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Impedance Changes in Biceps Brachii Due to Isometric Contractions and Muscle Fatigue Using Electrical Impedance Myography (EIM)

Travis Orth

IMPEDANCE CHANGES IN BICEPS BRACHII DUE TO ISOMETRIC CONTRACTIONS AND MUSCLE FATIGUE USING ELECTRICAL IMPEDANCE MYOGRAPHY (EIM)

by

TRAVIS D. ORTH

(Under the Direction of Mohammad Ahad)

ABSTRACT

Electrical impedance myography (EIM) is a non-invasive technique used to evaluate neuromuscular conditions by using a quantitative parameter called impedance (Z). It relies upon the application and measurement of high-frequency, low-intensity electrical current imbedded over a localized muscle to determine its opposition to current flow. In brevity, impedance determines the resistance of muscle fibers due to changes in muscle composition. The objective of this thesis was to establish a relationship between muscles at rest and during isometric contractions at various force levels (25%, 50%, and 100% of maximum voluntary contraction) of the biceps brachii over a multifrequency spectrum. Impedance measurements due to muscle fatigue was further studied and compared to muscles under static conditions. It was discovered that isometric contractions had a direct, but nonlinear effect on impedance measurements; as force

increased, resistance and reactance decreased on the bicep. On the other hand during muscle fatigue, only the resistance increased and the reactance saw a decline. Supporting data was presented for seven healthy males, with ages ranging from 22 to 26 years. Evidence justified that resistance at maximum voluntary isometric contraction (MVIC) correlated to the greatest difference of 12.80% whereas a percent difference of 4.63 was calculated for 25% of the MVIC. Reactance decreased from an average of 11.165 Ω at rest to 9.6025 Ω at 100% maximum isometric contraction. In a similar fashion, the resistance values saw a reduction during muscle fatigue of the biceps brachii with an 11.24% decrease. However, the average reactance increased 3.58% from the muscle at rest to the muscle during fatigue. This research study will provide an understanding of underlying muscle tissue composition during dynamic changes using a quick, pain-free, and portable bioimpedance device.

INDEX WORDS: Electrical impedance myography, Isometric contractions, Impedance, Muscle, Electromyography, Electrode, Bioimpedance

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DEDICATION

My wonderful mom and family, whose understanding and support have truly made everything possible.

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Electrical impedance myography (EIM) is a new, non-invasive method of assessing muscle tissue characteristics of a localized muscle or group of muscles through the use of electrical impedance. EIM takes advantage of a fundamental trait of skeletal muscle in order to measure tissue degeneration. Muscles are made up of many long bundled parallel fibers that degenerate in physiological and morphological vicissitudes according to different neuromuscular diseases, such as amyotrophic lateral sclerosis (ALS, commonly known as *Lou Gehrig's* disease), inflammatory myopathy, and muscular dystrophy (Chin, et al. May 2008). Muscle tissue is not perfectly cylindrical but rather is thicker in some areas than others; thus, when passing through it, an electrical current must flow both parallel and transverse to the bundles to reach all parts of the tissue (Goodman 2004). By using an application of high-frequency, low-intensity alternating electrical currents, the abnormalities in the muscle fiber and muscle membrane will result in impedance changes based on the accompanying neuromuscular disease or muscle property changes. The transverse current passes through a series of cell membranes, and in healthy cells, these act as insulators and delay the current (Goodman 2004). These alterations detected by EIM can help determine the resistance of

a muscle, which will ultimately provide an assessment of neuromuscular health and its progression or remission.

1.2 Current Clinical Neuromuscular Diagnosis Methods

During the past century, there have been many contributing methods of diagnosing neuromuscular diseases using bioelectrical assessments. Each has been successful in identifying various neuromuscular diseases, but they also deliver disadvantages in their own ways. The three main methods currently used in clinical environments are electromyography (EMG), ultrasound, and magnetic resonance imaging (MRI). Although they are effective diagnostics in the neuromuscular field, their limitations have pushed doctors and biomedical engineers to pursue new techniques and developments that will not only detect copious amounts of muscular diseases, but ultimately become more accurate in the precision/evaluation of assessing muscle conditions.

Electromyography (EMG)

Electromyography has been a well-established clinical application since the 1960s when Hardyck was the first practitioner to use this technique (Hardyck , Petrincovich and Ellsworth 1966). Unlike electrical impedance myography, EMG involves inserting a needle into the muscle and physically contracting it by exciting motor unit action potentials (MUAP) in order to observe the electrical activity of tissues when the nerves to the muscle are stimulated (Gekht 1990). EMG graphs appear as wavy and spikey lines of voltage over time domain (Kaplanis, et al. 2009). Although it is one of the most

common tests and has proven to be a useful clinical technique, EMG testing can be painful and discomforting to the patient and requires a hospital with a specifically designed room where no outside electrical interference can skew the results. EIM, on the other hand, is non-invasive and focuses more readily on muscle fiber organization and structure over a small area of interest via measurements of anisotropy (Rutkove December 2009). Alternately, a non-invasive form of electromyography has been developed called surface electromyography (sEMG) where small electrodes are placed on top of the skin over the muscle. Its drawbacks are that it has a narrow frequency spectrum as compared to EIM and is susceptible to a contamination of electrical activity due to interfering muscles in close proximity (Turker October 1993).

Ultrasound

In brevity, an ultrasound in the neuromuscular discipline involves a device that sends audible sound waves above the frequency range of human hearing to a desired muscle in order to detect the shape, size, structure, and composition. In typical ultrasonic sensing, the ultrasonic waves are travelling in a medium where an interaction of ultrasonic energy with an object are acquired as ultrasonic signals that are the waveform variations with transit time (Ihara 2008). It has been useful in detecting muscle tears and detecting healing problems such as fibrosis, cystic haematomas or myositis ossifican (Peetrons January 2002). Further research by Reimers *et al* proved that ultrasonic testing was successful in the detection of inflammatory myopathies of skeletal muscle (Reimers, et al. May 1993). A clear disadvantage of ultrasound in neuromuscular diagnosis is that it contributes more to *qualitative* data rather than *quantitative* data. It examines the

overall quality or structure of a muscle and can cause a subjective muscular image based on the technique of the physician.

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) testing is most commonly known as a method of detecting structural abnormalities of the entire body including the brain, spine, and heart. It utilizes radio frequency waves to produce an image of the overall body structure by distinguishing pathological tissue from normal tissue. Its major advantages are that the images produced from an MRI are very detailed and accurate and testing is virtually painless. As compared to EIM, MRI has numerous drawbacks that prohibit it as a benefactor in the detection of neuromuscular diseases. A distinct disadvantage is that an MRI only detects the general architecture of a muscle, rather than the muscle fiber properties. Unlike EIM which can examine a single muscle, an MRI requires the entire body to be scanned in an expensive apparatus under motionless conditions.

Electrical impedance myography differs from the current methods of diagnosing neuromuscular diseases in many ways. It actively measures the voltage drop of an individual muscle instead of analyzing electrical muscle activity in the passive form of electromyography. EIM does not require a specially designed room for testing and is completely painless. Ultrasounds and MRIs measure the overall structure of a muscle to detect tears or tissue loss and cannot accurately associate those with certain diseases. EIM has the potential ability to focus closely on the integrity of muscle fibers and their physiological changes within the muscle membrane. Although it is still in its early state of clinical use, electrical impedance myography has already proven to be a dependable

technology/successor in the detection of neuromuscular diseases by providing valuable quantitative data in a quick, painless, and straightforward manner.

1.3 Thesis Objectives and its Importance

By using closely spaced sensing electrodes placed directly on the skin and over the muscle group of interest, impedance measurements can be used to assess the resistive properties of the underlying muscle (Shiffman, Aaron and Rutkove February 2003). Many studies so far have demonstrated changes in impedance of normal and diseased subjects under *relaxed* muscle participation, but the objective of this research was to study impedance changes under *dynamic* muscle conditions in a multifrequency spectrum. In particular, the impedance signals i.e. resistance, reactance, and frequency during voluntary isometric contraction (VIC) of the biceps brachii of healthy individuals were experimentally studied and presented. Previous work has demonstrated positive changes in electrical impedance during isometric contraction of the anterior forearm at a single frequency, but had limited explanation of the phenomenon (Shiffman, Aaron and Rutkove, Electrical Impedance of Muscle During Isometric Contraction February 2003). Isometric contraction of muscles means the limb remains at a fixed position so the length of muscle fibers do not elongate or shorten, but is still activated due to an opposing force. Carrying an object, gripping a ball, or flexing particular muscles are examples of isometric contractions (Figure 1).

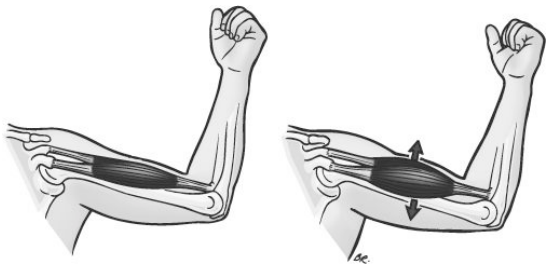


Figure 1: Isometric contraction on the bicep brachii (Jonas 2005)

Figure 1 demonstrates an isometric contraction of the biceps brachii with the elbow joint bent at 90°. However, for this research study the elbow was not bent so that the bicep and forearm remained in a straight line. An isometric contraction of the bicep in this manner acted as a cantilever beam with the shoulder fixating the arm and the palm of the hand represented the free end. A contraction was still able to be achieved due to a downward force in the hand, allowing the muscle fibers to create an equal and opposing force to sustain the weight.

The main hypothesis behind this study was that voluntary isometric contractions of human muscles are directly related to impedance changes; as force increases, resistance decreases, thereby having a negative effect on impedance. As muscles contract, blood flow increases steadily until muscle fatigue (Humphreys and Lind 1963); therefore changing morphological and physiological properties of muscle, and ultimately causing a drop in impedance. In general, the muscle weakness and slowing down is not only attributable to changes in muscle mass, but also associated with neurological changes. This has an impact on the force/torque production because force is modulated by the number and type of motor units recruited and firing rate of units (Merletti, et al. 2002). To illustrate the hypothesis, repeatable testing on 7 healthy subjects in static

positioning was done using 25, 50, and 100% maximum voluntary contraction (MVC) and was further compared to muscles at rest.

