EVALUATION OF SKELETAL MUSCLE ENDURANCE AND MUSCLE OXIDATIVE CAPACTIY USING ELECTRICAL TWITCH MECHANOMYOGRAPHY AND NEAR-INFRARED SPECTROSCOPY

by

LAUREN ASHLEY BROWN

(Under the Direction of Kevin McCully)

ABSTRACT

A skeletal muscle endurance test has been developed for use in clinical populations. **PURPOSE**: This study will evaluate two methods of generating an endurance index (EI) and compare these methods to mitochondrial capacity in skeletal muscle. **METHODS**: Healthy participants (n=15) were tested on two occasions. Surface electrodes and a tri-axial accelerometer were placed over the hamstring muscles. The stimulation induced skeletal muscle contractions (5Hz for 5 minutes). Maintenance of contraction acceleration was used to calculate an EI. Two analysis methods were used: peak-to-peak (P2P) and total (TLT). Each index was compared to mitochondrial capacity values measured by Near-Infrared Spectroscopy (NIRS). **RESULTS**: The P2P results were more reproducible (R^2=0.87 vs R^2=0.76) and had a stronger correlation with mitochondrial capacity (R^2 =0.96 vs R^2=0.36) than TLT ($p < 0.05$). **CONCLUSIONS**: The P2P analysis provided a reproducible measure of muscle endurance, which was consistent with mitochondrial capacity values.

INDEX WORDS: accelerometry, muscle fatigue, muscle metabolism, hamstring muscles, mitochondrial function, fatigability

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B.S., Elon University, 2017

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2019

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May, 2019

ACKNOWLEDGEMENTS

Foremost, I would like to express my very great appreciation to Dr. McCully for his invaluable guidance and mentorship over the years. His contagious passion and enthusiasm for problem solving kept me going during times of adversity. He has challenged me to think about new problems and ideas in a unique, meaningful way.

Thank you to my committee members, Dr. Jenkins and Dr. Call. Your time, patience, and intellectual contributions have helped me grow as a scientist.

Thank you to my lab-mates: Megan, Hallie, Uma, and Adeola. My experience in the lab was greatly enhanced by your friendship. I look forward to watching you all make your mark on this world.

Thank you to my family. You have always pushed me to strive for more and given me the tools to pursue my wildest dreams.

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CHAPTER 1

INTRODUCTION

Fatigue is a common symptom complaint in clinical practice and is associated with many health conditions; including multiple sclerosis, spinal cord injury, heart failure, and chronic obstructive pulmonary disease[1-3]. Metabolic dysfunction such as reduced mitochondrial capacity has been observed in these populations[2]. Fatigue related to metabolic dysfunction may contribute to reduced quality of life in these populations [2]. Interventions to reduce fatigue and improve muscle metabolism may have benefit in these populations.

Skeletal muscle fatigue has been defined as "failure to maintain the required or expected force or power output as a result of exercise"[4]. Physiological mechanisms responsible for fatigue may be central or peripheral in origin. This study will focus on peripheral fatigue. Peripheral fatigue is " a decline in the force generating capacity as a function of differences at the muscle level resulting from muscle contractile properties, intramuscular oxidative metabolism, or impaired excitability or excitation-contraction coupling"[5]. The primary metabolic changes associated with peripheral fatigue include the accumulation of inorganic phosphate and hydrogen ions, which are thought to act synergistically[6]. Impaired release and reuptake of calcium is also thought to be a mechanism for peripheral muscle fatigue. This production of metabolites is inversely related to mitochondrial capacity.

The concept of fatigue has been expressed with terms including fatigability and endurance. Fatigability refers to as the inability of muscles to maintain contractile function and is often used more in the context of an activity[7]. This is to distinguish fatigability from weakness, which is a more general reduction in contractile ability. Endurance is the inverse of fatigability

which is the ability of skeletal muscle to maintain contractile function. The results of this study will be presented using muscle endurance.

Muscle endurance/fatigue has been studied for a long time[8,9], and a large variety of protocols have been used to evaluate endurance/fatigue. A common approach to measuring muscle endurance/fatigue use voluntary contractions that require participants to perform maximal contractions until the contractions fall below target levels, or submaximal contractions to task failure which require maximal effort. A key aspect to these approaches is that it will be influenced by central fatigue: the participant must be motivated and have the ability to activate the appropriate group of muscles in what includes a maximal effort. Another limitation is that using maximal or near maximal contractions produce high force levels that can have a safety risk in some clinical populations. For example, people with motor complete spinal cord injuries have been shown to be more susceptible to muscle damage during high force contractions[10]. Endurance/fatigue protocols have also used electrical stimulation to perform endurance/fatigue tests [11]. Electrical stimulation can circumvent central fatigue. Endurance/fatigue tests using tetanic contractions produced by electrical stimulation can still produce force levels high enough to result in muscle damage, and for people with intact sensory systems, electrical stimulation sufficient to produce tetanic contractions can be uncomfortable.

A recently developed endurance test, electrical twitch mechanomyography (EMT), uses low intensity, low force twitch contractions and measures changes in twitch acceleration[12]. This approach has been applied to clinical populations[2, 13, 14]. An endurance index (EI) is calculated as the remaining acceleration during a 9 minute protocol (3 minutes each at 2 ,4 and 6 Hz). Recently a shorter protocol consisting of 5 minutes of stimulation at 5 Hz has been developed. Because the stimulation currents are submaximal, subject discomfort is limited compared to

other protocols that use tetanic contractions with higher current levels. In addition, twitch contractions produce much lower force levels that tetanic contractions, making them safer than higher force stimulation/exercise protocols. Because the ETM approach uses an accelerometer, it can target more muscle groups than approaches that use muscle ergometers. Although ETM is increasing in popularity among clinical populations, the method is still relatively new; careful evaluation of this method is needed.

The twitch endurance test has used an analysis program that measures the peak to peak changes in muscle acceleration due to the twitch contractions[3, 15]. This approach uses the data points that have the largest signal to noise ratios, but only uses the accelerations from the peak of each twitch. For example, if data is collected with a sampling frequency of 400 Hz, a 5 Hz twitch protocol would use 10 of the data points per second. This would leave any information from the remaining 390 points per second unanalyzed. An alternative analysis approach would use all the 400 Hz data points to obtain a total acceleration value. While many of the points have low signal to noise values, the total signal from 400 points per second might result in better signal to noise and more accurate assessments of changes in muscle contractile activity.

