deoxygenation was observed during repeated sprints alongside a significant increase in VO2. In another study using 30-second high-intensity Wingate tests on a cycle ergometer, Bae et al. (2000) found a decline in vastus lateralis muscle oxygen between seconds 3-15, with a plateau occurring after 15 seconds. The ability for NIRS to measure muscle oxygen patterns has been well covered in review papers by Bambhani et al. (2004) and Hamaoka et al. (2011).

In addition to examining muscle oxygenation patterns, NIRS has also been used to compare to other more established exercise measurements. Several studies have found an inverse relationship among muscle oxygen saturation and oxygen consumption (VO2). As previously mentioned, Racainas found an inverse relationship during repeated sprint tests. As muscle oxygen decreased, VO2 was found to increase. A 2003 study by Shibuya et al. examined the relationship between muscle oxygenation and VO2 max during incremental cycling. It found that a significant relationship existed between the percentage of muscle oxygenation and VO2 max. Shibuya also suggested that muscle oxygenation using NIRS technology could be a predictor of muscle oxygen diffusion capacity since the diffusion of muscle oxygen is a limiting factor for VO2 max. In an earlier study by Tesch et al. (1985), beta-blockers were used to lower heart rate in participants, thereby lowering VO2. Results found that oxygenation decreased, suggesting a correlation between the two variables.

The relationship between muscle oxygenation and blood lactate has also been examined. Studies have found that muscle oxygenation decreases as blood lactate increases. Grassi et al. (1999) found significant correlations between the onset of lactate acceleration and the onset of muscle deoxygenation during incremental exercise. Balsom et al. (1994) also found that diminished oxygen availability resulted in greater accumulation of blood lactate. Bhambhani et al. (2004) demonstrated the correlation between lactate threshold and VO2 max. Bellotti et al. (2013) found that a plateau in muscle oxygenation correlated with maximum steady state while cycling and suggested muscle oxygen monitoring may be a better means for monitoring aerobic fitness than VO2 max due to the cost effectiveness and ease of use. Overall, the ability for NIRS to measure muscle oxygenation patterns and correlate these findings to other measurements including VO2 and blood lactate has been adequately examined. To date, no research exists in assessing the MOXY monitor output along with VO2, HR and blood lactate.

Purpose

The purpose of this study was to assess the MOXY monitor as a potential exercise tool by evaluating SmO2 along with established exercise measurements including VO2, heart rate, and blood lactate and comparing to physiological expectations. This was done to establish relationships among the variables to explore the possibilities with NIRS technology. Our hypotheses were that heart rate and VO2 would increase to peak values during the 30-second intervals as SmO2 decreased to a trough. We also predicted that SmO2 would decrease as the interval testing progressed and that BL would show an increase from interval 1 to interval 6.

Significance

The validity of NIRS has long been examined and has shown its ability to produce useable estimations of muscle oxygenation. With the development of more portable and wireless

NIRS devices, the applications for use in exercise monitoring are numerous. Many papers have been written examining the output provided by various devices in a variety of settings. Studies have measured other parameters alongside muscle oxygen like VO2 and blood lactate and found inverse relationships. These findings suggest that SmO2 provided by the MOXY monitor may provide a noninvasive route for assessing exercise intensity and recovery as well as oxygen utilization.

Chapter 3: Methods and Materials

Participants

The participants in this study were 6 endurance-trained East Carolina University students aged 18-23 years. Inclusion criteria included regularly engaging in endurance exercise for at least 30 minutes a minimum of three times per week for the previous 6 months. Participants were also required to be between the ages of 18 and 30 years and have a minimum VO2 max of 30ml/kg/min in the VO2 max test (maximum oxygen consumption in milliliters per kilogram per minute). Participants were excluded if they had a history of cardiovascular disease, bleeding disorders, or a musculoskeletal injury over the previous 3 months. Written voluntary consent was obtained from all participants, providing necessary information regarding the purpose, procedure and risks of the study. The study procedure was approved by the ECU Institutional Review Board.

Experimental Protocol

This study was conducted in the Human Performance Laboratory (HPL) in room 363, located in the Ward Sports Medicine building at East Carolina University. The laboratory used was a biosafety level 2 certified laboratory. Participant's full participation was 90-minutes or less and required just one visit. During this visit, participants completed questionnaires on training including related injuries, typical exercise duration and intensity as well as exercise classes taken in school. The dietary questionnaire included a frequency questionnaire in which it was recorded how many times in the past month, week or day the participant consumed a variety of foods as well as dietary supplements.

VO2 max was assessed using a cycle ergometer to obtain a VO2 max value as well as a maximum wattage to later be used in calculating interval intensity for the participants. SmO2, HR, and VO2 were measured during the test as well as a BL measurement both before and after.

Finally, six 30-second high-intensity intervals were done on the same cycle ergometer while SmO2, VO2, HR were measured. BL was obtained a total of six times during the intervals, occurring within 5 seconds after the end of each of the six intervals. Anthropometrics measurements, including height, weight, and skinfold thickness were taken for each participant, followed by the placement of the MOXY monitors on each quad and the left deltoid.

Questionnaires

Participants completed questionnaires pertaining to training history, diet and supplement use. Examples of the training and dietary questionnaires can be found in appendix C. The training questionnaires provided information on the participant's training regimen such as type of exercise, frequency, duration, and intensity. Questions also included history of endurance training, number of miles run per week, number of training injuries in the last 12 months, number of exercise courses taken in school, and musculoskeletal injuries in the previous 3 months. The dietary questionnaire included a food frequency questionnaire in which participants responded with how many times per day or week they consumed the following foods: fruits, vegetables, whole grains, dairy, nuts, fish, poultry, lunch meats, pork and fish. The dietary supplement questionnaire included questions regarding frequency of use, including the following supplements: multivitamins, vitamin D, fish oil, protein powder, creatine/HMB, pre-workout stimulants, energy drinks, melatonin, and amino acids, among others.

Anthropometric Measurements

Both height and weight were obtained using a Detecto medical scale (Detecto Scale Company, MO) located in the human performance laboratory. Body fat was obtained using skinfold calipers (Lange, HealthCheck Systems, NY) and the 7-site skinfold method according to ACSM procedures (Thompson et al., 2010). All measurements were taken twice, with a third

attempt if there was greater than 2 cm difference between first two. Skinfold sites included triceps, chest, midaxillary, subscapular, suprailiac, abdominal, left thigh, and right thigh. All sites were taken in millimeters (mm). Calculations were done using the average between the left and right thigh measurements. Calculations for body fat were done using the Jackson and Pollock body density equation followed by the Siri equation (Jackson, A.S et al. 1978; Jackson, A.S et al. 1980; Siri et al. 1961).

Jackson and Pollock Equation:

Males: Body Density = 1.112 – (.00043499 x sum of skinfolds) + (.00000055 x square of the sum of skinfold sites) – (.00028826 x age yrs)

Females: Body Density = 1.097 – (.00046971 x sum of skinfolds) + (.00000056 x square of the sum of skinfolds sites) – (.00012828 x age yrs)

Siri Equation:

% Body Fat = (495/ body density) – 450

Placement and Internal Calibration of The MOXY Monitor

The MOXY monitor is a portable and wireless muscle oxygen monitor that uses near-infrared spectroscopy to assess skeletal muscle oxygen saturation (SmO2) and total hemoglobin (THb) in a specific muscle during exercise. The equation for SmO2 can be written as follows:

$$SmO2 = \frac{\text{Oxygenated hemoglobin and myoglobin}}{\text{Total amount of hemoglobin and myoglobin}}$$

MOXY uses near-infrared light between 670 and 810 nanometers to penetrate the muscle fiber and based on differing absorbencies between oxygenated/deoxygenated hemoglobin and myoglobin, an algorithm is used to calculate a percentage of muscle that is oxygenated. (https://www.moxymonitor.com/) As a small portable device, the MOXY monitor is advertised as an exercise tool to be used to monitor adaptations in muscle oxygen saturation to improve

muscular aerobic capacity. The purchase of the monitor comes with a software program called Peripedal that collects, displays and stores data on a computer directly from the monitors in real time.

Three MOXY monitors were used during testing and placed on the participants where they remained until all testing was completed. One monitor was placed on each subject's left and right quadriceps. Using a tape measure to measure the distance between the patella and the hip, a marker was used to mark a midway point for the monitor to be placed. This was done on both quadriceps. The muscle most likely being measured based on placement was the rectus femoris. A third monitor was placed on the midway point on the left deltoid as a control for a non-working muscle. The same monitors were used to measure the left and right quadriceps and deltoid for each subject to control for monitor variation. Each monitor was labeled with a manufacturer given number, with the three used in this study being 327, 524 and 525. Monitor number 327 was assigned to the left deltoid, monitor 524 to the subject's left quadriceps and 525 to the right quadriceps muscle. Each monitor was held to the skin using specific sticky pads that came with the purchase of each of the monitors. Black Powerflex tape was used to cover the monitors to ensure ambient light was unable to interfere with the monitor's near-infrared light. The MOXY monitors provided wireless data on every interval through testing and these data were stored in the Peripedal program for analyzing.

For the purpose of obtaining a minimum value for comparison with testing values, an occlusion calibration protocol was used on the monitors assigned to the participant's quadriceps. A large manual blood pressure cuff was placed around the subject's thigh, above the location of the MOXY monitor. The participant's testing leg was slightly elevated, held up by the researcher to promote occlusion. The blood pressure cuff was then inflated as tolerated by the subject.

SmO2 levels were monitored. Once SmO_2 plateaued for greater than 30 seconds or the subject became too uncomfortable to continue, the calibration ended and the lowest SmO_2 obtained was recorded. The procedure was completed on both legs. This procedure was recommended by Terence Ryan, an expert in NIRS technology who works at the ECU Heart Institute and specializes in NIRS technology and conduced similar procedures in previous research. (Ryan et al. 2013)

VO_2 Max Test

The VO_2 max protocol was adopted from the Storer Maximal Bicycle Test. (Storer et al. 1990). Testing was conducted on a Corival cycle ergometer using a Parvo Medics TrueOne 2400 metabolic cart. Standard facemask and VO_2 testing equipment was used with the metabolic cart to obtain VO_2 and the respiratory exchange ratio. Participant's shoes were taped to the pedals of the cycle ergometer to prevent the pedal straps from coming undone due to the intensity of the cycling.

Participants began the VO_2 max test at 50 watts on the cycle ergometer and were instructed to maintain 70 rpms. The metabolic cart was connected to the cycle ergometer and pre-programmed to begin at 50 watts and automatically increase by 15 watts every 60 seconds. The test was terminated when the rotations per minute (rpms) fell below 70rpm. The last fully completed interval wattage was designated the maximum power wattage and subsequently used to calculate the interval intensities. VO_2 max was calculated using ml/kg/min and provided by the metabolic cart. A 10-15-minute break was allowed between the VO_2 test and the high-intensity intervals.

In addition to SmO2 and THb provided by the MOXY monitors, heart rate, VO2 and blood lactate was also measured. Heart rate was taken continuously using a Garmin FR 70 watch

which was synced to the Peripedal program using ANT+ technology provided with the Garmin watch. Heart rate was displayed in the Peripedal program and stored with SmO2 and THb. The heart rate monitor used in this study contained a heart rate strap to be worn by all subjects during testing. The monitors were activated before testing and beats per minute were verified to be displayed on the laptop with SmO_2 and hemoglobin before continuing. VO2 was measured by the metabolic cart and was taken every 20 seconds. The Parvo Medic metabolic cart was calibrated prior to participant testing following the standard Human Performance Lab Parvomedics instructions.

Blood lactate was taken 30 seconds before testing began and immediately following testing using a Lactate Plus meter by Sports Resource Group, Inc. The meter used a single drop of blood on the end of a blood lactate strip, much like a diabetic checks their blood glucose. After the drop of blood was applied, the meter took 14 seconds to display the blood lactate reading. The principal investigator (PI) was the only researcher to conduct the blood lactate pricks. Blood absorbent cloths were places on the floor and around the cycle ergometer's handle bars to catch possible stray blood drops. The PI wore an appropriate laboratory coat and did all testing over a blood absorbent cloth covered table.

High-Intensity Intervals

Subjects completed six 30-second intervals with 90 seconds of rest between intervals. The intensities of the intervals were calculated based on the subject's maximum wattage during the VO2 max test. The intervals were conducted using 125% of maximum wattage obtained in the VO2 max test, while the 90-second rests between intervals were done at 20% maximum wattage. All subjects began and ended the high-intensity intervals with a 90-second rest. The cycle ergometer was programmed to automatically adjust to pre-calculated wattage using a

computer-generated protocol on the metabolic cart. Subjects were instructed to continue pedaling at all times and to pedal as hard as possible during the 30-second intervals with verbal encouragement.

Heart rate, SmO2, THb, VO2 and blood lactate were measured during the interval testing. Blood lactate was taken immediately concluding each interval, totaling 6 blood lactate samples. A second attempt at obtaining a lactate reading was immediately used with the subject's permission if the first attempt was unsuccessful. A third attempt was not used if the second attempt failed.

Data Analysis

Data collected by the Peripedal program, including SmO2, THb and heart rate were converted using Peripedal into Microsoft EXCEL spreadsheets, with data values displayed against time. Time measured by the metabolic cart displaying VO2, and blood lactate documented by the researchers were matched manually to align with the time scale used by the Peripedal program.

This pilot study was analyzed like a series of case studies to identify if the MOXY monitor output followed patterns of established physiological factors (VO2, heart rate, lactic acid). Graphs were created using Microsoft EXCEL for each participant. Line graphs were used to illustrate the change in measurements over time. A figure was created for each subject to display SmO2 and THb over time, each with its own y-axis, and time displayed on the x-axis. Another figure was created for each subject with SmO2 and VO2 having their own y-axis with time on the x-axis. A third figure displayed SmO2 and heart rate on two y-axes and time on the x-axis. A table was created for each subject to display SmO2 and blood lactate. Tables were also

used to display results of the nutritional and training surveys as well as results from VO2 max testing and calibration.

Chapter 4: Results

Participant Characteristics

A total of 6 East Carolina University students (2 males, 4 females) aged 18-23 years participated in the study. Table 1 shows descriptive characteristics for participants. Overall, participants were young and healthy, with body composition in the normal range per guidelines set by the American College of Sports Medicine (ACSM) (Dwyer et al., 2008).

Table 1. *Participant Demographics and Anthropometric Measurements*

Characteristic	Mean ± SD	Range
Age (yrs)	21 ± 2	18-23
Height (m)	1.69 ± .12	1.58- 1.88
Weight (kg)	69.2 ± 17	50-100
Body Fat (%)	18.4 ± 6.3	7.4-25.2
Sum of Skinfolds (mm)	104 ± 30	62-134

Table 2 shows training characteristics of participants. Five of the six participants reported having performed regular endurance training for greater than one year while one subject reported not having been performing regular endurance style training; however, this subject had a VO_2 max similar to the other endurance-trained participants and was permitted to participate in the study. Four out of the six participants reported an average exercise session lasting between 60-120 minutes while two participants reported typical exercise duration lasting greater than 120 minutes. Mean exercise intensity reported was 3.6 out of 5.

