Reproducibility and The Effects of Exercise on The Endurance Index

by

Michael Dean Smith, JR.

Bachelor of Science
University of South Carolina, 2017

Submitted in Partial Fulfillment of the Requirements
For the Degree of Master of Science in
Exercise Science
The Norman J. Arnold School of Public Health
University of South Carolina
2019

Accepted by:
Raymond Thompson, Director of Thesis
Troy Herter, Reader
Jay Patel, Reader

Cheryl L. Addy, Vice Provost and Dean of the Graduate School
ABSTRACT

Background: The Endurance Index (EI%) is a method to objectively assess a muscle’s ability to resist fatigue. The purpose of these studies was to determine the reproducibility of the EI% and determine the influence of muscular fatigue on the EI%.

Methods: Study 1: sixteen apparently healthy participants (18-30yrs) completed three nonconsecutive visits each with 3 bouts of 5-minute electrical muscle stimulations (EMS) and accelerometer-based mechanomyography (aMMG) of the vastus lateralis at 4 Hz and low amperage (25-35mA) on the hamstrings of the dominant leg; peak torque was measured at each visit. Study 2: fifteen apparently healthy participants (ages 18-30yrs) completed 3 bouts of 5-minute bouts of EMS and aMMG of the vastus lateralis at 4 Hz and low amperage (25-35mA) on both limbs to determine the effect of exercise on the EI%. Next the participants performed 50 maximal voluntary contractions (MVCs) with one limb and the contralateral limb serving as a control; peak torque was assessed for each limb. EMS and aMMG were repeated immediately following the second peak torque assessment. MVCs (60 degrees/sec) were collected via isokinetic dynamometry. The EI was calculated from aMMG data as a percent change from peak acceleration for each period. One-, Two-, and Three-way Repeated Measures ANOVA’s assessed main effects and interactions (p≤0.05) and Intraclass Correlations were used to determine reproducibility.

Results: Study 1: The EI% was not different between Trials 1, 2 and 3 (p>0.05), however the EI% changed significantly over time.
Post hoc analysis revealed D0 was different from D1, D2, and D3 (100±0.0, 61.3±3.1, 61.8±3.1, 60.3±3.1; p<0.001) but no other differences were found. Moderate reliability coefficients were computed for D1 (.445), D2 (.410), and D3 (.534) across trials for the EI% while strong reliability coefficients were computed for the strength data (0.96). Study 2: A significant Treatment by Trial interaction for peak torque (p=0.0003) followed by post hoc analysis revealed no difference in peak torque between control and exercise limb at baseline. Peak torque was significantly lower only in the exercise limb after 50 MVCs (p<0.0001) (Ex Pre 109.8±9.2, Ex Post 67.6±4.4, Con pre 105.9±11.5, Con Post 104.7±10.7 Nm). Treatment by Trial interaction followed by post-hoc analysis revealed that the EI% was significantly lower in the exercise limb after 50. **Conclusion:** The EI% appears to be moderately reliable, however it was sufficiently sensitive to detect local muscular fatigue indicating that the EI% is a valid measure of skeletal muscle endurance.
TABLE OF CONTENTS

Abstract .............................................................................................................................. iii
List of Tables ..................................................................................................................... vi
List of Figures ................................................................................................................... vii
Chapter 1: Introduction ...............................................................................................1
Chapter 2: Literature Review .......................................................................................4
Chapter 3: Methods ......................................................................................................14
Chapter 4: Results ......................................................................................................20
Chapter 5: Discussion ...............................................................................................34
References ..................................................................................................................40
LIST OF TABLES

Table 4.1 Study 1: Participant Demographics .................................................................23
Table 4.2 Study 2: EI% Reliability Analyses .................................................................26
Table 4.3 Study 2: Participant Demographics .................................................................28
LIST OF FIGURES

Figure 2.1 Endurance Index of the rectus femoris and the biceps femoris over three bouts of EMS ................................................................. 12

Figure 2.2 Peak extension torque and peak flexion at 60°/sec and 120°/sec .......... 13

Figure 2.3 Extension/Flexion of peak torque ratio ........................................ 13

Figure 4.1 Hamstring Strength Data ................................................................ 24

Figure 4.2 Hamstring EI% Over Time ............................................................... 25

Figure 4.3 Correlation of Hamstring EI% ......................................................... 27

Figure 4.4 Representative Data of Fatigue Protocol at 60 deg/sec .................. 29

Figure 4.5 Vastus Control and Exercise Limb Pre and Post Test .................... 30

Figure 4.6 Vastus EI% for Control Leg ............................................................. 31

Figure 4.7 Vastus EI% for Test Leg ................................................................. 31

Figure 4.8 Vastus Treatment by Trial Pre and Post Exercise ......................... 32

Figure 4.9 Vastus Trial by Time Pre and Post Exercise ................................. 33
CHAPTER 1

