MEASURING MUSCLE ACCELERATION USING CONSECUTIVE CORRELATION OF ULTRASOUND IMAGES

by

UMA SANDEEP DIXIT

(Under the Direction of Kevin McCully)

ABSTRACT

Twitch acceleration has been used to measure muscle endurance. **PURPOSE:** To evaluate the use of consecutive correlation to measure muscle acceleration twitch contractions in ultrasound videos. **METHODS:** Thirty-five healthy, able bodied participants were tested on one occasion. Surface electrodes and a triaxial accelerometer were placed over the hamstring muscles. The stimulation occurred for 5 minutes at five Hz. B-mode ultrasound images were recorded analyzed using a developed analysis program consisting of correlational methods. **RESULTS:** Optimization of consecutive correlations favored the 1:3 over the 1:2 sequence (ΔR^2 of 0.05±0.05 versus 0.03±0.01, p =0.001). No relationship between ultrasound endurance index and accelerometer index was found ($R^2 < 0.01$, $p = 0.5$). **CONCLUSION:** We were able to successfully develop and test the parameters of an analysis program, however we could not accept our hypothesis that ultrasound index and accelerometer index were similar.

INDEX WORDS: biomedical imaging, hamstring muscles, electrical stimulation, muscle mechanography, muscle endurance

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Muscle dysfunction can be identified in many diseases and medical conditions (28). In addition, muscle dysfunction can be seen as a result of inactivity and aging. Muscle dysfunction can also influence the progression of the disease or condition, such that the role of muscle dysfunction can play in the progress of type II diabetes (1, 2). It is caused by the interaction of local and systemic factors. Muscle dysfunction can be a direct result of a medical condition, or an indirect result of disuse or inactivity. Quantification of muscle dysfunction is important for identification and treatment.

Muscle endurance is an important characteristic of skeletal muscle, along with muscle strength and muscle motor control. Muscle endurance has been studied for a long period of time (3). Skeletal muscle fatigue is often referred to as "the failure to maintain the required or expected force or power output as a result of exercise" (4, 5). Throughout time, muscle endurance, which is defined as the ability to sustain muscle contractions has also been referred to as muscle fatigue, fatigability, and even characterized by measurements such as 'critical power' (3, 5-7). Other authors have used fatigue as a 'state' or condition of the body and fatigability as the inability to maintain force or exercise, the opposite of endurance (7). The mechanisms behind fatigue are central or peripheral in origin. In this specific study, peripheral fatigue will be observed, which is known as the decline in the force generated as a function of differences at a muscular

level. This difference results from muscular contractile properties, impaired excitability coupling, or intramuscular oxidative metabolism (5).

Various protocols have been developed to evaluate muscle endurance (8). The majority of them specifically use voluntary muscle contraction till exhaustion. However, in a recent study, twitch mechanomyography (ETM) and accelerometer-based mechanomyography (aMMG) based muscle endurance has been developed (9). ETM is a method that utilizes low intensity and force twitch contractions to measure changes in twitch acceleration (10). This approach is used to study clinical populations in regard to calculating endurance index (EI) values (11-21). The values are collected during a nine minute protocol, although recently a five-minute protocol (5 minutes at 5 Hz) has been developed (22). Because of the stimulation currents being submaximal, and the subject being in discomfort is not an issue in protocols that use tetanic contractions with higher current levels. In addition, the twitch contractions that are produced are of much lower force levels than those of tetanic contractions with higher current levels. Although ETM has become a more appreciated technique among the clinical research populations, the method is relatively new and needs further evaluation.

There is a potential limitation to the endurance test. This limitation is that the current method of analyzing muscle contraction is done by measuring contraction induced acceleration on the surface of the body over the contracting muscle with tri-axial accelerometry. This method does not consider the effects of adipose tissue thickness on accelerometer readings. A potential solution to maintaining this approach would be to directly measure endurance through the utilization of B-mode ultrasonography technology. The characteristics of muscle contraction have been measured previously

using ultrasound, based on work by Fukunaga et al (23). These studies typically took a time series of ultrasound images of skeletal muscles to study pennation angles using the echo reflected back from the fascicles (23). Similarly, this method can be used to non-invasively record images of muscle contractions through monitoring technical factors such as frequency, focal zone, and dynamic range (24).

Typically, the analysis of moving ultrasound images has involved using landmarks that are identified in consecutive images (25). The difficulty in using landmarks in rapidly moving images is that it may require other methodologies to monitor the high-speed muscle contractions. Autocorrelation has been used to measure the velocity of steady movement in images. However, studying the movement with twitch contractions is not considered to be steady. The statistical method of autocorrelation is the method of detecting randomness in a dataset. It is often used to determine whether a sample data was generated from a random process (26). Autocorrelation is used in multiple studies to specifically track non-randomness and in this scenario the first lag is the result of interest (26). In order to study the movement of muscle from an ultrasound image, the method of linear correlation or consecutive correlation can be used to evaluate consistency across the frames of the video. Measuring the strength and consistency of the linear relationships across the frames of the image is to the acceleration occurring at the area of the image taken. The utilization of this method opens up for more future work in this field.

Statement of the Problem

Muscle dysfunction is an important characteristic of skeletal muscle. Specifically, muscle endurance is a characteristic that is commonly associated with exercise intolerance and reduced functional ability. A number of different methods have been used to measure muscle endurance, including using twitch mechanomyography and accelerometry. A potential limitation to common adoption of twitch mechanography and accelerometry is the potential confounding influence of varying adipose tissue thickness. This study was designed to evaluate muscle twitch contractions directly using ultrasound with a consecutive correlation algorithm to track movement across the frames of the image taken.

Specific Aims

Specific Aim 1: Develop an analysis program for the ultrasound measurements and determine the operating parameters of the program.

Specific Aim 2: Perform endurance index tests on the hamstring muscles of young healthy men and women. Collect the data from an accelerometer and ultrasound machine.

Outcome and Hypothesis

I. Outcome: To develop a method that can determine the timing of the consecutive images to use within the analysis program as well as determine the sample area to use in the analysis (muscle or adipose tissue).