Several previous studies that used twitch acceleration to measure muscle endurance compared the EI results to muscle mitochondrial capacity measured with Near-infrared spectroscopy (NIRS). NIRS is an accessible, non-invasive method of measuring mitochondrial capacity[16]. NIRS has been demonstrated to have test-retest validity and correlates with Phosphocreatine recovery in the ^{31}P MRS, the "gold-standard" method of measuring mitochondrial capacity[12, 17]. NIRS has been used in various clinical populations including those with peripheral arterial disease, spinal cord injuries, and able-bodied humans[12]. Additional

studies that compare EI values to muscle mitochondrial capacity would strengthen the utility of EMT as a measure of muscle endurance.

Statement of the Problem

Careful evaluation of the analysis methods used to produce an EI value from electrical twitch mechanomyography needs to be performed. Comparisons of the EI values to NIRS measured mitochondrial capacity will further support the use of EI as a measure of muscle endurance, and can serve as a criterion for deciding which approach to analyzing EI data is best.

Specific Aims

Specific Aim 1: Measure the reproducibility of the endurance index values in the hamstring muscles by use of electrical twitch mechanomyography, and analyze the results using two different approaches.

Specific Aim 2: Measure mitochondrial capacity in the hamstring muscles of healthy participants using near-infrared spectroscopy.

Specific Aim 3: Compare the two measurement approaches for the endurance index with mitochondrial capacity.

Hypotheses

I. The peak-to-peak and total methods of analysis for the EI will have significantly different mean values, slope, and intercept values.

II. Both the peak-to-peak and total analysis methods for the EI will be reproducible (R^2 >0.80).

III. There will be a significant difference in the correlation values between the peak-to-peak and total methods of EI analysis with mitochondrial capacity values.

Significance of Study

Findings from this study will improve our ability to measure muscle endurance in clinical populations. Specifically, this study will improve our understanding of how best to analyze the data from the endurance test. It will also provide a better understanding of the physiological mechanisms that underlie the muscle endurance test.

CHAPTER 2

REVIEW OF LITERATURE

Skeletal muscle fatigue is a well-studied phenomenon for a long period of time[8, 9]. 'Fatigue' is an important clinical problem, and muscle dysfunction can be an important contributor to fatigue. Because of the importance of fatigue in clinical populations, improving methods of measuring fatigue, including fatigue resulting from muscle dysfunction is important. Specifically, improving methods to evaluate muscle related fatigue in clinical populations is important. This includes making measurements of fatigue safer and more comfortable, easier and cheaper to perform, and improving the links between muscle fatigue and the underlying physiological mechanism is important. This thesis will evaluate a simple, noninvasive measurement approach appropriate for clinical populations.

Although there are many definitions for fatigue, it is most commonly defined as the inability of a muscle to sustain repeated contractions[18]. Others define fatigue as a progressive decline of performance which largely recovers after a period of rest[18]. Although each accepted definition of fatigue has theoretical support, the lack of a consistent definition makes it more challenging to identify a method of assessment. The following study will focus on endurance, the ability to resist fatigue[15]. This definition is limited in its ability to assess changes in sensation, mechanism, or the interaction between the two. However, through focusing on potential decreases in movement, we are able to have a more general, applied understanding of changes that may be more easily applied to all populations.

Mechanisms of Skeletal Muscle Fatigue

There are a range of mechanisms that contribute to the progressive decline including effects of ionic changes, failure of the sarcoplasmic reticulum to release calcium, and the effects of reactive oxygen species[18].There are various differences in skeletal muscle cells including their speed of contraction, intracellular functions, glycolytic and oxidative capacity, and ability to resist fatigue[18]. Each of these differences gives further insight on function including fatigability.

There are a sequence of events leading to a skeletal muscle contraction. For simplicity, the process may be understood as the following: A signal is transferred from the brain down the spinal cord. These motor neurons excite muscle fibers, leading to the release of calcium into the cytosol. Calcium is then able to bind to troponin C. This allows cross bridges to be formed and create force or a power cross-bridge[19]. Fatigue may be a result of changes during any point of this cycle. For example, there may be a decrease in excitation rate from the motor neurons to the muscle fibers. Propagation of the action potential may be impaired by an imbalance of Na^+ and K^+ ions over the sarcolemma. A decrease in the calcium released into the cytosol will also attenuate the cross-bridge cycle. Lastly, an inadequate supply of ATP decreases the cross-bridge cycles ability to generate force[19]. Understanding the events of the muscle contraction help support the background of endurance analysis.

The expression of myosin heavy chain isoforms (MHC) characterizes muscle into Type I, IIa and IIb[20]. The expression of MHC isoform is the most important factor in determining the maximal shortening velocity .[18]Although there may be co-expression of different isoforms, it is generally accepted that type I, type IIa and type IIb represent slow, intermediate, and fast respectively[21]. The development of muscle biopsy and NMR further support the understanding of each fiber type[22, 23]. Fiber type classification is relevant to metabolic changes; fast twitch

fibers will consume ATP at a faster rate than slow twitch fibers[18]. Most critical to this study, slow type I fibers generally have a higher oxidative capacity compared to type II[24]. We will not be identifying fiber types in this study, however it is important to acknowledge the foundational role they play in muscular endurance.

High frequency and low frequency fatigue have different mechanisms. High frequency fatigue occurs when a muscle is stimulated continuously at a frequency close to that which gives maximal force, producing a rapid decline of force[19]. This type of fatigue occurs when performing tasks such as lifting a very heavy object; both onset and recovery of fatigue occur quickly[19]. Low frequency fatigue occurs when there is a much slower rate of fatigue (although it may persist longer) and simulates natural activities such as walking and breathing[18]. The protocol for inducing this type of fatigue may include changing the fraction of time during the muscle contraction or using a low stimulus frequency[18]. The protocol used for this study, 5 minutes and 5 Hz, is representative of performing day to day tasks[19]. Low frequency fatigue maybe related to calcium homeostasis in skeletal muscle and can take hours to resolve[25].