The distinct objectives of this work were:

- To study the effects of impedance changes during dynamic EIM at 10kHz, 50kHz, 100kHz, and 150kHz
- To implement various force levels on biceps brachii and antebrachium muscle
- To investigate impedance changes on fatigued muscle
- To analyze physiological changes on the muscle due to isometric contractions
- To support the importance of EIM in clinical use as a reliable technique when accessing the condition of a muscle
- To develop a Finite Element Model of the muscles using COMSOL MultiPhysics 4.2

1.4 Thesis Organization

The paper is organized as follows. Chapter 2 reviews all previous relevant literature in the subject of electrical impedance myography. It begins with an

introduction to the concept of impedance and its role in the biomedical field. Equations and important terms are introduced that quantify the new phenomena of EIM. Following suit is the topic which covers the composition of muscles by explaining physiological and morphological changes when muscles are at rest and at voluntary isometric contractions.

Chapter 3 covers the research methodology used in this study. It provides the experimental setup and procedure of the subjects used throughout this thesis. It explains in detail subject preparation and the apparatus used to measure resistance, reactance, phase and impedance. An overview of the electrode placement and array was thoroughly explained, as well as data acquisition software used to calculate EIM parameters.

Chapter 4 presents the detailed results and findings of the healthy subjects using Impedimed's Bioimp software. Tables and graphical interpretations of impedance versus frequency and phase versus frequency were presented in this chapter. A thorough discussion of the effects of isometric contractions and its repeatability was addressed. Changes in impedance for muscles at rest and at muscle fatigue condition were addressed with supporting explanations.

Finally, Chapter 5 summarizes and concludes the thesis research of the present work and provides a discussion of future work.

Appendix A provides raw data of individual subjects that were used during average impedance and phase calculations.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Concept of Impedance

Impedance is the measure of the degree to which an electric circuit resists current flow when a voltage is impressed across its terminals. All impedance methods rely upon the basic principle that if an alternating current is applied to a substance, energy will be dissipated as it travels through it, thus producing a measurable voltage. Electrical impedance methods can be applied to practically any substance or material, and consequently have found a prevalent use in disciplines as disparate as medicine, metallurgy, geology, and forestry. As the electrical current travels through the substance, it loses energy (due to the substance's inherent resistance), therefore reducing its amplitude (Rutkove December 2009).

Unlike pure resistance, which is a concept used in direct current (DC) applications, electrical impedance is the alternating current (AC) equivalent and takes into consideration the dynamic principles of AC circuits. The timing of the resulting voltage alternations is slightly delayed, and it no longer crosses the x-axis at the expected time due to the inherent capacitive and inductive characteristics of the substance (Rutkove December 2009). It is more complex than just resistance and is dependent on a property called reactance. Figure 2 depicts the key components of impedance and their relationships.

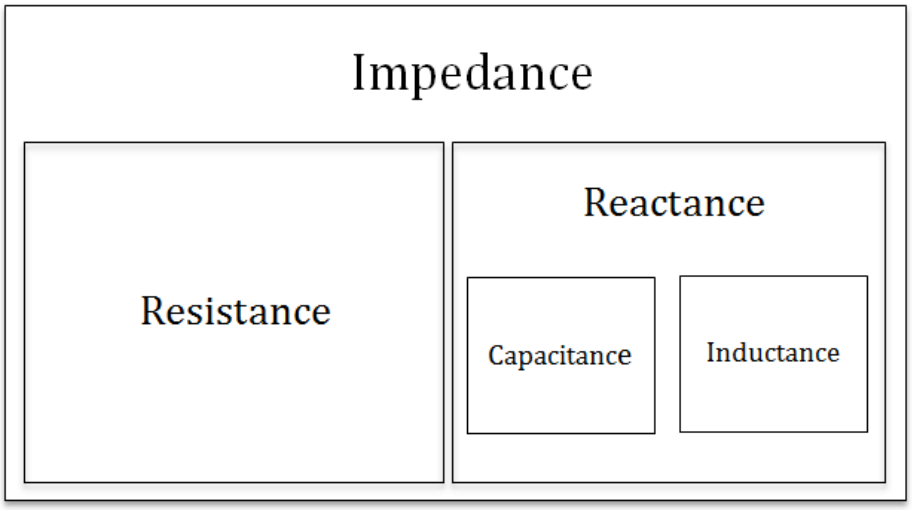


Figure 2: Measurable parameters of impedance

Mathematically, impedance is measured in a unit called the Ohm (Ω) and extends beyond the voltage-current characteristic of an ideal resistor in Ohm's law (Alciatore and Histand 2012).

Ohm's law states:

$$V = IR \tag{1}$$

where V is voltage, I is the current flow, and R is the resistance, but this equation only describes a pure resistive circuit, without capacitors or inductors.

Pure resistance is affected by conductivity and area of the circuit in which the current passes through. The mathematical expression for resistance is:

$$R = \left(\frac{\rho l}{A}\right) \tag{2}$$

where ρ is the resistivity of the medium, l is the length of the conductor, and A is the area of the circuit in which the current passes through.

Capacitors and inductors are known as active components, which mean they are able to change their resistance according to the input voltage or current. For a more complex circuit that includes those elements, Ohm's law can be rewritten as:

$$V = IZ \tag{3}$$

where Z represents the complex impedance of a circuit and is dependent on the obstruction of current flow due to capacitors and inductors in the circuit.

Since it is a complex number, impedance can be written in several forms:

$$Z = |Z| \angle \theta \longrightarrow \textit{polar form} \tag{4a}$$

$$= \frac{V}{I}\left(e^{j\theta}\right) \longrightarrow \textit{exponential form} \tag{4b}$$

$$= R + jX \longrightarrow \textit{rectangular form} \tag{4c}$$

with R being the real part and X as the imaginary part of the complex number Z (Dorf and Svoboda 2010). The magnitude of impedance (measured in Ohms) is

$$Z = \sqrt{R^2 + X^2} \tag{5}$$

and the phase angle (measured in degrees) is

$$\theta = \tan^{-1}\left(\frac{X}{R}\right) \tag{6}$$

The relationships of equations 4 and 5 can be illustrated in Figure 3.

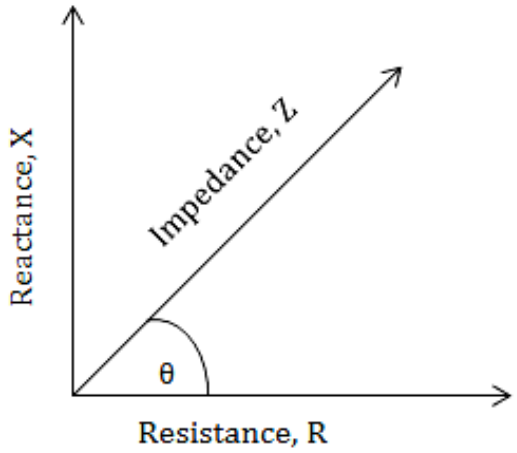


Figure 3: Graphical representation of impedance

Reactance is the opposition to a change in flow due to capacitance and is expressed by the equation:

$$X_C = \frac{1}{2\pi fC} \tag{7}$$

where X_C is the reactance in ohms, f is frequency in kHz, and C is capacitance in farads.

Mathematical expressions of capacitance and inductance can be further discussed, but are beyond the scope of subject for this thesis research.

2.2 Composition of Muscles

Muscles are made up of long bundled parallel fibers that have the ability to conduct electricity due to the biological properties of tissues. Healthy muscles are organized in an anisotropy manner meaning electric current flows easily along the muscle fiber rather than across them (Aaron, Huang and Shiffman 1997), but as neurogenic disease disorganizes fiber structure and replaces it with fatty tissue, the muscle tends to demonstrate isotropic properties resulting in disturbed impedance values. Everything that lies under and between the electrodes contributes to the measured impedance of a muscle, including a highly conductive saline solution. Various concentrations of intracellular and extracellular fluids along the muscle membranes will cause a change in lipid bilayers of muscles. These bilayers act as additional capacitors that will store and release charge with the reversing current flow during isometric contractions (Rutkove December 2009). Rutkove illustrates a simplified '3-element' circuit diagram of muscle tissue for the theory of electrical impedance in Figure 4. The capacitor represents the reactance of cell membranes and the resistors represent the extra and intracellular resistance of a skeletal muscle.

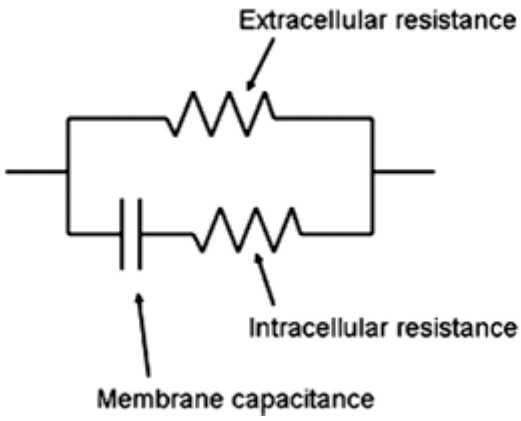


Figure 4: Circuit diagram of muscle tissue (S. Rutkove 2009)

Moreover, the capacitance varies on the frequency of the applied current, which in turn fluctuates the resistance of each muscle tissue. At lower frequencies, the current will flow initially through all three elements until the capacitor is fully charged. Once charged, the current will only flow across the extracellular resistor, but at higher frequencies of alternating currents, the current will be able to penetrate both the extra and intracellular resistors. A mathemathical relationship of frequency (in Hz), resistance, and capacitance is presented in equation 6. Significantly higher frequency values make the capacitance of cell membranes virtually obsolete, thus contributing to little change in impedance measurements.

$$f_{peak} = \frac{1}{R_i + R_e C^2} \tag{6}$$

Previous studies to date have concluded that a frequency of 50 kHz is sensitive enough to detect neurogenic and myopathic diseases using EIM (Shiffman, Kashuri and Aaron

2008; Rutkove, Fogerson and Tarulli 2008; Esper, et al. June 2006). As a result, much devoted research has been focused at 50 kHz, but using measurements over a full frequency spectrum will still produce contributable data to the field.