Table 2. *Training Characteristics*

Variable	Results
Participants who engage in endurance training ≥3 days/week for ≥30 minutes each session (n, %)	5 (83%)
Participants who ran regularly (n, %)	1 (17%)
Participants with injury in past year (n, %)	1 (17%)
Participants who complete exercise course in school (n, %)	4 (66%)
Mean Exercise Frequency (days/week)	5± 1
Mean Exercise Length (min/session)	90± 45
Mean Exercise Intensity (scale: 1=light; 5=hard)	3.6± 0.8

Last 3 results are mean ±SD

Table 3 shows the results of the dietary questionnaire. Participants reported meeting USDA guidelines for fruit and vegetable intake with mean consumption of at least 2.5 servings of fruits and 2.5 servings of vegetables per day (www.choosemyplate.gov). Dairy was reported to be consumed daily. Poultry was reported to be the most frequently consumed meat. One participant failed to complete the nutritional and dietary supplement portion of the survey.

Table 3 *Dietary Questionnaire*

Question	Lowest Value	Mean ± SD
Fruit servings per day	2.5	3.1 ± 0.8
Vegetable servings per day	2.5	3.1
Grain servings per day	0.2	2.3 ± 1.4
Dairy servings per day	1.0	1.9 ± 0.8
Nuts servings per week	0.5	2.1 ± 1.4
Red meat servings per week	0	0.7 ± 0.4
Poultry servings per week	2.5	3.7 ± 0.7
Lunch Meat servings per week	0	1.9 ± 1.9
Pork servings per week	0	0.9 ± 1.0
Fish servings per week	0.3	1.5

Only 5 participants completed questions on dietary intake

Table 4 shows the results of the dietary supplement questionnaire. Results showed an overall limited use of dietary supplements among participants. Two participants reported using a multivitamin daily; two reported using a single vitamin or mineral supplement 2-3 times per week; and 2 participants reported using a carbohydrate and/or electrolyte supplement 2-3 times per week. Other supplements that were used at least once a month included protein powder, amino acids, energy drinks, melatonin and joint formulas.

Table 4: *Supplement Use Questionnaire*

Frequency of Use	Results
Multivitamin (n, %)	2 (40%)
Single vitamin or mineral (n, %)	2 (40%)
Carbohydrate and/or electrolyte supplement (n, %)	2 (40%)
Protein Powder (n, %)	1 (20%)
Amino Acids (n, %)	1 (20%)
Energy Drinks (n, %)	1 (20%)
Melatonin (n, %)	2 (40%)

Joint Formulas (n, %)	1 (20%)

Only 5 participants completed questions on dietary supplements

Internal Calibration of MOXY Monitors

Table 5 shows the range of the MOXY monitors for each individual participant, establishing a minimum and maximum value. Minimum values were obtained from thigh occlusion and maximum values were obtained from the highest SmO_2 values achieved during the high-intensity intervals. Five out of the six participants achieved SmO_2 below 50% in the left and right quadriceps during calibration. During calibration, four out of the six participants achieved lower SmO_2 during calibration than during interval testing for both the left and right quadriceps. Participant 004 achieved lower SmO_2 in both quadriceps during the interval testing than during calibration. Participant 005 demonstrated the greatest range during interval testing, with changes during interval testing in left and right quadriceps SmO2 of -78% and 51%, respectively. Participants with the lowest obtained SmO_2 during calibration did not demonstrate the greatest range of SmO_2 or reach the highest SmO_2 during interval testing, when compared to other participants. It should be noted that subject 006 produced abnormally high SmO_2 values during the high-intensity intervals.

Table 5. *Lowest SmO_2 achieved before VO_2 Max testing and Highest/Lowest SmO_2 During 6 High-Intensity Intervals*

Subject ID #	Lowest Calibration L(%)	Lowest Interval SmO_2 L(%)	Highest Interval SmO_2 L(%)	Lowest Calibration R(%)	Lowest Interval SmO_2 R(%)	Highest Interval SmO_2 R(%)
001	45	64	99	28	63	84
002	28	30	73	41	45	75
003	30	68	92	41	50	90
004	39	27	61	85	55	87
005	55	21	99	39	45	96
006	36	92	97		96	99

Lowest right quadriceps SmO2 during calibration was unavailable for participant 006
L= left quadriceps MOXY monitor R= right quadriceps MOXY monitor D= deltoid MOXY monitor

VO_2 Max Testing

Tables 6 shows the results obtained from the VO_2 max test, including participant's VO_2 max, maximum heart rate, blood lactate levels before and after the VO_2 max test and maximum wattage. All subjects achieved a VO_2 max above the inclusion criteria minimum of 30 ml/kg/min, supporting their status as endurance-trained with all participants scoring in the categories of fair, good, excellent and superior (Heyward, 1998). Post VO2 max BL levels reflected maximum effort as defined as ≥7 mmol/l for participants 001, 002, 004, 005 and ≥9 mmol/l for participants 003 and 006 based on gender and age (Evardsen, 2014).

Table 6 *VO_2 Max Testing*

Subject ID #	VO_2 Max (ml/kg/min)	Max HR (bpm)	BL Before VO_2 Test (mmol/l)	BL After VO_2 Test (mmol/l)	SmO_2 D (%)	SmO_2 L (%)	SmO_2 R (%)	Max Wattage (W)
001	38.7	202	1.1	12	67	27	44	170
002	33.2	188	1.3	7.7	56	26	39	170
003	51.8	180	8.1	12.9	64	63	47	305
004	37.7	194	4.0	12.9	37	30	63	110
005	52.9	194	2.3	13.2	77	52	41	290
006	42.8	182	3.4	19.6	83	96	99	305

SmO_2 D= deltoid MOXY monitor SmO_2 L= left quadriceps MOXY monitor SmO_2 R= right quadriceps MOXY monitor
SmO_2 data taken at time VO_2 max was achieved
Max wattage reflects the wattage of the last fully completed 60-second interval

High Intensity Intervals

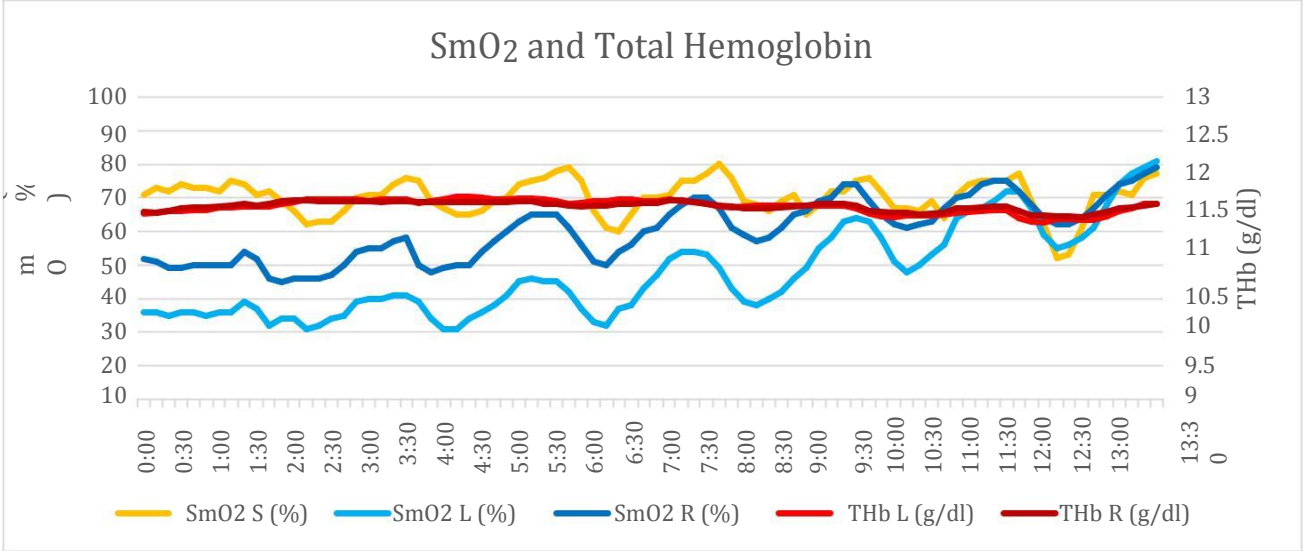
Three of the six participants were selected for the graphical illustrations and tables below. Two females and one male were selected (002, 003, 004) for analysis because their SmO2 was measurable throughout testing and blood lactate samples were adequately obtained for participant 002. Participant 003 had the highest maximum wattage during the VO2 max test and one of the highest VO2 max scores. Participant 003 was an ECU tennis player who reported performing a high volume of endurance training. The tables and graphs below illustrate the

relationships and patterns seen among the variables measured including SmO2 and VO2, SmO2 and HR and SmO2 and BL.

SmO2 and THb During Six High Intensity Intervals

Figures 1 a, b and c illustrate the pattern of SmO2 and THb during the six high-intensity intervals for participants 002, 003 and 004. Participants showed clear decreases in SmO2 during each of the 6 intervals. Notably, troughs in SmO2 were often seen to occur about 10 seconds after the end of the intervals. Progression through the 6 intervals revealed an overall increase in SmO2 for participant 002 while participant 004 had an increase in the right quadriceps and a decrease in the left quadriceps as the intervals progressed. Left quadriceps SmO2 decreased during each interval in all three participants but differed in the progressive pattern. SmO2 in the left quadriceps for participant 003 remained relatively stable, ranging between 87%-90%. Participant 004 showed an overall decrease in the left leg, falling from 54-35%. Right quadriceps SmO2 remained relatively stable in participant 003. THb in participants 002 and 004 remained stable with little overall change. Participant 003 demonstrated decreases in THb during each of the 6 intervals. Participant 002 showed a decrease in deltoid SmO2 during each interval but remained relatively stable overall, returning to prior values between intervals. Participant 003 also showed a decrease in deltoid SmO2 during intervals but with a slight overall increase, rising from 69% to 76% between intervals.

Figure 1a. *Participant #002 SmO2 (%) of the left and right quadriceps and THb(g/dl) across time*



Interval 1- 1:30-2:00 min Interval 2- 3:30-4:00 min Interval 3- 5:30-6:00 min Interval 4- 7:30-8:00 min Interval 5- 9:30-10:00 min Interval 6- 11:30-12:00 min SmO2 D= Deltoid MOXY monitor SmO2 L= Left Quadriceps MOXY monitor SmO2 R= Right Quadriceps MOXY monitor

Table 7a *SmO2 and total hemoglobin (THb) at start and end of intervals*

Subject #002	Deltoid SmO2 (%) Start/End	L Quad SmO2 (%) Start/End	R Quad SmO2 (%) Start/End	L Quad THb (g/dl) Start/End	R Quad THb (g/dl) Start/End
Interval 1	71/66	37/34	52/46	11.6/11.6	11.6/11.6
Interval 2	76/67	41/31	58/49	11.6/11.7	11.6/11.6
Interval 3	78/66	45/33	65/51	11.6/11.6	11.6/11.6
Interval 4	77/69	53/39	70/59	11.6/11.6	11.6/11.5
Interval 5	75/67	64/51	74/62	11.5/11.4	11.6/11.5
Interval 6	75/62	72/59	75/64	11.5/11.3	11.6/11.4
Avg Change Per Interval	-9.2%	-10.8%	-10.5%	-.05 mg/dl	-.04 mg/dl

Figure 1b. *Participant #003 SmO2 (%) of the left and right quadriceps and THb(g/dl) across time*

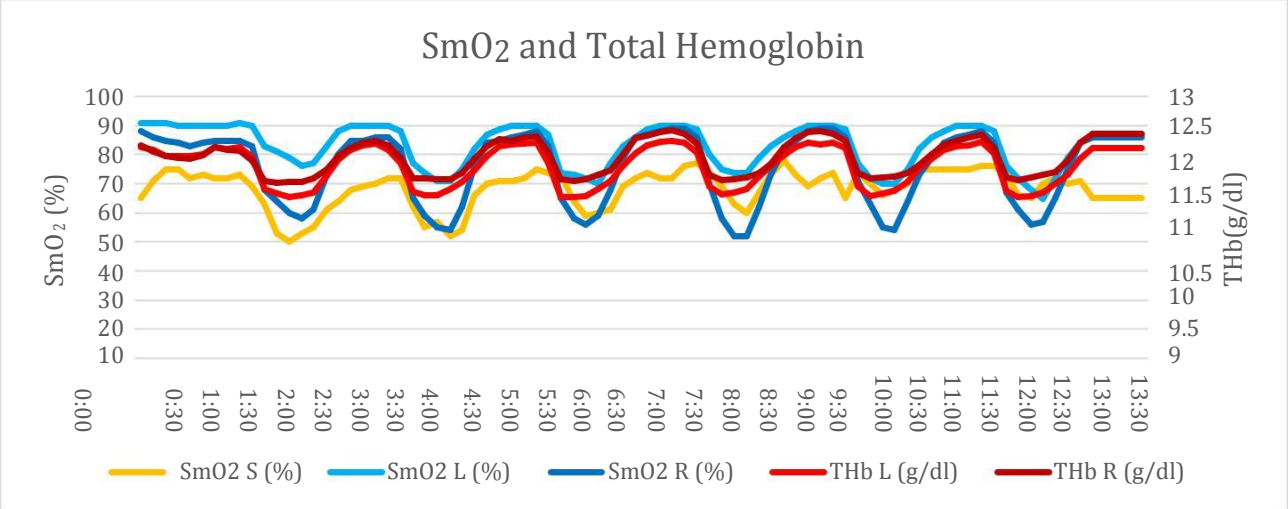


Table 7b *SmO_2 and Total Hemoglobin (THb) at Start and End of Intervals*

Subject #003	Deltoid SmO_2 (%) Start/End	LeftQuad SmO_2 (%) Start/End	Right Quad SmO_2 (%) Start/End	Left Quad THb (mg/dl) Start/End	Right Quad THb Start/End
Interval 1	69/50	90/79	83/60	12.1/ 11.5	12.0 / 11.7
Interval 2	72/57	88/71	82/55	12.0/ 11.5	12.1/ 11.7
Interval 3	74/59	87/72	83/56	12.0/ 11.4	12.1/11.7
Interval 4	77/63	89/74	86/52	12.1/11.5	12.3/11.7
Interval 5	65/66	89/70	86/55	12.2/11.5	12.3/11.8
Interval 6	76/65	88/68	84/56	12.1/11.5	12.2/11.8
Avg Change Per Interval	-12.2%	-16.2%	-28.3%	-.61 mg/dl	-.44 mg/dl