II. Hypothesis: The ultrasound analysis will significantly correlate to the results of the accelerometry method.

Significance of Study

Findings from this study will increase our ability to obtain muscle endurance values in clinical populations. The findings could reveal the potential limitations to the twitch endurance test when being performed using analysis of movement on the surface of the limb, in particular with subjects with varying amounts of tissue above the contracting muscle. In addition, the use of consecutive correlation may prove to be generally useful in the study of contracting skeletal muscle.

CHAPTER 2

REVIEW OF LITERATURE

Muscle Dysfunction

Muscle dysfunction is identifiable in multiple diseases and medical conditions. It can visibly be observed in groups of inactive or aged people (27). More specifically, the term associated with the muscle function loss due to aging is known as sarcopenia (28). Previous studies have further delved into the potential causes, clinical consequences, and the potential for intervention in regard to sarcopenia (29). The causes behind this are multifactorial, specifically the findings relate to apoptosis and mitochondrial decline. The interventions should be targeted around nutrition and exercise (29). The prevalence of muscle dysfunction has found an increase in the progression of Type 2 diabetes mellitus (T2DM). Through the altered glucose disposal due to low muscle mass and increase in localized inflammation, through inter/intra-muscular adipose tissue accumulation, sarcopenia contributes to the development of T2DM (30). Muscle dysfunction is a characteristic that needs to be quantified to find solutions accurately and efficiently for the medical conditions associated with it.

Muscle Fatigue

Muscle weakness, fatigue, fatigability, task failure, and endurance are all similar but different terms that help characterize muscle. Muscle weakness occurs when full effort does not produce a normal muscle contraction, whereas muscle fatigue is an exercise induced decrease in the ability to produce a force. Task failure is the point at

which a person is not able to maintain the level of force required by a task, and respectively muscle endurance is the ability of a muscle group to exert itself to sustain repeated contractions against resistance over a period of time.

It is recognized that fatigue occurs through multiple mechanisms related to both the contractile apparatus and how it behaves through the depolarization of muscle fiber membrane. The differences are known as peripheral and central fatigue (31). Fatigue through the process at or of distal to the neuromuscular junction is known as peripheral fatigue (31). Peripheral fatigue refers to the processes occurring at (or distal to) the neuromuscular junction and reflects a reduction of sarcolemma excitability, contractile properties, and excitation–contraction coupling (32). Neural drive on the other hand determines when and to what degree the muscle fibers are activated, a process that occurs within the central nervous system, known as central fatigue (31). Central fatigue has also been defined as a negative central influence that exists despite the subject's full motivation as a force generated by voluntary muscular effort that is less than that produced by electrical stimulation (33). Peripheral fatigue arises from the muscle and predominately involves muscle bioenergetics or excitation contraction (34).

Muscle fatigue can be quantified as the decline in the maximal force capacity of the muscle due to an impairment of either the activation signal or the function of the contractile proteins" (3). However, the mechanisms behind the decrease in maximal force are dependent on the details of the tasks being performed. Another method to study muscle fatigue is through observation of mechanisms responsible for the failure of specific tasks (35). According to a study conducted by Enoka,"the criterion for task failure was the inability to sustain target force or position for at least give seconds,

despite strong verbal encouragement to correct the deviation" (35). Task failure is an operational measurement. But most methods require voluntary and high efforts. The functional significance of this study was that the extent to which any single process leads to task failure likely depends on the relative demand placed on each of the processes that contribute to the force exerted by the muscle during a given task (36). Changes in neuromuscular, sensory, and homeostatic systems all contribute to fatigue with exercise (31). Calcium fatigue is seen in single muscle single fiber studies. While it may be seen in exercise in the body, perhaps it is also related to H_2PO_4.

Muscle electrical activity can be measured through a technique called electromyography (EMG), which measures the electric potential connected to muscular fibers' depolarization, which is the trigger signal for fibers' shortening and muscle contraction. This procedure requires the use of electrodes and biopotential amplifiers to record the surface level or intramuscular EMG (37). Measuring muscle contractions, force, or power is equivalent to energy output per unit of time, or "the rate of doing work" (38, 39). Power is calculated as work/time (i.e., torque x angular displacement/time), not simply torque/time (39). The mechanisms of fatigue that are included in the twitch endurance test are peripheral which is evidence that is related to the mitochondrial capacity and muscle endurance (11).

Muscle Endurance

To further evaluate skeletal muscle, we must consider factors such as general movement and force, work over a range of lengths, the ability to sustain movements, motor control, and repair. This study focuses on the maintenance of muscle contractions to further understand the properties of skeletal muscle. Muscle endurance is a significant

aspect of these contractions which is a topic that has been studied for a long period of time (3). Over time, muscle endurance has been defined in many different ways. Mostly it is known as muscle fatigue, fatiguability, and "critical power" (5). "The failure to maintain the required or expected force or power output as a result of exercise" can be used to define skeletal muscle fatigue (5). The mechanisms behind this physiological process are originally either central or peripheral (40-43). More specifically, this study consists of looking more closely at peripheral fatigue. Muscle endurance measured by twitch acceleration has been correlated to mitochondrial capacity, thus the mitochondria keeps proton and phosphorus levels lower, reducing fatigue.

Current Method of Measuring Endurance

There are numerous ways that muscle fatigue can be measured (3). Fatigue is most often measured dynamically through maximal torque, velocity, and power. These measures describe isometric force which are generated voluntarily. More recently, twitch-based muscle endurance tests have been developed to further study the contractions. Twitch mechanomyography is a technique that uses low intensity and force twitch contractions to measure the changes in twitch acceleration (11-15, 44). This approach has been used to further study clinical populations in regard to calculating their EI. The EI values within this study are collected during a five-minute testing procedure for five minutes at five Hertz. The stimulations are submaximal which allows the testing subject to not experience any discomfort. Twitch contractions produced are of a much lower force level than those of tetanic contractions with higher current levels. The ETM procedure has gained much popularity among the clinical researchers within the field,

however, the procedure is still a relatively new method and has many parameters that could be further evaluated.

Limitations to Twitch Acceleration Protocol

While the endurance test may be an easily practical method of further studying muscle capacity, there are a limitation that need to be further questioned. Through the ETM procedure, acceleration of the contracting muscle is measured through the use of surface tri-axial accelerometry. The instrumentation for this procedure consists of an accelerometer that is placed on the surface of the body over the contracting muscle. Though the accelerometer is a non-invasive and wireless device, it does not consider the effects of adipose tissue thickness on the directional readings.