CHAPTER 3

RESEARCH METHODOLOGY

Extensive testing was performed on human subjects to analyze the impedance changes during various levels of isometric contractions and muscle fatigue in the bicep brachii and antebrachium. All procedures and methods were ultimately reviewed by the Georgia Southern University Institutional Review Board who approves research involving human subjects. Signed consent was obtained from all individuals over the age of 18 in order to voluntarily participate in this study.

3.1 Normal Subjects

7 normal healthy subjects were recruited via advertisement and by word of mouth on the campus of Georgia Southern University. All participants had no history of neuromuscular diseases and denied having previous neuromuscular injuries in the upper limbs. A brief evaluation of muscle movement and strength of the bicep and forearm determined every subject used in this study was in overall good health. All subjects were of good health and demonstrated normal strength and movement of their bicep and forearm.

3.2 Subject Preparation and Initial Strength Measurement

All experimental procedures were performed in the research laboratory of the Allen E. Paulson College of Engineering and IT in Statesboro, Georgia. Subjects were required to wear loose clothing that allowed for easy access to the bicep and forearm muscle and to remove all metal jewelry along the upper limb i.e. ring, watch, bracelet. Excess hair on the arm was shaved in order to provide direct unobtrusive contact between the electrodes and skin. A preliminary set of EIM data was measured before any force exertion or strenuous activity transpired. The subject was required to sit in a chair and rest their arm on a table to ensure no opposing force due to gravity could manipulate the data. This would be called the "at rest" condition of the muscle. Patients were asked to stand in an upright position with their dominant arm positioned straight out and the palm of their hand faced up towards the ceiling. A slight bend in the elbow ($< 10^{o}$) was acceptable as long as the subject did not deviate from this position as any change would lead to a variation in muscle fiber length, invalidating the definition of isometric contractions. For an initial evaluation of maximum voluntary isometric contraction, subjects were asked to hold dumbbells consisting of free weights in their hand for 5 seconds. Weights were added until a vertical change in arm position was observed, thus making the former force as the maximum isometric contraction. Data collection began with measurements on the bicep holding 25% of the maximum voluntary isometric contraction, then 50%, then 100%. Adequate time was given between each measurement to ensure the muscle returned to rest condition. The applied technique and experimental

procedure of isometric contractions is shown in Figure 5 below. The electrodes remained in the same position throughout each experiment to ensure reproducibility during all muscle conditions.

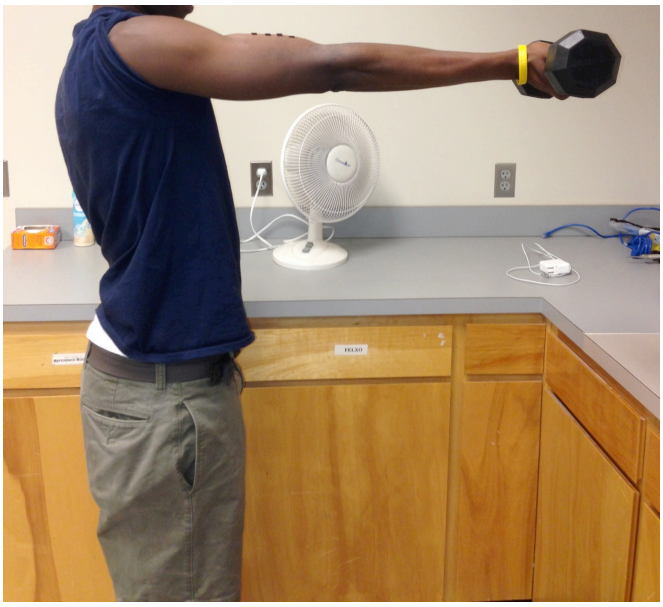


Figure 5: Experimental isometric contraction of the biceps brachii

Following the isometric contraction procedure, muscle fatigue was tested by curling the maximum isometric contraction force in sets of 10 repetitions until full movement could not be completed. EIM measurements were taken as soon as the subject completed all procedures while the muscle was fatigued in the "rest" condition. A correct bicep curl is shown in Figure 6 as the subject began curls with their maximum isometric contraction force.

Figure 6: Bicep curl for muscle fatigue

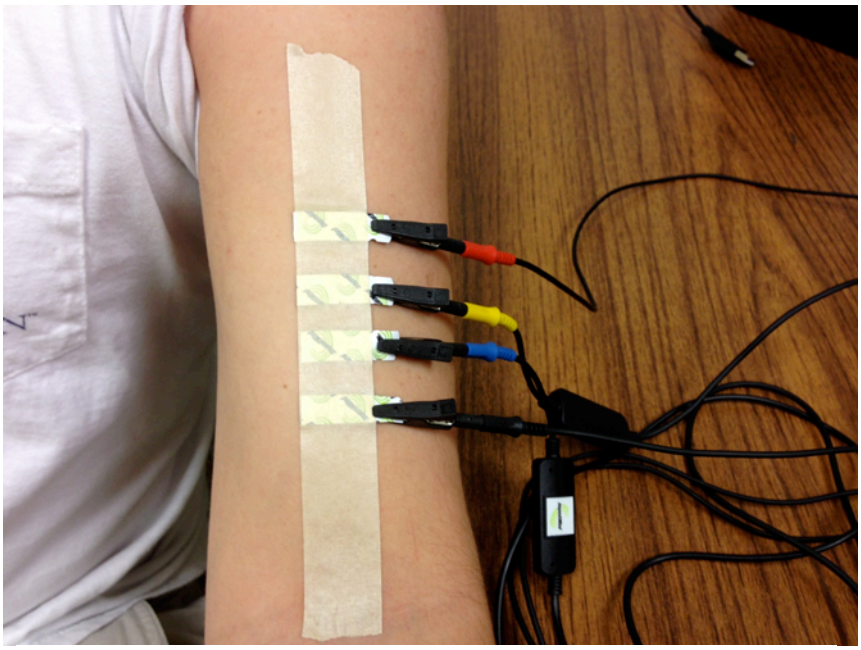
The subject maintained their elbow against their body and generated a full arm extension down by their quadriceps and slowly curled it towards their shoulder. Figure 6 was taken at the midpoint between one bicep curl for a 24 year old male.

3.3 Electrode Placement and Array

A four-electrode setup in a parallel arrangement was used where the outer two electrodes delivered a current at different frequencies and the inner two electrodes measured a resulting voltage. Each 23 X 25mm electrode strip (Part No. 292-STE; ImpediMed, Inc., Queensland, Australia) was resized down to a length of 10 x 25mm with a spacing of 10mm between each strip (Figure 5). A paper model with the electrode

Figure 7: Electrode placement and configuration on the biceps brachii

configuration was designed to guarantee reproducibility between data collection. Any change in the electrode array would produce inconsistent data between subjects. Rutkove, Partida, et al. 2005 saw the greatest stability in phase when the current electrodes were placed 10-15cm away from the voltage electrodes on both the bicep and forearm. The Ag-AgCl strips were manufactured with a conductive adhesive gel and were disposed of if firm contact to the skin was lost. To ensure good electrical contact and the absence of movement during measurement, 3M masking tape was placed over the array, affixing the electrodes firmly to the skin.



Careful consideration of the electrode position, size, and arrangement was taken but with a goal of fitting over the greater part of muscle fibers in the biceps brachii. The midpoint of the inducing current electrode was placed at a distance of 40mm from the bend of the inner elbow joint to the bicep for both isometric contractions and muscle fatigue.

3.4 EIM Data Acquisition

Multifrequency measurements were performed with an ImpediMed SFB7 ® device (ImpediMed, Inc., Queensland, Australia. http://www.impedimed.com). This single channel, tetra polar bioimpedance spectroscopy (BIS) device scanned 256 frequencies between 4 kHz and 1000 kHz; however, only measurements at 50, 100, and 150 kHz were used for this study (Figure 6).

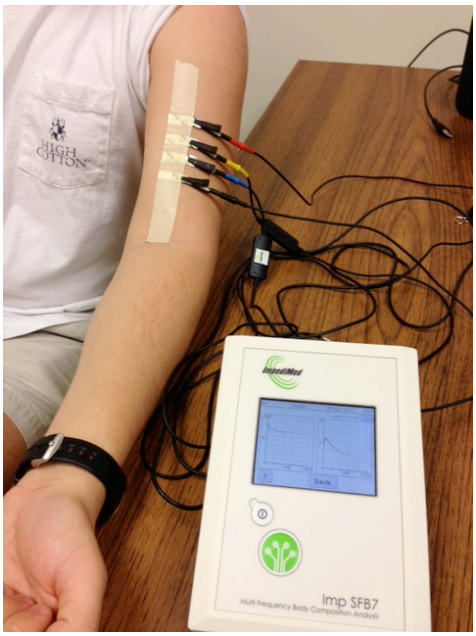


Figure 8: ImpediMed SFB7 device

The device was chosen for its reliability, portability, and ease of use in the biomedical field. Each measurement took less than three seconds to compute resistance (R), reactance (X), and impedance (Z) graphs that were stored directly on the instrument. Phase (θ) was calculated via the relationship in equation 5 using BioImp software for precise values of resistance and reactance at each frequency. A software illustration and its corresponding graphs can be seen in Figure 7.