Figure 1c. *Participant #004 SmO2 (%) of the left and right quadriceps and THb(g/dl) across time*

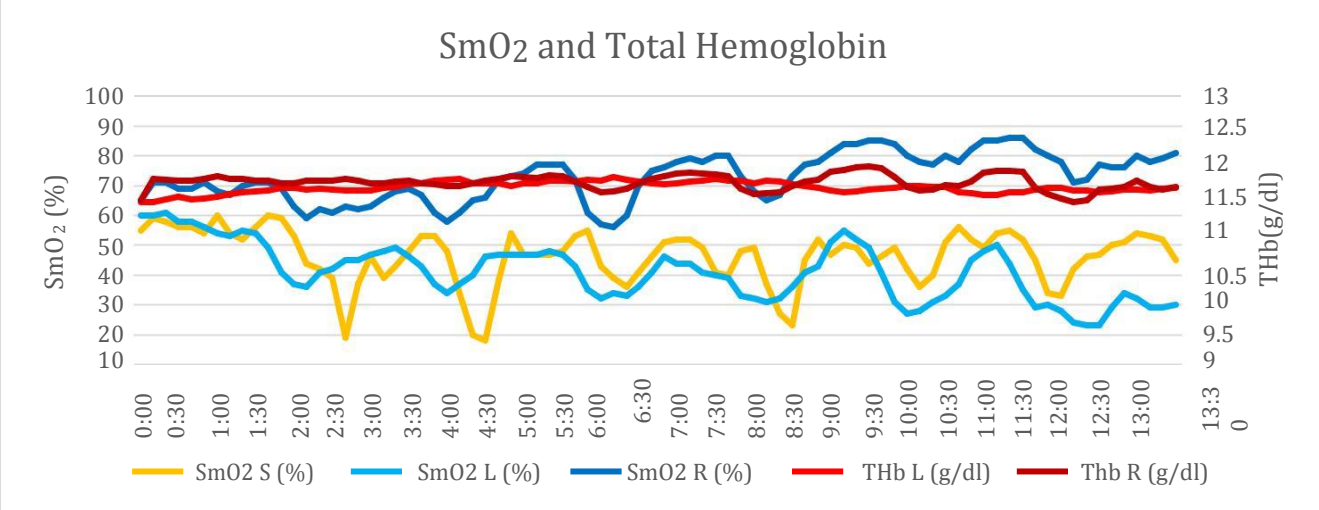


Table 7c *SmO_2 and Total Hemoglobin (THb) at Start and End of Interval*

Subject #004	Deltoid SmO_2 (%) Start/End	Left Quad SmO_2 (%) Start/End	Right Quad SmO_2 (%) Start/End	Left Quad THb (mg/dl) Start/End	Right Quad THb (mg/dl) Start/End
Interval 1	56/53	54/37	71/63	11.6/11.6	11.7/11.7
Interval 2	48/48	46/34	69/58	11.7/11.8	11.73/11.7
Interval 3	48/43	47/32	77/57	11.7/11.7	11.8/11.6
Interval 4	41/49	40/32	80/68	11.8/11.7	11.8/11.5
Interval 5	44/42	49/27	85/80	11.6/11.7	12.0/11.7
Interval 6	52/33	35/28	86/78	11.6/11.6	11.9/11.5
Avg Change Per Interval	-3.5%	-13.5%	-10.7%	+.03 mg/dl	-.22 mg/dl

Progressive Changes in Measured Variables During the Six High-Intensity Intervals

Tables 8a, b and c show the patterns seen among the measured variables. The BL values ≥7mmol for females and ≥9mmol/l for men demonstrate the maximal effort exerted by the participants. BL was shown to increase in participant 002 through the first 3 intervals, followed by slight decrease in the last 3 intervals. Only 2 BL measurements were obtained for participants 003 and 004, both showing an increase. HR also increased through intervals 1-6 showing an increase in effort and fatigue. VO2 reached between 86.3 and 92.6% VO2 max for participants, also demonstrating effort. SmO2 in the left quadriceps was shown to slightly increase for participant 002 while it decreased for participants 003 and 004. Right quadriceps SmO2 remained stable for participant 002, decreased in participant 003 and increased in participant 004. Deltoid SmO2 increased for participants 002 and 003 while decreasing for participant 004, suggesting there may have been upper body movement during the intervals. Participant 002 demonstrated exceptionally high BL levels, peaking at 19.8mmol/l at the end of interval 3. An increase over the first 3 intervals was seen in this participant followed by a slight decrease from interval 4 to 6. A pattern of decreasing SmO2 with increasing BL was not observed. Overall,

intensity and effort was confirmed by BL accumulation, increase in HR and %VO2 max achieved.

Table 8a. *Participant #002. Interval Ending Measurements*

Interval #	SmO_2 D(%)	SmO_2 L(%)	SmO_2 R(%)	Blood Lactate (mmol/l)	Heart Rate (bpm)	VO_2 (ml/kg/min)	% VO_2 Max
1	34	46	66	9.2	169	28.8	86.7
2	31	49	67	16.8	175	28.4	85.5
3	33	51	66	19.8	176	25.8	77.7
4	39	59	69	18.6	179	28.7	86.4
5	51	62	67	15.1	179	27.6	83.1
6	59	64	62	14.5	182	29.1	87.7

Table 8b. *Participant #003. Interval Ending Measurements*

Interval #	SmO_2 D(%)	SmO_2 L(%)	SmO_2 R(%)	Blood Lactate (mmol/l)	Heart Rate (bpm)	VO_2 (ml/kg/min)	% VO_2 Max
1	50	79	60		164	34.2	66
2	57	71	55	11	168	43.5	84
3	59	72	56		170	44.7	86.3
4	63	74	52		167	39.8	76.8
5	66	70	55		171	42.2	81.5
6	65	68	56	16.1	174	43.5	84

Table 8c. *Participant #004. Interval Ending Measurements*

Interval #	SmO_2 D(%)	SmO_2 L(%)	SmO_2 R(%)	Blood Lactate (mmol/l)	Heart Rate (bpm)	VO_2 (ml/kg/min)	% VO_2 Max
1	50	36	62	8.3	180	26.2	69.5
2	44	35	59	11.8	188	30.8	81.7
3	41	32	55		190	33.1	87.8
4	46	30	67		190	34.9	92.6
5	36	28	80		187	30.9	82.0
6	35	26	76		190	34.9	92.6

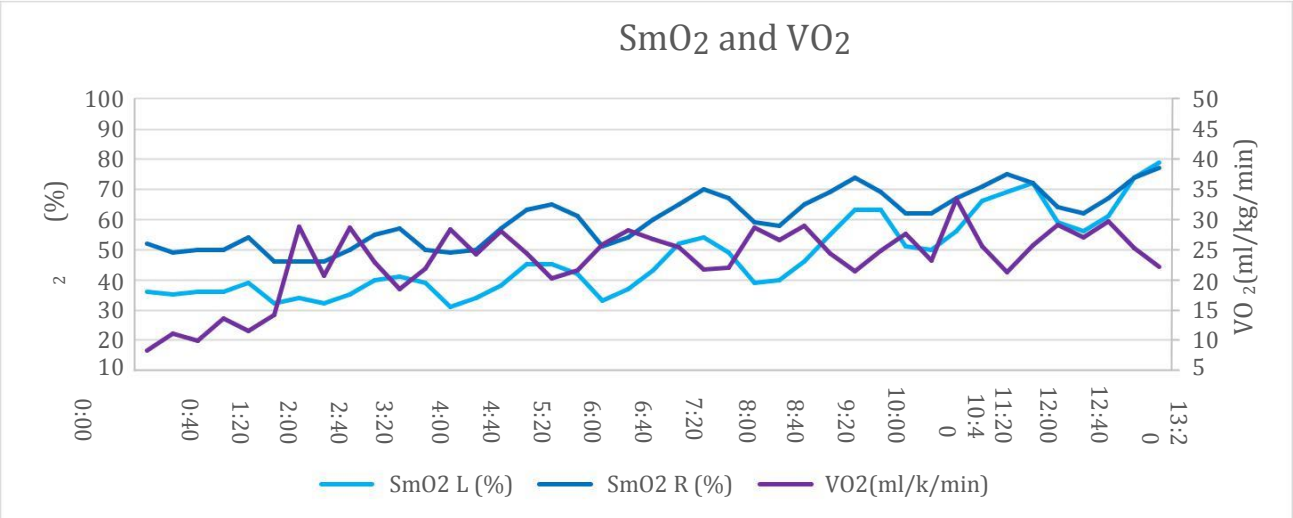
Blank spaces indicate unavailable data (lost MOXY monitor reading or blood lactate reading error)

SmO_2 S= Deltoid monitor SmO_2 L= left Quadricep monitor SmO_2 R= right Quadricep monitor

Relationship between SmO_2 and VO_2 During High-Intensity Intervals

Figures 2 a, b and c illustrate the inverse relationship seen among SmO2 and VO2 during each of the 6 intervals. Both VO2 and SmO2 were plotted every 20 seconds to allow comparison, as the VO2 value was taken by the metabolic cart every 20 seconds during testing. A decrease in SmO2 of the left and right quadriceps was consistently seen during each interval among the three participants illustrated in the graphs. Simultaneously, VO2 was shown to increase during each of the six intervals. Interval progression showed peak VO2 during interval testing occurred at the end of interval 6 for participants 002 and 004 and interval 3 for participant 003. Participant 002 demonstrated an overall increase in SmO2 in both quadriceps as the intervals progressed, while VO2 increased steadily. Peak VO2 during the intervals did not consistently occur at the end of the intervals. Participant 002 saw several spikes in VO2 between intervals, often occurring about 40 seconds after the end of the interval. For example, spikes are seen at 2:40, 4:40, 6:20, 8:40, 10:40 and 12:40. A similar pattern was seen in evaluating participant 003, with peaks in VO2 often occurring at 20 or 40 seconds after the end of the interval.

Figure 2a. *Participant 002. SmO2 (%) of the left and right quadriceps and VO2 (ml/kg/min) across time*



Interval 1- 1:30-2:00 min Interval 2- 3:30-4:00 min Interval 3- 5:30-6:00 min

Interval 4- 7:30-8:00 min Interval 5- 9:30-10:00 min Interval 6- 11:30-12:00 min

Figure 2b. *Participant #003 SmO2 (%) of the Left and Right Quadriceps and VO2 (ml/kg/min) across time*

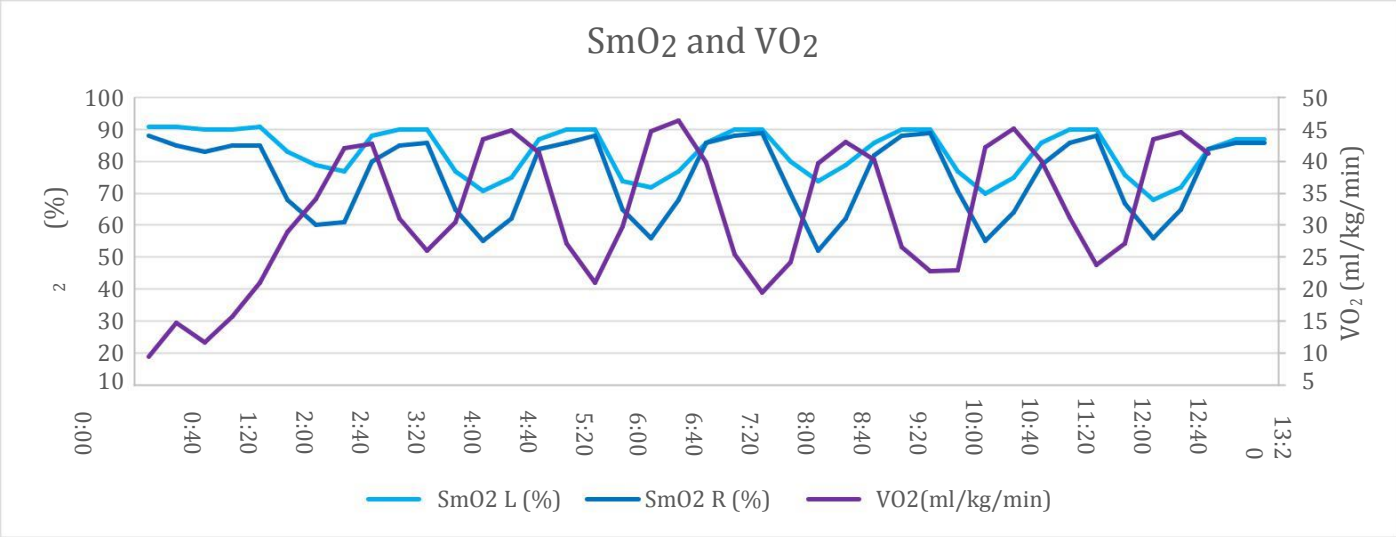
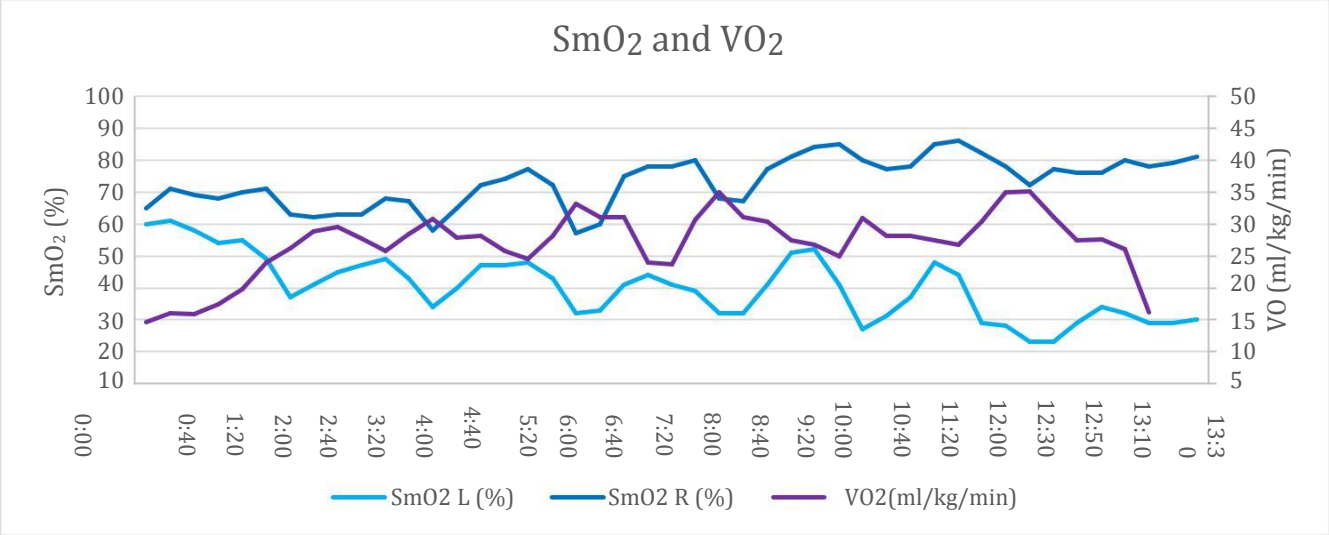