Current Measures of Fatigue in Clinical Populations

There have been a large number of methods used to evaluate muscle fatigue[26]. Most studies involve either maximal contractions or torque[27 ,28], where a decline in force over time is measured, or a submaximal effort, where the time to task failure is measured. These forces that are generated voluntarily can be limited by lack of motivation and inhibitory effects at various levels in the central nervous system[29, 30]. They also involve the development of high levels of force which can involve risk to some participants. Electrical stimulation using tetanic contractions can be used to perform fatigue test. The use of electrical stimulation can overcome the limitation of motivation. Ashraf et al., performed fatigue tests using tetanic stimulation

frequencies from 25 to 100 Hz[31]. Tetanic contractions from electrical stimulation involve high force contractions, which may be unsafe and uncomfortable for some participants.

Several traditional muscle endurance tests have been developed specifically for clinical populations[32]. Among these, gait speed, chair-rise, and grip strength tests are the most frequently used methods[33]. These measures have their own limitations, "they often only examine particular aspects of muscle function (e.g., balance, power, force) and few provide a quantitative measure (e.g., force, velocity, power)"[33]. Additionally, these tests are not often time specific to muscle. A quantitative, muscle-specific method needs to be explored.

Electromyographic (EMG) signals (compound action potentials from skeletal muscle when they contract) have been used to monitor electrical changes within the motor unit during muscular activity[34, 35]. Ibitoye et. al., found a positive correlation of up to R^2=0.90 ($p<0.05$) between the decline in the peak-to-peak amplitude of the eEMG and the decline in the force output during fatiguing isometric contractions[35]. However, there are significant limitations to the use of EMG to study muscle fatigue[36]. The primary limitation is that EMG signals reflect changes in muscle membrane potentials, which sometimes but not always reflect changes in muscle cross-bridge cycling (force generation)[35]. A similar, more comprehensive method of measuring fatigue is needed.

Surface Mechanomyography & Analysis

Tri-axial accelerometers have been used to measure muscle movement instead of torque or force with an ergometer[37]. The benefits of surface mechanomyography, such as low-cost, ease of use, minimal skin preparation, and negligible effect of skin impedance make this technique ideal for various clinical populations[37]. It is also suitable for skeletal muscle activity assessment and can be employed to investigate various muscle groups[38]. Surface

mechanomyography has also been employed to distinguish between functioning of muscles in subjects with Parkinson's Disease (PD) and healthy controls. Researchers found that it not only was able to depict differences between healthy controls and individuals with PD, but also was not affected by physiological postural tremor[39].

Electrical Twitch Mechanomyography Endurance Protocol

A muscle-specific endurance test has been developed. The protocol involved 9 minutes of twitch stimulation, 3 minutes each at 2, 4, and 6 Hz[40]. They used submaximal stimulation currents and found that the endurance index was not influenced by the stimulation current levels [40]. This protocol has been used to study a number of different patient populations. Bossie et al., used electrical twitch mechanomyography to study endurance in persons with Friedreich ataxia (FRDA) and able-bodied controls. Researchers found a lowered muscle endurance of 19.2 % in the FRDA group[2].

Near-infrared Spectroscopy (NIRS)

Skeletal muscle fatigue is largely dependent on the oxidative capacity of mitochondria within the muscle[12]. Many populations including those who suffer from neurologic and cardiovascular pathologies have displayed impairments in oxidative metabolism [12, 41, 42].Thus, the understanding of mitochondrial function is essential to understand fatigability. Mitochondrial capacity may be studied both *in vitro* and *in vivo*. Typically, *in vitro* measurements come from measuring rates of oxygen consumption in permeabilized muscle fibers or isolated muscles from muscle biopsies[12]. *In vivo* methods are commonly used, however they are invasive in nature. Near-infrared spectroscopy (NIRS) provides a non-invasive approach that will be used in this study. This method uses changes in muscle oxygenation to measure the changes in exercise

metabolism from exercise to rest. The rate constant of recovery is an index of mitochondrial capacity[2, 3, 12].

CHAPTER 3

EVALUATION OF SKELETAL MUSCLE ENDURANCE AND MUSCLE OXIDATIVE CAPACTIY USING ELECTRICAL TWITCH MECHANOMYOGRAPHY AND NEAR-INFRARED SPECTROSCOPY

Brown, Lauren. To be submitted to Medicine & Science in Sports & Exercise

Abstract

A skeletal muscle endurance test has been developed for use in clinical populations. **PURPOSE**: This study will evaluate two methods of generating an endurance index (EI) and compare these methods to mitochondrial capacity in skeletal muscle. **METHODS:** Healthy participants (n=15) were tested on two occasions. Surface electrodes and a tri-axial accelerometer were placed over the hamstring muscles. The stimulation induced skeletal muscle contractions (5Hz for 5 minutes). Maintenance of contraction acceleration was used to calculate an EI. Two analysis methods were used: peak-to-peak (P2P) and total (TLT). Each index was compared to mitochondrial capacity values measured by Near-Infrared Spectroscopy (NIRS). **RESULTS:** The P2P results were more reproducible (R^2=0.87 vs R^2=0.76) and had a stronger correlation with mitochondrial capacity (R^2 =0.96 vs R^2=0.36) than TLT ($p < 0.05$). **CONCLUSIONS:** The P2P analysis provided a reproducible measure of muscle endurance, which was consistent with mitochondrial capacity values.

KEY WORDS: accelerometry, muscle fatigue, mitochondrial function, fatigability

Introduction

Skeletal muscle fatigue, defined as the inability to maintain the required or expected force or power output, is a common symptom complaint in clinical practice and is associated with many health conditions including multiple sclerosis, spinal cord injury, heart failure, and chronic obstructive pulmonary disease[1-3]. Similarly, many of these clinical populations have demonstrated mitochondrial dysfunction and an overall reduced quality of life[2,4,5]. Taken together, fatigue is an important measure in the clinical population yet, there is an overarching lack of feasible methods to assess fatigue in clinical populations.

Skeletal muscle fatigue has been studied in the past using voluntary, maximal and submaximal endurance tests in clinical populations[6-9]. Each of these methods has limitations; the participant must have the ability to innervate the appropriate group of muscles in some capacity. Many clinical populations including aging populations, those with spinal cord injuries, or other neuromuscular system disorders may be unable to perform the suggested voluntary tasks, thus would need an alternative method to measure skeletal muscle fatigue[10].