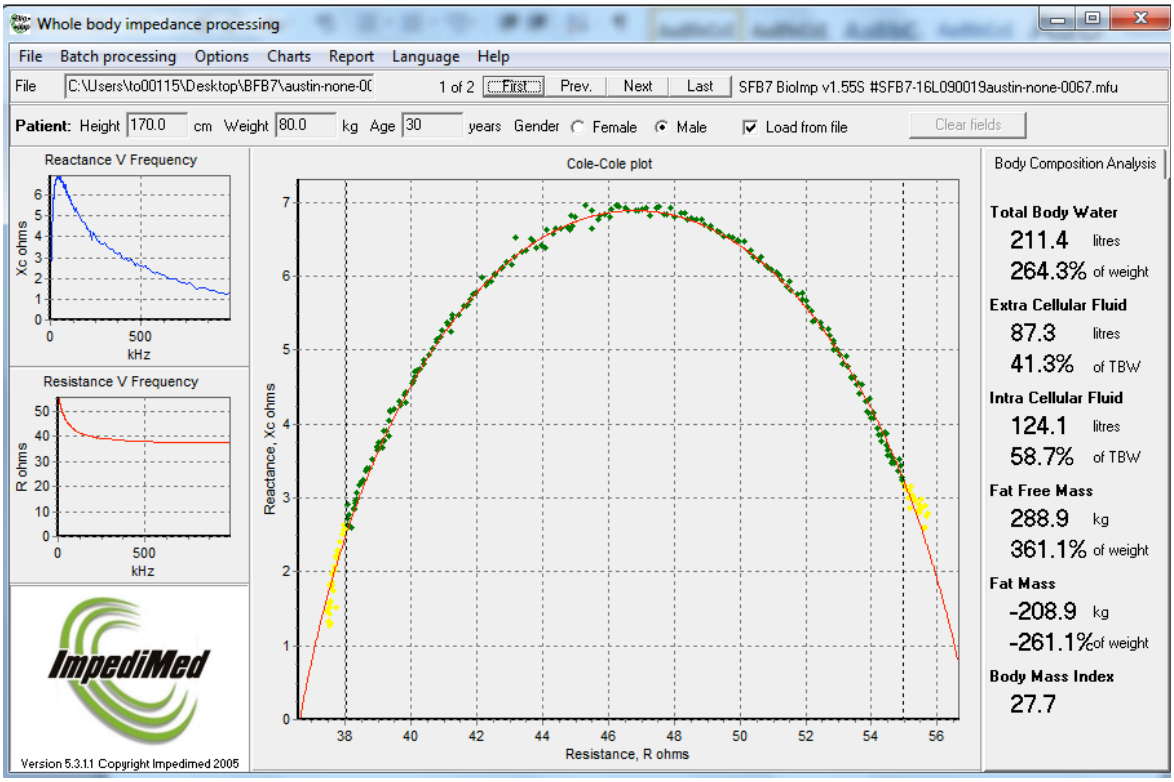


Figure 9: BioImp software plotting reactance vs. frequency, resistance vs. frequency and impedance graphs

CHAPTER 4

EXPERIMENTAL RESULTS AND DISCUSSION

4.1 Experimental Results

A series of structured data collection was performed on seven healthy males with a mean age of 23.3 years and a range of 22-26 years. For all subjects, the resistance (R) and reactance (X) were measured, resulting in calculations of impedance and phase using equation 4 for the former and equation 5 for the latter. An outlier set of EIM measurements was found on one subject for isometric contractions whose average standard deviation was 0.317 on impedance data. Isometric contractions at various forces had a minuscule effect on impedance, phase, resistance, and reactance values for this subject, therefore the measurements were not taken into consideration for experimental averages. To be consistent, the muscle fatigue data was unused for all average calculations as well. Graphs of resistance versus frequency, reactance versus frequency, and phase versus frequency were examined during both isometric contractions and after muscle fatigue.

4.2 Relationship of Isometric Contractions and EIM

Impedance graphs along the full frequency spectrum of 3kHz to 1000kHz were first introduced to illustrate the entire trend of each parameter. The group average of resistance, reactance, and phase versus frequency is shown in Figure 9.

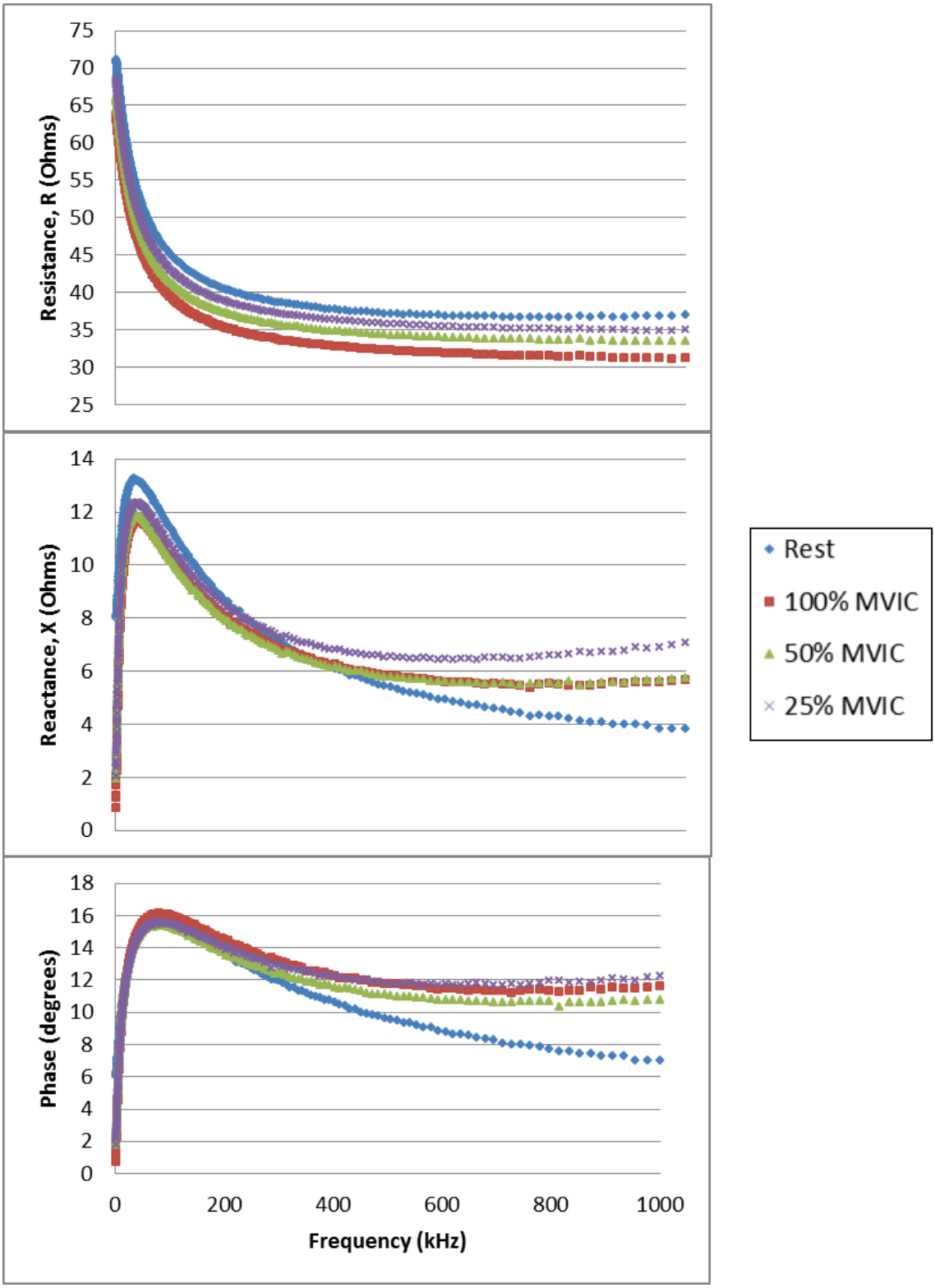


Figure 10: Resistance, Reactance, and Phase versus Frequency of Isometric Contractions on Biceps Brachii from 3kHz to 1000kHz.

In order to understand impedance changes during various muscle conditions, values at 10, 50, 100, and 150kHz were chosen and examined. These frequencies were selected because values beyond 200kHz are rarely studied due to unexplained activity of muscle at higher frequencies (Shiffman, Kashuri and Aaron, Electrical Impedance Myography at Frequencies up to 2 MHz 2008). Impedance at higher frequencies for both healthy and diseased muscle produces uncorrelated data in conjunction with the theoretical reasoning behind the changes. To incorporate all parameters of impedance measurements, the resistance, reactance, and phase versus frequency was analyzed and discussed. A graphical interpretation of these measurements at the four specific frequencies was shown in Figure 11. The muscle at rest was represented by diamonds, the muscle during 100% isometric contraction was shown as squares, the 50% MVIC was denoted by triangles, and the 25% MVIC was signified by x's. The corresponding lines are best-fit lines between each point and represent accurate data measurements at each frequency.