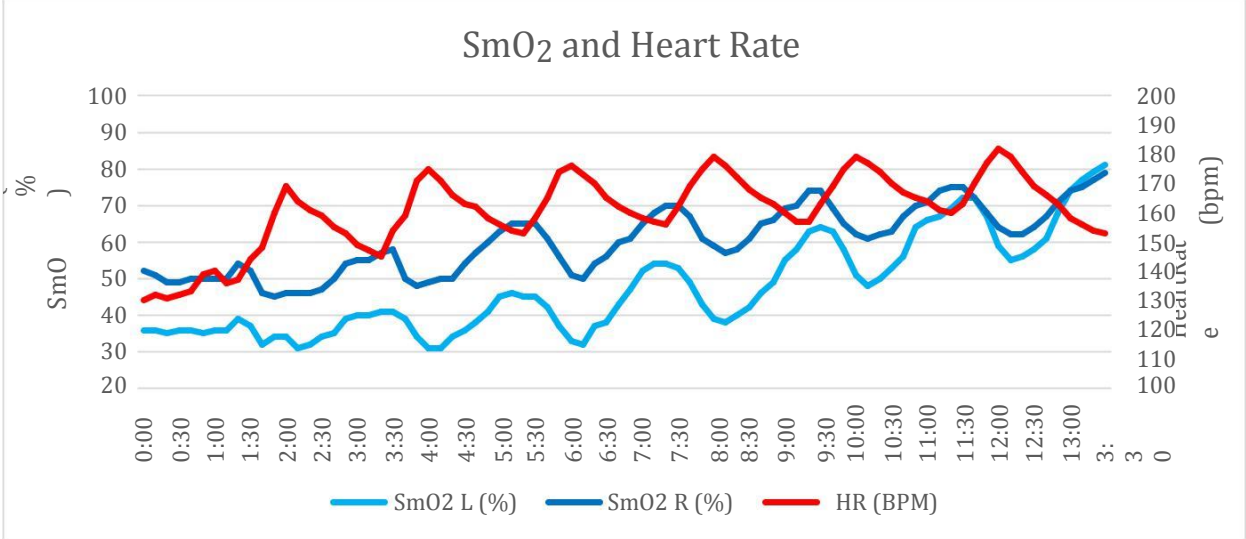
Figure 2c. *Participant #004. SmO2 (%) of the left and right quadriceps and VO2 (ml/kg/min) across time*



Relationship between SmO_2 and HR During High-Intensity Intervals

Figures 3 a, b and c illustrate the inverse relationship observed between SmO2 and heart rate among the three participants. During each interval, heart rate consistently increased while SmO2 in the left and right quadriceps decreased. As the intervals progressed, an increase in resting HR between intervals was seen in participants 002 and 003 while participant 004 showed a smaller range between 181-192 bpm with obvious decreases seen during the intervals. Peak HR was shown to consistently occur at the end of each of the intervals, with most occurring right at the end of the 30-seconds. However, SmO2 troughs were shown to consistently occur about 10 seconds after the end of the interval, showing peak HR and SmO2 troughs to not be exactly aligned.

Figure 3a. *Participant #002. SmO_2 (%) of the left and right quadriceps and heart rate (bpm) across time*



Interval 1- 1:30-2:00 min Interval 2- 3:30-4:00 min Interval 3- 5:30-6:00 min Interval 4- 7:30-8:00 min Interval 5- 9:30-10:00 min Interval 6- 11:30-12:00 min

Figure 3b. *Participant #003. SmO_2 (%) of the left and right quadriceps and heart rate (bpm) across time*

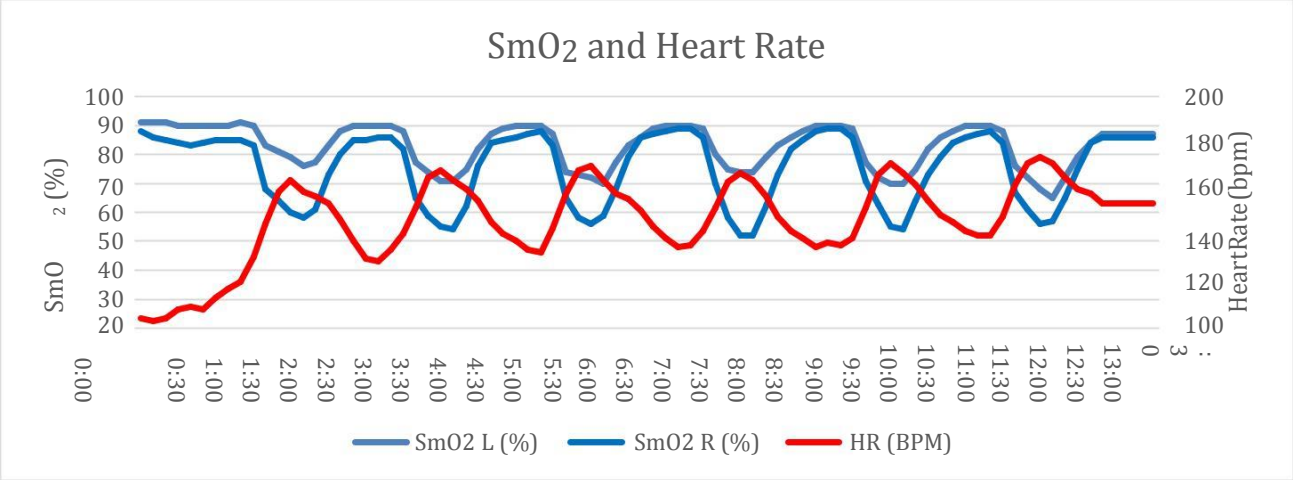
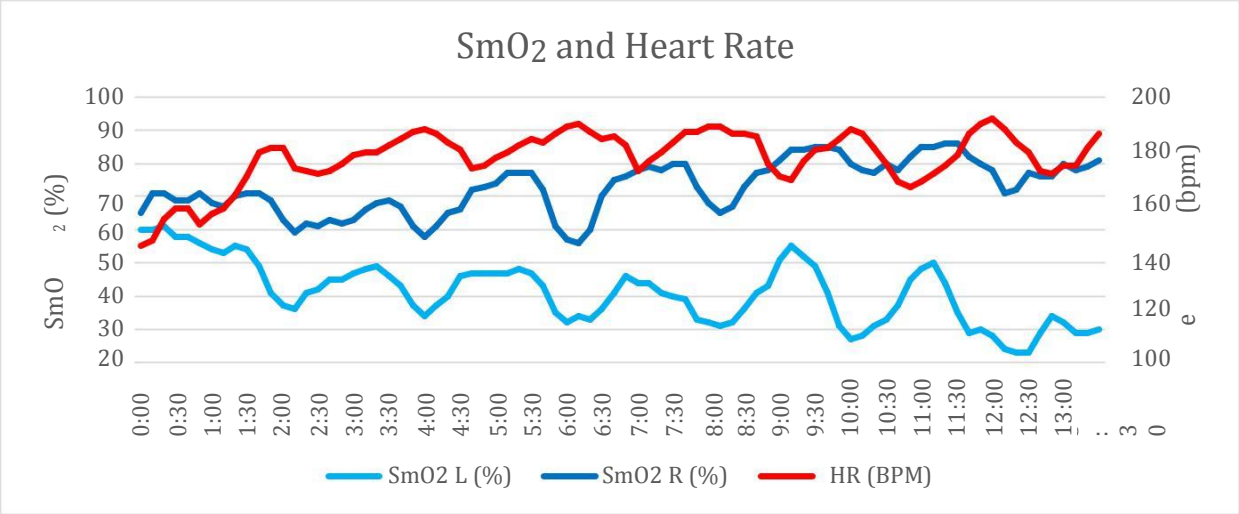


Figure 3c. *Participant #004. SmO_2 (%) of the left and right quadriceps and heart rate (bpm) across time*



Relationship between SmO_2 and Blood Lactate

Tables 9 a, b, and c present the changes seen in SmO2 of the right and left quadriceps as well as the blood lactate taken at the end of each of the 6 high-intensity intervals. Increases were seen in BL as both right and left quadriceps SmO2 increased and decreased. Peak BL did not correspond to troughs in SmO2 of either the left or right quadriceps.

Table 9a *Participant 002. SmO2 (%) of the left and right quadriceps and* b*lood lactate (mmol/l) taken at the end of each interval*

	Left Quad SmO_2 (%)	Right Quad SmO_2 (%)	Blood Lactate (mmol/l)
Interval 1	34	46	9.2
Interval 2	31	49	16.8
Interval 3	33	51	19.8
Interval 4	39	59	18.6
Interval 5	51	62	15.1
Interval 6	59	64	14.5

Table 9b *Participant 003. SmO2 (%) of the left and right quadriceps and* b*lood lactate (mmol/l) taken at the end of each interval*

	Left Quad SmO2 (%)	Right Quad SmO2 (%)	Blood Lactate (mmol/l)
Interval 1	79	60	
Interval 2	71	55	11
Interval 3	72	56	
Interval 4	74	52	
Interval 5	70	55	
Interval 6	68	56	16.1

Table 9c *Participant 004. SmO2 (%) of the left and right quadriceps and* b*lood lactate (mmol/l) taken at the end of each interval*

	Left Quad SmO2 (%)	Right Quad SmO2 (%)	Blood Lactate (mmol/l)
Interval 1	37	63	8.3
Interval 2	34	58	11.8
Interval 3	32	57	
Interval 4	32	68	
Interval 5	27	80	
Interval 6	28	78	

Chapter 5: Discussion

Improving the aerobic capacity of an athlete requires improvements in the ability to monitor and assess physiological adaptations created in response to training programs. Exercise tools, such as monitors used to measure heart rate, metabolic carts to measure the volume of oxygen consumed (VO2) and portable meters to measure blood lactate are just some of the ways these adaptations are measured and evaluated. Over the past few decades, near infrared spectroscopy (NIRS) has emerged as a technology that may serve as a useful exercise tool to monitor and evaluate muscle oxygenation patterns within working muscles of the athlete. With the emergence and increased accessibility of more portable and wireless NIRS instruments, the need for assessing the applicability and usefulness for obtaining muscle oxygen has increased. This study sought to identify if the MOXY monitor output followed patterns of established physiological factors, including heart rate, VO2 and lactic acid. The purpose of this study was to assess the output provided by the MOXY monitor, muscle oxygen saturation (SmO2), to compare with expected physiological changes. Eg. A decrease in SmO2 as VO2, heart rate, and blood lactate increase. Our hypothesis was that SmO2 would decrease during the 30-second intervals as VO2, heart rate and blood lactate increased and that troughs in SmO2 would coincide with peaks in the other variables.

This study relied on comparing observed measurements to expected physiological patterns. These patterns are centered around known physiological responses to exercise that have been well-studied in literature. Anaerobic exercise, done at higher intensities, creates a higher respiratory rate as the demand for oxygen increases. This increased need for oxygen causes an increase in heart rate to keep up with blood and oxygen delivery, thereby also creating an increase in the volume of oxygen consumed (VO2). As the body struggles to maintain adequate

oxygen delivery, the body begins to accumulate blood lactate as a product of anaerobic metabolism. This point at which blood lactate begins to increase exponentially is referred to as the lactate threshold (McArdle et al., 2015). As less oxygen becomes available during these high intensities and heart rate, VO2 and blood lactate increase, a decrease in muscle oxygen saturation in working muscles would be expected as oxygen is less available. Therefore, an inverse relationship between SmO2 and heart rate, VO2 and blood lactate would be in agreement with physiological expectations.

By testing six participants during 6 high-intensity intervals on a cycle ergometer, we found an inverse relationship among SmO2 when plotted against VO2 and heart rate. As SmO2 decreased during each of the 30-second intervals, both VO2 and heart rate increased. A notable finding was that many intervals demonstrated a 10-second delay among SmO2 troughs. Rather than reaching a trough at the end of the 30-seconds, lowest SmO2 occurred around 10-seconds after the 30-second interval ended. This pattern was observed in several intervals for the participants but was not seen in all intervals. The patterns illustrated by SmO2 supports our hypothesis that MOXY output would provide SmO2 values that would decrease during high-intensity intervals, as a high-intensity anaerobic environment has been shown to decrease muscle oxygenation in similar studies using other muscle oxygen saturation monitors.

Hypothesis 1: BL will illustrate an inverse relationship to SmO2 by increasing with each interval as SmO2 will decrease as the intervals progress from interval 1 to interval 6.

The support of this hypothesis was not able to be determined, due to clear patterns not being able to be evaluated as only two participants out of six had all 6 BL samples successfully obtained. These participants demonstrated similar patterns, increasing during the first 3-4 intervals before slightly decreasing in the remaining intervals. Participant 005 also showed a

similar pattern of increasing through the beginning intervals before decreasing, despite only having 5 BL values obtained. Both HR and VO2 were shown to increase during the last 2-3 intervals for these participants, demonstrating sufficient effort and intensity was provided by the participants, thereby disproving the assumption that the participants simply decreased their efforts. Blood lactate proved to be difficult to measure, as the lactate meter displayed an error message for twelve out of the total thirty-six readings attempted during the high-intensity intervals.

Hypothesis 2: SmO2 will illustrate an inverse relationship to HR by decreasing during the intervals while HR increases and peak HR values will coincide with troughs in SmO2.

The hypothesis was supported as the inverse relationship was observed in the present study, with SmO2 decreasing and HR increasing during the intervals for all 6 participants. A delay of about 10 seconds observed among some SmO2 troughs, compared to peak HR which consistently occurred at the end of the 30-second intervals. It is unclear as to whether this delay in SmO2 was due to a physiological delay in muscle deoxygenation or a delay in the MOXY monitor's ability to measure live values. Overall the hypothesis was supported that decreases in SmO2 were inversely related to increases in HR.

Hypothesis 3: SmO2 will illustrate an inverse relationship with VO2 by decreasing during the 30-second intervals as VO2 increases. Troughs in SmO2 will coincide with VO2 peak values.

The hypothesis was supported in the present study as SmO2 was shown to decrease during the intervals and VO2 was shown to increase. The degree to which the two variables changed did not seem to be related, as SmO2 decreased to varying degrees and did not necessarily correlate to a relative increase or decrease in VO2. Peak VO2 was shown to often

occur around 20 to 40 seconds after the end of the interval when SmO2 had begun increase. Thus, the hypothesis that peaks in VO2 and troughs in SmO2 would coincide was not supported. It should be noted that the metabolic cart was programmed to measure VO2 every 20 seconds, thereby limiting the available data to show a relationship among the two variables. SmO2 values were plotted every 20-seconds as well so as to allow comparisons between the two variables. Overall, the hypothesis was partially supported as SmO2 showed clear decreases during the intervals as VO2 increased, despite peaks and troughs failing to coincide.