Electrical twitch mechanomyograpahy has provided a feasible, non-invasive approach to support testing in clinical populations[3,11,12]. This method uses low frequency surface electrodes to produce twitch contractions and a tri-axial accelerometer to measure the acceleration of muscle movement[2].As fatigue of the muscle increases, the acceleration from the muscle movement decreases, generating an endurance index[13]. Although this technique is more affordable, portable, and potentially more applicable to clinical groups, there are no standard protocols used in the literature.

Another non-invasive technique may be employed to measure mitochondrial capacity in these populations. Near-infrared spectroscopy (NIRS) has been previously used and validated in many clinical populations[4, 5].

The purpose of this study was to evaluate two methods of generating an endurance index and comparing them to mitochondrial capacity. It was hypothesized that the peak-to-peak vs. continuous method of analysis will have significantly different mean values, slope, and intercept values. We also hypothesized that both the continuous and peak-to-peak analysis methods would be reproducible (R^2 value > 0.80). Finally, we hypothesized that there would be a moderate positive correlation between the continuous endurance index value and the mitochondrial capacity values.

Methods

Study Participants

Healthy, able-bodied participants were recruited for participation in this study (Table 3.1). This study was approved by the Institutional Review Board at the University of Georgia and all participants provided informed consent prior to data collection. Individuals were excluded from the study if they were unable to comfortably lay down for ten minutes, pregnant, or have fragile veins. Participation was voluntary and participants were able to stop taking part at any time.

Design and Procedures

This study consisted of two parts. Part 1: Evaluate the reproducibility of electrical twitch mechanomyography (5 min. 5 Hz). Measurements were taken on two separate days. Part 2: Evaluate the validity of the Endurance Index values through correlation of mitochondrial oxidative capacity, determined by the five minute five hertz analysis and NIRS testing respectively. All experiments were completed within 3 weeks for each participant and tested by the same researcher.

Measurements

Electrical Twitch Mechanomyograpahy (ETM)

The testing protocol consisted of 5 Hz electrical stimulation for 5 minutes, which is a shorter version of the 9 minute protocol using 2, 4, and 6 Hz stimulation 14, 4. Participants were positioned in a prone position on a padded table. Two aluminum foil electrodes (12.7 cm X 1.7 cm) were attached to a stimulator and were positioned over the hamstring muscles. A small amount of ultrasound gel was used as a conduction medium between the electrodes and the skin. One electrode was placed right below the gluteal fold, while the more distal electrode was placed right above the popliteal fold. The muscles were being stimulated using levels that ranged from 40 mA to 80 mA with pulse durations/intervals of 200 μs/50 μs. A wireless tri-axial accelerometer (WAX3; Axivity, Newcastle upon Tyne, UK) was used to measure the magnitude of muscle contractions.

Muscle acceleration data was transmitted by Bluetooth to a computer for analysis. The magnitude vector was calculated using the three axes in the following equation: Magnitude Vector= $\sqrt{}$ $(X^2+Y^2+Z^2)$.Two custom written analysis programs were used in MATLAB(Mathworks, Natick, MA). The peak-to-peak analysis used for each twitch, the magnitude of change from the highest acceleration to the next lowest acceleration vector. The total analysis method used the average change in acceleration for all 80 points in each twitch. The endurance index was calculated as the percentage of the end value divided by the maximal value. The end value was determined as the average of the last 20 seconds and the maximal value was the highest value for 3 seconds in the first 60 seconds. (Figure 3.2).

Near-Infrared Spectroscopy (NIRS)

The testing protocol consisted of resting metabolism measurements, muscle activation (stim) check, and four repeated mitochondrial capacity tests[15,16]. Participants were positioned prone on a padded table. The NIRS probe (PortaMon, Artinis Medical Systems, Inc.) was placed vertically over the hamstring muscles and secured on the leg with bi adhesive tape and pre-wrap. Two electrodes (11cm x 5 cm) attached to a stimulator were positioned proximal and distal to the probe. The electrode more proximal was placed right below the gluteal fold, while the more distal electrode was placed right above the popliteal fossa. The blood pressure cuff attached to a Hokanson AG101 Rapid Cuff Inflator was wrapped around the upper thigh, as high as anatomically possible, proximal to the NIRS probe. Electrical stimulation was used to activate the muscle. Stimulation intensity was selected based on the highest tolerable current which also produced a visible contraction. The stimulation intensity varied for each participant. Stimulation frequency was set at 5Hz.

The NIRS protocol consisted of five minutes of rest to assess muscle oxygenation levels. A 30 second cuff (300-310 mmHg) was performed. A 30 second rest period occurred followed by 30 seconds of electrical stimulation and an immediate inflation of the cuff. This was performed to determine resting metabolic rate and ensure adequate activation of muscle during stimulation.

Each mitochondrial test measurement consisted of electrically stimulating the muscle for 30 seconds, immediately followed by a 5 second arterial occlusion cuff to assess metabolic rate. There were a total of 6 metabolic rate measurements periods (5-second inflation followed by 5 seconds of rest). The final time point consisted of five minutes of rest followed by a 30 second cuff inflation.

The NIRS test was analyzed using a custom written Matlab program. Analysis included a blood volume correction to account for the redistribution of blood from high and low pressure arterioles during arterial occlusion[43]. Muscle metabolic rates were calculated from the slopes of corrected NIRS signals using linear regression. To determine oxidative recovery after electrical stimulation, slope measurements were fit to a monoexponential curve and rate constants were calculated.

Statistical Analysis

Descriptive statistics were measured and values are presented as means and standard deviations. A multivariate test was run on each of the four individual endurance index values. Comparisons between the average two endurance index values were made with a paired t-test (P2P EI vs. TLT EI). Comparisons within each testing analysis protocol were made using a paired t-test (P2P EI Test 1 vs. P2P EI Test 2). Significance was assumed at $P < 0.05$. The order effect between each testing day was measured as well as the coefficient of variation among each analysis.

Comparisons between the two endurance index values and mitochondrial capacity were correlated. Power calculations were performed to estimate an adequate sample size in order to detect a meaningful difference.

