

ABSTRACT

Visualizing Muscular Fatigue During Running Exercises with Electromyography-Derived Variables

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Runners commonly face injuries due to fatigue and overuse. However, it is difficult to know when the body is too fatigued to continue a task without damage. This study attempted to observe if fatigue occurred in the leg muscles, specifically both gastrocnemius muscles and the vastus lateralis, during a running trial using electromyography to record the muscle activation signals. Post-processing methods calculated four parameters to detect fatigue: mean frequency, root mean square amplitude, 2nd order Dimitrov spectral index, and fractal dimension. A validation trial with calf raises proved the reliability of these parameters. Results of the running trials showed that both gastrocnemius muscles had their mean frequency and fractal dimension increase, while the root mean square and the spectral index decreased. The vastus lateralis only differed in the spectral index parameter from the gastrocnemius results. These results indicate that muscle recruitment differs in low workload, endurance tasks than strength-based exercises, but also requires future work to corroborate these results.

Visualizing Muscular Fatigue During Running Exercises With Electromyography-Derived
Variables

by

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DEDICATION

To all the real ones. You know who you are.

CHAPTER ONE

Introduction

Humans are active creatures. They run, jump, and use their bodies for all sorts of high intensity activities. However, using these bodies too much can result in injuries that require downtime or even surgery. Knowing when the body is tired is imperative to avoiding bodily harm.

A common activity that can cause injury to anyone, from casual exercisers to professional athletes, is running. Running is one of the most popular forms of exercise today and is growing in popularity. According to statista.com, from 2006 to 2017, the number of people running in the United States has grown from 38.72 million to 55.9 million. This is an increase of 17.18 million participants in only eleven years [1].

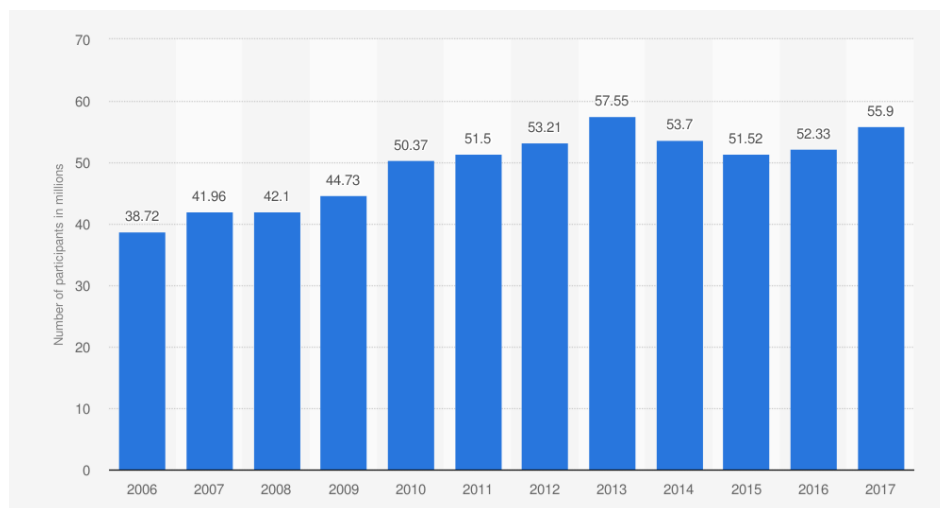


Fig.1.1. Number of participants in running, jogging, and trail running in the U.S. from 2006-2017 (in millions) [1]

In a given year, the possibility of the average runner developing an injury is between 37% and 56%. In fact, for every one-thousand hours of running that a runner completes, they will receive anywhere from 2.5 to 12.1 injuries. A majority of these injuries are overuse injuries, reported to be between 50% to 75% of all running injuries received [2]. Some of these injuries include stress fractures, shin splints, runner's knee, Achilles tendinitis, and iliotibial band syndrome [3].

Even though these injuries are overuse injuries, runners do not always know that these injuries are occurring while they are running. Running fatigue has a slow onset and results in a gradual weakening of proper form. While this weakened form can decrease the load on the body, it also decreases the ability of the muscles to absorb the impact of each step, which can develop injuries in the body [4].

It is not until after the run that the runner realizes that they pushed themselves too hard or broke form when they exhausted themselves and received an injury. If a runner knew that their body was tired, they could reduce the intensity of their run, or stop altogether, in order to avoid damage to their body. Since anywhere from 30% to 90 % of running injuries cause the participant to reduce or cease their training, it is imperative that the participants are aware of their body's exhaustion level in order to avoid injury and continue their training plan [2].

This research will attempt to bridge the gap between understanding muscle fatigue and observing its progression during running exercises. Utilizing a surface electromyography system, a device that can measure the brain's electric signals to the muscles, the key muscles in the leg will be measured during a fatiguing run to see the

changes in the signal. Also, this research will help to develop the tools that can make further advanced studies possible in the field of electromyography.

The upcoming chapters will explain the research in detail. Chapter Two will explain the background information and previous literature to explain this piece of research's place in the field, as a whole. Chapter Three will review the pilot testing involved in this research, its results, and how it impacted the core research. The next chapter, Chapter Four, will explain the methods used to conduct the research. The two following chapters, Chapters Five and Six, will contain the results of the research and discuss their impact, as well as future work and limitations of the research.

CHAPTER TWO

Background Info

Motivation

For runners, whether casual or athletic, the body's form during the exercise is pivotal to performance, as well as in injury prevention. Unfortunately, due to muscle physiology, this form tends to break down as the body fatigues over the course of the exercise. This can cause multiple injuries, such as tibial stress fracture and tendon damage [2],[3].

In this chapter, there will be a quick overview of muscle function from an anatomical perspective and its general behaviors and functions. This is followed by an explanation of muscle contractions, fatigue, and types of muscle twitch fibers. A summarized history of electromyography is then presented, along with a synopsis of how electromyography functions. Finally, the various processing methods are introduced, and a brief overview of background literature is discussed.

Muscle Anatomy

There are three categories of muscles: skeletal, cardiac, and smooth. Cardiac and smooth muscles are involuntary and control the contractions of the heart and internal organs. Skeletal muscles attach to bone and allow voluntary muscle contractions and locomotion [5].

Skeletal muscle components are arranged by size: muscle, fascicle, fiber, myofibril, and sarcomere, with each component comprised of a grouping of the lower

hierarchical muscle unit. For example, a muscle consists of several fascicles, and fascicles consist of several fibers. Sarcomeres, the smallest muscle component, is approximately 2-3 μm in length and consists of myosin filaments held between two actin filament caps, as well as a store of adenosine triphosphate (ATP) and glucose for energy.

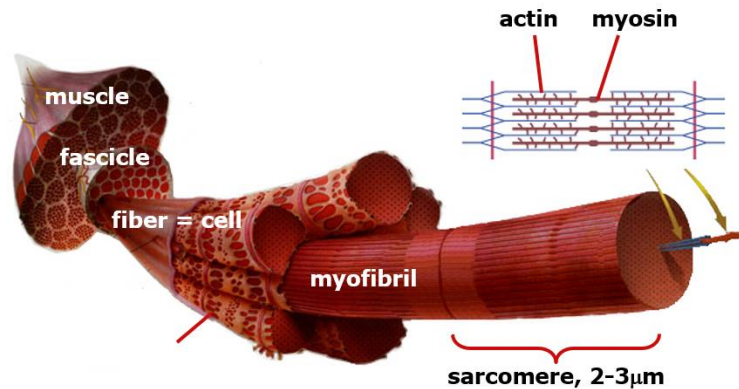


Fig. 2.1. Muscle anatomy hierarchy [6]

Muscles contract when a motor neuron from the brain sends an action potential down an axon to a motor end plate, which is where the motor neuron meets the muscle fibers assigned to it. This causes acetylcholine (ACh) to be released at the junction, opening up calcium channels in the muscle and filling it with calcium.

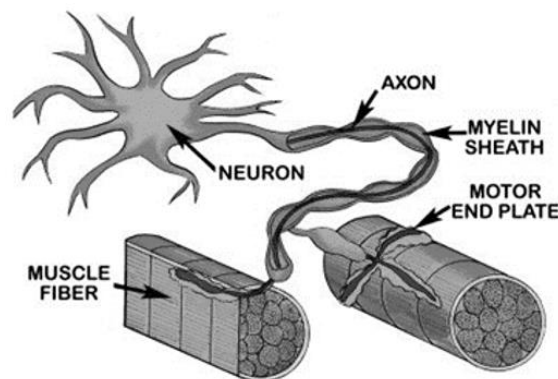


Fig. 2.2. Neuron-muscle connections of motor units [7]

The sarcomere, and thus the muscle as a whole, is able to contract when the stems from the myosin attach to the actin and use the cell's ATP as energy pull the two actin caps together. When the calcium channels open, the calcium binds with a protein inside the sarcomere called troponin, a protein that prevents myosin and actin from attaching to each other, and allows the actin and myosin to attach and contract. This allows the muscle to contract and become shorter. The contraction reaches a threshold when the myosin hits the end of the actin caps, preventing the sarcomere from getting any shorter.

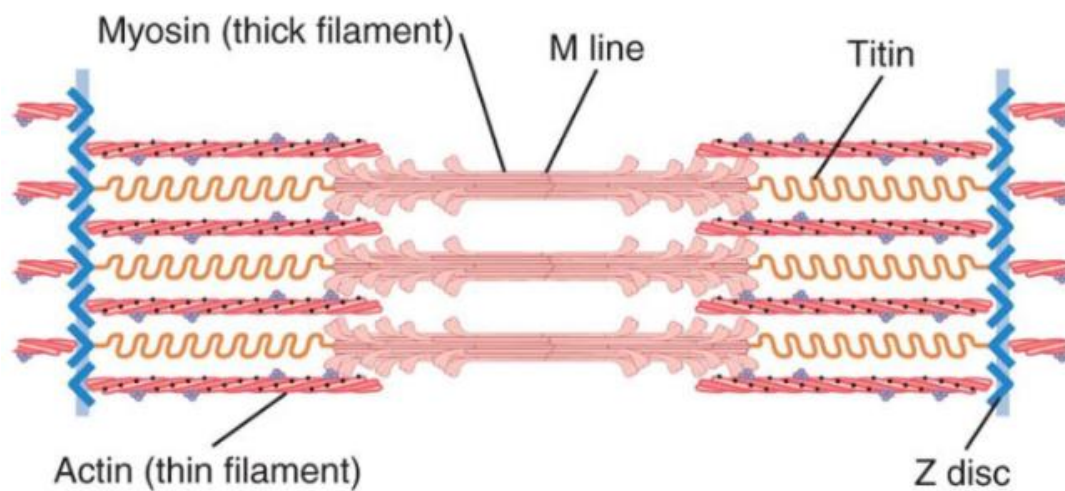


Figure 2.3. Sarcomere components [8]

Since the actin and myosin pull together during contraction and slip apart during relaxation, the muscle force output has a dependency on length. When the muscle is stretched, the myosin tip is pulled close to the opening of the actin cap. The myosin stems have little overlap with the actin and can only make a few connections. This decreases the available force until the muscle is able to contract enough to allow the myosin stems to make more connections. There is an optimal point during contraction where the myosin is contracted enough to make many connections to the actin with its stems but has not hit

the actin cap yet. The force tapers off, however, as the myosin reaches the end of the contraction and squeezes against the Z disc line, which is a protein band at the end of the actin cap [5],[7],[9].

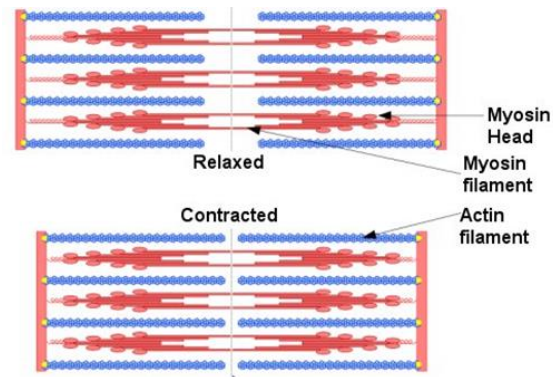


Figure 2.4. Sarcomere in relaxed state and contracted state [9]

Motor Units

Muscle fibers are controlled by many different motor neurons. A single motor neuron can control anywhere from ten to over a thousand muscle fibers at once. The less fibers a neuron controls, the finer the movement allowed from the muscle. For example, the neurons that control the muscles of the fingers, which need dextrous and fine movement, control much fewer muscles than the neurons that control the thighs, which do not need as much fine control as the fingers.