B-Mode Ultrasound Imaging

A potential solution to address these limitations would be to directly measure the endurance through the utilization of B-mode ultrasonography. Many studies have observed the characteristics of muscle contractions using ultrasound (45-48). Fukunaga et al conducted a study that took longitudinal ultrasound images of the vastus lateralis muscle to observe the pennation angles using B-mode ultrasound (20. B-mode is most commonly used to detect tendon and muscle injury. It is also used for locating various tissues. This method can similarly be used to non-invasively explore images of muscle contractions through factors of B-mode. This method of analysis has become a well-established technique for physiological measurements. Within this study, the images were obtained from a radiologist using a high-resolution ultrasound machine. It was found that the three most significant technical factors were frequency, focal zone, and dynamic

range, which were pre-determined prior to the testing sessions (24). Depending on whether a study keeps the voluntary contractions at a controlled and relatively slow rate, versus performing on a muscle at a fixed position, this affects the usage of landmarks in imaging analysis of muscle. Having pre-defined landmarks or markings within consecutive moving ultrasound images has been the most common method of analysis.

Auto Correlation and Cross Correlation

Far more efficient methods have been developed that can measure movement at a faster rate and also does not require a clear identifiable set of landmarks. The issue with using landmarks in rapidly moving images of muscle contraction requires the use of alternate image analysis methods to measure muscle endurance. Studies that focus on studying movement and tracking non-randomness have many a times used a statistical method of autocorrelation. Autocorrelation is used as a tool to detect any randomness within a given dataset (26). A previous study presented a two-frame motion estimation algorithm that estimated displacement within two consecutive frames. The results showed that with single iteration the results are accurate (49). In order to correctly assess a moving ultrasound image, the method of linear or consecutive correlation can be applied. This method has the capacity to measure consistency across the frames of the video. By measuring the strength of the linear relationship across the frames of the video will allow for one to find the muscle acceleration occurring at the area of the image analyzed using the pixel intensity values. According to a study done by Yen (26) the most widely recognized disadvantage of linear correlation using Pearson's coefficient is its computational intensity. Due to the complexities of interpretation, over-sensitivity to pixel noise, and gain variations, there could be possible bias. However, the advantages of

this method are that is condenses the comparison of two two-dimensional images into a single scalar, r (26) . Thus, the disadvantages addressed will try and be controlled as well as avoided in this study making the linear correlation an analysis with much potential for studying muscle.

CHAPTER 3

Introduction

Muscle fatigue is a hallmark of many diseases and conditions (3). Muscle fatigue is an important attribute that can be further studied to better understand muscle. Utilizing twitch acceleration as a measurement of muscle fatigue has expanded our knowledge on muscle (8). A number of methods have been developed to observe and study muscle endurance, all of which involve the exhaustion of voluntary muscle contraction (50). One approach uses twitch acceleration, non-invasively utilizes a low intensity current to generate force twitch contractions which are then stored and calculated as twitch acceleration values that are reflected as endurance values (16). The endurance index value can be used to further clinical populations.

An assumption of the twitch acceleration method of studying muscle endurance is that muscle contractions are faithfully represented by movement of an accelerometer placed on the skin above the muscle. It has not been established that differences in the amount of tissue between the contracting muscle and the accelerometer might influence the relationship between muscle contraction and measured acceleration on the skin over the muscle. To address this assumption, direct assessment of muscle contraction is needed.

The proposed image analysis method consisted of evaluating muscle contractions in a series of B-mode ultrasound images. Previous studies have used B-mode images to evaluate muscle contractions (25). These studied used image analysis programs that

incorporated a landmark feature to monitor and calculate movement. The landmark approach worked well when the movement was relatively slow and steady. Muscle twitch contractions are too fast and have associated vibration that makes the use of landmarks to monitor movement untenable (26). Thus, this study incorporated a statistical tool known as linear correlation to monitor muscle movement. Linear correlation, also referred to as consecutive correlation, can be used to evaluate whether there is consistency across a certain number of frames (26). The faster a muscle contracts the greater the reduction in the correlation of the pixels in the image. The assumption is that when fatigue reduces the speed of a muscle contraction this will appear as an increase in the consecutive correlation.

The aim of this study was to evaluate the use of consecutive correlations to monitor muscle movement, and to determine if direct measurement of muscle movement using ultrasound can address the issue of whether ATT above the muscle of interest influences the acceleration measured at the skin. A consecutive correlation analysis routine was developed and compared to muscle acceleration measured with a tri-axial accelerometer. Measurements were made on the hamstring muscles of young men and women using twitch electrical stimulation to produce muscle contractions.

Methods

Study Participants

Healthy, able bodied participants were recruited for participation in this study (Table 1). This study was approved by the Institutional Review Board at the University of Georgia and all participants were provided informed consent prior to the data collection procedure. . Individuals were excluded from the study if they were unable to lay

comfortably for ten minutes, were female and pregnant, or had fragile veins. Participation was voluntary, and participants were able to stop participation at any given time.

Study Design and Procedures

Data was collected in one test session on one group of participants. Data from the Ultrasound videos were evaluated to determine the optimal analysis parameters for the consecutive correlation analysis. Then the data from the ultrasound images was compared to data utilizing a triaxial accelerometer. All experiments were completed within one day for each participant and tested by the same researcher.

Measurements

Electrical Twitch Mechanomyography (ETM)

The ETM testing protocol consisted of a 5 Hz electrical stimulation which lasted 5 minutes which was a modification of the nine-minute protocol (22). Participants were positioned in a prone position on a padded table. Two sticky electrical stimulation pads were attached to a stimulator (Richmar, Chattanooga, Tennessee, USA) (13) and were placed over the hamstring muscles. One electrode was placed below the gluteal fold while the distal electrode was placed above the popliteal fold. The muscles that were stimulated ranged from levels of 35 mA to 75 mA with pulse durations/intervals of 200 uS. A wireless tri-axial accelerometer (WAX3; Axivity, Newcastle upon Tyne, UK) was utilized to measure the magnitude of the contractions on the skin over the muscle.