INTRODUCTION

Anterior Cruciate Ligament (ACL) injuries lead to a loss of playtime, increased health care expense, and an increased risk of osteoarthritis. In female athletes, 70% of ACL injuries are from non-contact, which indicates that it is a modifiable risk.\textsuperscript{1} The quadriceps and hamstring muscle groups are prime movers of the tibia and stabilizers of the knee joint\textsuperscript{42}. Evidence suggests that the strength between the quadriceps and hamstring muscles is a key factor for injury prevention with normal strength ratios between 0.50 to 0.80, with approximately 0.60 being ideal.\textsuperscript{23} When the hamstrings are significantly weaker than the quadriceps, the stress placed on the ACL can be increased during dynamic movement which can increase the risk of injury due to anterior translation of the tibia.\textsuperscript{3} Muscular fatigue appears to be a contributing factor in non-contact ACL injuries due to a reduction in force by the stabilizing muscles. Developing a screening tool to identify athletes at risk of ACL injury that can also be used as a return to play tool may reduce the number of non-contact ACL injuries and re-injuries. The Endurance Index (EI) is a tool to assess fatigue resistance of a muscle. However, the EI has not been validated or independently investigated for reproducibility. Therefore I assessed the strength and endurance of the quadriceps and the hamstring muscles and tested a model for assessing potential risk and return to play standards.
Aim 1. To determine the reproducibility of the Hamstring protocol for the Endurance Index.

**Rationale:** To determine the reproducibility of the Endurance Index, Accelerometer-based mechanomyography (aMMg) was used to measure the amplitude of the biceps femoris and the vastus lateralis movement. The Endurance Index (EI) measures the ability of a muscle to maintain muscular contractions as well as a series of contractions, which can be affected by the specificity of training, pattern of use/lifestyle, and injury status. In order for the EI to be a useful technique demonstrating that the results of the EI are reproducible for the same muscle groups and of different bouts within participants will validate the measuring technique. The Biodex will be used for the strength testing and correlation analysis for the EI.

**Hypothesis 1A:** Measurement of maximal muscle force of the Hamstring muscle group will be reproducible.

**Hypothesis 1B:** The Endurance Index for the Hamstring muscle group will be reproducible.

Aim 2. To determine whether a single session of strenuous exercise affects the Endurance Index.

**Rationale:** The Endurance Index is a measure of a muscle’s ability to maintain contractile activity. An exercised muscle may draw from its ATP stores faster than it replenishes ATP and be unable to maintain basal contractile activity. Localized muscle fatigue attenuates muscle force production and the rate of shortening.\(^4\).
Therefore, an exercised muscle may show reduced contractile activity, however no studies report the effect of prior muscle contractions on the EI%.

Fatigued skeletal muscle results in loss of force production and decreased shortening velocity of the muscle due to lack of ATP, which can be seen in the EI%. Previous studies have shown that oxygen supply is not inhibited by electrical muscle stimulation and is not a factor for fatigue and the EI% has been seen to decrease with time over bouts of electrical muscle stimulation.\(^5\) aMMG does not assess force or the shortening of the velocity of skeletal muscle, but aMMG does measure the acceleration of movement due to the contractile activity of the contracted muscle, which is likely due to the properties of fatigue. The acceleration (g) of the muscle is assessed by aMMG and the EI% expresses the change in g during a specific stimulation protocol.

To determine the effect of exercise on the EI%, the EI will be assessed immediately before and five minutes after a single session of exercise performed by the quadriceps muscles. The EI for the Vastus Lateralis will be assessed for both dominant and non-dominant limbs. The contralateral limb will not exercise and serve as a control. aMMG and the EI should be attenuated following a strenuous fatiguing bout of exercise.

**Hypothesis 2A:** A single session of strenuous exercise will reduce maximal voluntary skeletal muscle force production by the quadriceps muscle group.

**Hypothesis 2B:** A single session of strenuous exercise will induce a greater decline in the Endurance Index compared to baseline in the quadriceps muscle group than the control.
CHAPTER 2

LITERATURE REVIEW

Muscle Function and Activation

Skeletal muscle is necessary for the production of force, which results in movement of the body. The anatomy and physiology of how skeletal muscle works is essential for the study of muscular endurance and a muscles’ ability to resist fatigue. Likewise, understanding mechanisms of fatigue, a muscle’s response to stimuli is essential when interpreting results from previous studies and this study.

Skeletal muscle generates force and movement when the functional units (sarcomeres) shorten; this is referred to as muscular contraction. Sarcomeres are made up of myofibers and each myofiber contains myofibrils that are connected in series. Multiple muscle fibers make up a muscle fascicle, and multiple fascicles make up skeletal muscle as a whole unit, with connective tissue between each layer.

Myofibers house the sarcomeres, which are the functional units of skeletal muscles. The Sarcoplasmic Reticulum (SR) intergraded throughout the muscle as a whole to release and uptake calcium via the Sarco/endoplasmic reticulum calcium ATPase (SERCA). Mitochondria are within the muscle supplying ATP for cross-bridge cycling and for the sodium and potassium pump to restore resting membrane potential.
The sarcolemma surrounds the muscle and is the site for neuromuscular junctions and sodium voltage gated channels where Action Potentials (AP) are given and where AP will propagate to cause contraction muscle resulting in a new AP in the myofiber. This AP propagates down the membrane to the transverse tubular system of the sarcolemma, releasing Calcium.\textsuperscript{6,8} The Calcium ions bind to Troponin C that allows for the formation of cross-bridges between myosin and actin. Now cross-bride cycling can occur producing force and muscle shortening. Sarco/endoplasmic reticulum calcium ATPase (SERCA) results in the relaxation of the muscle.\textsuperscript{6,8,9}

Electrical stimulation initiates the gated ion channels to open and cause muscular contraction through excitation coupling (EC coupling), mimicking an AP. Short, low levels of external electrical stimulation can cause a single muscular twitch. Continued or prolonged stimulation with adequate frequency can lead to tetanus, which is the continued/maintained muscular contraction. For involuntary muscular contractions due to electrical stimulation, the AP is due to depolarization of the sarcolemma resulting in muscular contraction. A single AP results in a single muscular twitch, and if the frequency of AP is higher, tetany can be seen which the muscle is continuously contracting. Electrical stimulation bypasses voluntary muscle contractions and does not require the lower motor neuron for contraction to occur.