A motor unit is a set of a motor neuron and all the muscle fibers that the neuron controls. Therefore, a single muscle is made of many motor units. During a contraction, not all muscle fibers are taking part in performing the contraction. The brain determines how many motor units are needed to complete a task and activates them, keeping the rest in reserve to replace fatigued fibers. Small motor units are activated first, and then larger motor units are recruited when the muscle requires more force to complete the task.

When a motor unit contracts due to a single action potential, the contraction is called a muscle twitch. There are three sections of a muscle twitch: latent period, contraction period, and relaxation period. The latent period, usually just a few milliseconds, is the time period starting when the action potential reaches the muscle and ends just before observable muscle activity is observed. The contraction period is the time frame from when the muscle begins to contract and generates force up to the point before it relaxes. Finally, the relaxation period is when the muscle has ceased contracting and begins to return to its normal length. The total time length for this twitch could be anywhere from ten to a hundred milliseconds, depending on the muscle.

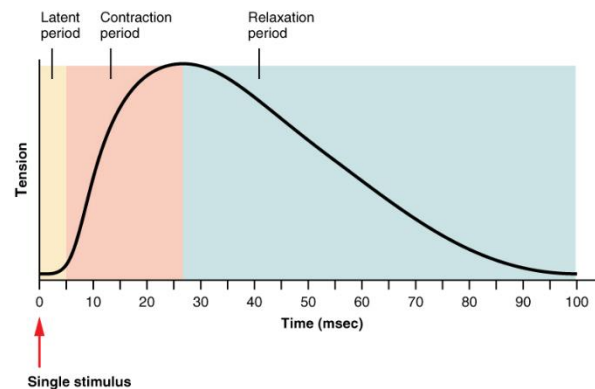


Figure 2.5. Twitch potential stages [10]

If a second action potential is received before the muscle can completely relax, the muscle will contract again, but stronger. More action potentials received before relaxation can occur results in larger and larger force outputs. If a muscle receives enough action potentials fast enough, it can reach its Maximum Voluntary Contraction (MVC), which is the maximum amount of force a specific muscle can output [7],[10].

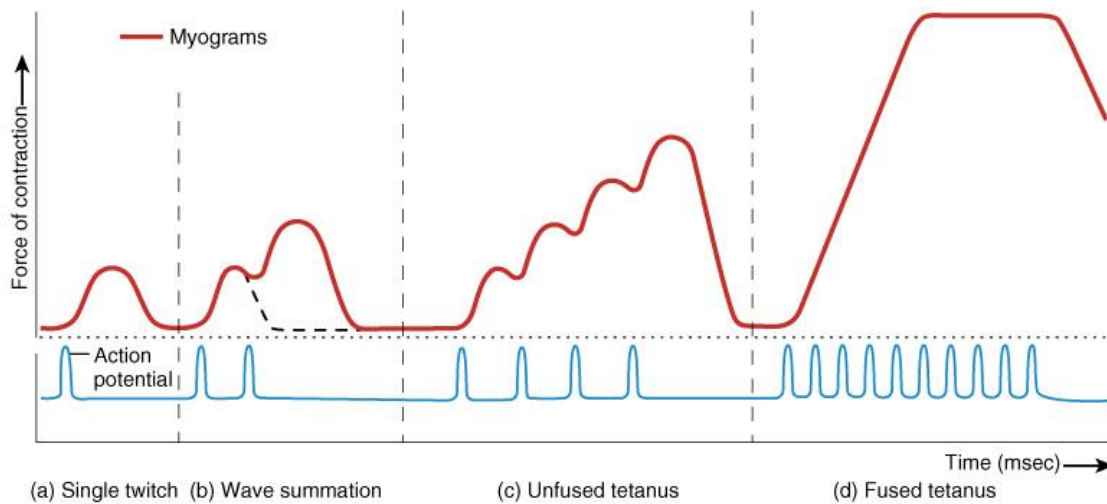


Figure 2.6. Force output with a) a single twitch action potential, b) a summation of two twitch potentials, c) several summed twitch potentials, and d) many twitch potentials with a high rate of stimulation [11]

Fatigue

The most common phenomena that prevents a muscle from contracting is when there is no more ATP left for the muscle to continue contracting with the current workload applied to the muscle. When the myosin stems attach to the actin, there is not enough power for it to pull at the actin and contract the muscle. This is known as muscular fatigue.

Muscle fibers are divided into two functional subgroups: Type I (slow-twitch) and Type II (fast-twitch). Every muscle in the body is comprised of a mix of both fiber types. Slow-twitch is an oxidative muscle fiber type, meaning it is able to efficiently convert oxygen to energy. This allows slow-twitch muscle fibers to continue to contract for long periods of time. However, slow-twitch fibers have the smallest diameter, and therefore have low power output.

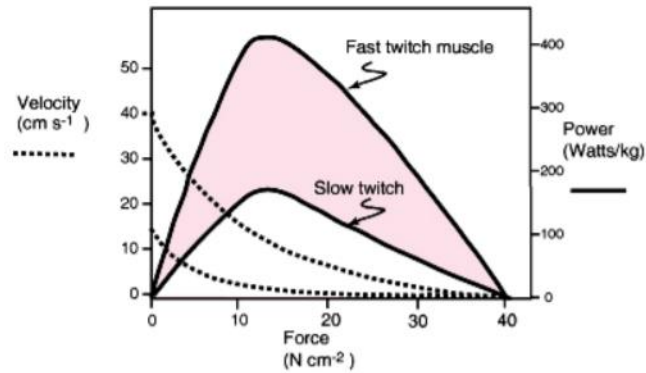


Fig. 2.7. Velocity and power for fast twitch vs. slow twitch muscle fibers [12]

Fast-twitch muscles are divided into Type IIa and Type IIb. Type IIb is glycolytic, meaning it gets its energy from stored glycogen. This means that the muscle fibers fatigue faster as it depends on energy stored that doesn't replenish quickly during activities. These muscle fibers create high force and speed compared to the other two fiber types. Type IIa are an "in-between" muscle fiber that relies on stored glycogen and oxygen intake. However, it isn't as efficient in using these energy sources as the other two muscle fiber types. The generated force and fatigue rate lie in the middle of Type I and Type IIb. Table 2.1 summarizes fiber type behavior.

Table 2.1. Muscle fiber characteristics

Characteristics	Slow Twitch, Type I	Fast Twitch, Type IIA	Fast Twitch, Type IIB
Twitch Speed	Slow	Fast	Fast
Twitch Force	Low	Medium	High
Resistance to Fatigue	High	Medium-High	Low

For most muscle contractions, the body relies on the Henneman's Size Principle, that states that a muscle relies first on small motor units, which are typically slow twitch

muscles, and slowly increases the amount and size of motor units to match the intensity of the current action. As the fibers begin to fatigue, the body starts to replace fatigued fast twitch fibers with multiple slow twitch fibers in order to continue to match the output force of the fatigued fast twitch fibers [10].

History of EMG

Electromyography (EMG) is a method of measuring the signals sent from the brain to the muscles and the muscles response to the electrical stimulation of the nerve endings. It involves placing a pair of electrodes, either metal plates or needles, over a muscle of interest, measuring the voltage potential between the two, and transmitting the signal to a receiver for recording.

The basics of nerves and their electrical potential started in the late 1700's when Luigi Galvani accidentally discovered that nerves, not only could be controlled via an outside electrical source but also conduct electricity on their own. Up to this point, the popular belief was that the soul controlled the nervous system. This work was furthered by Emil Du Bois-Reymond, who created a galvanometer sensitive enough to detect the electrical impulses travelling across a contracting muscle.

In 1842, Guillaume Benjamin Duchenne discovered that muscles could be locally stimulated and developed electrodes that could stimulate muscles at specific locations on the muscle's surface. Duchenne's research and the technology creation of the early 20th century would aid in creating more milestones on the road to functional electromyography. H. Piper was one of the pioneers of using muscle signals to determine muscle fatigue. In 1912, he wrote about his experiments where he performed a nerve conduction study on an ulnar nerve. He theorized that the signals he recorded were the

frequency of the signals received from the brain. This was expanded by the work of Joseph Erlanger and Herbert Gasser, who found that nerve fibers with different diameters have different conduction velocities. This work won them the Nobel Prize in medicine in 1944.

Throughout the early-to-mid 20th century, researchers used electromyographic methods to look at activation patterns of muscles during various activities. Several important muscle properties were discovered in this research period, such as the “all-or-none” law in skeletal muscles [12]. This law states that a muscle fibres response is either a complete response or not responsive at all and is independent of the strength of the stimulus signal. The first known use of EMG as a method of measuring muscle fatigue was by Friedrich Jolly. His work found that the orbicularis oculi muscle of patients with myasthenia gravis, an autoimmune disease that affects the skeletal muscle, would fatigue quickly when electrically stimulated.

In the early 1940’s, James Golseth made the first commercially available EMG machine system. This allowed EMG to be available for clinical use. In the 1960’s, John Basmajian realized that EMG could be used as a training feedback element, for as small as even a single motor unit. This started the use of using EMG in a clinical setting for diagnosing and treating various muscular disorders. Basmajian also helped found the International Society of Electrophysiological Kinesiology, which still exists as one of the only international forums to share information and research on EMG [14],[15].

SEMG Advantages, Disadvantages and Influencing Factors

Surface electromyography (SEMG) is different than standard EMG because it uses a sensor that sticks to the skin instead of a needle that penetrates the muscle. This

method is easier and less painful to use but comes with several advantages and disadvantages.

One clear advantage of SEMG is the attachment style. While standard needle EMG is stuck through the skin into the muscle, SEMG is normally joined to the skin with an adhesive gel. This requires less training than injecting a needle EMG, as properly piercing the skin and finding the motor unit is intricate, and creates less pain for the subject.

SEMG is a useful laboratory and clinical tool to observe the total pattern of a muscle's signal. Multiple SEMGs can be connected to the same muscle to observe pattern differences localized in different regions of a muscle over the course of a movement, whereas needle EMGs are meant only to measure single motor units. The general activation pattern obtained with an SEMG can also be used in a clinical setting as feedback to a patient to aid in neuromuscular reeducation.

However, there are a few disadvantages to SEMG. One such shortcoming is that skin has a natural resistance and capacitance. This creates a low-pass filter effect with a cutoff frequency around 100 Hz, which reduces received energy in the region above 100 Hz. This effect cannot be negated, but it can be reduced by proper skin preparation, such as abrasion of skin and rubbing alcohol, and a preamplifier, which is normally pre-built into the SEMG system.

Another disadvantage of SEMG is the possibility of "cross-talk". Since the SEMG sensor sits atop the skin, signals for other nearby muscles can leak into the recording for a muscle. Also, skin is loose and not firmly fixed to the body. It can "jiggle" during various motions, which can slightly move the SEMG from above the muscle being observed. The

likelihood of either of these occurring and the effect of each is decreased by placing the EMGs onto the correct placement sites for each muscle.

Finally, SEMG cannot obtain a signal more specific than the overall pattern. Since it is adhered to the top of the skin, it collects many motor units at once. Individual motor units can only be obtained with needle EMG.

SEMG signals can be influenced by several factors: level of contraction, localized muscle fatigue, thickness of body tissue, inter-electrode distance, artefacts and noises, and “cross-talk”. Level of contraction is the intensity that the muscle is working to complete a motion. The total magnitude of the SEMG output increases or decreases with the change in contraction level.

Localized muscle fatigue is the reduction in the local muscles ability to produce force. As the muscle uses up its energy stores and the oxygen intake increases, the fast twitch muscle fibers begin to fatigue and replace themselves with slow twitch muscle fibers. This phenomenon is characterized by a decrease of the median or mean frequency of the SEMG signal, as well as an increase in the overall magnitude.

The body tissue that affects SEMG output is any bodily material that gets between the muscle being measured and the SEMG sensor. This can be fat, ligaments, tendons, skin, nerves, and blood. This tissue acts as a low pass filter and will more dramatically affect the signal the thicker it gets.

Inter-electrode distance changes the signal by increasing the SEMG signal amplitude and lowering the mean and median frequency the further the electrodes are apart from each other. Distancing the electrodes allows measuring action potentials from a larger area but increases the likelihood of “cross-talk”.

Artefacts are consistent noise patterns that can manifest in an SEMG signal. The strongest artefact that can occur is the pattern of the heartbeat in a signal. Since the heart is a strong muscle that contracts regularly, this signal can interfere with the SEMG signal, becoming stronger as an SEMG is located closer to the heart.