B-mode Ultrasonography

To conclude the first session of measurements an ultrasound probe with gel (GE Healthcare UK Ltd., Chalfont, Buckinghamshire, England) was placed gently on

the hamstring muscle between the two electrical stimulation pads and away from the accelerometer to properly get a video image of the muscle contracting (Figure 3.1). Scanning depth was set to 4 cm with an apparent depth resolution of 80 μm/pixel. Adipose tissue thickness was measured at the starting in centimeters. One video was collected per subject in B-mode which contained 177 frames. The video clip had a duration of 4.2 seconds and a frame rate of 42.14 frames per minute. The video clip dimensions were 532 x 434 pixels.

Program and Statistical Analysis

Data analysis included the development and utilization of the written MATLAB program version 2019a (Mathworks, Natick, MA). The ultrasound images that were collected were computationally correlated using a MATLAB function to first determine coordinate points that described the area of the video frame being analyzed. After the crop dimensions were determined, the analysis was performed either with a 1:2 or 1:3 sequence for the image time separation. This corresponds to a time between frames of 23.7 and 47.5 ms, respectively. From these two steps, a 2-D correlation coefficient "R" was generated and stored within an array (Figure 3.2).

Muscle acceleration values from the accelerometer (WAX3; Axivity, Newcastle upon Tyne, UK) were transmitted via Bluetooth as CSV file to a computer. The muscles were being stimulated using levels that ranged from 35 mA to 80 mA with pulse durations/intervals of 200 μs/50 μs. Microsoft Excel was utilized to calculate a magnitude vector of the data collected in the X, Y, and Z directions. Excel was used to then find the highest acceleration magnitude vector within the first 60 seconds of stimulation as well as determine the average acceleration magnitude vector within the last 20 seconds (Figure 3.4). These values were used to determine a ratio to measure the endurance index (16).

Statistical Analysis

All data are presented as means and standard deviations. Comparisons were made with T tests and correlations. Significance was accepted with $p < 0.05$.

Results

Figure 3.1 shows a single frame from the collected ultrasound clip that was used in the analysis program. The flow of the code is mapped out in Figure 3.2, where each individual box represents a step or iteration within the script.

The duration of the endurance test in which B-mode ultrasonography was used on a single participant's hamstring was 4.2 seconds long. These R^2 values are generated from the previously developed analysis program and then plotted over the length of the array of the recorded clip. Figure 3.3a shows the plotted R^2 values over the entirety of the recording session. Figure 3.3b shows only 1 second of movement of the recorded clip. The endurance test data which involved electrical twitch mechanomyography was collected using a tri-axial accelerometer. This data can be seen in Figure 3.4a where the length of the test lasted approximately 5 minutes long. Figure 3.4b is a scaled plot of the previous that show the contractions for 1 second during the 5 minutes.

The first parameter being tested in the analysis was the sequence at which correlation occurs. Two image time separation sequences were computed and compared, 1:2 and 1:3. Figure 5 clearly shows the change in R^2 values per participant for both sequences. Optimization of consecutive correlations favored the 1:3 over the 1:2 sequence (ΔR^2 of 0.05±0.05 versus 0.03±0.01, $p = 0.001$). The final bar shows an averaged value for both sequences of all the subjects. When comparing the muscle area

and the ATT area outputs from the analysis program at 1:2 sequence it was seen that the average ΔR^2_{ATT} was a 0.01 where the average ΔR^2_{Muscle} showed to be 0.03. For the 1:3 sequence, the average ΔR^2_{ATT} stood at 0.03 whereas the average ΔR^2_{muscle} was 0.05.

A correlational plot between the EI values calculated for both B-mode ultrasound images (analyzed at 1:3 sequence of the muscle area) and the accelerometer results can be seen in Figure 3.6. The average EI value and standard deviation found for the accelerometry data was 92.2% ±9.6 whereas for the ultrasound EI it was 98.5% ±0.8. For the female subjects, the average stood at 94.5% ± 10.3 whereas for the male subjects the average was 98.7% ± 0.6, there was. The correlation between the two methods was weak and no relationship between ultrasound endurance index and accelerometer index was found ($R^2 < 0.01$, $p > 0.5$).

In comparing the EI values between the male and female subgroups of this study, it was seen that the male participant average ATT was 1.1cm ± 0.5 whereas the female average was 1.0 cm ± 0.4. When correlating these values to the MMg EI values, an R^2 less than 0.01 was observed. However, when correlating these values to the ultrasound EI, an R^2 of 0.13 was seen. These points can be seen in Figure 3.8.

Figure 7 depicts the change in pixel intensity over the range of ΔR^2 values of each participant. The pixel intensity values for each subject in this plot is determined as the average pixel intensity value. The ΔR^2 is calculated through taking the highest ten R^2 values and subtracting them from the lowest ten R^2 values.

Discussion

The present study evaluated the effectiveness of the developed MATLAB analysis program in using B-mode ultrasound clips and low frequency twitch electrical

stimulation as a measure to calculate muscle endurance. Our study was able to successfully develop and test the parameters of an ultrasound image analysis program with the parameters being the sequence of the images and the image size. The developed analysis program was able to generate numerical R^2 value with the ultrasound images. The parameter of time separation between frames was observed and showed that 1:3 gave more accurate results as opposed to 1:2. When spatiotemporal correlation between two pixels was done at two different points in time (1:2 vs 1:3), the R^2 value at 1:3 was higher because of the pixel values being significantly more similar or different to the pixel values in first frame, thus outputting a vector with higher correlation value differences. In essence, the 1:3 sequence was able to track more movement than 1:2 by observation of pixel intensity changes per frame.

Crop values were determined for each individual video based on the adipose tissue thickness and the muscle and then analyzed separately and compared for observation. It was evidently seen that the muscle specific cropped images had a high ΔR^2 value than the adipose specific cropped images. This was due to the observation that the muscle contracts at a faster rate than the adipose tissue (51).

In writing an accessible analysis program for the ultrasound data, the second aim was to find strong agreement between the EI values of the both methods. This aim was not achieved and there was a poor correlation between the EI values. The reason behind this result is due mainly to the fact that EI values from the accelerometer were taken over a 5-minute period whereas the EI values from the ultrasound were taken over a 4.2 second period. Since the accelerometry was taken over a longer period of time, fatigue

was more clearly visible, whereas for the ultrasound machine, the allotted duration only showed at the muscle movement occurring at the halfway point of those 5 minutes.