Cross-bridge cycling is adenosine triphosphate (ATP) and Calcium dependent. ATP is critical for the continuation of cross-bride cycling (muscular contraction) and maintaining membrane potentials.
ATP allows for the resetting of the myosin head after the power stroke and an
interruption in the availability of ATP would result in the muscle to not be able to
contract.\textsuperscript{6} Interruption in the ATP supply to the myosin heads can lead to less cross bridge
cycling and force production. ATP is also critical for restoring homeostasis in the resting
membrane potentials by powering the sodium and potassium pumps. ATP is also
important for the reuptake of Calcium so that muscle can relax. Interruption in the supply
of ATP in any of these systems can inhibit muscular contraction and force.

**Fatigue**

Muscular fatigue can be defined as having reduced contractile ability.\textsuperscript{10} Muscular
force declines when the demand for ATP exceeds the supply for muscular fatigue.\textsuperscript{11}
Likewise; interruption of ATP availability for the sodium and potassium pumps can
disrupt resting membrane potential and interfere with the propagation of action potentials
and muscle excitability, resulting in reduced muscle activation and force, which is
referred to as muscular fatigue/peripheral fatigue.\textsuperscript{11–13} Decreased motor drive of the
central nervous system CNS can result in decreased muscle activation and is referred to
as CNS fatigue or central fatigue.\textsuperscript{11}

CNS fatigue also refers to an inability to recruit all of the muscle’s motor units
voluntarily or the impulses are not being fired at the optimal frequency for the required
force.\textsuperscript{11,13} While both central and peripheral fatigue can result in reduction in force,
Central fatigue is not as well understood as peripheral fatigue, the impact of exercise on
the contractility of local muscles is well known and documented.\textsuperscript{14}
Furthermore, due to electrical muscle stimulation causing involuntary muscular contraction, CNS fatigue as a confounder is controlled for and is likely to affect outcomes in this study. Testing for CNS fatigue is difficult and the underlying mechanisms driving CNS fatigue are not well understood.\(^\text{15}\)

**Effects of Fatigue**

Muscular fatigue negatively impacts sports performance, stabilization of joints, proprioception of limb position, and may lead to increased risk of injury.\(^\text{16–18}\) It has been shown that after sports participation, when muscle is likely to be fatigued, that there is an increase in anterior, posterior, and total anterior-posterior laxity, which leads to a increase in anterior tibial translation.\(^\text{19}\) Muscular fatigue has been shown to alter knee joint position sense after a decrease in muscular strength.\(^\text{20,21}\) Following a soccer exercise protocol, it has been shown that there is a significant difference in quadriceps and hamstring strength difference when compared to prior values.\(^\text{22}\) These changes in strength ratio between the quadriceps and hamstrings, decreased knee stability when fatigued, and a change in proprioception may lead to increased ACL injuries.\(^\text{23}\)

It has also been shown that the change in force production ratio between the quadriceps and hamstrings when fatigued is greater among female athletes compared to male athletes which may contribute to the increased rate of ACL injuries seen among females.\(^\text{19,24}\) The ACL restrains the forward translation of the tibia in relation to the femur and when muscular fatigue occurs in the quadriceps and hamstrings, more stress is put on the ACL which often results in injury.\(^\text{25}\) Following injury performance in sports is often worse when compared to pre injury and total force production and strength is decreased.
Fatigued muscle can lead to a greater risk of injury along with previous injury status. Being able to measure fatigue by loss of force production is important when looking at return to play following injury, however being able to assess resistance to fatigue prior to injury may be able to help guide training protocols to help reduce risks of injury.

**Muscular Adaptation to Training**

With training muscle undergoes mechanical and oxidative stress, leading to adaptation in muscle fibrils, motor units, mitochondria, capillary density, and oxidative enzyme activity.\(^{13}\) Time frame for strength gains from neural adaptations are six to eight weeks to have increased strength independent from hypertrophy.\(^{13}\) Further increases to strength are from muscular hypertrophy, increasing the cross-sectional area of muscle from increased protein synthesis.\(^{13}\) The oxidative stress at the same absolute workload has been shown to be less in trained subjects when compared to untrained subjects.

During bouts of high intensity exercise, untrained subjects had higher levels of venous plasma ammonia, which has been suggested to play a role in fatigue, when compared to trained individuals.\(^{26,27}\) It appears that with training, skeletal muscle becomes more resistant to fatigue, which may decrease the risk of injury by having greater knee stability and less change in stance and biomechanics during activity.\(^{28,29}\) Fiber composition has been shown to affect fatigue where slow-twitch muscle fibers showed no or little signs of fatigue when compared to fast twitch muscle fibers when completing isometric contractions of the leg.\(^{30}\) Specificity of training will drive the physiological response to exercise.
With endurance training there is a volume overload to the muscle and with resistance training there is an intensity overload to the muscle. Endurance training and resistance training both will elicit muscle hypertrophy, however resistance training will have greater hypertrophy. Exercise training is believed to be associated with delayed muscular fatigue.