Results

A representative endurance index test is shown in Figure 3.1. Endurance Index measures were taken for 15 participants. The averages for two P2P analyzed tests were 55.5 ±17% and 56.5±19% respectively. The averages for two TLT tests were 63.0±25% and 66.0±20% respectively. Endurance index averages are compared in Figure 3.2. The P2P analysis EI values had a R^2=0.87 and a coefficient of variation of 7 ±5% (Figure 3.3). The TLT measurements had an R^2=0.76 and the coefficient of variation between the two EI TLT values was 12±10% (Figure

3.3). The average (2 tests each) EI values from P2P and TLT measurements were not significantly different; F=2.6, P=0.11, as demonstrated in Figure 3.4. There was no order effect for either the P2P or TLT EI values (P=0.55 and P=0.39, respectively).

A representative example of oxygen saturation values for a NIRS mitochondrial test is shown in Figure 3.5. Mitochondrial capacity measurements were made in 10 participants. The average mitochondria capacity value was 2.1±1.1 min^{-1}. P2P had a stronger correlation with mitochondrial capacity, compared to TLT (R^2 =0.96 and R^2=0.37, respectively). The correlations are shown in Figure 3.6.

Discussion

The main finding of our study was that the P2P analysis approach had a stronger correlation with mitochondrial oxidative values than the TLT approach. Previous studies have reported correlations between muscle specific endurance and mitochondrial capacity[2,4,13,]. The stronger correlation suggests that the P2P method more accurately reflects muscle endurance than the TLT method. Previous studies have used the P2P method[2,4,14]. We are not aware of any studies that have used an analysis approach similar to the TLT method.

Our study also found that the P2P method was less variable compared to the TLT method. The coefficient of variation for the P2P method in this study (7%) is similar to the 6-9% values for coefficients of variation found in a previous study using the P2P method[13]. These findings are consistent with reproducibility values found in the literature[2, 3, 13, 17]. Because the TLT method uses more data points, 400 per second rather than 10 per second for the P2P method, it was possible that analyzing more signals would increase reproducibility. However, this was not supported by our study.

The endurance index values calculated with both methods were not different. In addition, there was a strong correlation between the two methods. This suggests that both analysis methods produced similar results.

The study used the hamstring muscles to test the two analysis approaches for endurance index. The hamstring muscles were selected based on a previous study that showed that EI values were 78.0±13.3%[14]. Studying muscles with high EI values such as the vastus lateralis muscles would have made it more difficult to detect differences between the two programs because of the small amount of both fatigability reported in this muscle[13]. A potential limitation to our study is the use of one muscle group, the hamstring muscles. Future studies may consider evaluating muscle in clinical populations with difference muscle characteristics[3, 13].

We found NIRS mitochondrial capacity values for the hamstring of 2.0±1.1. This is the first time to our knowledge that mitochondrial capacity of the hamstring muscles has been reported. Our values are slightly lower than previous values for the vastus lateralis muscle[3]. This is consistent with the differences in endurance index values seen between the VL and the hamstrings[14]. Given the possibility that hamstring muscles may have reduced endurance after knee reconstruction surgery[14], future studies measuring mitochondrial capacity of the hamstring muscle are needed.

In this study, we recruited individuals with a continuum of endurance index values. A potential limitation is that some participants have endurance index values below the lowest participants in our study. It is possible that the relationship between the two analysis methods would be different in those populations. Another potential limitation of the study was that we evaluated two analysis programs. It is possible that other analysis programs may work better.

Conclusions

The P2P analysis approach appeared to be better than the TLT analysis approach for analyzing muscle specific endurance with the twitch stimulation method. This was based on higher correlations with muscle mitochondrial capacity, as well as greater reproducibility. The TLT approach appeared to be less reproducible and less associated with muscle mitochondrial capacity, even though both approaches had the same EI values and did correlate with each other. This study could be tested on other muscle groups, or modifications to the analysis approach to confirm the findings. The correlation between muscle endurance and muscle mitochondrial capacity was consistent with previous studies and supports the use of the endurance test to evaluate skeletal muscle endurance related to metabolism.

Figure legends

Figure 3.1: A) A representative endurance test showing the resultant vector during 5 minutes of electrical stimulation at 5 Hz. The EI value for the P2P analysis was 43.4%. B) A representative twitch during the time used to calculate a maximal value. C) A representative twitch during the time used to calculate the end value. The red diamonds indicate data used for P2P analysis.

Figure 3.2: Endurance index test values for the two testing days and two testing methods. Data are mean and standard deviation.

Figure 3.3: Relationship between P2P test one and test two and relationship between TLT test 1 and 2. R^2=0.87 and R^2=0.76

Figure 3.4: Relationship between P2P EI and TLT EI. The average of two test days was used for each protocol. There was no statistical difference(F=2.61, P=0.112).

Figure 3.5: A representative mitochondrial capacity test showing changes in oxygenation (O_2Hb) levels during the duration of the test.