Limitations to the study included insufficient frame rate speeds of the ultrasound machine. The sampling rate of the ultrasound was 23.7 ms, compared to 2.5 ms for the accelerometer. Contraction speeds within human hamstring muscle were set to 5Hz/second. Isometric twitch contractions in human muscle are around 80 ms. Although acceleration is more complex that isometric force. Another problem could be blurring of loss of focus in the ultrasound image dominating the change in image intensities. This might be different than the movement and actually 'dominate' the changes seen. The researcher collecting the ultrasound data may have also faced issues in holding the probe steady in order for there to be no external movement in the recordings.

Conclusion

Developing non-invasive and user-friendly methods to assist in quantifying muscular endurance is critical to evaluate muscle disease progression in all clinical populations. The present study attempted to demonstrate the viability of the B-mode ultrasound analysis program that non-invasively analyzes muscle movement. It was found that the sequence at which the images were compared affected the programs ability to pick up on muscle movement using the pixel intensity values. The image time separation of 1:3 showed a larger magnitude within the correlation results. The user determined dimensions (of either muscle or adipose tissue) also affected the programs ability to calculate an accurate R^2 value of the cropped area.

Figure Legends

Figure 3.1 Representative B-mode ultrasound image, A single image from a 4.2-second-long ultrasound clip measurement during the 5 minutes of electrical stimulation. The yellow box signifies the area cropped for ATT and the red box shows the area used for muscle for this particular subject.

Figure 3.2 MATLAB analysis program flowchart, A representative diagram of the data flow within this developed ultrasound image assessment program.

Figure 3.3 A) Endurance test of ultrasound (4 seconds), A representative endurance test showing the R^2 value between two sequential frames during a 4.2-second-long ultrasound clip of electrical stimulation at 5 Hz. B) Endurance test of ultrasound (60 milliseconds), A representative endurance test showing the R^2 value between two sequential frames during a 60-millisecond long ultrasound clip of electrical stimulation at 5 Hz.

Figure 3.4 A) Endurance test (5 minutes), A representative endurance test showing the resultant vector during 5 minutes of electrical stimulation at 5 Hz. B) Endurance test (1 second), A representative twitch during the time used to calculate a maximal value.

Figure 3.5 Difference between sequence of correlation and ΔR^2 values at the muscle, An overall view of all subject ΔR^2 data at 1:2 or 1:3 sequencing in the analysis program. An averaged dataset is shown at the end.

Figure 3.6 St. Dev % over ΔR^2 relationship between st. dev % of pixel intensity average and the change in R^2 for each subject. No statistical difference was seen between the variables ($p > 0.05$).

Figure 3.7 Accelerometer EI with respect to ATT value. No statistical difference was seen between the variables ($p>0.05$).

Table 3.1 Participant Characteristics

Participants	Age	Height (m)	Weight (Kg)	BMI (Kg/m^2)	ATT (cm)
Females (n=17)	26±3	1.66±0.08	63.51±11.97	23.03±4.03	0.99±0.44
Males (n = 18)	25±2	1.75±0.06	73.51±11.28	23.84±3.16	1.11±0.49

Values are presented as mean ± SD. ATT refers to the skin and adipose tissue between the ultrasound probe and the skeletal muscle.