**Electrical Muscle Stimulation**

Electrical Muscle Stimulation (EMS) uses a external electrical current to skeletal muscles fibers through electrodes, causing involuntary induction of EC Coupling resulting in muscular contraction.EMS is able to induce an involuntary contraction with consistent stimuli through the delivery of an AP to the muscle, that allows the study of muscle twitch to be studied when combined with aMMG.

EMS has been used in clinical populations to induce muscular contractions in paralyzed muscle because of its safety and ability to contract muscle independently of neural input. EMS is able to externally depolarize the resting membrane of skeletal muscle by creating an AP resulting in consistent muscular contractions through EC coupling and the amount of muscle activated is dependent upon the stimulus of the frequency and the amperage, which is constant with EMS. The independent nature of EMS allows for the objective study of muscular contractions free from neural factors.

The frequency affects the amount of calcium released from a stimulated muscle while the amperage affects the quantity of myofibers stimulated, all of which affect the amount of force produced.
aMMG provides a means of acquiring reproducible data of twitch acceleration and amplitude of muscular contractions that correlates with muscle contractile strength.\textsuperscript{32} Using aMMG it may be possible to access muscular endurance and time to fatigue by the change in the amplitude of the muscular twitch.

**Twitch Interpolation / Force Measurement**

Twitch interpolation has been used to access central and peripheral (muscular) fatigue as there is a decline in the amplitude of the muscular twitch with the same stimuli over time.\textsuperscript{34} It has been shown that during isometric elbow contractions the myoelectric amplitude are correlated with contraction level and endurance time during different exercises.\textsuperscript{35}

It has also been reported that in light dynamic contractions there is a linear relationship between EMG, force, and oxygen uptake.\textsuperscript{35} The research suggests that measuring the amplitude of muscular contractions it is possible to correlate amplitude to endurance of the muscle and the force production of a muscle.

**Pilot Data**

Approximately 30\% of all anterior cruciate ligament (ACL) injuries in female athletes are due to physical contact. The majority of ACL injuries does not involve contact and occur late in games when muscles are fatigued. Muscular fatigue reduces force and joint stability, which leads to greater translational movement and increased risk of injury.
To determine the Endurance Index (EI) for the rectus femoris (RF, quadriceps) and the biceps femoris (BF, hamstrings), 19 college-aged females (20.6±1.2 yrs) completed accelerometer-based mechanomyography (aMMG) and strength testing. aMMG requires electrical muscle stimulation (EMS) at low frequency (4 Hz) and amperage (25 mA) for three 5-min periods and contractions were recorded using an accelerometer. The EI was calculated from aMMG data as the percent change from peak acceleration for each period and muscle. An isokinetic dynamometer was used to assess strength (60 deg/sec) of the quadriceps and hamstring muscles. Data were analyzed using 2-way repeated measures ANOVA with preplanned comparisons.

A significant interaction between muscle and time (p=0.013) indicates that the EI for the BF (D0 100%, D1 61.4±18.3%, D2 54.6±20.2%, D3 52.8±21.9%) declined significantly more over the periods of stimulation than the RF (T0 100%, D1 76.7±11.6%, D2 70.2±14.3%, D3 70.5±28.6%) (Figure 2.1). The quadriceps muscle groups were 2.2-fold stronger than the hamstrings (90.9±14.9 vs 42.2±10.2 Nm; p<0.001) though there was no relationship between strength and EI (Figure 2.2). The shortening velocity diminishes with the onset of muscular fatigue and may help explain the decline in acceleration in the EI as shown in Figure 2.2. These data suggest that the BF may fatigue before the RF during athletic events in healthy females, and the resistance to fatigue is unrelated to muscle strength.

Figure 2.3 shows that the H/Q strength ratio is below desirable, 50-60%, at both velocities. Subjects with poor H/Q strength ratios are at greater risk of non-contact ACL injury and the lower EI of the hamstring relative the quadriceps may increase the risk of ACL injury.
Figure 2.1: Endurance Index of the rectus femoris and the biceps femoris over three bouts of EMS. Standard error bars are displayed with significance marked between D0 and D1 and D0 and D2.
Figure 2.2: **Peak extension torque and peak flexion at 60°/sec and 120°/sec.** Peak torque for extension is higher at both velocities with higher values at 60 deg/sec compared to 120 deg/sec are shown with standard error bars.

Figure 2.3: **Extension/Flexion of peak torque ratio.** Lower ratio of extension to flexion are shown with lower velocities than at higher velocities are displayed with standard error bars.
CHAPTER 3

METHODS

The long-term goal of this line of investigation was to determine the relationship between muscle fatigue and the risk of Anterior Cruciate Ligament (ACL) injury. However, this study specifically sought to determine the validity and reproducibility of the EI% test, a potential tool to assess a muscle’s resistance to fatigue. Validation of the EI% was done by inducing local muscular fatigue or by inducing mechanisms to resist local muscular fatigue. The Biodex was used to access voluntary peak force but aMMG was used to assess the EI% and is involuntary muscle contraction in order to minimize the impact of central fatigue on the EI%.