“Cross-talk”, as discussed earlier, is when a signal from one muscle bleeds into the reading of another muscle. This is especially noticeable when the two muscles are antagonist pairs, that alternate “on-off” cycles [15], [16].

Processing Methods

Overview

There are several processing methods that have been used in past and current research: mean and median frequency, amplitude of signal, Dimitrov’s spectral index, and fractal dimension.

Mean and median frequency, two very similar methods that trend identically, is an original processing technique used in the electromyography field. In terms of the frequency spectrum of the signal, fast twitch fibers correlate to high frequencies, and slow twitch fibers correlate to low frequencies. Since fast twitch fibers fatigue quickly, when the mean and median frequency decreases, it is usually indicative of a trend toward slow twitch fiber recruitment. If the frequency increases, this would indicate a trend toward fast twitch recruitment.

Amplitude changes simply observe the trends in the EMG signal’s overall amplitude. While this can be represented in many different formats, the most common is finding the root mean square. Amplitude and mean/median frequency typically go hand-

in-hand when processing signals, as an amplitude change can mean different things for different frequency trends. Without a change in frequency, an amplitude increase indicates an increase in muscle fiber recruitment, and a decrease suggests decruitment. When frequency and amplitude increase together, the body is relying on fast twitch muscle and increasing force output. However, if the frequency drops with an amplitude increase, this would indicate fatigue, as many slow twitch fibers are being recruited to make up for the loss in power of the fatigued fast twitch fibers.

Spectral index is a more complex variable. Since it is composed of both frequency and amplitude components, it is tied to changes in both. In a typical fatiguing situation, when the frequency drops and the amplitude increases, the spectral index increases dramatically. It is utilized for its high sensitivity to changes in fatiguing.

A novel parameter in EMG signal processing is fractal dimension. Since fractal use is new, its usage in literature is limited. In currently trending fractal-EMG research, it would seem that a change in fractal dimension, even just by a few percentage points, is a significant shift. An increase in fractal dimension seems to suggest that there is an increase in muscle recruitment and an increase in central fatigue [17]. An increase in muscle recruitment means that the body is having to recruit more muscle fibers in order to replace fatigued ones, or it is having to recruit more muscle fibers to meet a new demand in muscular output. Central fatigue means muscle force declines due to a reduction in the body's motoneuronal output.

Alternatively, a decrease in fractal dimension shows an increase in motor unit synchronization and an increase in local muscle fatigue. Motor unit synchronization is when the body adapts to a repetitive, rapid task and starts to synchronize the firing of the

neurons of different muscle fibers at similar times in order to aid force development. At the beginning of an exercise, there is a varied timing recruitment of muscle fibers to keep force output smooth and consistent. As fatigue increases, synchronization increases as well [18]. This results in a reduction in the independent recruitment of motor units and a larger dependence on a “common motor pool”. However, it does not affect the average strength of the output; it increases the overall variance of the signal. Motor unit synchronization is also accompanied by a decrease in mean frequency and an increase in amplitude. However, the output force of the muscle becomes unsteady and “tremulous” due to gaps in the neuron firings [19]. For example, this motor synchronization is evident during strength exercises, such as a leg press. At the beginning of the exercises, when synchronization is low, the motion is smooth and controlled. However, as fatigue and synchronization increase, the exercise is still able to be completed, but the muscles shake due to the gaps in neuron firings.

Mean and Median Frequency

One the earliest and most common processing method for electromyography is finding the mean and median frequency. This traditionally involves performing a Fourier transform on the signal and obtaining the power spectrum of the signal.

$$F(\omega) = \int_{-\infty}^{\infty} f(t)e^{j\omega t} \partial t$$

$$P(\omega) = F(\omega)^2$$

This spectrum shows how much power is contained in each frequency level. The median and mean frequency can be then be found from this spectrum and can be used to identify various muscle phenomena based on their shift.

$$\text{Mean Frequency (MNF)} = \frac{\int_0^{\infty} \omega P(\omega) d\omega}{\int_0^{\infty} P(\omega) d\omega}$$

$$\text{Median Frequency (MDF)} = \int_0^{f_{med}} P(\omega) d\omega = \int_{f_{med}}^{\infty} P(\omega) d\omega = \frac{1}{2} \int_0^{\infty} P(\omega) d\omega$$

As a muscle contracts, it continues to recruit motor units until the desired action can be performed. The mean and median frequency increase until the end of this recruitment process, at which point they should stay constant, or slightly decrease, while attempting to maintain the same isometric level of contraction [17],[19].

Another processing method commonly performed in conjunction with frequency analysis is amplitude analysis. This is normally achieved by integrating the signal to find the area under the signal's curve or by taking the root mean square of the signal by using the equation:

$$\text{Root Mean Square (RMS)} = \sqrt{\frac{1}{T} \int_0^T f^2(t) dt.}$$

The change in the amplitude of either the RMS or the integration of the signal can characterize the signal and its fatigue level [17].

A processing method devised in the early 2000's by Dimitrov et. al. involves taking the spectral moment of the signal for an order k. The spectral moment of zero is then divided by the spectral moment of k to create the Dimitrov spectral parameter FI_{nsmk} [17],[21].

$$\text{Spectral moment of order } k = \int_{f_1}^{f_2} f^k P(f) df$$

$$FI_{nsmk} = \frac{\int_{f_1}^{f_2} f^{-1} P(f) df}{\int_{f_1}^{f_2} f^k P(f) df}$$

A more recent method that has gained traction in the last decade, especially for brain signal analysis, is fractal dimensions. This method is derived from the study of geometric fractals. Geometric fractals look at the reoccurrence of patterns in a shape. Specifically, fractals have two properties: self-similarity and non-integer dimensions. Self-similarity refers to the existence of the overall pattern of the shape occurring no matter the scale. The shape can be magnified multiple times and still have the same pattern as before. This can be illustrated using the Sierpiński Triangle.

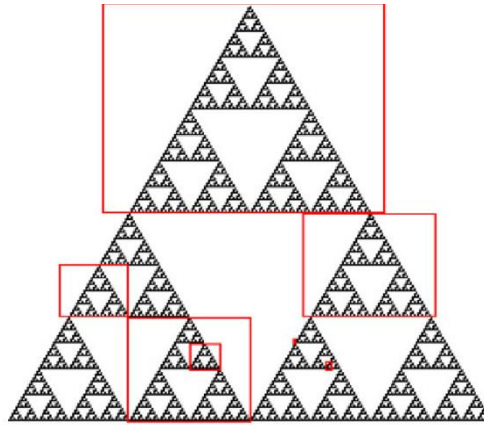


Figure 2.8. Sierpiński triangle [22]

Fractals also exhibit non-integer dimensions. While classical geometry deals with integer dimensions, such as one-dimensional lines and two dimensional planes, fractal dimensioning explains various subjects with a dimension between two whole numbers. A fractal curve, for example, can have a dimension between 1 and 2, but a straight line is one dimensional. As a fractal curve takes up more space and becomes more complex, the dimension will approach the 2nd dimension.

Several mathematicians have derived various equations for finding the fractal dimension of a waveform, such as Higuchi, Katz, and Petrosian. Higuchi's equation starts

by creating a kmax value of new time series from an original time series with the equation

$$X_k^m = \left\{ x[m], x[m + k], x[m + 2k], \dots, x \left[m + \text{floor} \left(\frac{N - m}{k} \right) * k \right] \right\},$$

where k is the current iteration of kmax and m=1, 2, ..., k is the initial time value. The value of k is iterated after every new time series.

Next, the length of each new time series is found by using the equation

$$L(m, k) = \frac{\left(\sum_{i=1}^{\text{floor} \left(\frac{N-m}{k} \right)} |x[m + ik] - x[m + (i - 1) * k]| \right) (N - 1)}{\text{floor} \left(\frac{N - m}{k} \right) * k}$$

where N is the length of the original time series and (N-1)/{floor[(N-m)/k]*k} is a normalization factor. The length of the curve of the time interval k is the average of the k values L(m,k), for m = 1, 2, ..., k, found by the equation

$$L(k) = \frac{1}{k} * \sum_{m=1}^k L(m, k).$$

Finally, the fractal dimension is found by plotting L(k) against 1/k on a double logarithmic scale, with k = 1, 2, ..., kmax. The data should be a straight line, with a slope equaling the fractal dimension of the original time series [22],[23].

Central Fatigue vs Peripheral Fatigue

Muscle fatigue, as described by Place et al., can be simplified as a reduction in maximum voluntary force of a muscle due to exercise [24]. However, fatigue itself is not a simple state of exhaustion. Fatigue can manifest in a multitude of complex ways in the human body. For example, an endurance exercise fatigues the body differently than a

weightlifting session [25]. In general, this can be broken down into two categories: peripheral fatigue and central fatigue.

Peripheral fatigue is localized to the muscle being observed. It is a change in the biochemical function of the muscle, causing altered and reduced metabolic capability. These biochemical changes could be several different cellular changes, such as a reduction in calcium release in the sarcoplasmic reticulum and a decrease in muscle fiber conduction velocity, which causes the muscle to shift to slow twitch fibers instead of fast twitch [26]. The resulting output is a reduction of the individual muscle's ability to react to neural stimulation [27]. Peripheral fatigue typically is exhibited during strength-based tests, such as weightlifting and sprinting [25]. In this case, the muscle is usually isolated with the rest of the body relaxed.

On the other hand, central fatigue stems from anything neurological. This can be a combination of problems, such as Renshaw cell inhibition, a lack of neuronal drive from supraspinal positions, or even increased serotonin in the brain, that affect anything between the central nervous system to the point of contact with the muscle [26],[27]. It does not occur in the muscles themselves, but it affects their control and output. This results in a reduction in stimulation from neurons that control the muscle which creates less overall muscular force [28]. Central fatigue can occur during endurance exercises, such as distance running or cycling, or maximal contractions that are sustained for long periods of time [26],[29].

Previous Research

Electromyography has been investigated in its use of detecting muscle fatigue for several decades. However, it is not the only method that has been explored for observing

muscle behavior. Alongside electromyography, several well-documented variables for detecting muscle fatigue are VO₂ and blood lactate.

A person's VO₂ measures the volume of oxygen that your body uses to create ATP energy. As an aerobic endurance activity continues, the muscles begin to fatigue and require more oxygen to maintain current endurance levels. The VO₂ Max is the maximal amount of oxygen that a person's body can intake while performing intense exercise and can dictate someone's endurance time. This max value can be improved with aerobic endurance training. As a person fatigues, their VO₂ increases until it hits the VO₂ max. At this point, current exertion of the body cannot exceed what it is currently outputting [30].

Another reliable test for fatigue is checking blood lactate levels, as were mentioned above. When the body engages in an endurance activity, the body begins to rely on stored glycogen. As the body breaks down the stored glycogen, lactic acid and pyruvate are released into the blood stream. Pyruvate, when oxidized by an increased breathing rate, turns into energy for aerobic muscles. However, as the pyruvate continues to build up, the oxygen intake can no longer keep up with the lactic acid and pyruvate production. The lactic acid, once it builds up, can then impede the breakdown of glycogen and create a burning sensation in the muscle. This will eventually cease the increase in exercise intensity [31].

VO₂ measurement has become a popular method for measuring endurance times. Several studies have evaluated using VO₂ in various endurance settings. Takaishi et. al. found that as subjects used a bicycle ergometer to exhaustion, VO₂ not only increased over time, but also increased when the workload intensity was increased [32]. This is

corroborated by other articles as well. Barstow found that VO₂ increased with work rate and is “statistically associated with the rate and magnitude of increase in blood lactate” [33]. Jones et. al. also discovered a similar relationship between blood lactate and VO₂, where both variables would increase at similar trends with an increase in running speed [34]. The performance of these variables can be improved with training, as learned by Casaburi, et. al and Phillips et. al. Casaburi’s eight weeks of cycle ergometer endurance training was able to raise the test subjects’ VO₂ max by 15% on average, as well as reduced the level of blood lactate at the end of the exercise [35]. Likewise, Phillips’ thirty days of endurance training was able to reduce mid-exercise blood lactate concentrations and increase VO₂ max by 10% [36].

In a connection with EMG, it was found by Horita and Ishiko that muscle lactate levels correlate with changes in EMG median frequency [37].