Figure 3.1 B-mode Ultrasound Image

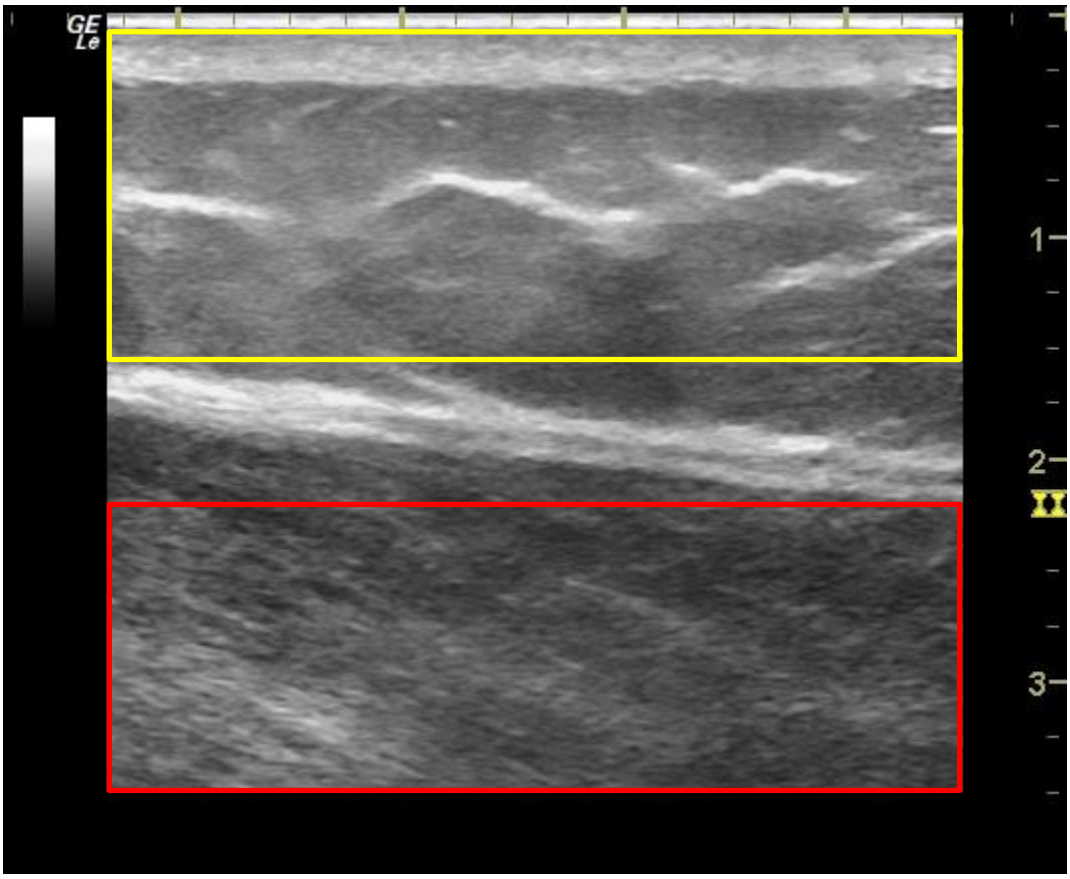


Figure 3.2 MATLAB Analysis Program Flowchart

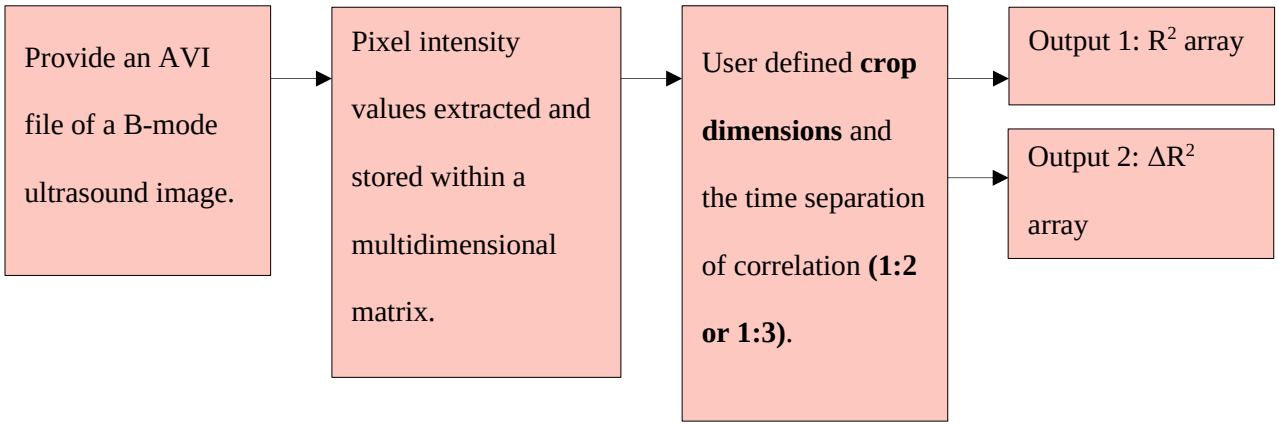


Figure 3.3 Endurance Test of Ultrasound