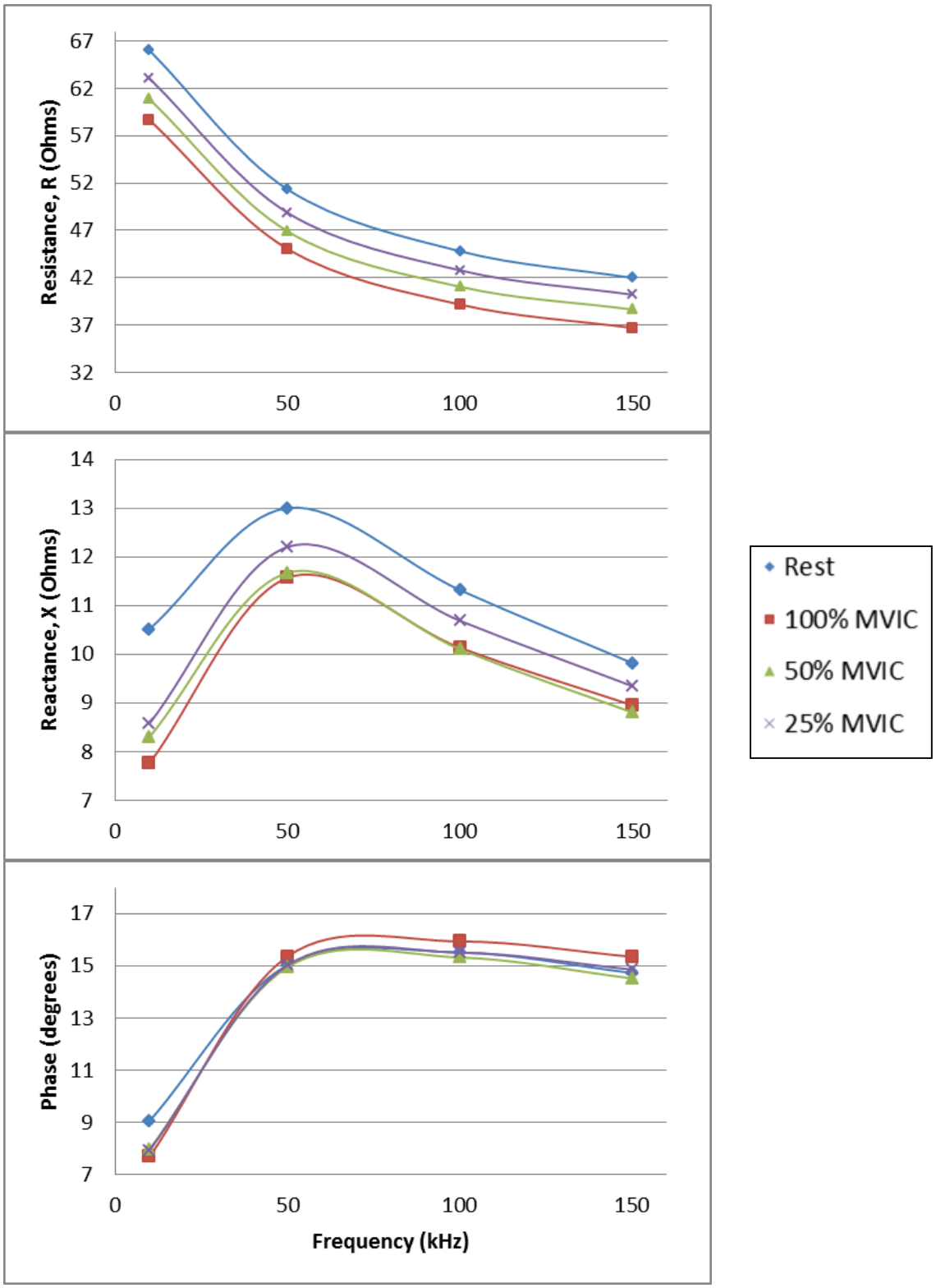


Figure 11: EIM at 10kHz, 50kHz, 100kHz, and 150kHz for six healthy males during isometric contractions on the biceps brachii. At rest measurements are represented by diamonds, 25% MVIC is (x's), 50% is triangles, and 100% is squares.

A direct, but nonlinear correlation between isometric contractions and resistance was discovered. The data confirmed with substantial evidence that as isometric contraction force increased, the resistance decreased on the biceps brachii muscle. 100% of the maximum isometric contraction yielded the greatest difference on resistance values from the bicep at rest, whereas 25% of the MVIC saw the least change. An average percent difference of 12.80, 8.51, and 4.63 was calculated between the bicep at rest and at 100%, 50%, and 25% MVIC respectively. Although the change was minor, there was nonetheless a noticeable discrepancy amongst the various muscle conditions on the biceps brachii. Table 1 shows the average resistance of the six individuals during rest stage, 100%, 50%, and 25% MVIC at different frequencies.

Table 1. Average Resistance for Biceps Brachii during Isometric Contractions

Muscle Condition	*Frequency*			
	10 kHz	**50 kHz**	**100 kHz**	**150 kHz**
Rest	66.018 Ω	51.327 Ω	44.792 Ω	41.975 Ω
100% MVIC	58.673 Ω	45.038 Ω	39.162 Ω	36.688 Ω
50% MVIC	60.88 Ω	46.893 Ω	41.04 Ω	38.637 Ω
25% MVIC	63.057 Ω	48.858 Ω	42.772 Ω	40.193 Ω

One predominate trend for healthy muscles is that resistance peaks at lower frequencies and declines as it approaches higher frequencies, thus producing a slight concave-down curvature (Shiffman, Kashuri and Aaron, Electrical Impedance

Myography at Frequencies up to 2 MHz 2008). The decline can be attributed to biological properties of muscle tissue membranes that are less affected at higher frequencies (Esper, et al. June 2006). This can be seen in Table 1 for each muscle condition that was tested. Out of the four frequencies denoted for this study, the greatest impedance value was found at the lowest frequency point, 10 kHz and the smallest impedance value was found at the highest frequency point, 150 kHz.

The drop in resistance during dynamic EIM cannot be explained in purely quantitative terms, but moreover in qualitative relationships that occurred during isometric contractions. The muscle undergoes numerous physiological and morphological changes as it transforms from a static (at rest) condition to a dynamic (isometric contraction) condition. When muscles are at rest, it experiences a similar state to atrophy where a loss of mass and strength takes place due to immobility. At this phase, a buildup of fat tissue and non-muscle tissue between muscle fibers emerge that act as resistors during measurements. The muscle shifts from an anisotropic form to an isotropic state that directly affects impedance data (Chin, et al. May 2008). The current moving parallel to the muscle fibers is restricted by these factors, consequently producing higher resistance and impedance values. However, during muscle contraction, blood flow increases allowing cell membranes to expand (Humphreys and Lind 1963), which break up and reduce the amount of resistive cell walls along the muscle fiber. Various force levels will determine the amount of muscle motor units needed fire to produce an opposing contraction that will sustain the force (Ahad, et al. 2012). Even the force at 25% of the maximum voluntary isometric contraction produced enough change in muscle

activity to decrease the resistance. Another biological factor that contributes to resistance changes during isometric contractions is the electrical properties of blood. Blood is made up of saline that is highly conductive (McComas, et al. 1968) and during contractions, blood flow increases to activate more muscle fibers. This increase in blood flow leads to an increase in conductance; resulting in a decrease in resistance. From the results, it is proven that the maximum isometric contraction had the greatest contribution to increased blood flow, whereas the isometric contraction at 25% had the least influence.

Table 2 shows the average reactance versus frequency at 10kHz, 50kHz, 100kHz, and 150kHz at various muscle conditions during isometric contractions. The reactance decreased during isometric contractions for each muscle condition, but at different amounts. The muscle during 25% isometric contraction yielded the smallest percent difference of 9.0%, the 50% had a 13.86% difference, and the 100% contraction produced a change of 15.03% as compared to the muscle at rest. Regardless of muscle condition, the reactance of healthy muscles generates a peak between 30-50kHz (Shiffman, Kashuri and Aaron, Electrical Impedance Myography at Frequencies up to 2 MHz 2008) that was noticed in this research study. The average reactance at 10kHz was 8.79 Ω, then it peaked at 50kHz with an average of 12.11 Ω, then it steadily decreased at higher frequencies.

Table 2. Average Reactance for Biceps Brachii during Isometric Contractions

Muscle Condition	*Frequency*			
	10 kHz	**50 kHz**	**100 kHz**	**150 kHz**
Rest	10.517 Ω	13.00 Ω	11.317 Ω	9.815 Ω
100% MVIC	7.757 Ω	11.57 Ω	10.132 Ω	8.95 Ω
50% MVIC	8.3 Ω	11.67 Ω	10.102 Ω	8.803 Ω
25% MVIC	8.577 Ω	12.203 Ω	10.687 Ω	9.342 Ω

The decrease in reactance can be explained in both quantitative and qualitative terms. Similarly to resistance, when blood flow increases, more metabolic ions stick to the intracellular and extracellular membranes of the muscle fibers. These provide additional capacitive ions along the muscle that consequently increase capacitance. Referring to equation 7 from Chapter 1, capacitance and reactance are directly related; an increase in capacitance will result in a decrease in reactance. Out of the three tested isometric contractions, the 25% MVIC had the smallest influence on reactance since its blood flow was the least. At 50kHz, the reactance was 12.203 Ω as compared to the 13.00 Ω measurement at rest. During 50% of the maximum isometric contraction, the reactance was 11.67 Ω and the 100% MVIC resulted in the largest discrepancy of 11.57 Ω.

Table 3 shows the average phase versus frequency at 10kHz, 50kHz, 100kHz, and 150kHz and various muscle conditions during isometric contractions. The trend of a phase graph is similar to reactance where it steadily increases at lower frequencies then

decreases at higher frequencies. Instead of peaking between 30-50kHz, it was noticed that the phase peaked between 50-100kHz. At 10kHz, the average phase slope was 8.15°, at 50kHz it was 15.09°, 100kHz had an average value of 15.57°, and 150kHz produced an average slope of 15.11°.

Table 3. Average Phase for Biceps Brachii during Isometric Contractions

Muscle Condition	*Frequency*			
	10 kHz	**50 kHz**	**100 kHz**	**150 kHz**
Rest	9.062°	15.025°	15.51°	14.727°
100% MVIC	7.688°	15.357°	15.935°	15.347°
50% MVIC	7.982°	14.948°	15.322°	14.518°
25% MVIC	7.939°	15.023°	15.508°	15.858°

4.3 Relationship of Muscle Fatigue and EIM

The effects of muscle fatigue on impedance was the second significant muscle condition that was studied. Muscle fatigue is the inability of a muscle to generate a normal force (Kent-Braun 1999). It occurs when the body temporarily exhausts its supply of energy after strenuous exercise or overuse. The strenuous activity in this research came about from a repetitive upper limb movement designed to fatigue the biceps brachii. The impedance graphs over the full frequency spectrum are first presented in Figure 12 to show an overall trend. Then for discussion purposes, the impedance graphs at 10, 50, 100, and 150kHz are illustrated in Figure 13.