One of the most popular early studies was completed by Petrofsky that explored frequency and amplitude changes. For a dynamic fatiguing exercise, Petrofsky had his subjects use a bicycle ergometer for eighty minutes at different workloads. He found that, as a subject gets fatigued at workloads above 40% of maximum voluntary contraction, as verified with VO₂ and lactate level changes, the root mean square amplitude increases and the median frequency decreases [38]. He verified this again later with handgrip muscles, showing that the mean frequency decreased linearly during a fatiguing isometric contraction when tension had to be held above 25% MVC [39].

It is theorized that this is occurring because the fast twitch muscles work at a faster frequency. As they fatigue, ATP stores deplete, and the body must rely on aerobic energy. Slow twitch muscles, which are aerobic, start to replace the fast twitch muscles,

which decreases the frequency of the signal. However, since slow twitch muscles produce less power output than fast twitch muscles, more slow twitch muscles need to be recruited, which will increase the amplitude of the signal. The methods he promoted would later become known as the “Traditional Methods”, as these were the first processing techniques widely used for EMG [38].

Since the Petrofsky paper, several others have tried using similar methods to measure muscle fatigue with bicycle cyclometers. Several studies found that RMS amplitude increased in the gluteus maximus and the muscles of the thigh and decreased in the gastrocnemius and tibialis anterior [32],[40]. A study that used an elliptical, instead of a cyclometer, showed similar amplitude results, along with a median frequency regression line decrease of about 30 Hz [41]. Researchers estimate that this is due to the body adapting to a different mechanical pattern as the body tires. Specifically, the muscles of the upper leg and the gluteus maximus are used for propulsion power when the knee flexor muscle of the lower leg fatigues [42]. One group even found that higher pedal speeds lengthen the time it takes to fatigue, as shorter contraction times and lower pedal forces at high pedal speeds allows for better blood flow, which would allow more muscle fiber types to be recruited [43].

Some studies used the traditional methods in more unique settings. One study measured the upper trapezius muscles during a typewriting exercise. They found that median frequency dropped 10.6%, and the RMS amplitude increased 14.6% [44]. In another study, the trapezius muscles were also recorded, while the subject held a backpack of varying loads. For backpack loads above 15% of bodyweight, IEMG increased, and median frequency decreased up to 22.1% of the initial value [45].

Another exercise method used in conjunction with the traditional methods is isokinetic extensions. These studies normally evaluate the vastii muscles and perform a knee extension. One study, which divided subjects up as either slow twitch dominant or fast twitch dominant, found that torque output dropped 60% in fast twitch dominant subjects and only about 47% in slow twitch dominant subjects. In fast twitch dominant subjects, IEMG declined significantly and median frequency was reduced by approximately 25%. However, slow twitch subjects showed much better fatigue resistance. There was a slight, but not significant decline in IEMG, and a roughly 12% reduction in median frequency [46]. Horita and Ishiko had a similar study, but they found unique implications from the data. Interestingly, they discovered that as the vastus lateralis tired, the torque output lagged the electrical activity. This was found to correspond with muscle lactate accumulation and was assumed to be a sign of peripheral fatigue of the muscle.

These traditional methods have also been used in long distance endurance running scenarios. An investigation that utilized these methods for a ten-kilometer run attached EMGs to the subjects' vastus lateralis, biceps femoris, and gastrocnemius. The run was preceded by a twenty-meter maximal run and was succeeded by an additional twenty-meter maximal run. Each footstep's EMG activation was divided into two halves: pre-contact and initial contact phases in the first half and total contact, braking and propulsion phases in the second half. The results showed a 28.5-57.2% IEMG decrease in the first half and an IEMG decline of 13.5-35.1% in the second half of the footstep compared to the initial values [47]. Another study increased the running distance to a full marathon and performed pre and post isometric strength tests instead of the maximal

runs. The findings revealed an IEMG decrease in the vastus medialis by $36 \pm 26\%$, and in the vastus lateralis by $42 \pm 25\%$, as well as a $26 \pm 14\%$ drop in max torque [48].

However, in dynamic exercises, traditional methods could be less reliable when measured during the activity. For example, Manero et al. created a pair of leggings with EMGs embedded in the material to allow for easier attachment and implementation. The EMG data was processed by finding the average value of the rectified signal in a rolling 0.2 second window. The output showed varying levels of increase and decrease in the average rectified signal with no signs of reliability of the traditional method to work in this fashion [49]. Table 2.2 shows a summary of key papers and their trends.

Table 2.2. Key papers and their trends for frequency and amplitude. Exercise types with an orange star were papers with a strength exercise, and types with blue stars were papers with an endurance exercise.

Exercise	Frequency	Amplitude	Authors	Exercise Type
Isometric				
Bicycle Exercise	↓	↑	Petrofsky (1979)	★
Handgrip	↓	↑	Petrofsky (1980)	★
Bicycle ergometer		↑	Takaishi et al. (1996), Castronovo et al. (2012)	★
Elliptical	↓	↑	Chang et al. (2012)	★
Typewriter Exercise	↓	↑	Kimura et al. (2007)	★
Knee Extension	↓	↓	Komi and Tesch (1979)	★
Knee Extension	↑ (Low force loads), ↓ (High force loads)		Arendt-Nielsen et al. (1989)	★ ★
Cycling	↑ (Low force loads), ↓ (High force loads)		Petrofsky (1979)	★ ★
Isotonic Shoulder Hold	↑		Hägg and Ojok (1997)	★
10K Distance Run		↓	Paavolainen et al. (1999)	★
Marathon Run		↓	Nicol et al. (2007)	★

A recent development in EMG processing is the creation of “spectral indices” by Dimitrov et al. in 2006. These researchers were trying to create a new index that was more sensitive to change than mean or median frequency. As described previously, Dimitrov’s spectral index was found by finding the spectral moment of a desired order “k” and dividing the spectral moment of order zero by the spectral moment of the desired order “k”. The study specifically looked at order 2 through order 5 [21].

After seven subjects performed ten sets of fifteen repetitions of knee extensions, the subjects were placed into three muscle fatigability subgroups based on their results: low, medium, and high fatigability. The low fatigability group was determined to contain the highest level of muscle endurance. The median frequency and the new spectral index were taken from the sEMG data and compared. The average maximal median frequency decrease was 27% for the high fatigability group, $19 \pm 2\%$ for the medium fatigability group, and $6 \pm 2\%$ for the low fatigability group. However, the average maximal spectral index increases for spectral moment of order 5 was 602% for the high fatigability group, $300 \pm 17\%$ for the medium fatigability, and $162 \pm 16\%$ for the low fatigability group. Thus, the new spectral index was seen to be potentially more sensitive to muscle fatigue than median frequency [21].

Several studies have used Dimitrov’s spectral index for measuring upper arm muscles. One study measured the biceps brachii, triceps brachii, and rhomboideus on thirty-seven subjects performing CPR chest compressions for five minutes. The biceps and triceps had RMS increase of 28% and 38%, median decrease by 9% and 8%, and a Finsm5 increase of 71% and 64%, respectively. The rhomboideus saw no significant change, as it was a control muscle with no activation during the exercise [50]. Another

upper body study created a wearable shirt, with sEMGs embedded inside, and measured the biceps brachii from six subjects during four sets of fifteen repetitions of bicep curls. After each set, the spectral index was processed from the sEMG data. The first set had a relative change of >225%. The second set had a relative change of 350%. The third set had a relative change of 400%. Finally, the fourth set had a relative change of 475%. These results allowed these researchers to conclude that Dimitrov's spectral index is a good indicator of strength-based peripheral fatigue [51].

A study performed with a dynamic lower body exercise measured the vastii muscles of the quadriceps and the biceps femoris of thirteen test subjects during ten repetitions of a leg press, as well as blood lactate levels. After ten repetitions the blood lactate level increased from $1.1 \pm 0.2 \text{ mmol/l}$ to $4.8 \pm 0.9 \text{ mmol/l}$. Each repetition was split into 90° - 67° knee angle range and 23° - 0° knee angle range. The average median frequency experienced an 11-15% decrease for the vastii muscles at both angular positions and decreased in the biceps femoris by 8% for the 90° - 67° knee angle range. However, the spectral index in the vastii muscles increased by 79-92% at both angular positions and increased in the biceps femoris by 63% in the 90° - 67° knee angle range [52].

Dimitrov et al. had found in their experiments increases in the spectral index by upwards of 602%. However, most studies, as evidenced above, do not seem to find as great of results. Many studies seem to find increases of about 60-90%, with some outliers showing higher results. This appears to show that while the Dimitrov spectral index may be more sensitive than mean or median frequency, it still is not quite as good as had been

originally indicated, especially during dynamic studies [53]. A summary of papers using spectral index is shown in Table 2.3.

Table 2.3. Key papers and their trends for spectral index. Papers that included mean frequency and amplitude are also shown. Exercise types with an orange star were papers with a strength exercise.

Exercise	Spectral	Frequency	Amplitude	Authors	Exercise Type
Knee Extension	↑			Dimitrov et al. (2006)	★
Chest Compressions	↑	↓	↑	Lee et al. (2014)	★
Leg Press	↑	↓		Gorostiaga et al. (2011)	★
Bicep Curls	↑			Pino et al. (2018)	★

One of the newest processing methods used in the biological realm is fractal dimensions. Fractals, as described earlier, looks at the self-similarity and non-integer dimensions. One of the most popular and most widely used mathematical estimations of fractal dimensions of a waveform was created by Dr. Tomoyuki Higuchi [54],[55]. Higuchi's method has the benefit of being accurate regardless of the nature of the signal being observed, whether it is stationary, non-stationary, deterministic, or stochastic [55]. This mathematical equation, which originated from chaos theory, was originally intended to be used for describing the earth's changing magnetic field. However, it has been expanded to be utilized in many biomedical applications [56].

One such area being investigated using fractals is Electroencephalogram (EEG) signals, or brain activity. Researchers have discovered and utilized fractal behavior of brain signals to be able to observe a multitude of brain functions and dysfunctions. Several areas that Higuchi's method is applied to are sleep onset, level of pain experienced, the progression of traumatic brain injury, predicting epileptic seizures, and diagnosing major depressive disorder [55].

A study performed specifically to investigate the fractal behavior of the brain found evidence for structures in the brain that are similar to Sierpiński triangles that makeup the physical architecture of the brain [57]. Other researchers took short EEG recordings of subjects and found a total fractal dimension of around 5 with increases up to 6.9 in the Wernicke areas when the subjects were reading [57]. However, another group found a baseline reading of 6.5 for healthy subjects, which could decrease to 5.3 in patients with Parkinson's disease and 4.4 for patients with various forms of dementia [58]. This is supported by other claims that the brain loses complexity during neurodegeneration or injury [55].

While EEG is a vast area to be explored by fractals, the specific subject of muscle fatigue has been briefly studied. For EMG, fractal dimensions of muscle signals have shown to be, and should always be, values between 1 and 2, because, as Klonowski says, it characterizes complexity of the curve representing the signal under consideration on a two-dimensional plane [59]-[61].

Several studies have used fractals in the estimation of various workloads in static exercises. It was found that an increased workload increased the fractal dimension for both EEG and EMG, with the EMG signal's fractal dimension increasing from 1.1 to 1.4. This suggests that force output is linearly proportional to brain activation and neuron firing rate [62],[63].

Another research group recorded the vastii muscles of thirty-one female subjects and split the subjects into three subgroups: power athletes, recreationally active, and endurance athletes. The recreationally active subjects saw an average fractal dimension decrease of 4% during an isometric 60% MVC knee extension held to exhaustion.

Interestingly, power weightlifter athletes saw a larger decrease in their fractal dimension compared to the recreationally active, but endurance athletes saw an overall increase in their fractal dimension. The researchers concluded that an increased motor unit synchronization of the muscle decreased the fractal dimension and thus that the slope of fractal dimensions are an indicator of central fatigue when measured during a task requiring endurance. However, this study was limited by only having female subjects, as females are proven to be superior at sustaining muscle contractions for low and moderate intensities, which could alter the fractal dimension compared to males [26].

While not many papers have investigated dynamic endurance tasks, Mesin et al. performed a cumulative fatigue processing study on simulated muscle signals. They acknowledged all twelve currently used processing methods of indicators of fatigue, including RMS, mean and median frequency, and fractals, and determined which were most accurate for detecting fatigue. It was found that fractals are least affected by changes in conduction velocity and are not strongly altered by the fat layer that lies between the muscle belly and the skin. They also have shown a strong indication of being able to estimate a muscle's motor unit synchronization, which suggests that fractals can be used to detect central fatigue. Measuring conduction velocity, on the other hand, was best for measuring peripheral fatigue. It was suggested, then, that a combination of fractal dimensions and conduction velocity can describe a muscle's fatigue [20].