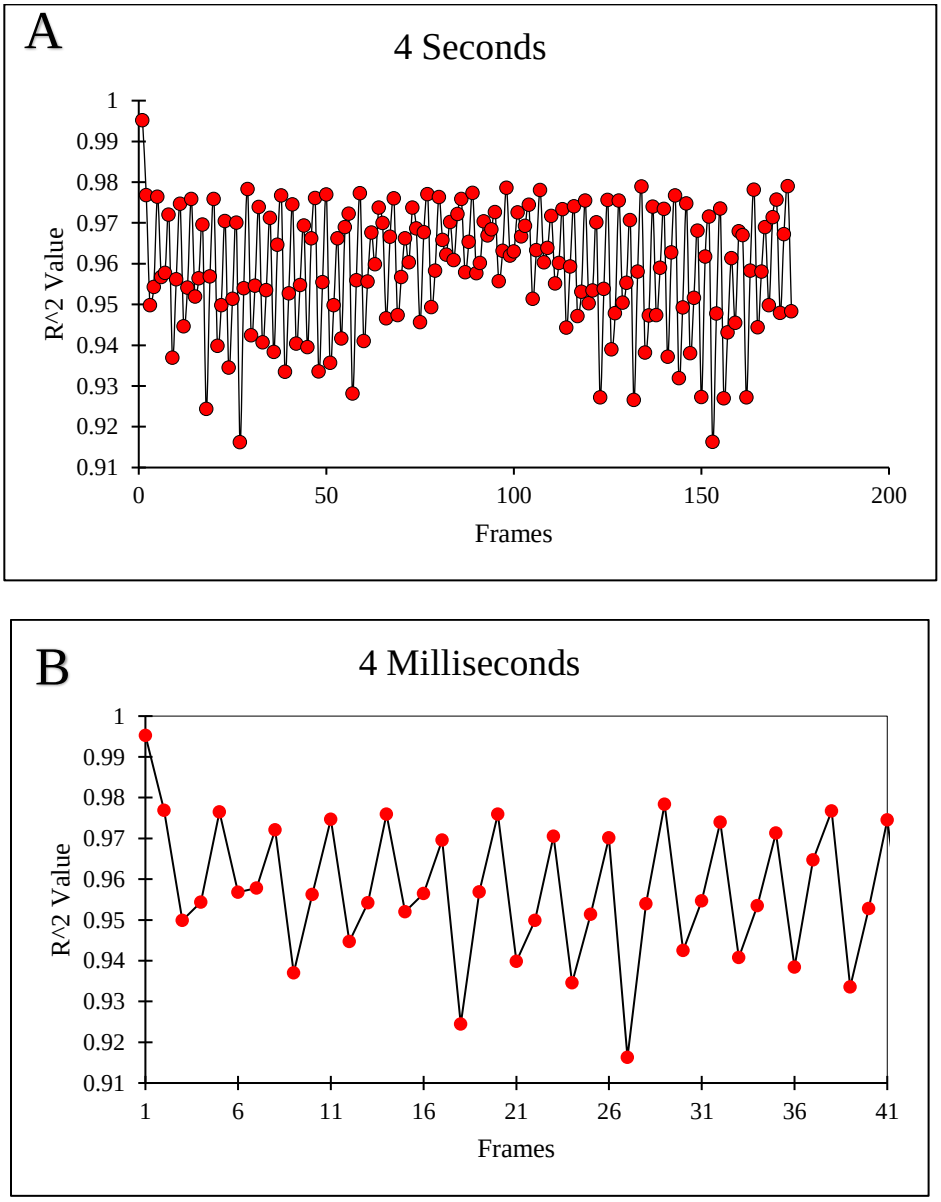


Figure 3.4 Accelerometer movement during muscle contractions

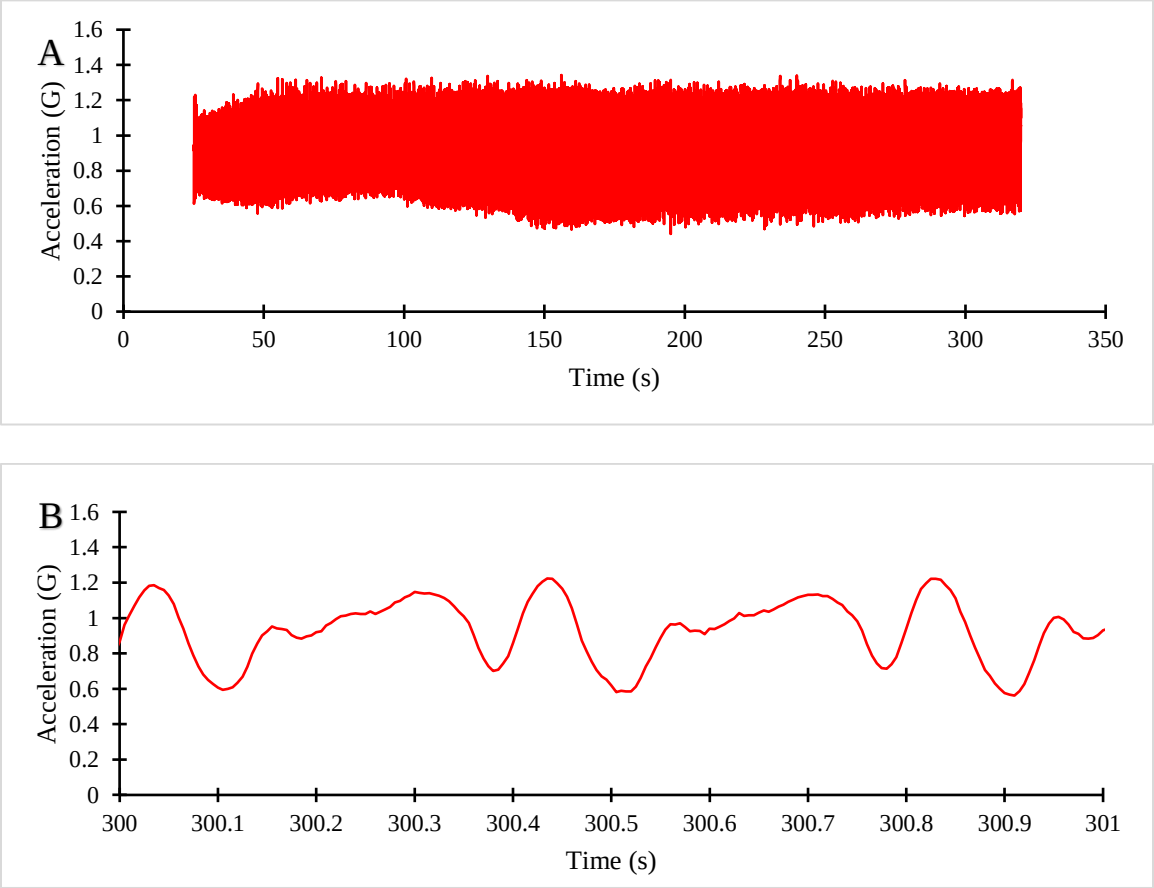


Figure 3.5 Difference Between Sequence of Correlation and the Δ R^2 Values at the Muscle