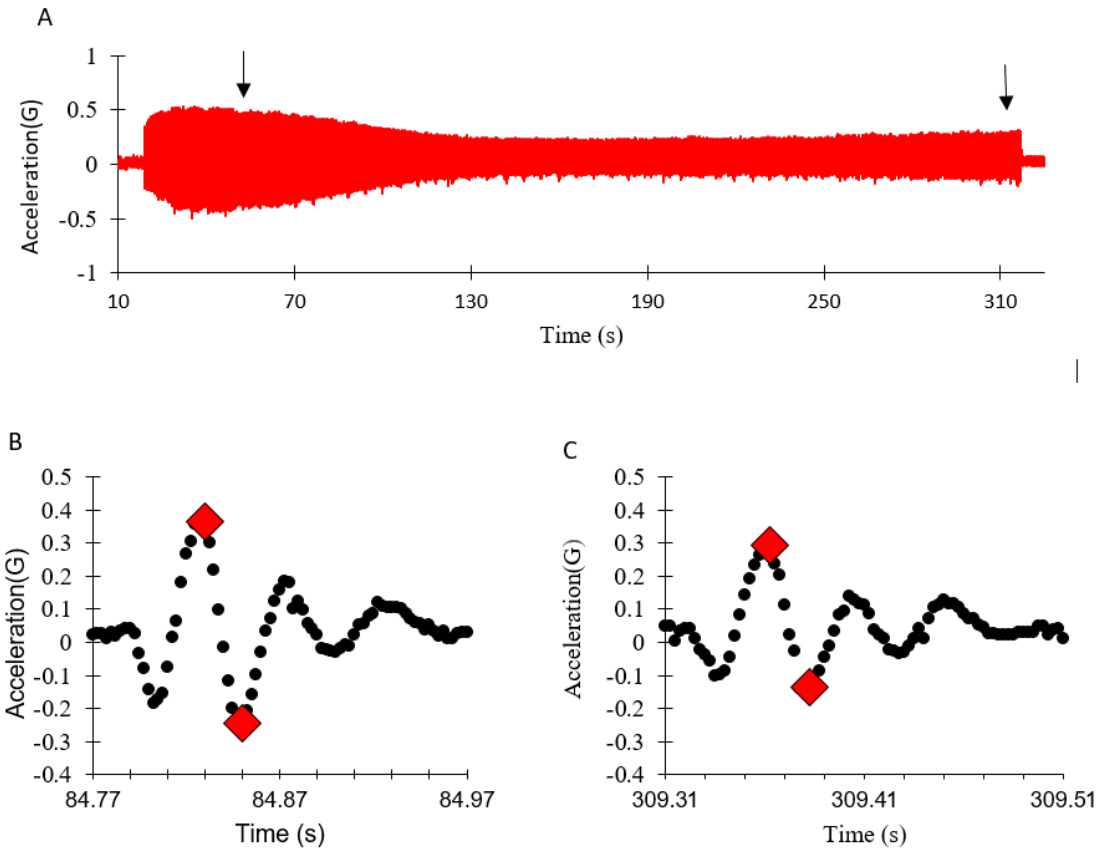
Figure 3.6: Relationship between the P2P EI value and mitochondrial capacity and the relationship between TLT and mitochondrial capacity. An average of two test days were used for both the P2P and TLT. An average of 4 mitochondrial test were used for the mitochondrial capacity values.

Table 3.1 Participant Characteristics

Participants	Age (yrs)	Height (m)	Weight(kg)	BMI (Kg/m^2)
Males (n=4)	21.0±0.8	1.79±0.71	73.4±4.1	22.9±1.7
Females (n=11)	22.2± 1.7	1.65±0.62	64.1±6.4	23.3± 2.8

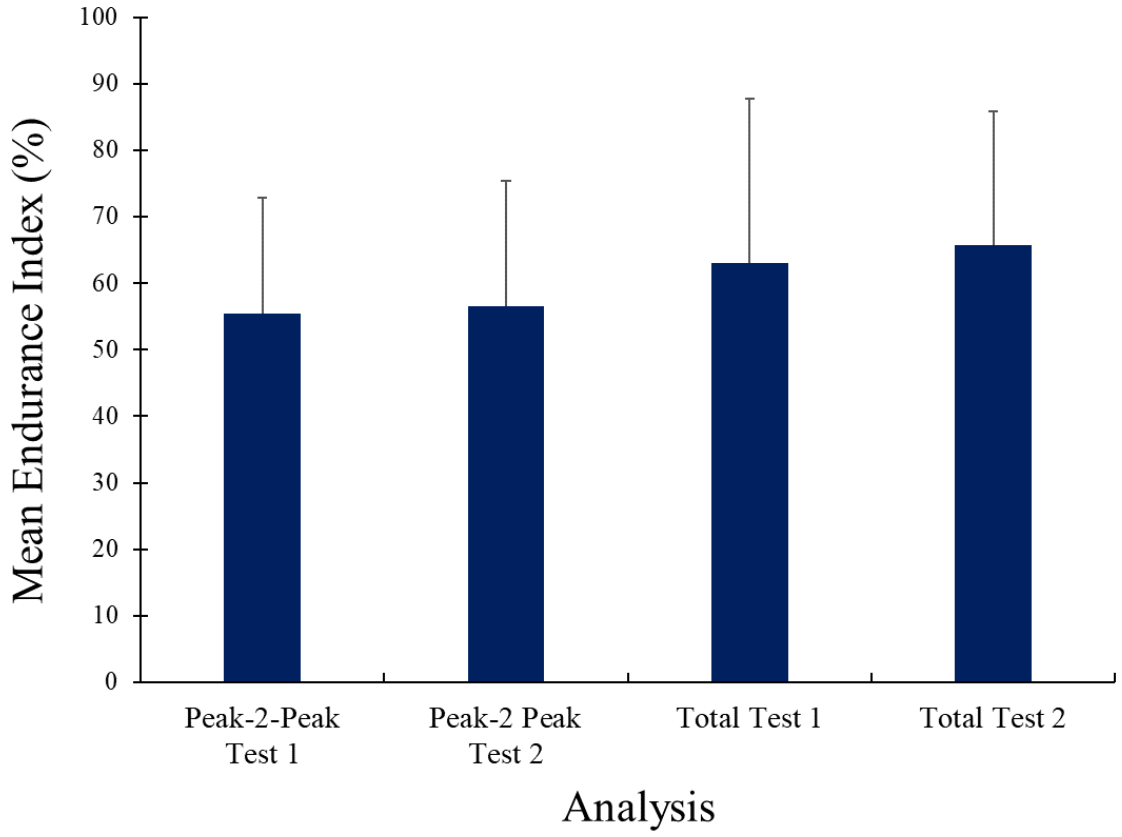
Values are presented as mean ± SD.