Comparison of MOXY Monitor Data to other NIRS monitors

As NIRS evolves into a more commonly used tool for monitoring exercise adaptations it is imperative that supporting research continues to provide relevant information that positions muscle oxygenation in its appropriate place among exercise tools. While much research has been done in validating NIRS as an accurate way to measure muscle oxygenation, less research is available in assessing the more commercial NIRS devices, such as MOXY. To our knowledge, no studies have been done examining the output provided by the MOXY monitors compared to more established variables like VO2, HR and BL. With differing numbers of light sources and detectors, as well as algorithms and calculations, it is wrong to assume all new NIRS instruments will provide useful data. This study sought to address this gap in MOXY literature by assessing the data provided by the MOXY monitor compared to other parameters, including HR, VO2 and BL.

The major findings in the present study were the inverse relationships observed between SmO2 and heart rate and SmO2 and VO2, which fall in line with expected physiological patterns based on anaerobic metabolism. This relationship suggests SmO2 output provided by the MOXY monitor may provide reliable muscle oxygenation patterns. Racinais et al. (2007) also found

significant increases in VO2 with repeated 6-second sprints on a cycle ergometer that corresponded to increases in muscle deoxygenation; thereby supporting an inverse relationship between muscle oxygenation and VO2. Racinais used a validated non-portable NIRO 300 (Hamamatsu, Japan) NIRS instrument to assess muscle oxygenation. A pattern seen among some of our participants was an increase in muscle oxygenation as the intervals progressed. Both HR and VO2 remained elevated, even peaking in some participants, during the final 3 intervals, suggesting that adequate effort was given by the participants. In the ten 6-second sprints conducted by Racinais, muscle oxygenation decreased with the repeated sprints; however, 30-second recovery periods were used between sprints. It is likely that the 90-second rest periods used between the intervals in the present study allowed participants to enter a recovery period, in which previous research has observed increases in SmO2. Studies by Bellardinelli et al. (1995) and Bhambhani et al. (1997) both observed rapid increases in muscle oxygenation during the 1-2 minutes following incremental exercise. This suggests that the 90-second rest periods were sufficient as to allow recovery and led to the progressive increase in SmO2 seen among some of the participants. This finding in the present study is of interest, as the recovery in muscle oxygenation may be an area of future significance. Studies by Chance et al. (1992) and Ding et al. (2001) found that well trained athletes exhibited faster recovery in muscle deoxygenation than untrained participants. It may be a focus of later studies to examine the use of MOXY as a means of assessing reoxygenation patterns as a response to various training programs. Similar to heart rate variability, SmO2 recovery between bouts of exercise may be an area worth further exploration.

The present study had similar findings to previous studies using portable NIRS. A 2013 study by Jones et al. (2013) examined muscle oxygenation changes in gastrocnemius muscle in 6

university rugby players during repeated shuttle sprints using a wireless PortaMon monitor while heart rate and blood lactate were also measured. Mean BL taken immediately after the shuttles tests was 14.3 mmol/l and mean heart rate was 182bpm. The present study observed a post interval testing BL mean of 13.4mmol/l (5 participants had interval ending BL obtained) and a mean HR of 182.0 bpm at the end of the intervals. Muscle oxygenation in the study by Jones decreased by a mean of 24.4% during each interval whereas the present study saw a mean decrease in SmO2 of 13.2%. Findings in VO2, HR and muscle oxygenation from the present study were similar to findings obtained by Jones, with some difference seen in the degree of deoxgenation using the different protocols. This suggests that VO2 and HR increase while muscle oxygenation decreases as measured by the PortaMon NIRS monitor.

Although the current study was unable to determine a relationship between blood lactate and muscle oxygenation, other studies have supported such a relationship. Miura et al. (2000) examined muscle oxygenation using NIRS and blood lactate during five, 6-minute cycling intervals. Blood lactate was measured at the end of each interval using a fingertip method similar to the present study. A correlation was observed between the percentage of oxygen saturation and blood lactate accumulation, suggesting that monitoring muscle oxygenation using NIRS may be a preferred method over blood collection as it is less invasive. Many other studies using NIRS have also supported the relationship between muscle oxygenation and blood lactate (Grassi, 1999 & Bellotti, 2013).

The findings obtained in the present study demonstrate a pattern of continuing decreases in SmO2 during each of the 30-second intervals accompanied by increases in HR and VO2. With these observed changes, troughs in SmO2 and peaks in HR and VO2 did not always coincide. Further studies will need to examine the timing of the changes in these variables. Inadequate BL

collection prevented conclusions from being made about patterns in BL; however, the BL values obtained in 5 out of the 6 participants showed an increasing pattern as the intervals progressed.

Limitations

The site with which the MOXY monitors were placed likely had the MOXY monitors assessing the rectus femoris muscles. Previous research examining cycling exercise has identified the vastus lateralis as one of the more active muscles during cycling exercise and is often chosen as the site measured using NIRS technology during cycling exercise (Miyashita, M., 1981). This limits the present study in being compared to other literature as the exact muscle being measured may differ. Muscle oxygenation of the rectus femoris and the vastus lateralis muscles should be similar as the muscular physiology should remain constant despite differences in degree of use during cycling exercise.

The calibration protocol used in the present study was adopted by the recommendations of Terence Ryan, an expert in NIRS technology. In his own studies, an occlusion protocol was used with a blood pressure cuff to occlude the testing muscle so as to achieve a minimal value for comparison, thereby calibrating the NIRS instrument. This procedure was limited in the present study by the availability of an appropriate cuff capable of occluding above the thigh. Additionally, the protocol included increasing pressure as tolerated by the participant and waiting until SmO2 values plateaued for greater than 30-seconds. One participant, 006, did become uncomfortable during this testing procedure and chose to stop the calibration. The cuff also came undone numerous times, causing the procedure to be restarted and possibly influencing the results. The application of this protocol became very difficult and provided little insight into the ability for MOXY to measure extremely low values. Further testing protocols

using MOXY may wish to use a more appropriate blood pressure cuff capable of occluding the quadriceps muscles or test a smaller muscle easily occluded using a standard sized cuff.

As previously mentioned, BL proved to be very difficult to obtain. Twelve BL measurements were unavailable as the lactate meter displayed an error message upon applying the blood samples. Possible reasons may have included sweat interference, calloused hands or application error by the researcher applying the blood sample to the meter. It was noted that participant 003 had very calloused hands, causing inadequate amounts of blood to be obtained. The missed samples were important in having the ability to observe patterns in BL throughout the intervals. The issue with obtaining BL values resulted in only 2 participants having a complete BL panel and two participants having only 2 values. Further studies may want to explore other means for obtaining BL or improved protocols making BL collection easier.

The computer program used to set the cycle ergometer to adjust wattage during interval testing automatically, proved to take longer than expected to reach ideal wattage. The high-intensity intervals were completed at 125% of maximum wattage achieved during the VO2 max test. For participants with a high maximum wattage (2 participants had a maximum wattage of 305W) it took between 10-15 seconds for the cycle to reach the desired value. This means the participants may have only cycled for about 15 seconds at the desired wattage which may have limited the decreases in SmO2 and increases in HR/VO2/BL. Future studies may wish to adjust the cycler ergometer protocol to account for the time needed to reach desired wattage.

Another important limitation in this study was the apparent loss in signal obtained by the MOXY monitor, evident by a reading of 0% in the SmO2 values produced by the Peripdal program. This was seen among participants 001, 005 and 006 and led to 002,003 and 003 being used in the results section of this paper. It is not apparent what led to these missing values but

possible suggestions include a lost connection or inappropriate taping of the monitor allowing ambient light to interfere with the NIRS signal. It is important for future researchers using MOXY monitors to ensure the monitors are firmly taped to the participant's skin as shifting of the monitor during exercise may lead to errors in the readings. Further research will be needed to explore this concern in MOXY output.

In addition to the 0% readings, abnormally high SmO2 values were seen among participant 006. SmO2 values remained well above 90% during all intervals which is highly unlikely given the high HR, VO2 and BL values, demonstrating adequate effort was provided by the participant. These values are skeptical as they do not agree with the other participants in the present study. Further research is needed to explore abnormally high SmO2 values provided by the MOXY monitor as they do not follow observed values in otter participants.

THb was predicted to remain consistent throughout testing and was supported in the illustrations provided by participants 002 and 004. Participant 003 demonstrated decreases in THb with each of the six intervals, which is contrary to expectations as THb would be predicted to remain consistent. The reason for this finding is unknown and warrants further exploration.

Participants were instructed to not exercise or eat in the hours before testing. However, participant 003 admitted to having completed an upper body workout on the morning of testing after testing had been completed. This may explain the above normal BL reading of 8.1mmol/l prior to the VO2 max test. Findings and values obtained on participant 003 may be have been affected by the early morning exercise causing increased BL.

Practical Implications

The benefits of being able to use a wireless NIRS instrument to monitor muscle oxygenation include having the ability to monitor exercise intensities, determine lactate

threshold, assess oxidative metabolism and estimate blood lactate production without the use of blood samples (Bhambhani et al., 2004; Ding et al., 2001; Miura et al., 2000). Wireless NIRS technology allows researchers to further explore the ways in which muscle oxygenation changes with differing exercise modalities and in various environments. Portable NIRS technology allows researchers the ability to monitor improvements in oxygen utilization in a variety of settings to develop an optimal training program for all types of athletes. Some research has suggested that the ability to recover between high-intensity intervals, referred to as reoxygenation may be more indicative of the aerobic training state of the athletes and demonstrate a greater oxygen utilization capacity (Ding 2001). This would prove to be valuable information for anyone looking to improve aerobic fitness, including endurance athletes, athletes performing repeated sprints and even military personnel. Military training places a major emphasis on aerobic fitness testing, especially running, which when done excessively has been shown to increase training injuries (Knapik, 2009). As the Physical Readiness Program (PRT) implements new training programs incorporating varying exercise to increase physical fitness (Knapik, 2009), it may be worthwhile for the military to explore using portable NIRS monitors like MOXY to monitor muscle oxygen utilization due to its correlations with VO2, heart rate and blood lactate.

Future Research

NIRS monitors have made major leaps in usefulness as they have become more mobile, easier to use and less expensive. As more monitors touting superiority in measuring precision become available in the commercial market, the need for validation and assessment will remain a priority. Future studies may wish to compare the MOXY monitor to more clinically established monitors for validation. As the MOXY monitor is marketed as a tool to monitor and guide training, more studies are needed to evaluate the monitor's ability to detect positive changes in

muscle oxygen utilization from various types of training. For example, observing overall increases in SmO2 with a given amount of work after completion of a specific training program. Further studies may also wish to evaluate more anaerobic styles of training on changes in muscle oxygenation including sprinting programs and resistance training.

As aerobic exercise is based on oxidative metabolism and reliant on mitochondrial capacity, using MOXY to monitor mitochondrial biogenesis may also be worthy of exploration. Previous work has found NIRS devices capable of accurately and reliably measuring mitochondrial function after exercise (Ryan et al. 2014). This certainly warrants further studies as mitochondria is a focal point for endurance monitoring.

As heart rate evolved into a more popular exercise tool, the attention shifted from monitoring heart rate peaks and troughs during exercise to monitoring heart rate variability. The same focus has been suggested for muscle oxygenation. Rather than focusing on the peaks and falls of muscle oxygenation, it may be more useful to focus on the speed of reoxygenation from repeated bouts of exercise. This area of research warrants further exploration and may be a focus for future studies using the MOXY monitor.

With the increase in popularity of wearable technologies like the Fitbit and Garmin watches, changes in the way NIRS is used may be on the horizon. A review by Hamoaka et al. (2011) suggests wearable NIRS technology in the form of clothing may be next for NIRS. A NIRS device embedded into the clothing with the ability to use portable solar energy sources may be the next step for a company like MOXY.

Conclusion and Summary

The major findings of the present study were the inverse relationships observed between SmO2 and heart rate and SmO2 and VO2 using the MOXY monitors as they agree with

physiological expectations. SmO2 was shown to decrease during the high-intensity intervals while VO2 and HR increased. Troughs in SmO2 were seen at the end of the intervals, with several occurring about 10 seconds after the interval. HR consistently peaked at the end of the interval while VO2 increased at the end of the interval and often achieved peak values at around 40 seconds after the interval. A pattern among BL was not able to be determined as only 2 participants had all 6 BL samples collected. These two participants, as well as another with 5 collected samples, demonstrated a progressive increase in BL from intervals 1-3 followed by a slight decrease in the last 2-3 intervals. As exercise intensity increases and reaches an anaerobic state, it would be physiologically expected for heart rate, VO2 and blood lactate to increase while muscle oxygen decreases. It was hypothesized that the MOXY monitors would provide data supporting these assumptions, suggesting that MOXY is a reliable tool for exercise. In evaluating 6 East Carolina University students during six 30-second intervals on a cycle ergometer, inverse relationships between SmO2 and heart rate and SmO2 and VO2 were observed among all participants. The patterns identified illustrate the ability for the MOXY monitors to observe changes in SmO2 during high-intensity cycling intervals that coincide with physiological expectations. This study suggests MOXY may be a useful tool that can be used to monitor muscle oxygen utilization in a more noninvasive manner that may simultaneously reflect VO2, heart rate and blood lactate values with less intrusion.

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Appendix A. ECU IRB Approval

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** · **www.ecu.edu/irb**

Notification of Initial Approval (Committee)

From:	Biomedical IRB
To:	Justin Simmons
CC:	Laurel Wentz
Date:	9/23/2015
Re:	UMCIRB 15-001021 The Assessment of Muscle Oxygen Saturation in Students

I am pleased to inform you that at the convened meeting on 9/23/2015 at 12:15 PM of the Biomedical IRB, the committee voted to approve the above study. Approval of the study and the consent form(s) is for the period of 9/23/2015 to 9/22/2016.

The Biomedical IRB deemed this study Greater than Minimal Risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Document	Description
Data Spreadsheet (0.01)	Data Collection Sheet
Informed Consent- Greater Than Minimal Risk (0.13)	Consent Forms
Nutrition Survey(0.01)	Surveys and Questionnaires
Pervomedic Protocol (0.01)	Study Protocol or Grant Application

Physical Training Survey (0.02)	Surveys and Questionnaires
Recruitment E-mail(0.02)	Recruitment Documents/Scripts
Recruitment Flyer(0.02)	Recruitment Documents/Scripts
Study Procedure(0.12)	Study Protocol or Grant Application

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:

None The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: None

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IRB00000705 East Carolina U IRB #1 (Biomedical) IORG0000418
IRB00003781 East Carolina U IRB #2 (Behavioral/SS) IORG0000418

Appendix B: Informed Consent

East Carolina University

Consent to Take Part in Research that has Potentially Greater than Minimal Risk

Information You Should Think About Before Agreeing to Take Part in This Research

Title of Research Study: The Assessment of Muscle Oxygen Saturation in Students During Maximal VO_2 Exercise and High Intensity Intervals

Principal Investigator: Justin Simmons
Institution, Department or Division: Nutrition Science
Address: 388 Human Performance Lab Suite Ward Building Greenville NC 27858 Telephone #: (804) 244-1263

Researchers at East Carolina University (ECU) study issues related to society, health problems, environmental problems, behavior problems and the human condition. To do this, we need the help of volunteers who are willing to take part in research.