Study Design

Study A. Reproducibility of the Endurance Index of the Biceps Femoris

For the EI% to be a useful measure of muscular endurance it must be shown to be a valid and reproducible measure. The EI% consists of the percent decline in EMS induced acceleration over 3 consecutive 5-min stimulation periods. The assessment was made on 3 nonconsecutive days within a 15-day period. Low frequency (4 Hz) EMS induced muscle twitch contractions in the rectus and biceps femoris over a period of 3 bouts that are each 5 minutes long. This longitudinal study yielded evidence to determine if the EMS protocol gives reproducible results within subjects.
The EI% for the Hamstring muscles group was accessed having participants lay on the floor supine with their feet supported and their knees at a 90-degree angle, the Biceps Femoris was used for assessment of the hamstring muscle group. At each visit the participant performed 5 maximal voluntary concentric contractions each for the quadriceps and the hamstring muscles. Peak torque was assessed by an isokinetic dynamometer and reported as the highest value for each muscle at each visit.

**Study B. Effect of a single session of strenuous exercise on the EI%.**

The EI% is a measure of a muscles ability to maintain contractile activity. EI% declines across the stimulation periods relative to baseline suggesting that the stimulated muscle fibers experience some degree of fatigue. Fatigue attenuates skeletal muscle force production and rate of shortening. Therefore, an exercised muscle may show reduced contractile activity, however no studies report the effect of prior muscle contractions on the EI%. To determine the effect of exercise on the EI%, the EI% was assessed immediately before and immediately after a single session of exercise performed by the quadriceps muscles. A Pretest-Posttest design was employed to assess the influence of a single session of exercise on EI%. The participants’ EI% was assessed on Vastus Lateralis of both limbs before and immediately after a brief session of strenuous exercise. The contralateral limb was not exercise and served as a Control. Pilot data indicates that there is no difference in the EI between the Dominant and non-Dominant leg.
Participants

This study recruited apparently healthy men and women between 18 and 30 years of age who self-reported no previous ACL injury. Participants were screened further for contraindications to exercise prior to inclusion in the study including orthopedic, cardiovascular and neurological diseases and disorders. Participants also self reported activity levels.

Procedures

Participants came in before base line testing to receive informed consent, introduced participants to the Biodex, and received baseline participant data (weight, height, Biodex settings). Participants completed the EI and maximum force production protocol for the Vastus Lateralis prior to and after the bout of exercise. The EI was determined using aMMG of the Vastus Lateralis through three five-minute bouts of EMS. Maximum concentric force production was determined through two sets of five voluntary maximum knee extensions using the Biodex at 60 degrees/second separated by approximately the same time as the fatiguing protocol. The bout of exercise to induce local muscle fatigue was the Thorstensson protocol.

Protocol to induce local muscle fatigue

The Thorstensson testing protocol consists of 50 maximal voluntary contractions of the knee extensors using an isokinetic dynamometer at 60 degrees/second. This protocol can access maximal muscular force production pre and post fatigued muscle.
Maximal force production was assessed before and after the fatiguing protocol by five maximal voluntary muscular contractions using the isokinetic dynamometer at 60 degrees/second.

**Accelerometer-based mechanomyography (aMMG)**

aMMG involves electrically stimulating a skeletal muscle and measuring the subsequent muscle contractions with a triaxial accelerometer (MetaMotionC, Mbientlab). The data collected using the accelerometer was compiled into an excel file and presented on the x,y, and z axis as an numerical value change from baseline.

The EI% was determined on the selected leg of each participant using data collected from accelerometry data. Electrical Muscle Stimulation (EMS) was utilized to deliver a low frequency, low amperage current to the Vastus Lateralis using two-inch surface electrodes. A Mettler Electronics Sys-Stim 294 electrical stimulator was used to deliver the stimulus to the desired muscles. The length of each muscle was determined on each participant through palpation of the muscles and the center of each muscle was determined with the location of electrodes and distance between recorded for consistency. Twenty-five to thirty-five milli Amps of current was delivered for three five-minute bouts via a biphasic cycle at 4 Hz. Specific amperage was determined for each individual and was maintained for each trial. Body composition, tolerance, and a visible twitch factored into the set value. This study will use controls, pre, and post exercise data to observe the difference in endurance index between participants after an acute bout of exercise and observe the differences after an acute bout of exercise after resistance training.
Quadriceps Strength Assessment

The peak force and the angle of peak force was collected for the isometric data collected on the Biodex Isokinetic (System 3) dynamometer. Data for maximal force production (power) of the Quadriceps and Hamstring muscles was collected using a Biodex Isokinetic (System 3) dynamometer. Maximal force was considered the peak torque from five trials of leg extensions and flexion at 60 degrees per second through 90-degrees of motion.

Endurance Index

The triaxial acceleration data collected via aMMG was analyzed in Excel. The root mean square (RMS) of the triaxial acceleration was calculated for each measurement (200 Hz). The amplitude of each electrically stimulated contraction cycle was determined from the RMS data. The peak amplitude during the first 5-min contraction period served as baseline (E₀). The average peak RMS amplitude of last 3 electrically stimulated contractions from each 5-min stimulation period was used to determine the Endurance Index (EI) (E₁, E₂, E₃).