An important part of calculating fractal dimensions with Higuchi's method is the k_{max} variable, which is a value chosen by the researcher. Several papers have looked at the sensitivity of the k_{max} variable and their influence on the fractal dimension. One study using Higuchi's method made a simulated signal with a fractal dimension of 1.3.

They found that a chosen kmax value of 6 produced a result closest to 1.3 for all lengths of simulated signal up to the max tested of 1000 sample points. This was verified by testing a range of signals that had fractal dimensions between 1.1 and 1.9 with a kmax value of 6, which produced an accurate output [64]. Another paper checked a multitude of kmax values, along with differing overlap and segmentation percentages and found that a kmax value of 44, with a segmentation length of 10% and an overlap of 30% was the most reliable with the least error. However, they did acknowledge that other papers were using a kmax value of 6 [60].

The results of these studies have led researchers to believe that fractal dimensions provide a more reliable estimation of central muscle fatigue and can yield more information than traditional methods [20],[64]. A summary of key papers for fractal dimension is shown in Table 2.4.

Table 2.4. Key papers and their trends for fractal dimension. Exercise types with an orange star were papers with a strength exercise.

Exercise	Fractal	Authors	Exercise Type
Knee Extension	↓ (>60% MVC)	Beretta-Piccoli et al. (2015)	★
Simulated Signal	↑ (with recruitment number)	Xu and Xiao (1997)	
Simulated Signal	↑ (conduction velocity and firing rate)	Mesin et al. (2016)	

Purpose

The research presented in this thesis will attempt to observe lower limb fatigue progression using EMG signals over the course of a running trial. Since the EMG data was collected during the course of an exercise, it shows that fatigue can be measured while an exercise is being performed. This was accomplished by utilizing a combination

of the four previously presented EMG parameters: mean frequency, RMS amplitude, 2nd order spectral index, and fractal dimension. In the research field, this will stand out due to its application during the exercise trial, instead of measures taken before and after, and its use of experimental parameters, spectral index and fractal dimension, for endurance activities.

CHAPTER THREE

Pilot Tests and Results

Purpose

This chapter is meant to present the preliminary test results that could verify the collection of the different parameters from EMG signals during various exercise conditions. Crucially, this will aid researchers by beginning with known data and conditions from literature and bridging to novel conditions. Specifically, the data presented in this chapter will move from using EMG with static upper body exercises to dynamic lower body exercise.

Isometric Testing

To begin the investigation into measuring dynamic motion muscle fatigue, some initial first steps had to be taken. First, initial research had to be taken to understand how EMGs work and what current popular processing techniques were available. An initial pass of the literature revealed what is called “traditional methods”, which involves taking the amplitude measurement as well as the Fourier transform of the signal to find the mean or median frequency.

There was a desire for these first initial tests to be simple and easy to perform, so the muscle of choice for the tests was the biceps brachii of the upper arm. This muscle was chosen because it is large, easily accessible, and exercises that fatigue this muscle can be performed without much difficulty.

The first test's setup began by placing a single surface EMG, or sEMG, on the biceps brachii according to SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) recommendations, which is a European project group that contains standardized protocols for sEMG sensor placement. The test subject was then instructed to hold a fifteen-pound dumbbell in their hand, with their arm bent at a 90° angle in front of them. The test subject held their arm in this position until they could not keep the weight in this position any longer, as the sEMG data was transmitted to a computer and recorded. The software used to collect the data is Nexus Vicon 2.5 and the sEMG sensors are the Noraxon Desktop Direct Transmission Sensors. The Noraxon sensors were collecting at 1500 Hz. Nineteen trial segments were recorded with 30,000 sample points in each trial segment. After collecting the data, signals were prefiltered between 10 Hz and 400 Hz, and a Fourier transform was applied to the sEMG data.

In the frequency band, the signal decreased for most subjects, as seen in Fig. 3.1. In the power amplitude, shown in Fig. 3.2, the signal showed a large increase in power by the end of the trial.

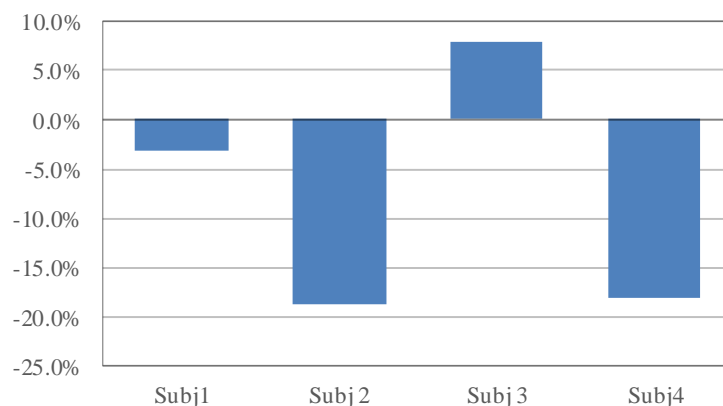


Fig. 3.1. Mean frequency percent change in biceps brachii during isometric hold. Key takeaway: most subjects decreased in frequency, showing that the results were repeatable.

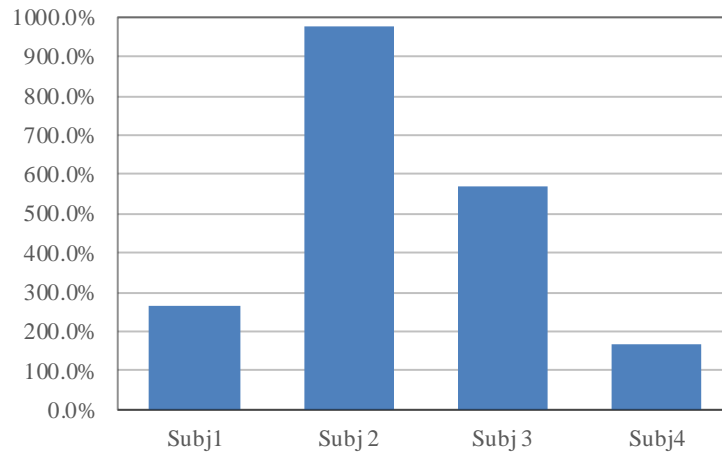


Fig. 3.2. Root mean square amplitude percent change in the biceps brachii during isometric hold. Key takeaway: all subjects increased in amplitude, showing the results were repeatable.

While the mean frequency decreased an average of 8%, the amplitude of the power increased by an average of 495%. At the time of performing this study, it was expected that there would be a clear downward trend of high frequencies, and thusly fast twitch muscles, and an upward trend of low frequencies with slow twitch muscles. Similar trends were noticed in papers that used static exercises, several of which were mentioned in the previous chapter.

The next step was to move to dynamic motion in the same biceps with a curl exercise. Curls were completed in a cadence of 84 beats per minute. This cadence is equal to half of a quick running pace of ~170 steps per minute divided by two, which gives the cadence for each leg. Four trials were completed with a two-minute rest in between each set. Each set was completed until exhaustion. Trial data was divided into 12,500 samples per segment, and then mean frequency, total power amplitude, and frequency band power amplitude were then extracted from the data. Results are shown in Fig. 3.3.

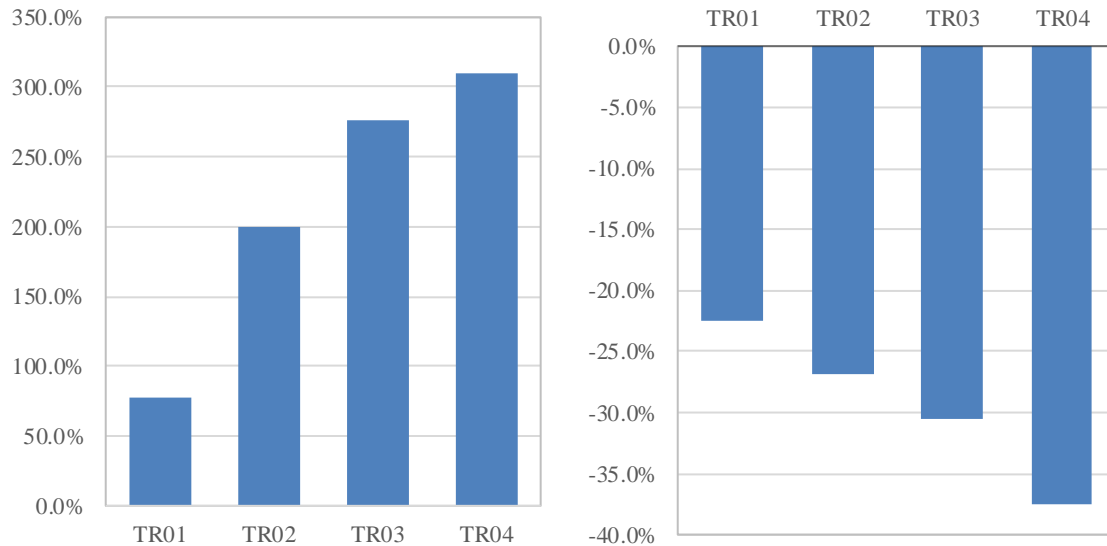


Fig. 3.3. Amplitude (left) and mean frequency (right) change of dynamic bicep trials for one subject. Key takeaway: all subjects increased in amplitude and decreased in mean frequency, showing the results were repeatable for dynamic actions.

Fig. 3.3 demonstrated a clear decrease in the mean frequency and a large increase of power amplitude. Each subsequent trial had a sharper slope of decrease in the mean frequency and a larger overall achieved power amplitude. This seemed to indicate that mean frequency shifts and amplitude increases were indicators of muscle fatigue.

Another idea that was tested was splitting each pulse in half, where one half is the power phase and the other is the “reset” phase. This is meant to mimic an actual running footstep, where the leg absorbs the body’s weight from heel strike to braking phase, and then uses power to push the leg through the propulsion phase and toe-off. In a bicep curl the upward stroke would be the power phase and the downward absorption would be the “reset” phase. After splitting the first three dynamic trials into the two phases, the mean frequency was extracted, as displayed in Fig. 3.4.

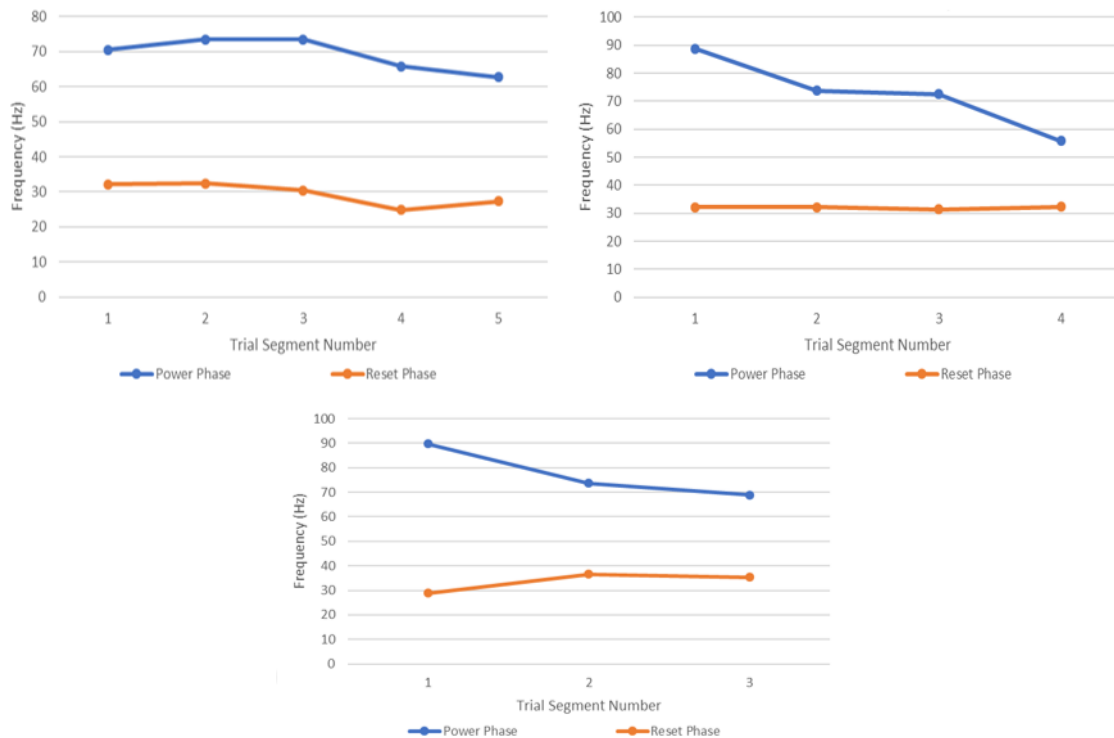


Fig. 3.4. Mean frequency across exercise phases in dynamic arm trials for trial 1 (top left), trial 2 (top right), and trial 3 (bottom)

As shown in Fig. 3.4, the frequency of the power phase starts in a higher frequency than the reset phase and decays considerably over the course of the exercise. The reset phase's frequency, however, stays consistently around 30 Hz. These results make sense as slow twitch muscle do not fatigue quickly, whereas fast twitch muscles fatigue rapidly. This is evidenced by the power phase decreasing an upwards of 37%, but the reset phase remaining unchanged.