Figure 3.1



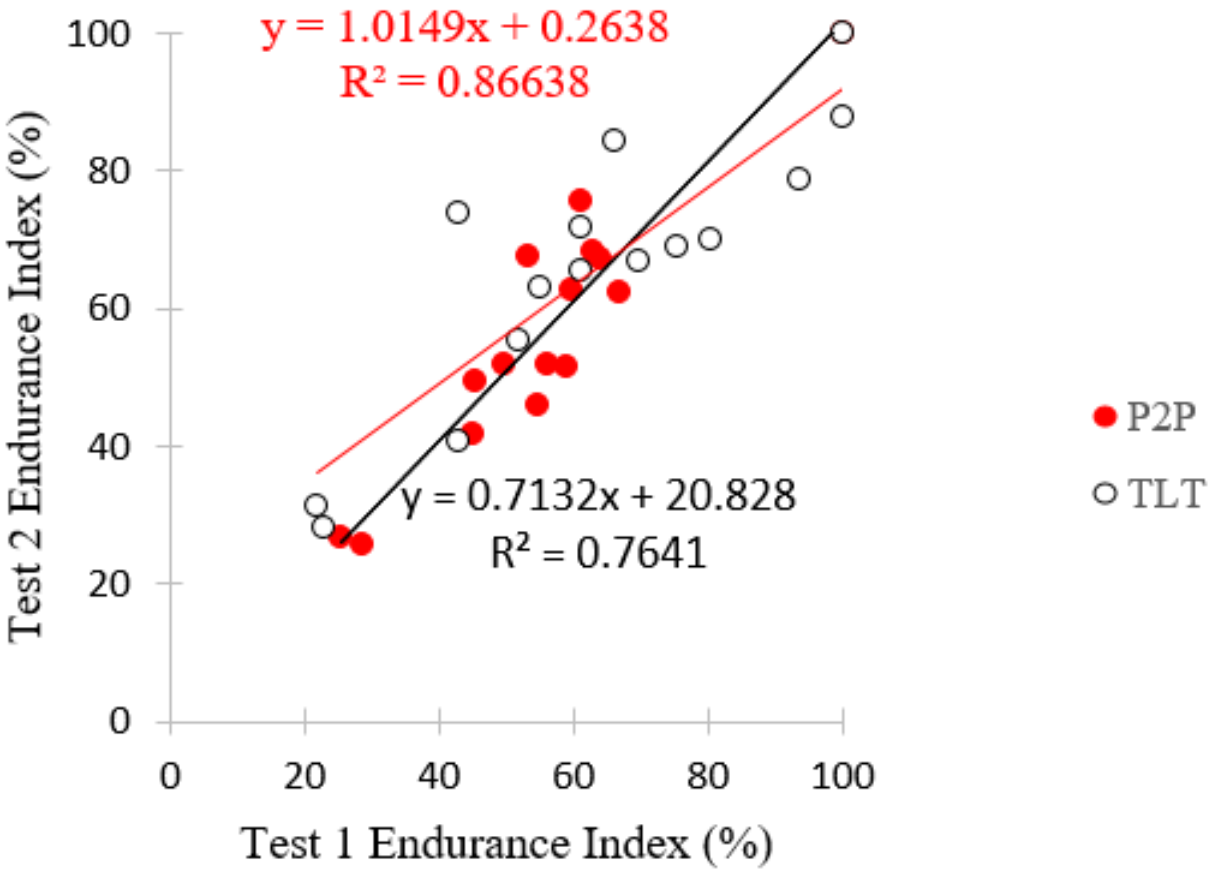
A: Representative Endurance Test
B: Individual Muscle Twitch Acceleration at Start of Test
C: Individual Muscle Twitch Acceleration at End of Test