The percent of the EI sustained across the 5-min contraction periods was calculated as \((E₀ − Ex)/E₀ *100\) in study 1. For study 2 Control Pre-Exercise and Post-Exercise limb Pre-Exercise were calculated as in Study 1. However, Post-Exercise EI was calculated using the Pre-Exercise aMMG data (E₀) for the Control limb and the Exercise limb, respectively.
Statistical Analyses

Descriptive statistics (mean ± standard deviation or standard error of measurement) was used to describe the data. Data were analyzed by one-, two-, or three way repeated measures of analysis of variance (ANOVA) as appropriate. Greenhouse-Geisser correction was applied to where the assumption of Sphericity was violated. Post-hoc with Bonferroni correction was used to determine differences for main effects and interactions. Reliability coefficients were calculated with McDonald’s Omega.

Setting

All measurement and exercise sessions will take place in the Public Health Research Center (PHRC) at the University of South Carolina (921 Assembly Street, Columbia SC 29208)

The University of South Carolina, Columbia Internal Review Board (IRB) Pro00089482, approved this study
CHAPTER 4
RESULTS

Study 1

The reproducibility of the EI% was evaluated for the hamstring muscle groups by testing the biceps femoris (BF). Sixteen participants completed study 1 to determine the reproducibility of the BF endurance index protocol. The population consisted of 8 males and 8 females, 14 were right leg dominant and 2 were left leg dominant all between 18 to 30 years old (Table 4.1). Strong reliability coefficients were computed for knee flexion peak torque (0.96) (Figure 4.1). Two-way repeated measures analysis revealed there were no significant differences across the 3 trials for the EI% (p=0.90) or an interaction between trial and time (p=0.97) (Figure 4.2). One-way repeated measures analysis of peak torque for knee flexion revealed that there were no significant differences between the 3 trials (p=0.16). EI% changed significantly from baseline over the 3 periods of aMMG (p<0.001) (Figure 4.2). Post hoc analysis revealed Delta 0 was different from Delta 1, Delta 2, and Delta 3 (100±0.0, 61.3±3.1,61.8±3.1 60.3±3.1; p<0.001). No other significant differences were found between D0 through D3 across all three trials. Reliability coefficients were calculated for the EI% and weak Intra-class correlations were found between t for D1, D2, and D3 (Figure 4.3), however stronger correlations were found within trials 1, 2, and 3 for D1 through D3 (Table 4.2).
Study 2

To evaluated the affect of a strenuous bout of exercise on the EI% on the quadriceps muscles by testing the Vastus Lateralis. Fifteen participant completed study 2 consisting of 7 males and 8 females, 14 were right leg dominant and 1 was left leg dominant, all between 18 and 30 years old (Table 4.3).

A two-way repeated measures analysis revealed a significant Treatment by Trial interaction for peak torque (p=0.00008) where peak torque was not different between control and exercise limb at baseline (p=0.99). No difference between pre and post-test of control leg was found (p=0.99) and significant difference between pre and post-test of the exercise limb after 50 MVCs (p>0.05) was found. Figure 4.5 demonstrates the fatiguing protocol and the decreased force over time with knee extensions performed at 60 deg/sec. There was also a significant difference between post for control and post for the exercise limb (p>0.001) (Figure 4.5).

Three-way repeated measures analysis revealed there was no 3- way interaction (p=0.41) though 2 two-way interactions were observed. The EI% did not change from Pre-exercise to Post-exercise in the control limb (p=0.601) (Figure 4.6) however; the EI% was significantly lower in the exercise limb after 50 MVCs than in the control limb (p<0.001) (Figure 4.7).
A significant interaction between Treatment and Trial revealed that the EI% was not different between control and exercise limb at baseline (p=0.999). However, there was a significant difference between pre-exercise and post-exercise for the exercise limb (stimulated) (p=0.001) (Figure 4.8). A significant interaction between Trial and Time (p=0.00006) revealed significant differences between Pre and Post Exercise at D0 (p=0.000001) and D1 (p=0.0065) (Figure 4.9). However, D0 was significantly different from D1 (p<0.0001), D2 (p<0.0001), and D3 (p<0.0001) for pre-exercise but not for post exercise (p>0.05).
Table 4.1 Study 1: Participant Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>± StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>75.0</td>
<td>± 21.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.9</td>
<td>± 10.1</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.4</td>
<td>± 5.5</td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Gender (Male/Females)</td>
<td>8/8</td>
<td></td>
</tr>
<tr>
<td>Dominate Leg (Right/Left)</td>
<td>14/2</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.1: Hamstring Strength Data. (A) shows the correlation between trial 1 and 2. (B) shows the correlation between trial 1 and 3. (C) shows the correlation between trial 2 and 3.
Figure 4.2: Hamstring EI% Over Time. EI% is displaced for trial 1 (dashed line), trial 2 (gray line), and trial 3 (black line) with standard error bar and significance marked between D0 and D1. *denotes significance
Table 4.2 EI% Reliability Analyses

<table>
<thead>
<tr>
<th>Time</th>
<th>Average Intraclass correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta 1</td>
<td>0.445</td>
</tr>
<tr>
<td>Delta 2</td>
<td>0.408</td>
</tr>
<tr>
<td>Delta 3</td>
<td>0.534</td>
</tr>
<tr>
<td>Delta 1-3 Trial 1</td>
<td>0.943</td>
</tr>
<tr>
<td>Delta 1-3 Trial 2</td>
<td>0.701</td>
</tr>
<tr>
<td>Delta 1-3 Trial 3</td>
<td>0.916</td>
</tr>
</tbody>
</table>