Research then progressed to including three test subjects to repeat the dynamic bicep test to see if the results were repeatable. First, subjects had to perform the same biceps brachii exercise as the previous test, with the same conditions for the dynamic measurements.

The dynamic tests showed in Fig. 3.5 and Fig. 3.6 an overall decrease of the mean frequency and an overall increase in the power amplitude. It appears that the shifts in mean frequency and power amplitude corroborated the results of the initial test and showed good indication of using these variables in detecting muscle fatigue.

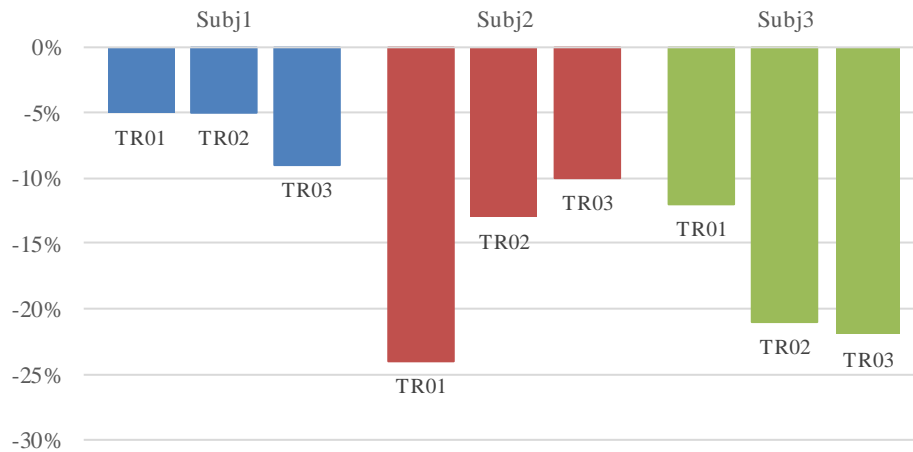


Fig. 3.5. Mean frequency as a percentage of initial value in the biceps for each of the three subjects in each of the three trials. Key takeaway: all subjects decreased in frequency, showing the results were repeatable for dynamic actions.

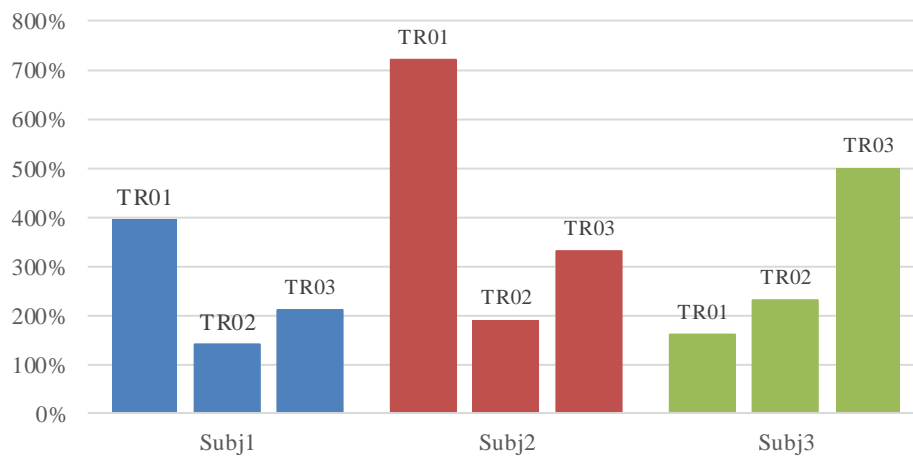


Fig. 3.6. Root mean square amplitude as a percentage of initial value in the biceps for each of the three subjects in each of the three trials. Key takeaway: all subjects decreased in frequency, showing the results were repeatable for dynamic actions.

Another method was tested with Dimitrov's spectral index explained in chapter 2. The arm dynamic test and a new calf dynamic test, which required the subjective to perform repetitive calf raises from a standing position, were performed while processing for mean frequency, median frequency, spectral index 2, and spectral index 5, as shown in Table 3.1.

Table 3.1. Frequency and spectral index results for five subjects during multi-repetition dynamic bicep curls. Key takeaway: spectral results all increased, showing an agreement with previous literature.

Subjects	Median Freq	Mean Freq	FInsm2	FInsm5
Subject 1	-63%	-35%	78%	257%
Subject 2	-36%	-34%	287%	1266%
Subject 3	-56%	-35%	111%	269%
Subject 4	-54%	-32%	167%	1100%
Subject 5	-57%	-38%	87%	175%

A “pulse finding” code was used to filter raw EMG waveforms down to just the moments when the muscle was activated. The processing methods were then applied on the pulses. The mean and median frequency results showed excellent downward trends that correlated with previous arm tests. The Dimitrov spectral index values also showed dramatic increases, climbing up approximately 250% for the second order spectral index and up to 1266% for the fifth order spectral index. Since these results were across five subject trials, this indicated that these results might be repeatable and trustworthy for indicating muscle fatigue in arm muscles for a curling exercise. This processing method was then applied to a dynamic calf raise test. These results are shown in Table 3.2 and Table 3.3.

Table 3.2. Frequency and spectral index during dynamic calf raises from the standing position

Muscles	Median Freq	Mean Freq	FInsm2	FInsm5
Gastroc Medialis	38%	0%	0%	15%
Gastroc Lateralis	13%	17%	-23%	-31%

Table 3.3. Frequency and spectral index during dynamic Biodex 3 calf raises at 45° per second. Key takeaway: the trends were the same between both standing calf raises and Biodex calf raises, showing that measuring dynamic lower body action was possible.

Muscles	Median Freq	Mean Freq	FInsm2	FInsm5
Gastroc Medialis	25%	11%	-8%	-16%
Gastroc Lateralis	24%	82%	-62%	-84%

The results showed an almost opposite reaction than the arms. The mean and median frequency increased while the Dimitrov spectral indexes decreased in the gastrocnemius lateralis. While this behavior could not be explained in the moment, it opened the possibility to explore this with more test subjects to observe a more repeatable data set.

To validate the results, the calf test was repeated with an isokinetic calf raise exercise performed in a Biodex 3 system. The torque was recorded by the Biodex to ensure that a reduction in output torque, a common mark of muscle fatigue, occurred during the exercise. These results showed similar results to the previous calf results. This showed an opposite reaction than was expected and brought doubt that these variables were exclusive markers of fatigue, especially in the legs. It was assumed that these markers are better for peripheral fatigue, but not central fatigue.

There were also some slight inconsistencies between the standing calf raises and the Biodex results. The Biodex results, at first, seemed to be more repeatable, but the Biodex system itself was much more difficult to use and adapt to different body types and sizes. The calf raises were much easier to implement and instruct the subject to perform. Even though the results were not originally consistent, it showed potential that with more control and instruction it could achieve similar consistency to the Biodex system.

Another processing method that was investigated was fractal dimension. Fractal dimension of an EMG looks at the partial dimension that the waveform exists between the first and second dimensions and the increase or decrease in complexity of the waveform. A change in the fractal dimension can indicate central fatigue [20],[26].

Data from eight subjects from a previous study was procured where subjects had to run a five-kilometer distance on a treadmill with sEMGs connected to their gastrocnemius medialis, gastrocnemius lateralis, tibialis anterior, and biceps femoris.

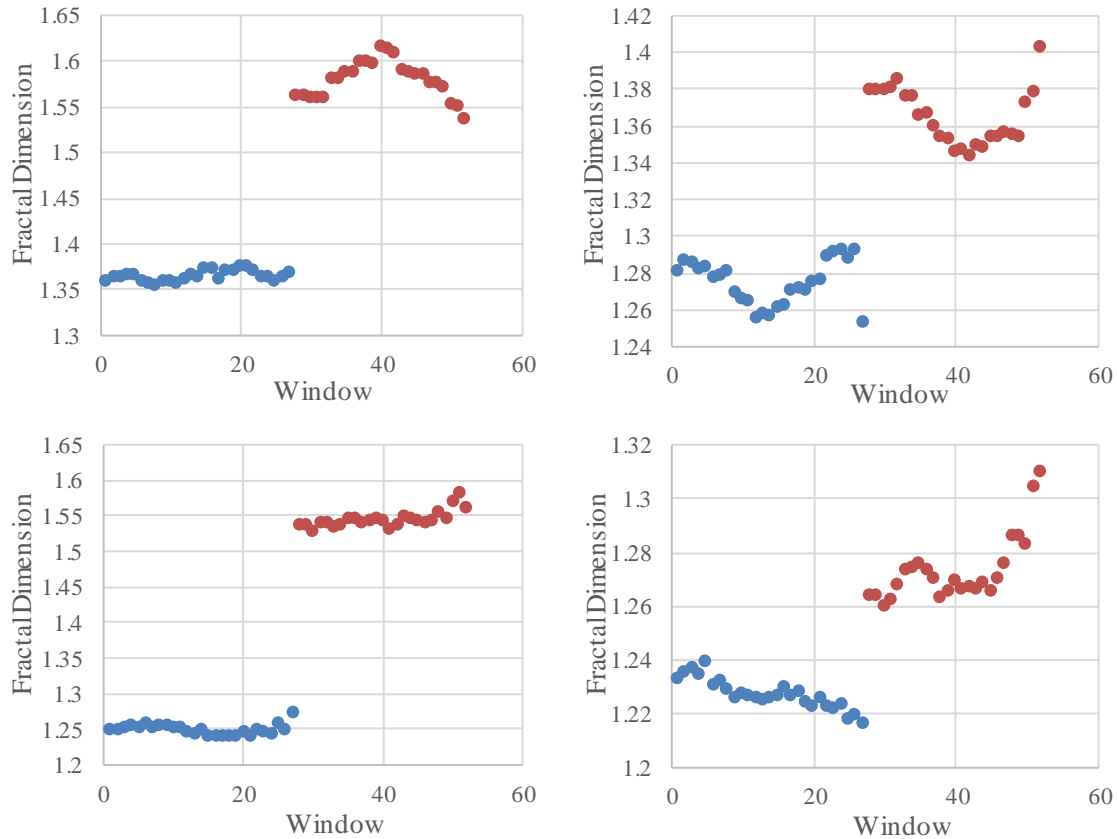


Fig. 3.7. Exemplary data of fractal dimension of four leg muscles for one subject at the beginning and end of a 5k run from left to right: 1) gastrocnemius medialis, 2) gastrocnemius lateralis, 3) biceps femoris, and 4) tibialis anterior. Key takeaway: all subjects showed an increase in fractal dimension with fatigue.

This showed a consistent increase of fractal dimension for the leg muscles in the subjects observed. The repeatability of the fractal dimension increase was encouraging as a possible variable to observe for muscle fatigue during fatiguing endurance-based exercises.

Key Outcomes

This chapter was designed to support the methodology for the larger study presented in future chapters. The testing described in this preliminary study demonstrated that each of the four parameters could be processed and recorded. One major takeaway

from this chapter is that each parameter was able to adequately exhibit changes when the measured muscle was fatigued, from the traditional methods of median frequency and amplitude changes to the experimental methods of spectral index and fractal dimension.

Also, it is seen in these pilot tests that mean and median frequency both had the same trends. Since median frequency added no new information, it was removed from the research parameters for the larger study. Another takeaway is that processing only on portions of the signal when the muscle was active decreased noise in the output. Previously, signal processing was implemented over the full signal, which included short rest periods between repetitions.

Finally, the last takeaway is that any order over the second order Dimitrov spectral index seemed to be superfluous and even detrimental at times. Higher orders simply exponentially increased the same trend, which also increased noise in the output by the same amount.