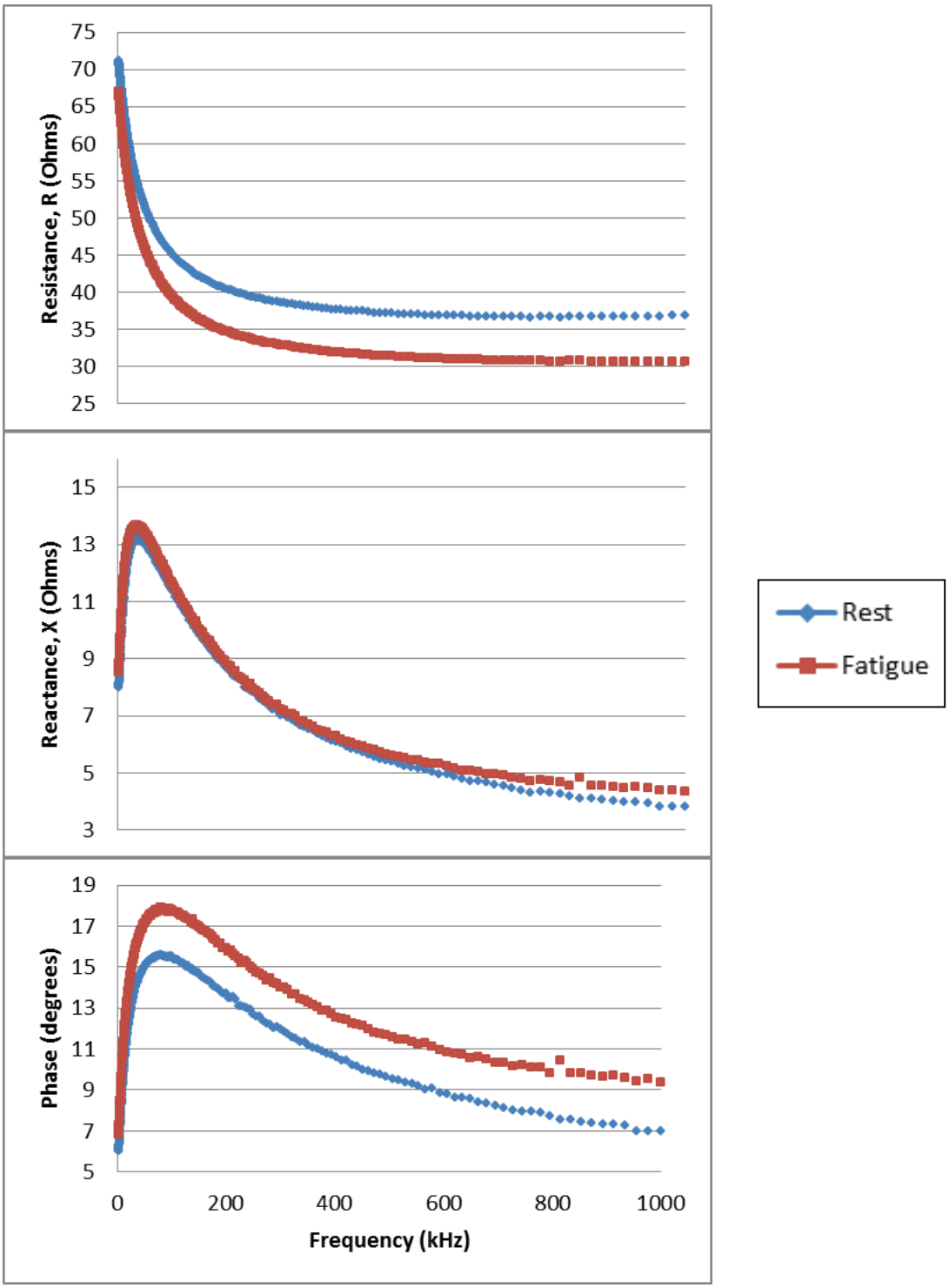


Figure 12: Resistance, Reactance, and Phase versus Frequency of Muscle Fatigue on Biceps Brachii from 3kHz to 1000kHz.

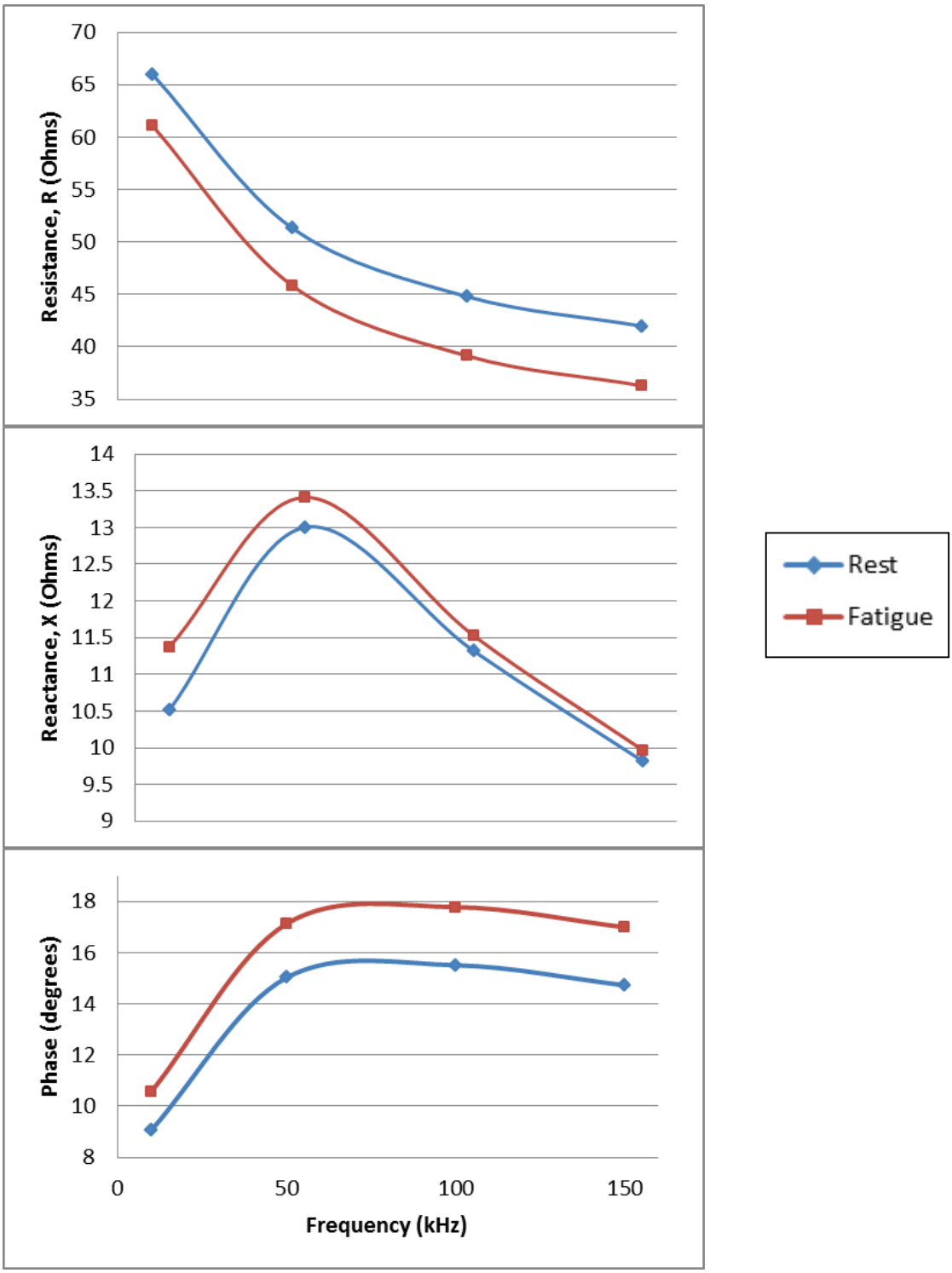


Figure 13: EIM at 10kHz, 50kHz, 100kHz, and 150kHz for six healthy males during muscle fatigue on the biceps brachii. At rest measurements are represented by diamonds and muscle fatigue is denoted by squares.

From the results, it was concluded that there was an incongruity of impedance between muscle fatigue and muscles at rest similar to the effects of isometric contractions. From Table 4, there was a drop in resistance values during muscle fatigue for all four frequencies. The average decrease in resistance during muscle fatigue was 11.24% from the muscle at rest. The highest resistance values were calculated at the 10kHz frequency whereas the lowest resistance values were found at 150kHz.

Table 4. Average Resistance for Biceps Brachii during Muscle Fatigue

Muscle Condition	*Frequency*			
	10 kHz	**50 kHz**	**100 kHz**	**150 kHz**
Rest	66.018 Ω	51.327 Ω	44.792 Ω	41.975 Ω
Fatigue	61.12 Ω	45.842 Ω	39.135 Ω	36.29 Ω

This phenomenon solidifies the theories presented in section 4.2, but further justifications can be explained since it is in a completely different stage than isometric contractions. Before impedance data was collected, bicep girth was taken at rest and during muscle fatigue using a flexible tape measure. The average bicep girth at rest was 12.375" whereas the average girth measurement after muscle fatigue was 12.92". This increase in muscle area is the most influential cause of a decrease in resistance. From equation 2 [$R=(\rho l)/A$], there is a direct relationship between resistance and area of the muscle. As area increases, the resistance decreases. The growth in bicep girth came about from the expansion of muscle fibers with increased blood flow. Muscle fibers are

flexible and can expand to allow more blood and oxygen to enter during exercise (McComas, et al. 1968). An excess amount of enlargement will result in an exponential growth that can be measured physically.

Table 5 provides reactance values at 10, 50, 100, and 150kHz for the bicep at rest and during muscle fatigue. Unlike the decrease of reactance for isometric contractions, muscle fatigue produced an increase in reactance as compared to rest. The reactance at each frequency increased slightly with an average difference of 3.58% throughout the four data points. A peak reactance was observed at 50kHz with a value of 13.412 Ω during fatigue. It decreased to 11.525 Ω at 100kHz and dropped even lower at 150kHz with a reactance of 9.967 Ω.