Average intraclass correlations are displayed for each time point (D1, D2, and D3). Intraclass correlations are also displayed for D1-3 for each trial.
Figure 4.3: Correlation of Hamstring EI%. (A) Correlation T2 D1 and Trial 1 D1. (B) Correlation T3 D1 and T1 D1. (C) Correlation T3 D1 and T2 D1. (D) Correlation T2 D2 and T1 D2. (E) Correlation T3 D2 and T1 D2. (F) Correlation T3 D2 and T2 D2. (G) Correlation T2 D3 and T1 D3. (H) Correlation T3 D3 and T1 D3. (I) Correlation T3 D3 and T2 D3.
Table 4.3 Study 2: Participant Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>± StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>73.6</td>
<td>± 17.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.6</td>
<td>± 9.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0</td>
<td>± 4.1</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Gender (Male/Females)</td>
<td>7/8</td>
<td></td>
</tr>
<tr>
<td>Dominate Leg (Right/Left)</td>
<td>14/1</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4:4 Representative Data of Exercise Protocol at 60 deg/sec. Peak torque throughout the 50 MVCs are represented above showing the decline of max torque during the fatiguing protocol.
Figure 4.5 Vastus Control and Exercise Limb Pre and Post Test. The solid black line represents the peak torque for the control leg pre and post-test. The gray line represents the peak torque for the exercise limb pre and post-test.

*denotes significance between post-test for control and exercise
†denotes significance between pre-test and post-test for exercise
Figure 4.6 Vastus EI% for Control Limb. The EI% for the control limb is represented above as a percent of the Control at D0. The black line and the gray line represent the pre and post exercise over time, respectively.

Figure 4.7 Vastus EI% for Test Leg. The EI% for the test limb is represented above as a percent of the Control at D0. The black line and the gray line represent the pre and post exercise limb over time, respectively.
Figure 4.8: Vastus Treatment by Trial Pre and Post Exercise. The black line represents the control limb pre and post exercise with no significant marked. The gray line represents the exercise limb pre and post exercise with no significant differences between pre for control and exercise and a significant marked.

* denotes significance between post controlled and exercise
† denotes significance between pre and post for exercise
Figure 4.9: Vastus Trial by Time Pre and Post Exercise. Pre exercise limb EI% is represented by the black line over time and the gray line represents the EI% of the exercise limb post exercise. Significance is marked for differences between pre and post and differences between deltas.

* denotes significance between Pre and Post at D0 and D1
! denotes significance between D1, D2, and D3 from D0
CHAPTER 5
DISCUSSION

This study specifically sought to determine the validity and reproducibility of the EI%, a potential tool to assess a muscle’s resistance to fatigue. The strength and endurance of the quadriceps and the hamstrings were investigated in order to test a model for assessing potential risk and return to play standards. The model had not previously been validated through investigation for reproducibility.

The results from Study 1 support the first Aim of the study. In Study 1 maximal muscle force of the Hamstring muscle group were tested on an isokinetic dynamometer and were found to be highly reliable for peak torque; these data support the hypothesis 1A (Figure 4.1). This is consistent in previous reports in the literature that using an isokinetic dynamometer produced reliable measurements of muscular strength.47 Figure 4.2 shows the decrease in the EI% over time for the hamstring muscle group with differences only seen within trials for D0 and D1. D1-D3 shows a consistent pattern with previous reports and pilot data (Figure 4.2).5,32 The drop in the EI% was less than initial pilot data and may be due to the change in muscle position which can affect the oxygen saturation this study was made up of both males and females (Figure 2.1,4.2).50
The results also show that the reproducibility of the EI% in the Hamstring muscles groups showed moderate reliability coefficients were computed for D1 (.445), D2 (.410), and D3 (.534). With lower than expected coefficients it cannot be said at this time the EI% for the hamstring muscles groups is reproducible (Figure 4.3). The data from this study showed weak correlations between trials for D1, D2, and D3 however stronger correlations were found within trials 1, 2, and 3 for D1 through D3 (0.94, 0.70, 0.92) (Table 4.2). This shows that within subjects there is higher reliability for the EI%; however, hypothesis 1B is only partially supported.

The EI% trend is similar to previous reported studies and similar to results from preliminary data and data from Study 2. Previous research has shown the reproducibility of different muscles groups using similar techniques. The reliability coefficients found in this study and for the hamstring muscle group is much lower than that which is reported by other research. However the reproducibility of the hamstring muscle group has not been thoroughly study as other muscle group. Further research with larger samples sizes may compensate for the moderate reproducibility.

Hypothesis 2A was supported by the research showing that a single session of strenuous exercise reduced peak torque in the exercise limb. Peak torque was significantly lower only in the exercise limb after 50 MVCs as seen in Figure 4.4. Figure 4.5 suggest that subjects experienced fatigue following the exercise protocol. The results also show that there was no difference in the exercise limb and the control limb of the individuals at baseline, which is supported by other studies.
Previous studies also report a significant decline in peak torque following an exercise protocol of similar methodology in the exercise limb only. Other studies have measured EMG activity during leg extensions and found the vastus lateralis was the most active of the quadriceps muscles. During a knee extension activity, the vastus lateralis is active and an exercise protocol would fatigue the vastus lateralis along with the other quadriceps muscles.