CHAPTER FOUR

Methods

After conducting multiple pilot studies, a finalized study procedure was confirmed. Since most studies had anywhere from two to thirty subjects, with a majority less than ten subjects, an acceptable sample size was an amount of ten or greater. For this study, eleven healthy subjects were recruited, but due to data corruption, only ten were used (six male, four female, ages 18-28). These subjects were asked to take part in the study and were briefed on lab procedure. Subjects had to be in a healthy condition with no current injuries and with a current running exercise regimen of no less than two miles a week. After signing a consent form, placement areas for the sEMGs were abraded and cleaned with rubbing alcohol. A Noraxon surface EMG system was used for the study, sampling at 1500 Hz. Fig. 4.1 is a picture of the electrode system.



Fig. 4.1. Noraxon Electrode System

Electrodes for the sEMGs were placed on the gastrocnemius medialis, gastrocnemius lateralis, and vastus lateralis of the dominant leg according to the SENIAM recommendations [65]. Once the sEMGs were placed, the sensors were held in place with cohesive bandage.

The test subjects were then placed in a Biodex 3 Multi-Joint System to perform maximum voluntary contraction isometric calf raises. Fig. 4.2 is a picture of the Biodex system.



Fig. 4.2. Biodex 3 Multi-Joint System

The Biodex 3 is a clinical research and rehabilitation dynamometer system that allows for isokinetic, passive, isometric, isotonic, and reactive eccentric exercises to be performed on knees, ankles, hips, shoulders, elbows, back, forearms, and wrists. Subjects were placed face down on the patient table, with their leg extended straight onto a foot

plate placed at a neutral angle. Fig. 4.3 illustrates how a subject is placed in the Biodex for isometric calf raises.



Fig. 4.3. Patient performing isometric calf raise in a Biodex 3 Multi-Joint System

When instructed, subjects would contract their gastrocnemius muscles to perform an isometric calf raise. Subjects would hold the contraction for three seconds and then relax for three seconds, repeating for a total of six repetitions. The accompanying software for the Biodex 3 system would record the output torque over time. The peak torque recorded was considered the maximum voluntary contraction for that subject.

Next, the subject had motion capture markers placed on their body according to the “plug-in gait” protocol. Measurements were also taken of the subject’s body, such as height, weight, and various joint lengths and widths, in order to calibrate the system to each subject. The Vicon Nexus 2.5 software and Vicon infrared motion capture cameras sampling at 240 Hz were utilized for the motion capture protocol.

Upon completion, subjects put on a heart rate chest strap, were placed in a treadmill, and told to run at a steady, fatiguing pace without changing the speed. Ten second data segments were recorded from the motion capture markers and the sEMGs every 45-60 seconds of elapsed time. Once a minute, the subject would give their rating on the Borg Scale of Perceived Exertion, which is a relative scale from 6 to 20, with 6 being no exertion and 20 being the most exertion. Heart rate data was also collected from the chest strap attached to the subject at these intervals. Both Borg scale and heart rate were utilized to determine fatigue level. If a subject had a Borg rating of 17 or a heart rate of at least 85% of the subject's maximum heart rate, the subject was considered fatigued [66],[67]. Fig. 4.4 shows a patient performing the running exercise on the treadmill.



Fig. 4.4. Patient running a 5K on a treadmill

Once a five-kilometer distance was reached, subjects were asked to repeat the Biodex isometric exercise once again. These results would be used to compare to the pre-treadmill results.

The sEMG data was processed using a Python 3 script that extracted the sEMG data, obtained the pulses, and found the mean frequency, second order Dimitrov spectral index, fifth order Dimitrov spectral index, and fractal dimension. Fractal dimension was specifically computed with the Higuchi's method with a k_{max} value of 6. Results were outputted to a comma separated value file. A two-tailed T-Test was then performed on the data to check for statistical significance.

After the running trials, the trend of the processed data was unexpected and required a validation of the collection and post-processing methods of the data. A few weeks later, a follow-up study using calf raises was conducted with eight returning subjects.

The subjects had sEMGs placed on the gastrocnemius medialis and the gastrocnemius lateralis of both legs, in order to ensure the trend was similar for both legs. The subjects were then instructed to perform thirty-five calf raises on each leg individually to a metronome beat of 70 beats per minute. This value was chosen in order to simulate the jogging pace of a subject [68],[69]. A setup was created for their feet that included a railing for balance and a target bar. The target bar was placed above the ball of the subject's foot and was designed to be touched by their foot near their maximum calf raise height, so that the full range of motion could be controlled. A patient performing this exercise is shown in Fig. 4.5.



Fig. 4.5. Patient performing calf raise exercise

The inactive leg was suspended into the air, not bearing weight. After a leg was completed, the subject switched legs and started again. After the subjects had completed two sets on each leg, the output was then processed by the Python script and graphed. Finally, a two-tailed T-Test was performed on the data to check for statistical significance.

The Python script was created using Anaconda, a distribution of Python with packages built-in for data processing. The script creates a graphical user interface that allows the user to choose multiple files for processing, as well as the EMGs that were active and the pulse definition parameters. The script then reads the EMG values from

each file for the active EMGs. An example of one of these waveforms is shown in Fig. 4.6.

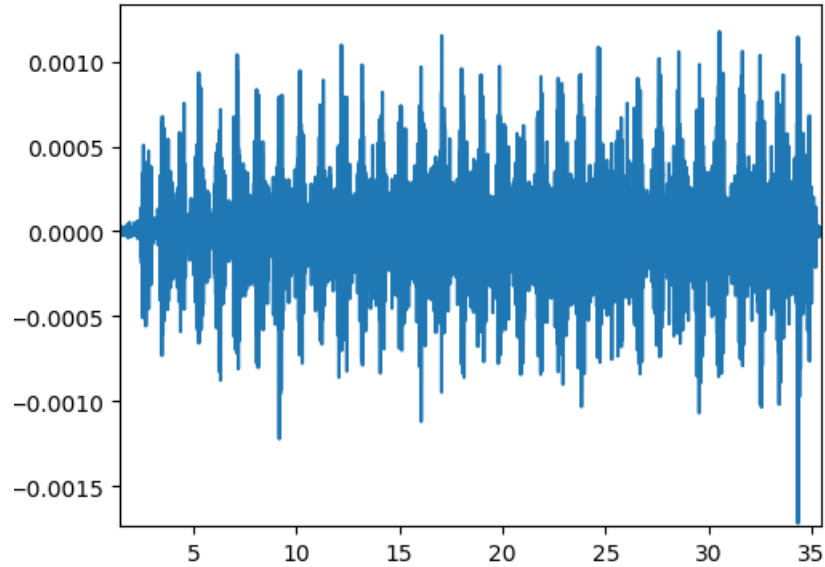


Fig. 4.6. Example of raw EMG signal

The signals are filtered between 20 Hz and 400 Hz and then put through a savgol filter to get the envelope of the EMG signal. The script then finds each pulse and processes the mean frequency, RMS amplitude, 2nd order Dimitrov Spectral Index, and fractal dimension for each pulse. An illustration of this process is shown in Fig. 4.7.

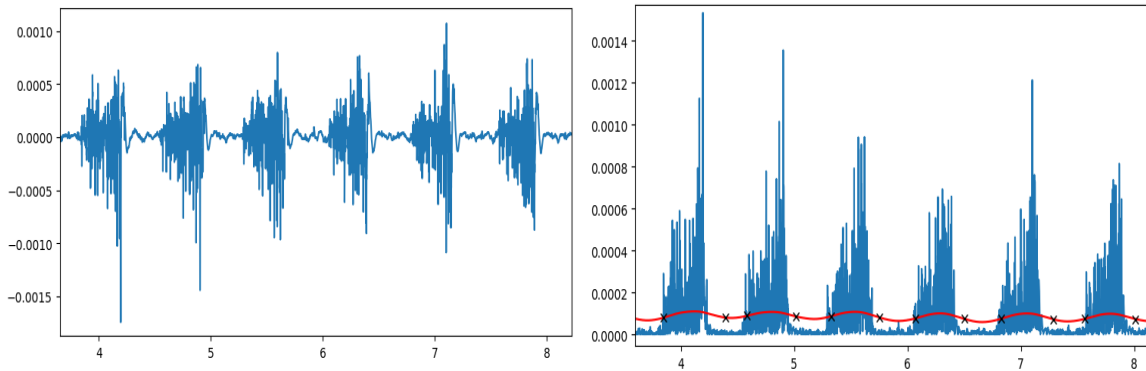


Fig. 4.7. Left) Example of raw signal zoomed in on a five second segment, Right) five second EMG signal rectified, filtered and pulse detected. The “x” points are where the program detected the start and end of the pulses. These were the pulses that were processed for the four parameters.

The first and last pulse needed to be deleted for most trials, especially the calf raise trials, due to the subject accustoming to the exercise at the beginning and then breaking form at the end. An excel file is then created with the graph of the output values.

Parameter Changes

An important factor in understanding the results obtained from processing is understanding what the changes in each of the parameters indicates. For mean frequency, when the frequency decreases, this indicates a switch from using fast twitch muscles to slow twitch muscles. If the frequency increases, this indicates changing from slow twitch muscles to fast twitch muscles. Since fatigue is typically signaled by the body changing from using anaerobic fast twitch muscles to aerobic slow twitch muscles, a frequency decrease would be assumed when there is a fatigue onset.

Amplitude, as a parameter, has meanings that are tied very closely with frequency, and they are usually utilized in conjunction. Amplitude of the EMG signal normally suggests power output, as long as no other parameter changes. An increase in amplitude is an increase in power output, and a decrease in amplitude is a decrease in

power output. However, if frequency changes with amplitude changes, this changes the interpretation of the signal. For example, if mean frequency goes up but amplitude decreases, this could indicate a swap to fast twitch muscles with a constant power output. Since fast twitch muscles have a high force output, less of them are needed to output the same force as slow twitch muscles. The opposite reaction would be a mean frequency decrease and amplitude increase, which indicates a larger amount of slow twitch muscles taking over for fatigued fast twitch muscles.

Spectral index changes are unique and not a lot is known about the intricacies of their alterations over a signal. Since frequency and amplitude are included in the calculation of the parameter, changes in one or both parameters will affect the outcome of spectral index. However, in general, when a muscle experiences fatigue, the spectral index will increase.

Fractal dimension is also a novel parameter that is seeing interest in the field. Fractals are a parameter that is greatly affected by the change in complexity of a waveform. If fractal dimension decreases, this is due to the reduction in the complexity of the waveform, which, in muscles, likely corresponds with an increase in synchronization of motor unit activations and a decrease in mean frequency. Synchronization increases as fatigue increases, usually within strength-based exercises, to keep muscle output constant. Mean frequency decreases due to a switch from fast twitch fibers to slow twitch fibers. In the case of a fractal increase, the muscles show an increase in motor unit recruitment and conduction velocity. This motor unit recruitment is to increase force due to growing demand or to replace fatigued fibers. Conduction velocity changes are due to exhaustion at low force levels.

CHAPTER FIVE

Results

Introduction

This chapter will present the results of the two experimental exercises that were performed. The calf raise experiment results will be introduced first because it is a validation experiment that will reinforce the testing and processing methods for the running section. The results of the running exercises are presented next, followed by a final comparison of the two experiments.

Calf Raise Trials

In the follow-up calf raise study, the nine subjects completed all thirty-five calf raises satisfactorily for both legs and both sets, with one subject's data corrupted. On visual observation, all subjects were able to lift their heel completely off the floor, leaving just the ball of their foot on the floor. Mean frequency and fractal dimension were calculated for each subject's gastrocnemius muscles.

Mean Frequency

The mean frequency data for each subject was averaged together after deleting the first and last point, in order to account for the subject acclimating to exercise protocol and then breaking form on the last repetition. The first three points were averaged to find the percent change from the beginning to the end with the average of the last three points.