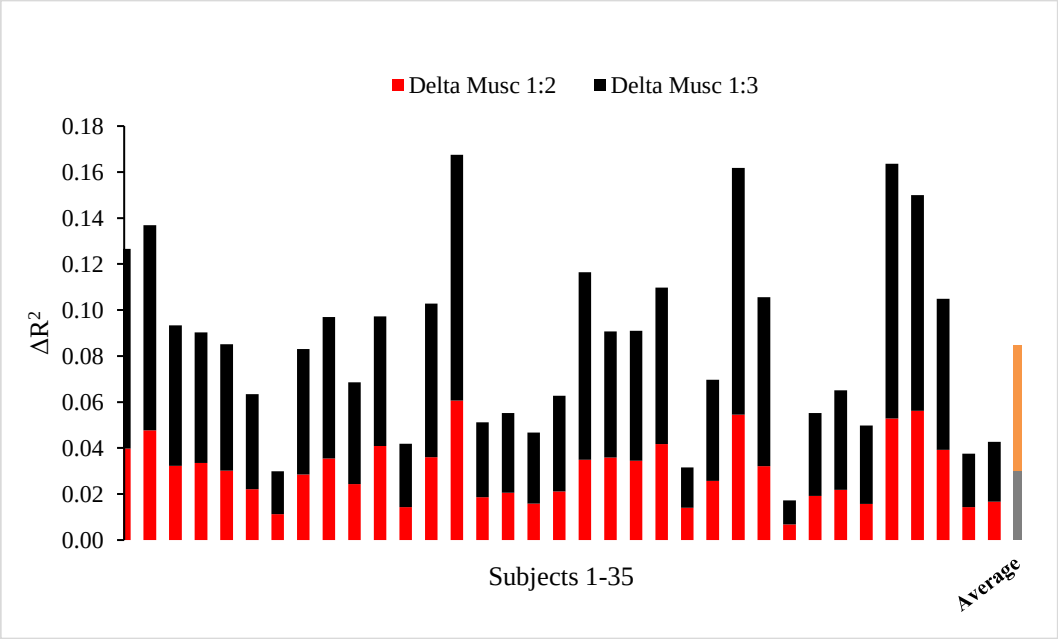


Figure 3.6 St. Dev % over ΔR^2

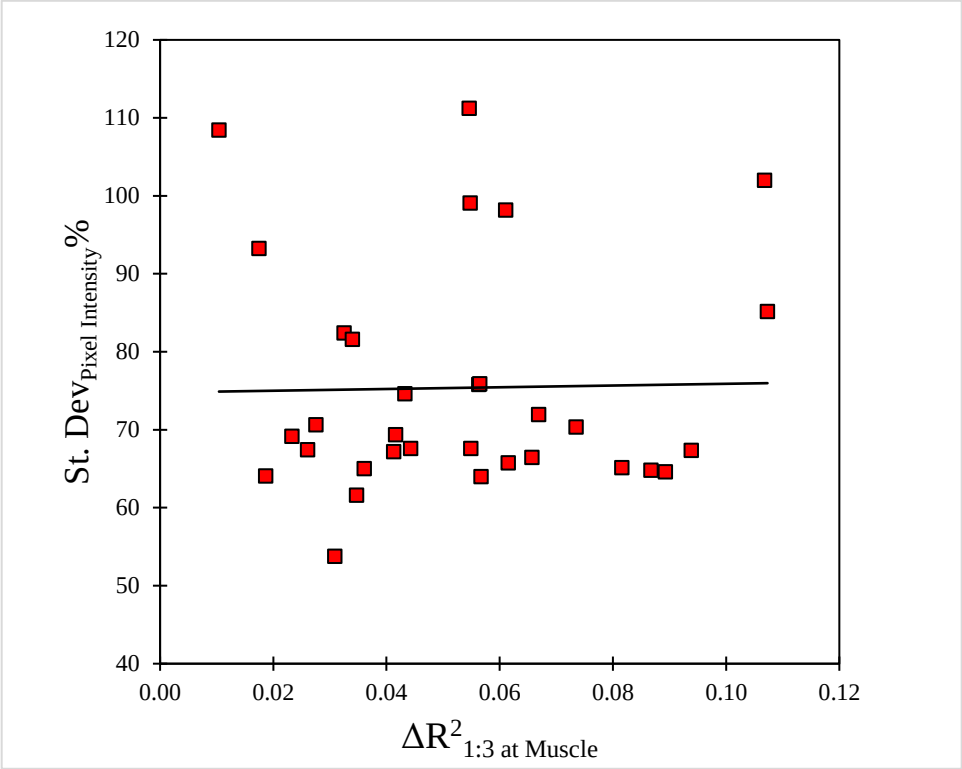
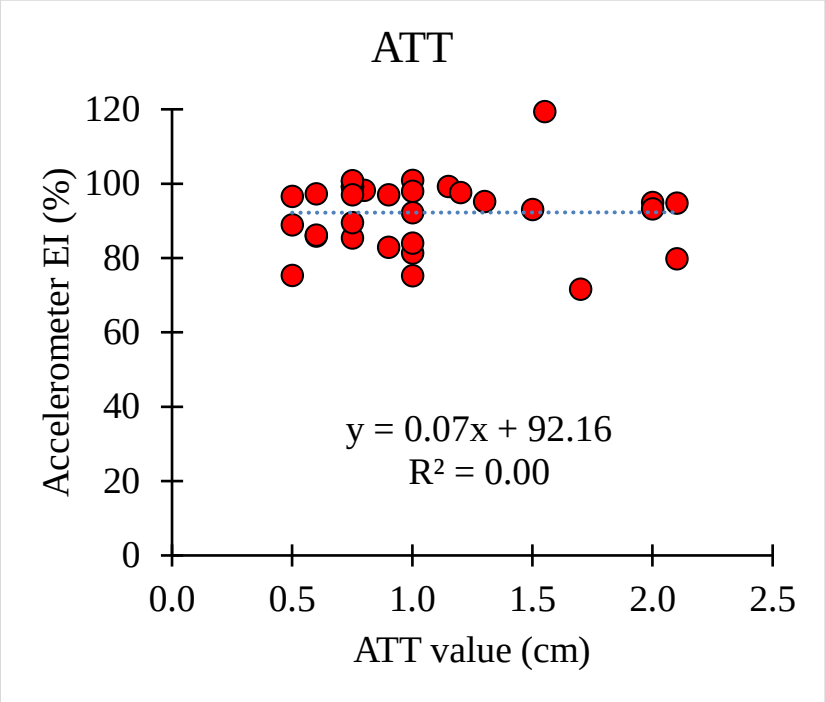


Figure 3.7. Accelerometer EI with respect to ATT value



CHAPTER 4

SUMMARY AND CONCLUSION

The use of aMMg and twitch electrical stimulation has been developed as a way to evaluate the endurance or fatiguability of skeletal muscles. There has been a number of papers published using this method. These studies have shown good reproducibility, strong correlations with related variables such as muscle mitochondrial capacity and walking speeds. The unanswered question addressed in this study was whether muscle endurance could be measured directly in skeletal muscle without the potential complication of varying amounts of adipose tissue laying between the accelerometer and the muscle that is contracting. This goal was not achieved in this study. Given the importance of determining the potential complication of adipose tissue, the project should be continued. Previous studies (Jones, et al, unpublished observations) attempted to answer the question by adding a gelatin phantom over the top of a contracting muscle. This study showed that additional tissue (the phantom) did not alter the endurance index, although it did alter the magnitude of the twitch accelerations. For the time being, that is the only experimental evidence that addresses the potential impact of adipose tissue on the endurance index.

Images collected from all subjects were properly obtained after each testing session and successfully analyzed using the developed ultrasound analysis program. Though the developed program was successful in giving an output of an EI value with the defined parameters of image size and image sequence, the resolution of the images

collected were not. In having a slow frame rate, the images collected did not provide the analysis program with enough numerical data during the time of muscle contraction, thus causing gaps within the plotted consecutive correlations. Placement of the ultrasound probe by the researcher could have also restrained the muscle movement, which would affect the pixel intensity values of the images collected.

For future studies, it would be suggested to use a recording device with higher resolution, faster framerate, and non-contact capabilities, as it would also be a viable input for the analysis program. The output of these images should be compared with results of the a MMg and twitch electrical stimulation method. The parameters that were assessed in this study should still be tested as it was seen that at crops taken at the muscle versus the adipose tissue, the change in R^2 values were drastically different. When testing the parameter of image time separation, the sequence of frame 1 to frame 3 gave higher ΔR^2 values. It is not clear if longer sequence separations helped the results, as it might be possible that the R^2 value is dominated by loss of focus rather than movement. These observations should continue to be evaluated in future studies using a more efficient recording method and device.

The development of the analysis program in MATLAB was successful as we were able to generate values that represented the movement that occurred within the input ultrasound video files. However, certain techniques could be considered in future development to speed up the performance of the code. Using more localized functions over nested function could be practiced. Pre-allocation of correlation array sizes could be implemented to improve memory usage and save time. Vectorization should be implemented as well to make the code more accessible and easier to understand, as well

as shorter, meaning fewer opportunities for programming errors to occur. Lastly, vectorization helps improve speed as opposed to code that contains loops. Future studies should implement the following changes to improve the performance of the analysis program.

In conclusion, MMg and electrical stimulation provides an adequate, muscle-specific, non-invasive measurement for obtaining skeletal muscle endurance. This innovative technology can continue to be used to evaluate endurance and fatigue in clinical populations. Further evaluation with clinical populations and different muscle should be explored as well as studies directly linking the mechanisms to these methods.

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