Following the exercise protocol there was a significant decline in the EI% in the exercise limb when compared to controls supporting hypothesis 2B. The results demonstrate that there is a decrease in the magnitude of acceleration (EI%) in the post testing for both the control and treatment limb however this was not significant in the control leg as seen in Figure 4.6,4.7. The control limb does experience a decrease in the EI% pre and post showing that the EMS did have an effect on the EI% at D0 and D1 but less on an effect at D2 and D3 (Figure 4.6). The EI% decrease at D0, D1, D2 and D3 are 17.8%, 4.73%, 3.29%, and 1.9% respectively for the control limb (Figure 4.6). The exercise limb saw a more drastic decrease in the EI% over all time points which suggest that the changes seen in the EI% are not only due to the EMS protocol but also the exercise protocol is responsible to the decrease in the EI% (Figure 4.7). The EI% decrease at D0, D1, D2, and D3 are 41.87%, 29.27%, 18.74%, and 17.45% respectively for the exercise limb (Figure 4.7). Trail by time analysis shows a greater decline in the EI% of the exercise limb at Delta 0 and 1 and meaningful differences between delta 1, 2, and 3 for the pre-exercised limb.
Low frequency stimulation has been seen to causes local muscular fatigue.\textsuperscript{50} The results from this study see a decline in the EI\% for the control limb indicating that there is an affect of the EMS protocol. However, the EMS protocol does not account for the greater decline in the EI\% for the exercise limb when compared the controls (Figure 4.7). Previous research has shown that muscular twitches recorded after a 60 second maximal voluntary contraction, there was no difference in the contraction time but there is increased time for relaxation by 50\%.\textsuperscript{11} The research has also shown that there is a decrease in the motor-neuron firing rates in exercised muscles, which may help to explain the decrease in the magnitude of acceleration seen in both the control and exercise limb post exercise testing.

The EI\% for the vastus treatment by trial pre and post exercise reveals a very similar trend seen in peak torque output (Figure 4.5,4.8). Pre control limb EI\% and pre exercise limb EI\% had similar values, however there was a significant difference in the pre and post for the exercise limb EI\%; this difference was not seen in the control limb pre and post (Figure 4.8). There was also a meaningful difference in the post-exercise EI\% when compared to post controlled limb (Figure 4.8). This is a new and novel finding that has not been shown before that the EI\% is sensitive enough of a test to measure fatigue. These outcomes are also seen in peak torque data for control and exercise limb pre and post (Figure 4.5). One limitation of this study is that EMG activity was not measured/recorded during the leg extension to ensure that the vastus lateralis was activated. This limitation is mitigated by previous research that has shown during leg extension the vastus lateralis was active.\textsuperscript{51}
It was also found that the vastus lateralis was the most valid individual muscle to measure with EMG due to its linearly relationship with force production with knee extension.\textsuperscript{51}

Figure 4.9 shows the interaction of trial by time with significant differences being seen of D1, D2, and D3 from D0 for pre and post exercise limb. There also appears to be a meaningful difference between D0 and D1 for pre and post for the exercise limb. These data suggest that the most meaningful drop in the EI\% is from D0 and D1, which may be useful when designing future protocols on the quadriceps muscle group.

Study 1 sought to determine the reproducibility of the EI for the hamstring muscle group and found that within trials there is a moderate to moderately strong intraclass correlation. Study 2 sought to determine if the EI was a potentially useful tool to measure muscular fatigue and if the EI was a sensitive enough test to do so through EMS. The EI\% may be a potential tool for accessing risk for non-contact ACL injuries due to its sensitivity of measuring local muscular fatigue and its ability to detect declines in muscle shortening velocity.

Future directions of this line of research are to determine the trainability of muscle against declines in the EI\%, which is a measure of fatigue, through different modes of exercise training. Future studies also seek to collect cross-section data between healthy individuals and patients who have had previous ACL injuries to establish EI\% profiles. Other directions are conducting a longitudinal study looking at the risk of injury and the change in the EI\% to use the EI\% as a screening tool and return to play tool.
Conclusion

The reliability index appears to be reproducible and suggests that within subject measures are more variable than in the torque data for the hamstring muscle group. The EI% appears to be moderately reliable for the quadriceps muscles, however it was sufficiently sensitive to detect local muscular fatigue indicating that the EI% is a valid measure of skeletal muscle endurance.
REFERENCES


45. Hagberg M. Muscular endurance and surface electromyogram in isometric and

46. Doucet, B. M., Lam, A., & Griffin, L. Neuromuscular electrical stimulation for

47. Sparks, J., Carter, J., & Brooks, K. Comparing dominant and non-dominant torque
and work using biodex 3 isokinetic protocol for knee flexors and extensors. 2011.
*International Journal of Exercise Science: Conference Proceedings*

48. McCurdy, K. and Langford, G.. Comparison of unilateral squat strength between the

49. Alkner BJA, Tesch PA, Berg HE. Quadriceps EMG/force relationship in knee

50. Matsunaga T, Shimada Y, Sato K. Muscle fatigue from intermittent stimulation with
low and high frequency electrical pulses. *Archives of Physical Medicine and

51. Miura H, Mccully K, Nioka S, Chance B. Relationship between muscle architectural