Table 5. Average Reactance for Biceps Brachii during Muscle Fatigue

Muscle Condition	*Frequency*			
	10 kHz	**50 kHz**	**100 kHz**	**150 kHz**
Rest	10.517 Ω	13.00 Ω	11.312 Ω	9.815 Ω
Fatigue	11.375 Ω	13.412 Ω	11.525 Ω	9.967 Ω

An initial expectation of resistance and reactance for muscle fatigue was to perceive the same changes as isometric contractions; however, there was a positive observed change in reactance during muscle fatigue. This contradictory evidence posed many intrinsic implications that could help explain the relationship of reactance and muscle fatigue. The most fundamental explanation is to investigate the reactance formula

in equation 7 [$X_c=1/(2\pi fC)$]. With frequency termed as an obsolete variable, an increase in the reactance must result from a decrease in capacitance. During isometric contractions, the capacitance increased due to an increased blood flow that allowed more capacitive ions to stick to the muscle membranes. Contrary to what was discovered in that muscle state, the capacitance during muscle fatigue decreased even though both muscle conditions underwent dynamic changes. Closer analysis deemed the reactance changes to be purely physiological that required a great extent of human skeletal muscle research during fatigue. The biceps brachii experiences a significant transformation of muscle fiber composition during muscle fatigue, which distinguishes itself from isometric contractions. A muscle is fatigued when it can no longer generate a force. A muscle can no longer generate a force when its fibers are pushed beyond its maximum capacity and begin to produce minor tears. These minor tears result in the impaired function of contractile proteins along the muscle fiber (Allen, Lamb and Westerblad 2008) which prohibit the muscle to perform a contraction. With a loss of muscle fiber connections, the amount of capacitive ions decreases, consequently increasing the reactance of the biceps brachii.

Table 6 shows the average phase values at each frequency during muscle fatigue. The relationship between phase and fatigue is more prominent than the study performed during isometric contractions. Phase increased after muscle fatigue for all four frequency values with an average amplification of 13.69%. It peaked between 50kHz and 100kHz and decreased at higher frequencies. When the muscle was at rest, the phase was 15.025° at 50kHz, and increased to 17.128°.

Table 6. Average Phase for Biceps Brachii during Muscle Fatigue

Muscle Condition	*Frequency*			
	10 kHz	**50 kHz**	**100 kHz**	**150 kHz**
Rest	9.062°	15.025°	15.51°	14.727°
Fatigue	10.565°	17.128°	17.778°	16.995°

Mathematically, phase is directly affected by reactance and resistance. When the reactance increased and resistance decreased during fatigue, it maintains an aggregate positive ratio. For example, the ratio between resistance and reactance at 50kHz was 0.255 during rest, whereas the ratio for fatigue was 0.293. This ratio increase at each frequency produced a positive upshot in phase through the muscle fatigue condition.

CHAPTER 5

CONCLUSION AND FUTURE WORK

5.1 Summary of Research Study

Electrical impedance myography (EIM) is a new technique used for diagnosing the conditions of muscles. It relies on a high-frequency, low intensity current to derive the parameter of impedance that is affected by resistance and reactance. With the human muscle naturally organized in an anisotropic structure, a voltage measurement can be calculated to determine the muscle fiber composition during isometric contractions and fatigue. It was observed that resistance and reactance both decreased steadily as the isometric contraction increased. 25% of the maximum isometric contraction contributed to the least amount of change as compared to the muscle at rest, whereas the full 100% maximum isometric contraction had the greatest influence of resistance and reactance changes. This can be explained by changes in the physiological properties of muscles as they undergo isometric contractions. Increasing the force will lead to increased blood flow to provide an equivalent amount of muscle activation to sustain the force. Blood consists of saline which is a conductive substance. When an isometric contraction forces more blood flow, the conductance will increases, resulting in a decrease in resistance. Similarly, it provides more capacitive metabolic ions that stick to intercellular and extracellular membranes along the muscle fibers. These ions increase the capacitance of the bicep, consequently decreasing the reactance.

During muscle fatigue, the bicep experiences dynamic changes in both physiological and morphological forms. A muscle is fatigued when it has exhausted all of its energy and can no longer generate a normal force. During this research, the muscle became fatigued after repetitive bicep curls were performed by the subject until full movement could not be completed. Impedance data was collected in the same position when the muscle was at rest to ensure no force would interfere with measurements. It was observed that resistance decreased during muscle fatigue, but reactance increased. Moreover, the bicep girth enlarged from 12.375" at rest to 12.92" after fatigue. This amplification in muscle area causes a decrease in resistance that can be comprehended in equation 2. The bicep girth increase came about from the muscle membrane expansion that occurred to allow maximum blood flow for all active fibers. As muscles reach fatigue, more fibers are recruited to utilize its full potential, thus increasing muscle mass. Contrary to what was observed in the reactance of isometric contractions, the reactance during muscle fatigue increased at all frequencies. This increase in reactance was not expected but can be explained by qualitative relationships within muscle tissue. It was studied that muscle fibers are pushed beyond its limits and begin to produce minor tears during fatigue. The torn fibers lead to the impairment of contractile proteins that allow the muscle to contract. A loss of muscle fibers reduced the amount of muscle capacitance of the bicep, ultimately increasing the reactance.

5.2 Future Research with EIM

This research study on isometric contractions and muscle fatigue using electrical impedance myography has created a precedent of groundbreaking knowledge that opened an avenue of future research. A discussion of physiological and morphological changes on the biceps brachii has been explained, but it may not be the sole cause of impedance changes. One particular observation that may have contributed to the change in resistance and reactance was the increase in skin temperature from rest to after both exercises were completed. The spike in temperature was more noticeable after muscle fatigue, but a temperature difference was felt in both conditions. It was difficult to determine if the temperature increase spawned from the underlying muscle or only the skin surface, but both studies should be researched. Using isometric contractions and muscle fatigue, an investigation on various temperatures of the biceps brachii could produce substantial information to EIM changes.

REFERENCES

Aaron, Ronald, M Huang, and Carl Shiffman. "Aniostropy of Human Muscle via Non-Invasive Impedance Measurements." *Physics in Medicine and Biology 42*, 1997: 1245-1262.

Ahad, Mohammad A, Travis D Orth, Nazmul Islam, and Mohammed Ferdjallah. "Simulation of EMG Signals for Aging Muscle." *IEEE.* 2012. 5.

Alciatore, David G, and Michael B Histand. *Introduction to Mechatronics and Measurement Systems.* New York, NY: McGraw-Hill 4th ed., 2012.

Allen, D G, G D Lamb, and H Westerblad. "Skeletal Muscle Fatigue: Cellular Mechanisms." *Physiological Reviews*, 2008: 287-332.

Chin, Anne B., Lindsay P Garmirian, Rui Nie, and Seward B Rutkove. "Optimizing Measurement of the Electrical Anisotropy of Muscle." *Muscle & Nerve 37, no. 5*, May 2008: 560-565.

Dorf, Richard C, and James A Svoboda. *Introduction to Electric Circuits.* Hoboken, NJ: John Wiley & Sons, Inc. 8th ed., 2010.

Esper, Gregory J, Carl A Shiffman, Ronald Aaron, Kyungmouk S Lee, and Seward B Rutkove. "Accessing Neuromuscular Disease with Multifrequency Electrical Impedance Myography." *Muscle & Nerve 34*, June 2006: 595-602.

Gekht, B. *Theoretical and Clinical Electromyography.* Leningrad: Nauka, 1990.

Goodman, Laurie. "Adding Muscle to Neuromuscular Degeneration Investigation." *Journal of Clinical Investigation 114, no. 3.* August 2004. http://www.jci.org/articles/view/22641 (accessed May 20, 2013).

Hardyck , C, L Petrincovich, and D Ellsworth. "Feedback of Speech Muscle Activity During Silent Reading: Rapid Extension." *Science*, 1966: 154.

Humphreys, P, and A Lind. "The Blood Flow Through Active and Inactive Muscles of the Forearm During Sustained Hand-Grip Contractions." *Journal of Physiology 166*, 1963: 120-135.

Ihara, Ikuo. "Ultrasonic Sensing: Fundamental and its Applications to Nondestructive Evaluation." *Lecture Notes Electrical Engineering 21*, 2008: 287-305.

Kaplanis, PA, CS Pattichis, LJ Hadjileontiadis, and VC Roberts. "Surface EMG Analysis on Normal Subjects Based on Isometric Voluntary Contraction." *Journal of Electromyography and Kinesiology 19*, 2009: 157-171.

Kent-Braun, Jane A. "Central and Peripheral Contributions to Muscle Fatigue in Humans During Sustained Maximal Effort." *European Journal of Applied Physiology*, 1999: 57-63.

McComas, A J, K Mrozek, D Gardner-Medwin, and W H Stanton. "Electrical Properties of Muscle Fibre Membranes in Man." *J Neurol Neurosurg Psychiatry*, 1968: 434-440.

Merletti, R, D Farina, M Gazzoni, and M Schieroni. "Effect of Age on Muscle Functions Investigated with Surface Electromyography." *Muscle & Nerve 25, no. 1*, 2002: 65.

Peetrons, P. "Ultrasound of Muscles." *European Radiology 12, no. 1*, January 2002: 35-43.

Reimers, Carl D, James L Fleckenstein, Thomas N Witt, Wolfgang Muller-Felber, and Dieter E Pongratz. "Muscular Ultrasound in Idiopathic Inflammatory Myopathies of Adults." *Journal of Neurological Sciences 116, no. 1*, May 1993: 82-92.

Rutkove, Seward B. "Electrical Impedance Myography: Background, Current State, and Future Directions." *Muscle & Nerve 40, no. 6*, December 2009: 936-946.

Rutkove, Seward B, Patricia M Fogerson, and Andrew W Tarulli. "Reference Values for 50-kHZ Electrical Impedance Myography." *Muscle & Nerve 38, no. 3*, 2008: 1128-1132.

Rutkove, Seward B, Ramon A Partida, Gregory J Esper, Ronald Aaron, and Carl A Shiffman. "Electrode Position and Size in Electrical Impedance Myography." *Clinical Neurophysiology 116*, 2005: 290-299.

Shiffman, Carl A, H Kashuri, and R Aaron. "Electrical Impedance Myography at Frequencies up to 2 MHz." *Physiological Measurement 29, no. 6*, 2008: 345-363.

Shiffman, Carl A, Ronald Aaron, and Seward B Rutkove. "Electrical Impedance of Muscle During Isometric Contraction." *Phiological Measurement 24*, February 2003: 213-234.