This form explains why this research is being done, what will happen during the research, and what you will need to do if you decide to volunteer to take part in this research

The person who is in charge of this research is called the Principal Investigator.
The person explaining the research to you may be someone other than the Principal Investigator.
Others who may be asking you to take part in this research include:
- Study Advisor: Dr. Laurel Wentz

There may be other research staff members who perform some of the procedures. You may have questions that this form does not answer.
If you have questions, feel free to ask those questions to the person explaining the study, as you go along.
You may also have questions later; feel free to ask those questions, as you think of them. There is no time limit for asking questions about this research.
Take your time and think about the information that is provided.
If you want, have a friend or family member go over this form with you before you decide. If you choose to be in the study, then you will be asked to sign this form when you feel you understand the information provided.
If you do not want to take part in the study, tell the person explaining the research that you do not want to sign this form.

You do not have to take part in this research study. That decision is yours and it is okay to decide not to volunteer.

Why is this research being done?

The purpose of this research is to evaluate a new technology called MOXY as a potential training tool for the military. You are being invited to take part in this research because you are a healthy endurance

trained ECU student and/or a ROTC cadet who closely mimics the physical training of the military. The decision to take part in this research is yours to make. By doing this research, we hope to learn more about muscle oxygen patterns during exercise and whether or not MOXY can be used as a training tool.

Why am I being invited to take part in this research?

You are being invited to take part in this research because you are a healthy endurance trained ECU student and/or in the ECU ROTC program and between the ages of 18 and 30 years. You are a healthy and physically active individual who does aerobic exercise for at least 30 minutes, 3 times per week over the previous 6 months. If you volunteer to take part in this study, you will be one of about 20 people to do so.

Are there reasons I should not take part in this research?

I understand I should not volunteer for this research study if I am not at least 18 years of age or if I am over the age of 30. Also, I should not participate if I am not healthy enough to cycle on a bike at a high intensity for more than 30 minutes. I understand that if I don't reach at least 40ml/kg/min on the VO_2 max test, I will not continue the study to the high intensity intervals. I should not participate if I have had a musculoskeletal injury within the past 3 months, have a history of cardiovascular disease or bleeding disorders.

What other choices do I have if I do not take part in this research?

You have the choice of not taking part in this research study.

Where is the research going to take place and how long will it last?

The research will be conducted in the Biosafety Level 2 Certified (BSL2) Human Performance Lab (Room 363) in the Ward sports medicine building at East Carolina University. You will need to come to room 363 Ward Sports Medicine Building located on the 3rd floor just one time during the study. The total amount of time you will be asked to volunteer for this study is 90 minutes.

What will I be asked to do?

The following procedures will be done strictly for research purposes.
You will be asked to do the following:

After completing this consent form, you will complete a food and training survey, which will include questions regarding previous training and eating habits.

Once all forms have been completed, the researcher will measure your height, weight, and body fat. Body fat will be calculated using a 7-site skin fold in which 7 anatomical landmarks will be measured on your upper and lower body.

A MOXY monitor will be taped to the front of both of your quadriceps as well as one taped to your left shoulder. A blood pressure cuff will be placed around your leg and pressure will be applied to attempt to have the MOXY monitor read 0%. This is to calibrate the monitor and will be stopped when the monitor reaches 0% or the reading remains the same for 10 seconds, indicating it has plateaued.

All testing will be conducted on the same day.

Before VO_2 max testing begins, a needle-size drop of blood will be taken using a blood lactate analyzer to measure a baseline blood lactate level. This will provide a baseline for comparison. The analyzer requires a needle-size drop of blood using a finger prick and

takes just 13 seconds to display the blood lactate reading. The device and procedure is very similar to a finger prick used for blood glucose testing.

You will perform a VO_2 max test in which you will cycle on a bike for approximately 8-12 minutes. The resistance and difficulty of pedaling will increase every minute until you can no longer maintain 70 rotations per minute (rpm). The test will then be completed.

- A VO_2 max test is a way to assess aerobic fitness level. It is a physically progressive testing protocol that increases in intensity with duration until you cannot maintain 70 rpms on the bike. This enables researchers to quantify your endurance capacity. Data from this evaluation will be used to determine intensity during the high intensity intervals and provide the 40ml/kg/min cutoff for participants.

Headgear will be worn that will include a headpiece and mouthpiece that may be uncomfortable at first.

A nosepiece will also be worn to close off nose breathing.

Your feet will be taped to the bike pedals to ensure the pedal straps do not come undone while cycling at high intensities.

VO_2 max testing protocol:

- You will begin pedaling at a rate of 70 rpm and 50 watts (W)
- After each minute, the watts will be increased by intervals of 15. The watts will be increased as follows: 50W, 65W, 80W, 95W, 110W, 125W, 140W, 155W, 170W, 205W, 220W, 235W, 250W etc.
- You will continue this pattern until you can no longer maintain 70 rpms. Once the rpm drops below this number, a blood lactate prick will be taken and the VO_2 max test is over. You may then take a break and hydrate.
- The wattage achieved in your last fully completed 60-second interval will be used to calculate the intensities for the high intensity intervals.

The length of time (break) between testing will be a minimum of 10 minutes and a maximum of 15 minutes; the MOXY and heart rate monitors will remain attached and continue monitoring during this break as well.

After the break and hydration, you will be set up once again on the stationary bike, per protocol. The high intensity intervals will then begin.

You will then undergo a second testing procedure. This will include 6 30-second intervals at a high intensity with each being followed by a 90-second interval at a lower intensity. You will wear the VO_2 mask for this portion as well.

High intensity protocol (HIIT): The HIIT protocol will consist of 6, 30-second intervals at high intensity as well as 6, 90-second recovery intervals at much lower intensities. These intervals will be based on the wattages calculated from the VO_2 max test. During the intervals the MOXY monitors will be placed on both quadriceps muscles as well as the left shoulder exactly as they were during the VO_2 max test. The heart rate monitor will also be used during this portion of the study. Blood lactate will be evaluated at the end of each high-intensity 30 second interval by using a finger prick and blood lactate analyzer. The percentage of maximum wattage will be as follows:

- 90 seconds @ 20% max VO_2 wattage
- 30 seconds @ 125% max VO_2 wattage
- 90 seconds @ 20% max VO_2 wattage
- 30 seconds @ 125% max VO_2 wattage
- 90 seconds @ 20% max VO_2 wattage

- 30 seconds @ 125% max VO_2 wattage
- 90 seconds @ 20% max VO_2 wattage
- 30 seconds @ 125% max VO_2 wattage
- 90 seconds @ 20% max VO_2 wattage
- 30 seconds @125% max VO_2 wattage
- 90 seconds @20% max VO_2 wattage o

30 seconds @ 125% max VO_2 wattage

- Cool down until you fully recover from the intervals.

Once the HIIT intervals are complete, the testing for this study is complete. All equipment will be taken off and your participation is complete.

What possible harms or discomforts might I experience if I take part in the research?

There are always risks (the chance of harm) when taking part in research. We know about the following risks or discomforts you may experience if you choose to volunteer for this study. These are called side effects. The following side effects are known to occur in some people:

Physical exhaustion
Muscle soreness
Rapid heartbeat
Heart arrhythmia
Vomiting
Shortness of breath
Discomfort from wearing the VO_2 mask
Gagging on the mouthpiece while performing the testing exercises

A finger prick will be used to draw blood at 8 different times. Each prick will use a small drop of blood to analyze lactate levels. Total blood drawn in 8 pricks will equal less than 6 needle-size drops of blood. This may briefly sting and may be sore.

The testing procedure may involve risks to the participants, which are currently unknown and unforeseeable

This study will have no impact on reproductive issues.

There is always a chance that you may experience some discomfort or harm when taking part in a research study, this study is no different. We will do everything possible to keep you from being harmed. There may be other risks or side effects that occur which we do not know about at this time.

It is important for you to tell us as quickly as possible if you experience any discomfort, harm or side effect as a result of taking part in this study.

Are there any reasons I might want to withdraw from the research or you might take me out of the research?

There may be reasons we would need to take you out of the study, even if you want to stay in the study. Some of these reasons include:

It is not safe for you to stay in the study;

You do not reach the cutoff VO_2 max minimum of 40ml/kg/min.

If we find that the research might harm you or that it is not providing enough of a benefit to justify the risks you are taking, we will stop testing immediately. Any data we had obtained up until that point will be made available to you upon request. If you would like a copy of the data, simply ask the principal investigator for a copy. They will then write down your data on a piece of paper and seal it in an envelope and personally give it to you.

What are the possible benefits I may experience from taking part in this research?

We do not know if you will benefit by taking part in this study. Other people who have taken part in this study have experienced learning about their VO_2 max, muscle oxygen patterns, resting/exercising heart rate and exercising blood lactate. This may be of interest to you as it reflects your current fitness training level. By participating in this research study, you may also experience these benefits.

Will I be paid for taking part in this research?

We will not be able to pay you for the time you volunteer while being in this study.

What will it cost me to take part in this research?

It will not cost you any money to be part of the research.

Who will know that I took part in this research and learn personal information about me?

To do this research, ECU and the people and organizations listed below may know that you took part in this research. They may also see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

The research team, including the Principal Investigator, study coordinator, and all other research staff.

All of the research sites' staff. This includes the research and medical staff at each site.

Any agency of the federal, state, or local government that regulates this research. This may include the Department of Health and Human Services (DHHS), the Food and Drug Administration (FDA), the North Carolina Department of Health, and the Office for Human Research Protections

The ECU University & Medical Center Institutional Review Board (UMCIRB) and the staff who have responsibility for overseeing your welfare during this research;

ECU office staff who oversee this research.

How will you keep the information you collect about me secure and how long will you keep it?

The data will be kept secure on a web-based drive as well as a metabolic cart located in the Human Performance Laboratory (HPL) lab and will be stored for 3 years. The data will be coded and secure so that no one except the PI can access it. The data will only be released if the participant wants to know their own data. If the subject does wish to know, the data will be sealed in an envelope and then given to the participant by the PI.

What if I decide I do not want to continue in this research?

Taking part in this study is voluntary. If you decide you no longer want to be in this research after it has started, you may stop at any time. You will not be penalized or criticized for stopping. You will not lose any benefits that you should normally receive by quitting.

What if I get sick or hurt while I am in this research?

If you need emergency care:
Call 911 for help. It is important that you tell the doctors, the hospital or emergency room staff that you are taking part in a research study and the name of the Principal Investigator. If possible, take a copy of this consent form with you when you go.

Call the principal investigator as soon as you can. He/she needs to know that you are hurt or ill. Call Justin Simmons (804) 244-1263 or Dr. Laurel Wentz at (717) 870-9082.

If you do NOT need emergency care, but have been hurt or get sick:
Contact Justin Simmons at (804) 244-1263.
Call the principal investigator as soon as you can. As necessary, go to your regular doctor. It is important that you tell your regular doctor that you are participating in a research study. If possible, take a copy of this consent form with you when you go.

If necessary, the Principal Investigator or your regular doctor can assist you in identifying the appropriate place to get care.

If you are harmed while taking part in this study:

If you believe you have been hurt or become sick because of something that is done during the study, you should contact the PI, Justin Simmons, immediately. There are procedures in place to help attend to your injuries or provide care if needed. Researchers are both CPR and blood pathogen certified in case of emergency during your testing. The proper authorities including 911 will be contacted for immediate assistance if necessary.

Who should I contact if I have questions?

The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator, Justin Simmons any day at (804) 244-1263. (nights and weekends).

If you have questions about your rights as someone taking part in research, you may call the ECU Office of Research Integrity & Compliance (ORIC) at phone number 252-744-2914 (days). If you would like to report a complaint or concern about this research study, you may call the Director of ORIC, at 252-744-1971

I have decided I want to take part in this research. What should I do now?

The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

I have read (or had read to me) all of the above information.

I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.

I understand that I can stop taking part in this study at any time.

By signing this informed consent form, I am not giving up any of my rights.

I have been given a copy of this consent document, and it is mine to keep.

_____

_____

Participant's Name (PRINT) **Signature** **Date**

I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above and answered all of the person's questions about the research.

_____

_____

Researcher's Name (PRINT) **Signature** **Date**

Appendix C: Physical Training and Dietary Questionnaires

Subject ID#____

The Assessment of Muscle Oxygen Saturation in Students During Maximal VO_2 Exercise and High Intensity Intervals

Physical Training History

1. How long have you been in the ROTC program?
    a) 0-1yr
    b) 2yr
    c) 3yr
    d) 4yr
    e) I am not in the ROTC program

2. How long have you been performing endurance training at least 3+ days/ week for 30min or more for each workout?
    a) <1yr
    b) 1-2yrs
    c) 2-3yrs
    d) 3-4yrs
    e) >4yrs
    f) I do not perform endurance training at least 30 minutes 3 times per week

3. How many miles per week on average do you run?
    a. I do not run
    b. 0-5miles
    c. 5-10miles
    d. 10-15miles
    e. 15-20 miles
    f. 20+ miles

4. Have you had any training related injuries within the last 12months?
    a. Yes
    b. No

5. Have you ever taken an exercise course in school?
    a. Yes
    b. No

6. On a scale of 1-10 with 10 being the least prepared and 10 being the most prepared, how well do you believe the ROTC training program prepares you for physical fitness testing and overall soldier physical readiness? (Put N/A if you are not in the ROTC program)

_____

7. Do you currently have or ever had a heart related condition or bleeding disorder?
   ____ Yes ____No
8. Have you had a musculoskeletal injury within the last 3 months?