Figure 3.2



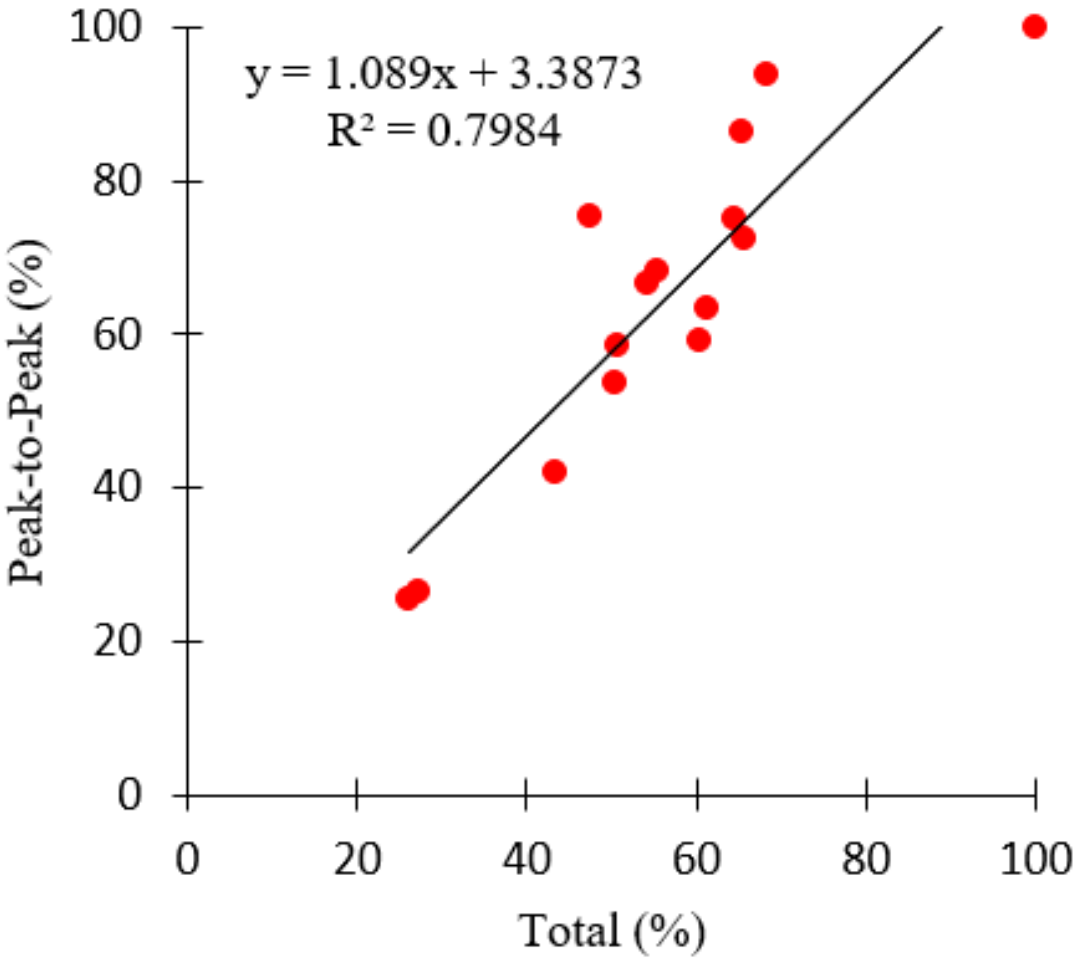
Mean Endurance Index Values

Figure 3.3



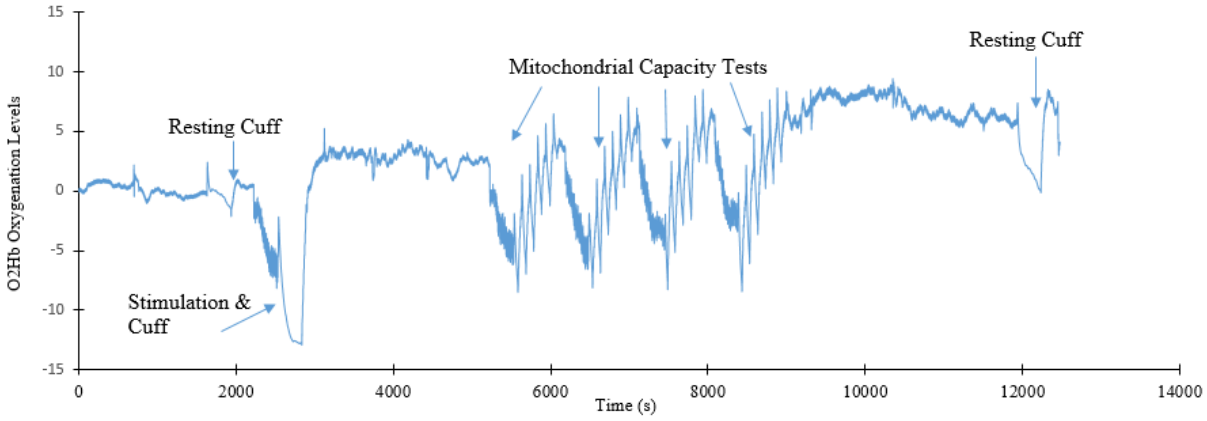
Correlation Between Endurance Index Test 2 and Test 1

Figure 3.4



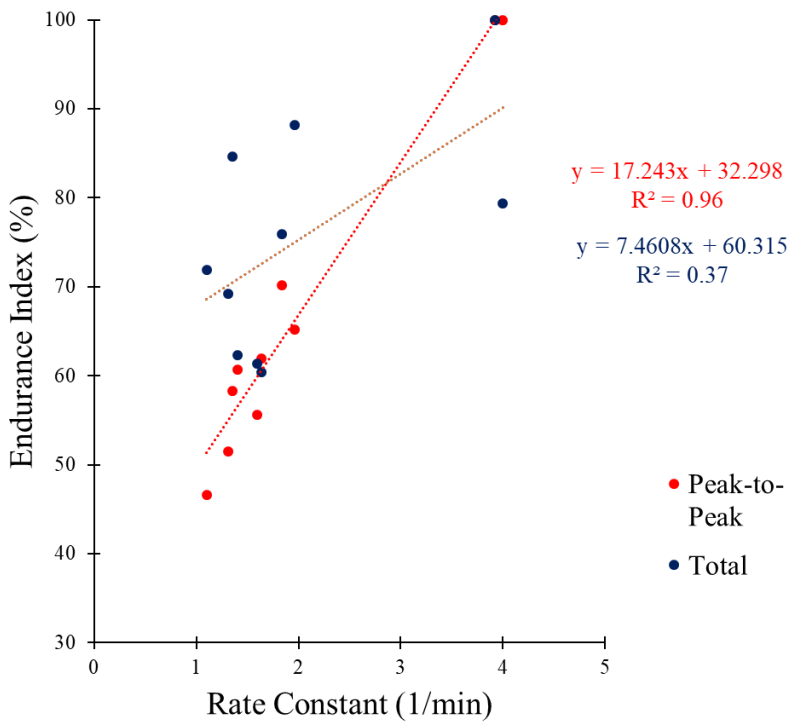
Correlation Between Peak-to-Peak and Total Analysis Program

Figure 3.5



Representative Mitochondrial Capacity Test

Figure 3.6



Correlation Between Endurance Index and Mitochondrial Capacity

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CHAPTER 4

SUMMARY AND CONCLUSION

Electrical twitch mechanomyography is still a fairly new technology and our findings support the utility of the measure. The main findings of our study was that the P2P analysis was superior to the TLT analysis program, due to the stronger correlation with mitochondrial oxidative values. The secondary finding was that the P2P method was also less variable compared to the TLT method. Although other studies in our lab have successfully used the P2P method of analysis, we tested whether a different approach, using more data points, could have an advantage. We believed using more data could potentially give us more valid results with the assumption that there would be less outliers and a decreased signal to noise ratio. Thus, the total analysis method would give us results more consistent with metabolic changes that occur in the muscle. Changes in specific metabolites have not been measured, yet would provide further validation of the appropriate method.

The hamstring muscles were selected based on our desire to find a fatigable, easily accessible muscle. Although the hamstring muscles are not as heavily researched as other primary movers, they provide a unique perspective on the overall muscle function of the individual. Findings in the hamstring have been similar to other muscles studied in our lab. Both the forearm muscles, trapezius, and quadriceps have been studied and have demonstrated reproducible fatigability [3, 15] .

Potential limitations of our study include the distribution of subjects without varying mitochondrial capacity values. It was difficult to recruit individuals on a continuum of mitochondrial capacity values. Although there were individuals much higher than the expected

range, our results were unaffected by the overall correlation values. Another potential limitation was the use of that this test was only done in a healthy population. Although this test would benefit to clinical populations, we need to include that population in order to further the utility. Another potential limitation of the study was the specific focus on two analysis programs. There may be other methods that provide similar or better endurance index values, more consistent with metabolic changes.

A key implication of these findings is that the conclusions drawn from EI need to be very specific and include the analysis method used. P2P and TLT both provide reliable results, yet the P2P analysis method had a stronger correlation with mitochondrial capacity values. These findings support the use of the P2P analysis protocol in clinical populations.

In conclusion, electrical twitch mechanomyography provides an adequate, muscle-specific, noninvasive measurement for obtaining skeletal muscle endurance. This innovative technology may be used over a wide range of performance levels across functional abilities. The peak-to-peak analysis program has results consistent with oxidative metabolism measures and should be used. Further evaluation with clinical populations and different muscles should be explored as well as studies directly linking the changes in metabolites to these protocols.

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