Turker, Kemal S. "Electromyography: Some Methodological Problems and Issues." *Journal of the American Physical Therapy Association 73, no. 10*, October 1993: 698-710.

APPENDIX A

RAW DATA OF IMPEDANCE, PHASE, RESISTANCE, AND REACTANCE FOR EACH SUBJECT

Subject 1		10kHz	50kHz	100kHz	150kHz
Bicep: Rest	Z (Ω)	80.04	63.72	57.48	54.84
	Phase (°)	11.56	12.36	10.82	9.52
	R (Ω)	78.42	62.24	56.46	54.08
	X (Ω)	16.04	13.64	10.79	9.07

Subject 1		10kHz	50kHz	100kHz	150kHz
Bicep: 100%	Z (Ω)	62.29	51.27	45.7	43.05
	Phase (°)	6.69	11.85	11.66	10.78
	R (Ω)	61.87	50.18	44.76	42.29
	X (Ω)	7.25	10.52	9.24	8.06

Subject 1		10kHz	50kHz	100kHz	150kHz
Bicep: 50%	Z (Ω)	67.29	53.57	48.14	45.98
	Phase (°)	9.08	11.79	10.47	9.2
	R (Ω)	66.45	52.44	47.34	45.39
	X (Ω)	10.62	10.95	8.74	7.35

Subject 1		10kHz	50kHz	100kHz	150kHz
Bicep: 25%	Z (Ω)	75.22	60.13	54.2	51.68
	Phase (°)	10.33	12.23	10.96	9.67
	R (Ω)	74	58.76	53.21	50.94
	X (Ω)	13.49	12.74	10.3	8.68

Subject 1		10kHz	50kHz	100kHz	150kHz
Bicep: Fatigue	Z (Ω)	63.7	50.67	44.32	41.45
	Phase (°)	8.1	13.61	13.59	12.45
	R (Ω)	63.07	49.25	43.08	40.47
	X (Ω)	8.97	11.92	10.41	8.94

Subject 2		10kHz	50kHz	100kHz	150kHz
Bicep: Rest	Z (Ω)	75.02	57.92	49.7	46.07
	Phase (°)	8.58	15.38	15.29	13.97
	R (Ω)	74.18	55.85	47.94	44.71
	X (Ω)	11.19	15.36	13.11	11.13

Subject 2		10kHz	50kHz	100kHz	150kHz
Bicep: 100%	Z (Ω)	57.19	44.22	37.87	35.1
	Phase (°)	0.26	15.4	17.74	18.61
	R (Ω)	57.19	42.63	36.07	33.26
	X (Ω)	0.26	11.76	11.54	11.2

Subject 2		10kHz	50kHz	100kHz	150kHz
Bicep: 50%	Z (Ω)	62.04	48.21	42.06	39.42
	Phase (°)	2.04	14.32	15.84	16.02
	R (Ω)	62	46.71	40.46	37.89
	X (Ω)	2.21	11.92	11.48	10.88

Subject 2		10kHz	50kHz	100kHz	150kHz
Bicep: 25%	Z (Ω)	65.67	51.23	44.77	41.91
	Phase (°)	0.942	14.24	15.91	16.25
	R (Ω)	65.66	49.66	43.06	40.24
	X (Ω)	1.08	12.6	12.6	11.73

Subject 2		10kHz	50kHz	100kHz	150kHz
Bicep: Fatigue	Z (Ω)	71.4	57.8	44.38	40.86
	Phase (°)	14.69	18.62	18.08	16.75
	R (Ω)	69.07	50.03	42.19	39.12
	X (Ω)	18.11	16.86	13.77	11.78

Subject 3		10kHz	50kHz	100kHz	150kHz
Bicep: Rest	Z (Ω)	55.81	45.7	40.49	38.19
	Phase (°)	7.07	12.21	12.23	11.22
	R (Ω)	55.39	44.66	39.57	37.46
	X (Ω)	6.87	9.67	8.58	7.43

Subject 3		10kHz	50kHz	100kHz	150kHz
Bicep: 100%	Z (Ω)	53.19	42.71	37.92	35.8
	Phase (°)	7.78	12.4	11.99	10.83
	R (Ω)	52.7	41.72	37.09	35.16
	X (Ω)	7.2	9.17	7.88	6.73

Subject 3		10kHz	50kHz	100kHz	150kHz
Bicep: 50%	Z (Ω)	53.64	43.72	38.72	36.53
	Phase (°)	7.13	12.23	11.8	10.72
	R (Ω)	53.23	42.73	37.9	35.89
	X (Ω)	6.65	9.26	7.92	6.8

Subject 3		10kHz	50kHz	100kHz	150kHz
Bicep: 25%	Z (Ω)	53.98	44.28	39.21	36.88
	Phase (°)	7.13	12.33	11.99	11.06
	R (Ω)	53.56	43.25	38.35	36.2
	X (Ω)	6.7	9.45	8.14	7.07

Subject 3		10kHz	50kHz	100kHz	150kHz
Bicep: Fatigue	Z (Ω)	52.41	42.17	37.07	34.76
	Phase (°)	7.8	13.24	13.16	12.32
	R (Ω)	51.93	41.05	36.1	33.96
	X (Ω)	7.11	9.66	8.44	7.42

Subject 4		10kHz	50kHz	100kHz	150kHz
Bicep: Rest	Z (Ω)	60.38	45.27	37.18	33.3
	Phase (°)	10.23	19.54	21.03	20.1
	R (Ω)	59.42	42.66	34.7	31.27
	X (Ω)	10.73	15.14	13.34	11.44

Subject 4		10kHz	50kHz	100kHz	150kHz
Bicep: 100%	Z (Ω)	58.99	42.35	34.6	31.17
	Phase (°)	12.11	20.58	20.87	19.36
	R (Ω)	57.67	39.65	32.34	29.41
	X (Ω)	12.38	14.89	12.33	10.33

Subject 4		10kHz	50kHz	100kHz	150kHz
Bicep: 50%	Z (Ω)	59.69	43.4	35.39	31.84
	Phase (°)	11.73	20.31	20.89	19.56
	R (Ω)	58.45	40.7	33.07	30
	X (Ω)	12.13	15.07	12.62	10.66

Subject 4		10kHz	50kHz	100kHz	150kHz
Bicep: 25%	Z (Ω)	60.12	43.66	35.58	31.96
	Phase (°)	11.72	20.59	21.44	20.22
	R (Ω)	58.87	40.87	33.12	29.99
	X (Ω)	12.21	15.35	13	11.05

Subject 4		10kHz	50kHz	100kHz	150kHz
Bicep: Fatigue	Z (Ω)	55.71	40.53	32.34	28.27
	Phase (°)	11.34	22.53	25.14	24.9
	R (Ω)	54.63	37.44	29.28	25.64
	X (Ω)	10.96	15.53	13.74	11.9

Subject 5		10kHz	50kHz	100kHz	150kHz
Bicep: Rest	Z (Ω)	43.68	33.09	27.01	23.69
	Phase (°)	9.64	20.78	24.09	24.51
	R (Ω)	43.06	30.93	24.66	21.56
	X (Ω)	7.31	11.74	11.02	9.83

Subject 5		10kHz	50kHz	100kHz	150kHz
Bicep: 100%	Z (Ω)	41.02	29.93	24.04	21.25
	Phase (°)	11.5	22.1	24.44	24.44
	R (Ω)	40.19	27.73	21.89	19.34
	X (Ω)	8.17	11.26	9.95	8.79

Subject 5		10kHz	50kHz	100kHz	150kHz
Bicep: 50%	Z (Ω)	41.73	30.65	24.73	21.68
	Phase (°)	10.97	21.79	24.55	24.12
	R (Ω)	40.97	28.46	22.5	19.79
	X (Ω)	7.94	11.38	10.27	8.86

Subject 5		10kHz	50kHz	100kHz	150kHz
Bicep: 25%	Z (Ω)	42.37	31.39	25.41	22.3
	Phase (°)	10.7	21.58	24.5	24.54
	R (Ω)	41.64	29.19	23.12	20.29
	X (Ω)	7.87	11.55	10.54	9.26

Subject 5		10kHz	50kHz	100kHz	150kHz
Bicep: Fatigue	Z (Ω)	45.8	32.74	25.91	22.66
	Phase (°)	12.44	23.45	26.1	25.94
	R (Ω)	44.72	30.03	23.27	20.38
	X (Ω)	9.87	13.03	11.4	9.91

Subject 6		10kHz	50kHz	100kHz	150kHz
Bicep: Rest	Z (Ω)	86.34	72.7	66.35	63.56
	Phase (°)	7.29	9.88	9.6	9.04
	R (Ω)	85.64	71.62	65.42	62.77
	X (Ω)	10.96	12.47	11.06	9.99

Subject 6		10kHz	50kHz	100kHz	150kHz
Bicep: 100%	Z (Ω)	83.19	69.33	63.58	61.27
	Phase (°)	7.79	9.81	8.91	8.06
	R (Ω)	82.42	68.32	62.82	60.67
	X (Ω)	11.28	11.82	9.85	8.59

Subject 6		10kHz	50kHz	100kHz	150kHz
Bicep: 50%	Z (Ω)	84.8	71.24	65.67	63.4
	Phase (°)	6.94	9.25	8.38	7.49
	R (Ω)	84.18	70.32	64.97	62.86
	X (Ω)	10.25	11.45	9.58	8.27

Subject 6		10kHz	50kHz	100kHz	150kHz
Bicep: 25%	Z (Ω)	85.21	72.34	66.46	64.04
	Phase (°)	6.81	9.17	8.25	7.41
	R (Ω)	84.61	71.42	65.77	63.5
	X (Ω)	10.11	11.53	9.54	8.26

Subject 6		10kHz	50kHz	100kHz	150kHz
Bicep: Fatigue	Z (Ω)	84.35	68.59	61.94	59
	Phase (°)	9.02	11.32	10.6	9.61
	R (Ω)	83.3	67.25	60.89	58.17
	X (Ω)	13.23	13.47	11.39	9.85