   ____ Yes ____No

9. Are you between the ages of 18 and 30 years?
   ____ Yes ____No

Subject ID: _____

Nutrition and Exercise Survey

Dietary Supplements

***In the <u>past month</u>*, how often did you take the following dietary supplements?**						
For each category, examples are provided but are not all-inclusive	**Never**	**1 time per <u>month</u>**	**2-3 times per <u>month</u>**	**1 time per <u>week</u>**	**2-3 times per <u>week</u>**	**Every day**
Multivitamin (*Centrum, One-a-Day, Mega-Men, etc.*)						
Vitamin D (*vitamin D_2 or D_3*)						
Individual vitamins and/or minerals (*vitamin C, vitamin E, B-complex, zinc, magnesium, etc.*)						
Fish oil (*containing omega-3 such as EPA/DHA*)						
Protein powders (*whey, casein, soy*)						
Creatine, HMB (*hydroxymethyl butyrate*)						
Amino acids (*branched chain amino acids, leucine, glutamine, beta-alanine, arginine*)						
SARM's (*Selective Androgen Receptor Modulator: Enobosarm, Triple Stack, Ostarine, S-4, Andarine*)						
Testosterone Pro-hormones/Boosters (*DMZ, ZMA, tribulus, dimethazine, methylsten, androstenedione, DHEA)*						
Pre-workout stimulants (*Jack3d, Superpump, NO Xplode, NO2 enhancers, Cellucor, Hemo Rage Black, Muscle Warfare, Naplam, Nitric Blast, etc.*)						
Thermogenics/weight loss aids (*Hydroxycut, Oxyelite, Thermoburst Hardcore, Lipo-6, Burn 60, RoxyLean, SlimQuick, Xenadrine, Ephedra, Green*						

tea extract, etc.)						
Energy drinks (*Red Bull, Monster, 5-hr energy, Rip It, Rock Star, etc.*)						
Melatonin						
Herbs (*fenugreek, milk thistle extract, saw palmetto, echinacea, ginkgo biloba, turmeric, ginger extract, St. John's wort, flaxseed, etc.)*						
Joint Formulas (*Glucosamine, chondroitin*)						
Carbohydrates/Electrolyte Formulas (*Endurox, Hammer products, Accelerade, Gatorade, Powerade Right Stuff, UCAN, Gu gels etc.*)						
OTHER (*please list individually*)						

Dietary Intake

***In the <u>past month</u>*, how often did you eat/drink each of the following foods?**						
Only a few examples from each category are listed, but more foods are available	Rarely or never	1-2 servings <u>per week</u>	3-6 servings <u>per week</u>	1 serving <u>per day</u>	2-3 servings <u>per day</u>	4 or more servings <u>per day</u>
FRUIT: *fresh, frozen, canned, dried, 100% fruit juice.* ***1 serving = 1 cup of fruit, ½ cup fruit juice***						
VEGETABLES: *fresh, frozen, canned, cooked, or raw.* ***1 serving = 1 cup of raw vegetables, ½ cup of cooked vegetables***						
WHOLE GRAINS: *whole wheat bread, pasta, brown rice, rye, oatmeal, corn tortillas* ***1 serving = 1 slice of bread, ½ cup of grains***						
DAIRY PRODUCTS: milk, yogurt, cottage cheese, deli cheese, ice cream/frozen yogurt ***1 serving = 8 oz. liquid, 1 oz. of cheese, ½ cup ice cream***						

***In the <u>past month</u>*, how often did you have each of the following foods?**						
	Rarely or never	1 time per <u>month</u>	2 times per <u>month</u>	1 time per <u>week</u>	2-3 times per <u>week</u>	4 or more times per <u>week</u>
Nuts						
Red meat						

Poultry (*chicken, turkey*)						
Lunch meats (*i.e., deli meats*)						
Pork (*pork, ham, sausage, bacon*)						
Fish (*tuna, salmon, tilapia , etc.)*						

Exercise Regimen

Please answer the following questions regarding your <u>current</u> exercise regimen	
How often do you exercise?	
What type of exercising are you doing? *(Crossfit-style, weightlifting, cardio, etc.)*	
How long are you exercising per session?	
On a scale of 1-5, what is the intensity of your usual workout? *(1-Leisure walking, 5-Feelings of nausea)*	

Appendix D: Additional Tables and Figures for Remaining Participants

Figure 1d. *Participant 001 SmO_2 (%) and total hemoglobin (g/dL) in the left and right quadriceps across time*

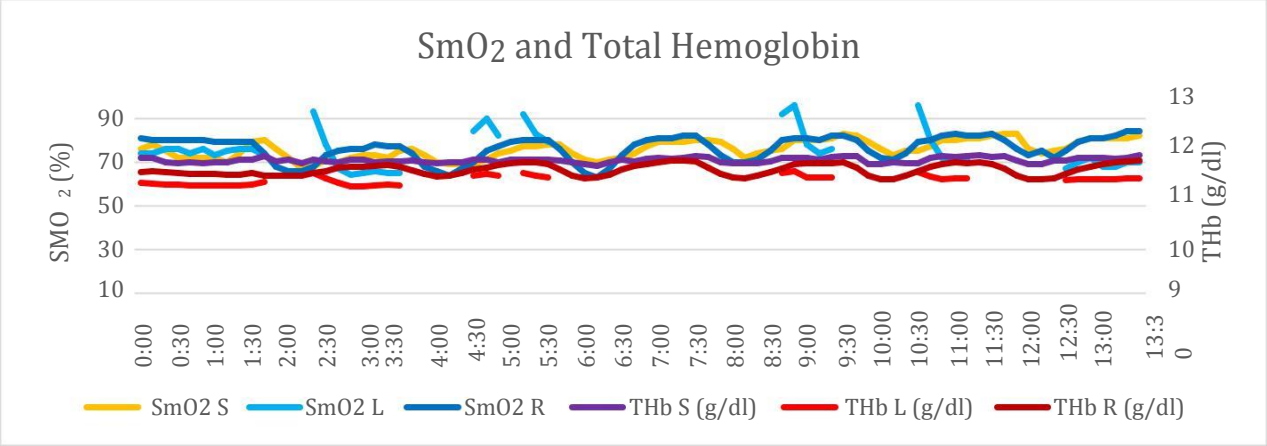

Table 7d. *Participant 001. SmO_2 and Total Hemoglobin*

Subject #001	S SmO_2 (%) Start/End	L SmO_2 (%) Start/End	R SmO_2 (%) Start/End	S THb (g/dl) Start/End	L THb (g/dl) Start/End	R THb (g/dl) Start/End
Interval 1	79/72	76	79/66	11.7/11.7	11.2/11.4	11.4/11.4
Interval 2	75/70	65	77/66	11.7/11.6	11.2	11.6/11.4
Interval 3	78/71	79	80/65	11.7/11.6	11.4	11.6/11.3
Interval 4	80/76		82/70	11.8/11.6		11.7/11.4
Interval 5	83/76	87	82/72	11.8/11.6	11.5	11.7/11.3
Interval 6	82/76	71	83/73	11.8/11.6	11.3	11.6/11.3
Avg Change Per Interval	-6%		-11.8%	-.11		-.25

Figure 1e. *Participant 005. SmO2 (%) and total hemoglobin (g/dl) in the left and right quadriceps across time*

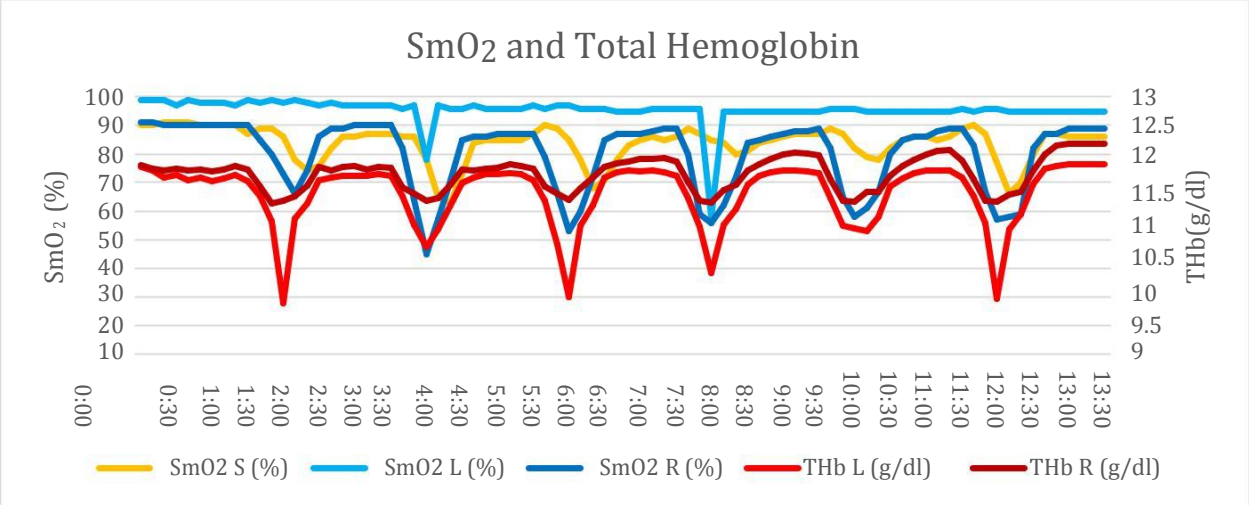

Table 7e. *Participant 005. SmO2 and Total Hemoglobin*

	Deltoid SmO_2 (%) Start/End	Left Quad SmO_2 (%) Start/End	Right Quad SmO_2 (%) Start/End	Left Quad THb (mg/dl) Start/End	Right Quad THb Start/End (mg/dl) Start/End
Interval 1	87/86	99/98	90/73	11.7/9.8	11.9/11.4
Interval 2	87/78	97/78	90/45	11.8/10.7	11.9/11.4
Interval 3	87/85	97/97	87/53	11.7/9.9	11.9/11.4
Interval 4	86/85	96/58	89/56	11.8/10.3	12.0/11.4
Interval 5	87/82	95/96	89/58	11.8/11.0	12.1/11.4
Interval 6	89/77	96/96	89/57	11.8/9.9	12.0/11.4
Avg Change Per Interval	-5%	-9.5%	-32%	-1.5 mg/dl	-.6 mg/dl

Figure 1f. Participant 006. SmO_2 (%) and total hemoglobin (g/dL) in the left and right quadriceps across time

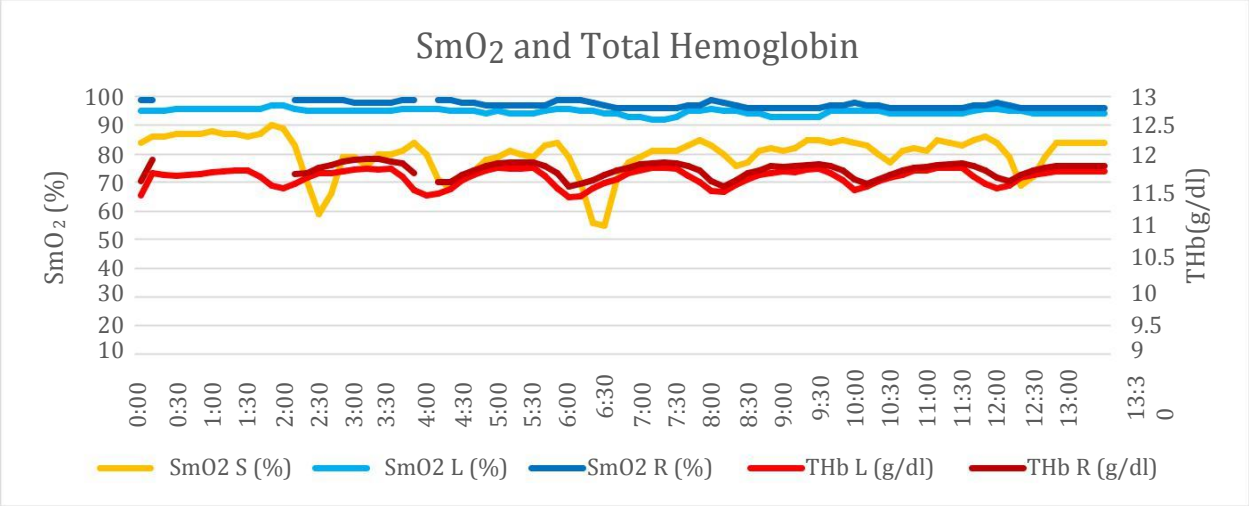

Table 7f. *Participant 006. SmO2 and Total Hemoglobin*

	Deltoid SmO_2 (%) Start/End	Left Quad SmO_2 (%) Start/End	Right Quad SmO_2 (%) Start/End	Left Quad THb (mg/dl) Start/End	Right Quad THb Start/End
Interval 1	86/89	96/97	/99	11.85/11.58	/11.80
Interval 2	80/80	95/96	98/99	11.88/11.46	12.0/
Interval 3	79/79	94/96	97/99	11.89/11.44	11.98/11.60
Interval 4	81/83	93/96	96/99	11.88/11.54	11.97/11.69
Interval 5	85/84	93/95	96/98	11.88/11.55	11.95/11.71
Interval 6	83/84	94/96	96/98	11.9/11.58	11.96/11.75
Avg Change Per Interval	+.83%	+1.8%	+1.7%	+1.7 mg/dl	-.31 mg/dl

Table 8d. *Participant 001. Interval Ending Measurements*

Interval #	SmO_2 S(%)	SmO_2 L(%)	SmO_2 R(%)	Blood Lactate (mmol/l)	Heart Rate (bpm)	VO_2 (ml/kg/min)	%VO_2 Max
1	70		66	7.2	182	20.0	51.7
2	68		64	8.9	190	23.9	61.8
3	71		64	9.2	194	21.8	56.3

4	74		69	10.3	194	28.8	74.4
5	74		71	8.3	195	28.0	72.3
6	73		72	9.8	196	28.8	74.4

Blank spaces indicate unavailable data

Table 8e. *Participant 005. Interval Ending Measurements*

Interval #	SmO_2 S(%)	SmO_2 L(%)	SmO_2 R(%)	Blood Lactate (mmol/l)	Heart Rate (bpm)	VO_2 (ml/kg/min)	% VO_2 Max
1	84	98		11.9	180	43.1	81.5
2	75	78	55	13.1	180	43.8	82.8
3	84		53	15.3	182	44.3	83.7
4	85	96	57	15.3	183	45.1	85.3
5	81		58		182	44.5	84.1
6	74		58	15.1	182	43.5	82.2

Blank spaces indicate unavailable data

Table 8f. *Participant 006. Interval Ending Measurements*

Interval #	SmO_2 S(%)	SmO_2 L(%)	SmO_2 R(%)	Blood Lactate (mmol/l)	Heart Rate (bpm)	VO_2 (ml/kg/min)	%VO_2 Max
1	86	96	99		168	29.0	67.8
2	76	96	99		172	32.7	76.4
3	75	96		12.8	170	31.5	73.6
4	81	96	99		170	31.6	73.8
5	84	96	98	12.0	167	30.8	72.0
6	81	96	98	11.6	168	31.4	73.4

Blank spaces indicate unavailable data

Figure 2d. *Participant 001. SmO_2 (%) of the left and right quadriceps and VO_2 (ml/kg/min) across time.*

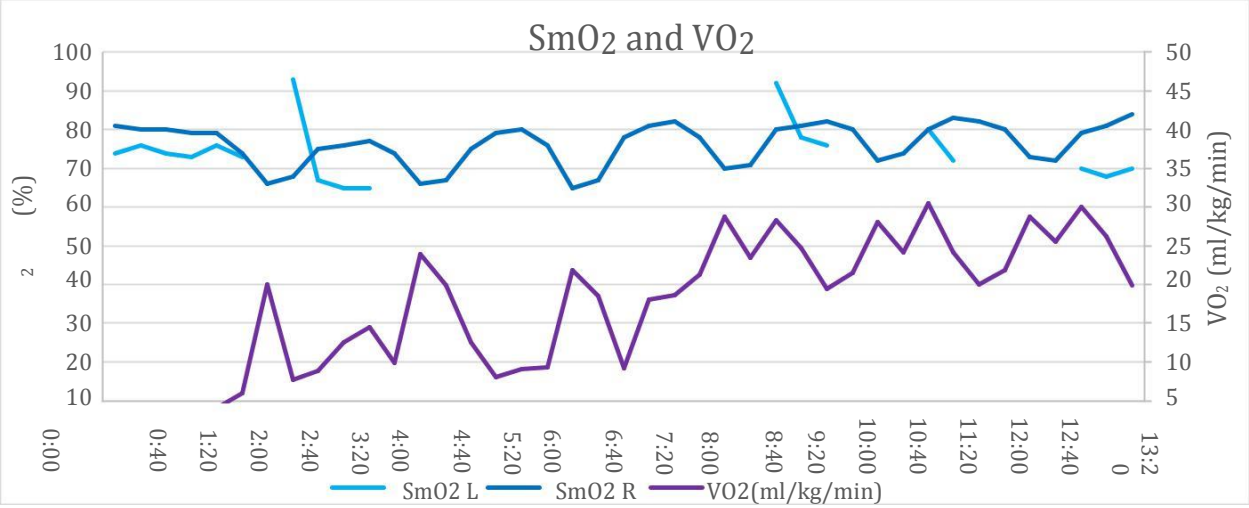

Figure 2e. *Participant 005. SmO_2 (%) of the left and right quadriceps and VO_2 (ml/kg/min) across time*

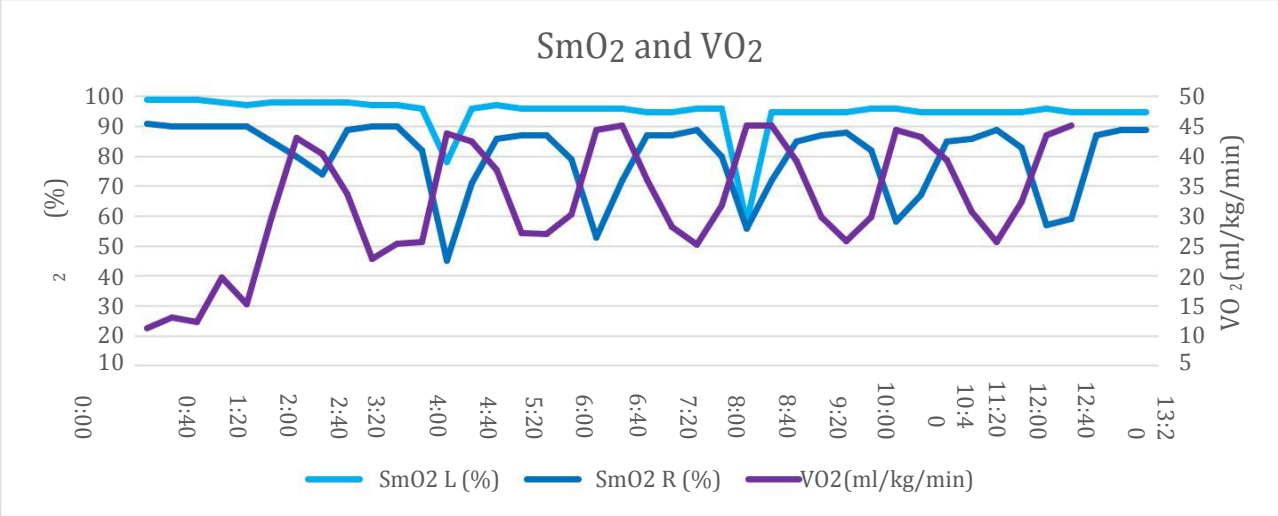

Figure 2f. *Participant 006. SmO_2 (%) of the left and right quadriceps and VO_2 (ml/kg/min) across time*

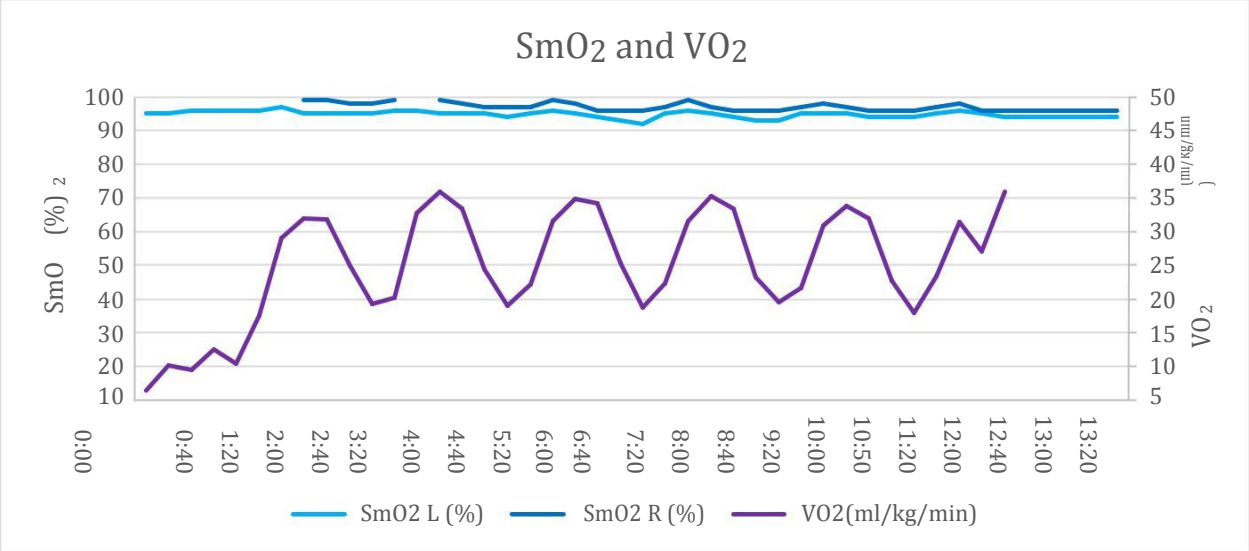

Figure 3d. *Participant 001. SmO_2 (%) of the left and right quadriceps and heart rate (bpm) during the six high-intensity intervals.*

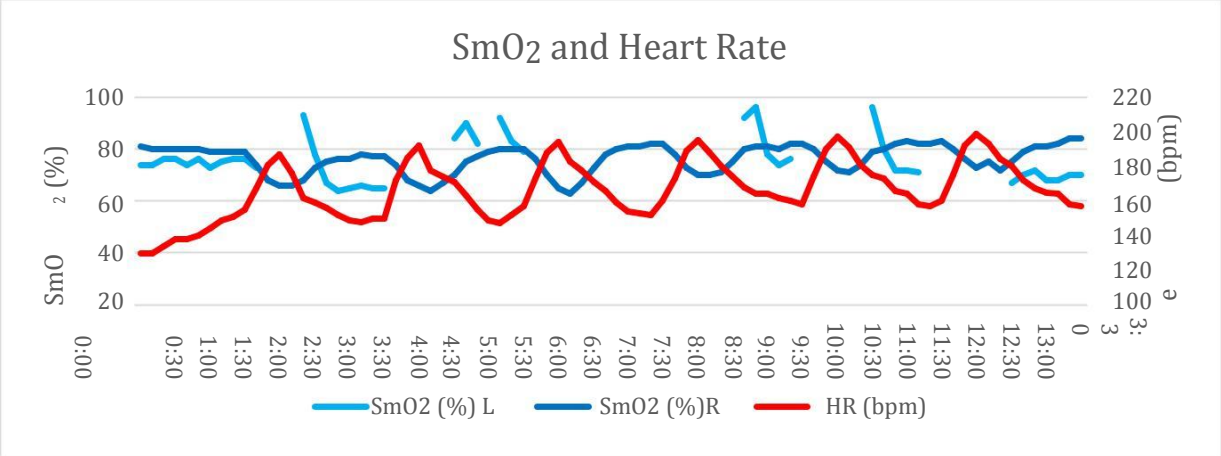

Figure 3e. *Participant 005. SmO_2 (%) of the left and right quadriceps and heart rate (bpm) during the six high-intensity intervals.*

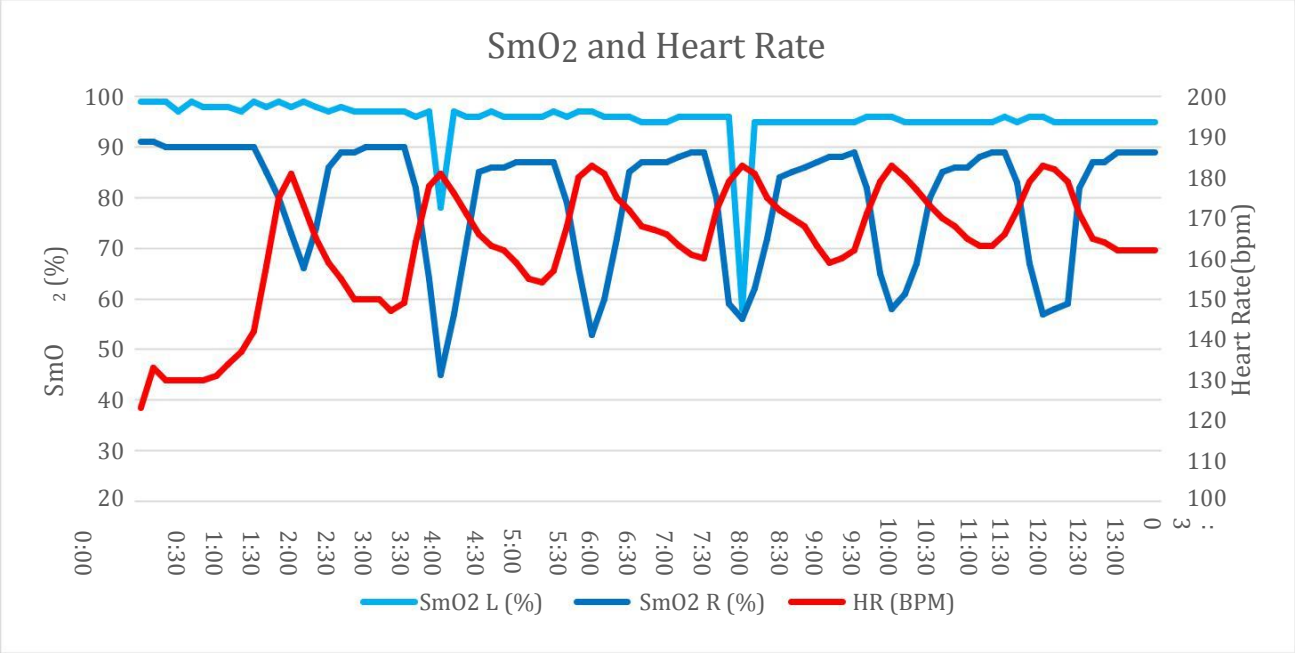

Figure 3f. *Participant 006. SmO_2 (%) of the left and right quadriceps and heart rate (bpm) during the six high-intensity intervals.*

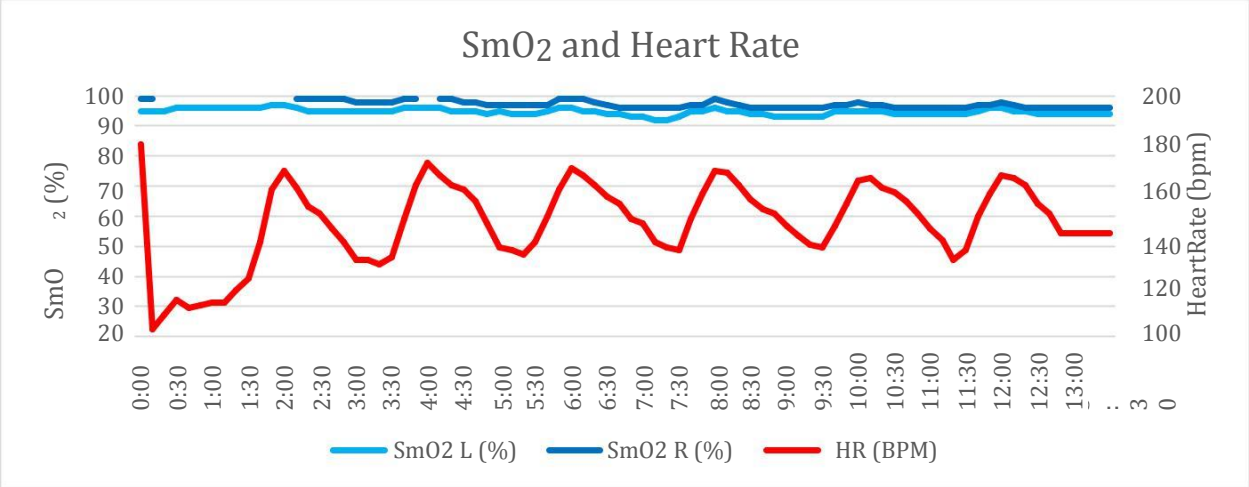

Table 9d. *Participant 001. Blood Lactate Taken at the End of Each Interval*

	L SmO2 (%) Start/End	R SmO2 (%) Start/End	BL (mmol/l) End
Interval 1	76	79/66	7.2
Interval 2	65	77/66	8.9
Interval 3	79	80/65	9.2
Interval 4		82/70	10.3
Interval 5	87	82/72	8.3
Interval 6	71	83/73	9.8

Blank spaces indicate unavailable data

Table 9e. *Participant 005. Blood Lactate Taken at the End of Each Interval*

	Left Quad SmO2 (%) Start/End	Right Quad SmO2 (%) Start/End	Blood Lactate (mmol/l) End
Interval 1	99/98	90/73	11.9
Interval 2	97/78	90/45	13.1
Interval 3	97/97	87/53	15.3
Interval 4	96/58	89/56	15.3
Interval 5	95/96	89/58	
Interval 6	96/96	89/57	15.1

Table 9f. *Participant 006. Blood Lactate Taken at the End of Each Interval*

	Left Quad SmO2 (%) Start/End	Right Quad SmO2 (%) Start/End	Blood Lactate (mmol/l) End
Interval 1	96/97	99	
Interval 2	95/96	98/99	
Interval 3	94/96	97/99	12.8
Interval 4	93/96	96/99	
Interval 5	93/95	96/98	12.0
Interval 6	94/96	96/98	11.6