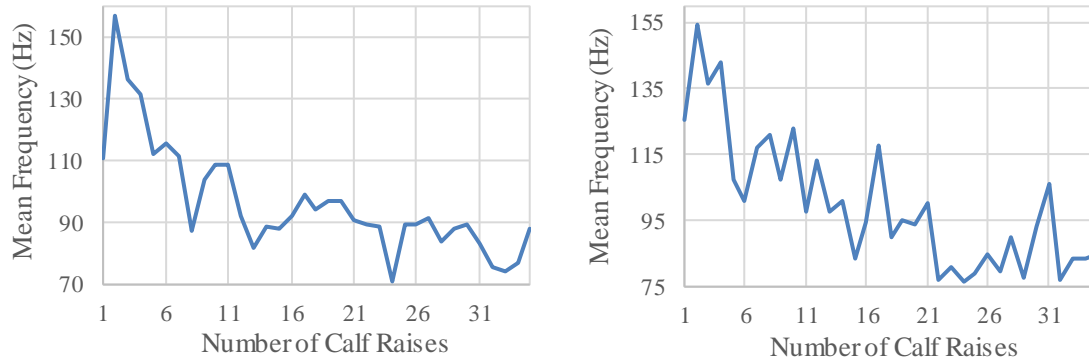


Fig 5.1. An example of calf raise mean frequency, (Left) subject 10 right leg gastrocnemius lateralis mean frequency, (Right) subject 10 right leg gastrocnemius medialis mean frequency

The percent change for each subject ranged from 7.8 – 42.6% and 5.9 – 26.8% for the left gastrocnemius lateralis and medialis, respectively, with the right gastrocnemius lateralis ranging from 3.2 – 40.8% and 0.1 – 41.4% for the medialis. For the left leg, every subject went down over the exercise except for one subject who went up. In the right leg, all subjects went down in mean frequency. The graphs of the average mean frequency is shown for both the left and right legs in Fig. 5.2 and Fig.5.3. The average percent change was found to be $-13.0 \pm 8.4\%$ and $-13.8 \pm 4.4\%$ for the left gastrocnemius lateralis and medialis, respectively. For the right gastrocnemius, the average percent change was found to be $-21.6 \pm 4.5\%$ for the lateralis and $-15.6 \pm 5.1\%$ for the medialis. These values are shown in Fig. 5.4 and Table 5.1.

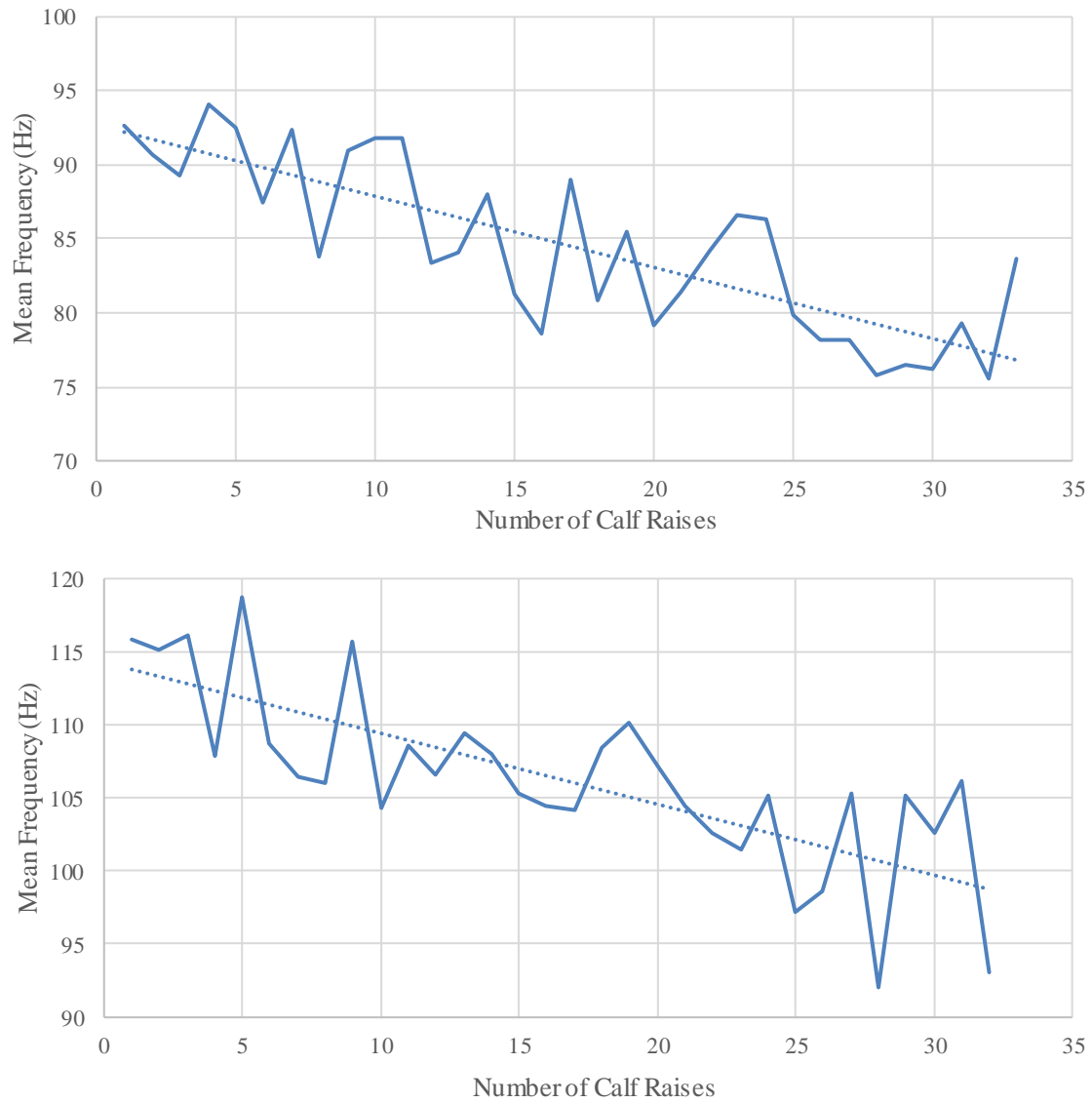


Fig 5.2. Average mean frequency for all subjects in the left leg for (top) gastrocnemius lateralis and (bottom) gastrocnemius medialis. Key takeaway: consistent decrease in mean frequency across subjects.

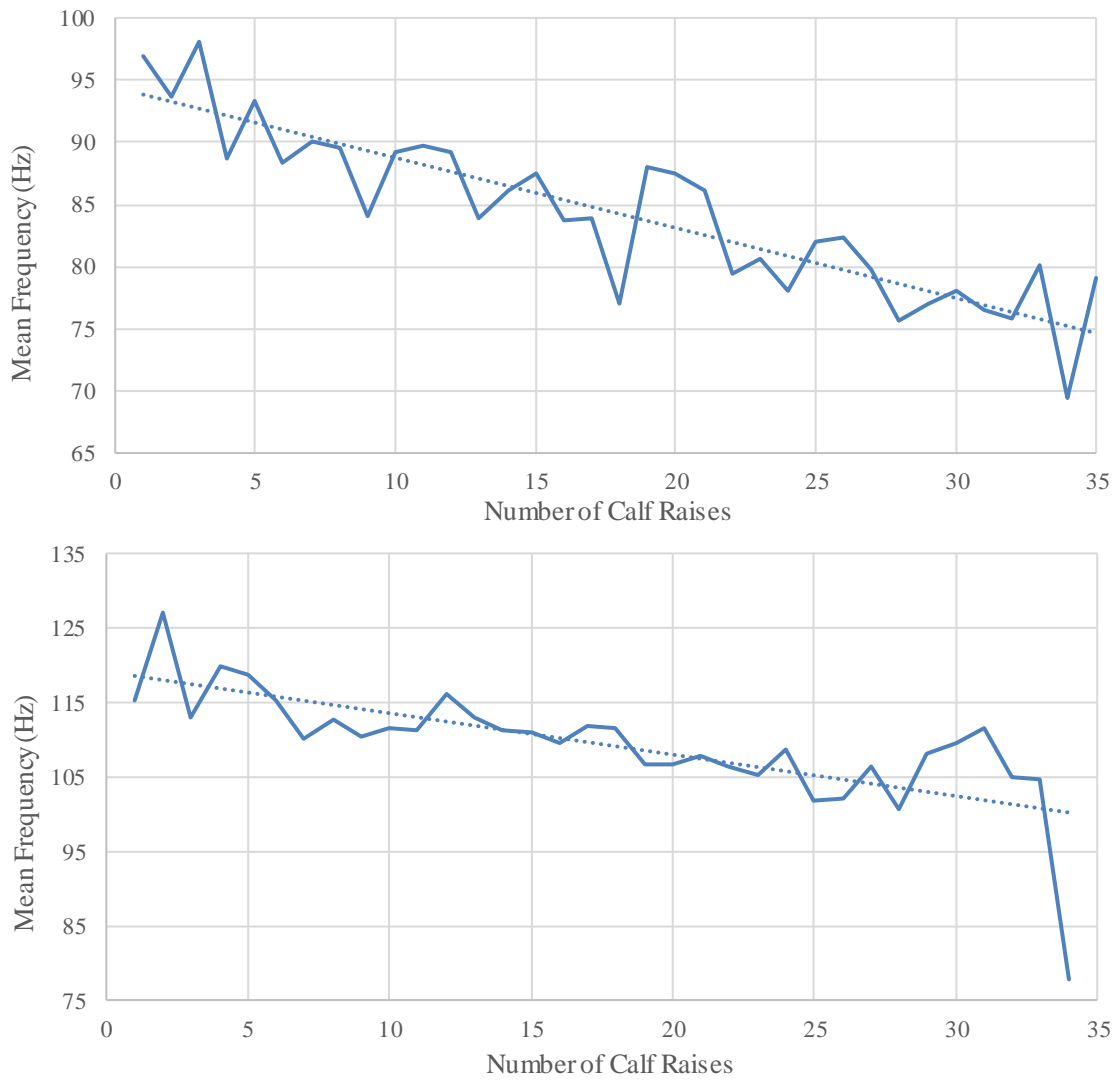


Fig 5.3. Average mean frequency for all subjects for (top) right gastrocnemius lateralis and (bottom) right gastrocnemius medialis. Key takeaway: consistent decrease in mean frequency across subjects.

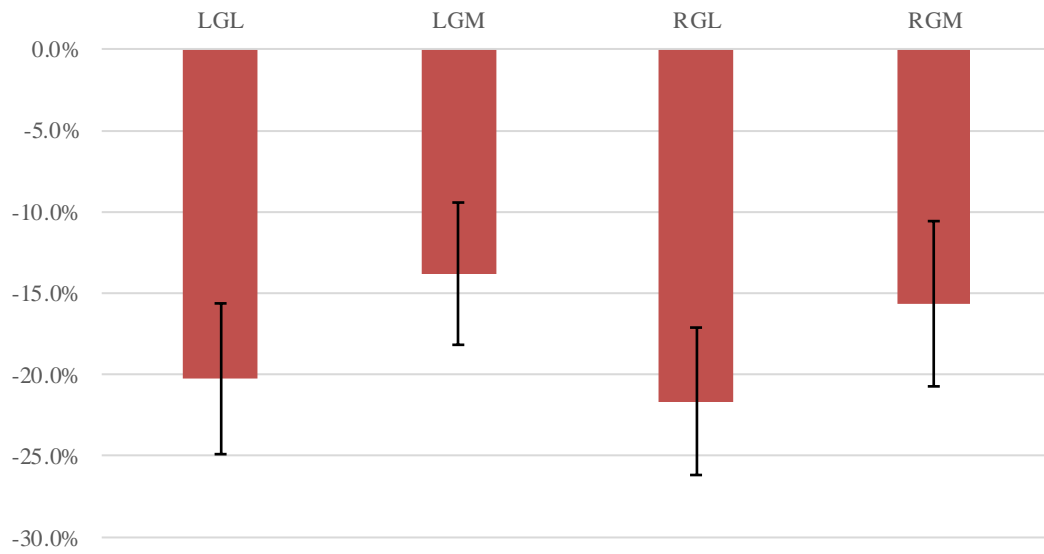


Fig. 5.4. Average mean frequency percent change in each leg muscle during calf raise exercise with standard error. Key takeaway: all muscles decreased in mean frequency.

Table 5.1. Average percent change of mean frequency for each muscle in each subject. Averages and frequency change in hertz are shown with standard error. A two-tailed t-test's p values are shown in the last column.

Muscles	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	AVG	Frequency Change (Hz)	P-Value
LGL	-7.8%	-	-18.0%	-23.8%	-17.4%	-11.9%	-	-42.6%	-20.3 ± 4.6%	-19.5 ± 4.5	0.015
LGM	-19.7%	5.9%	-9.2%	-10.8%	-26.8%	-9.3%	-	-26.5%	-13.8 ± 4.4%	-16.5 ± 4.6	0.016
RGL	-24.2%	-4.5%	-30.4%	-3.2%	-19.1%	-40.8%	-21.7%	-29.2%	-21.6 ± 4.5%	-23.0 ± 6.1	0.007
RGM	-10.3%	-6.4%	-9.6%	-4.7%	-0.1%	-41.4%	-30.6%	-22.1%	-15.6 ± 5.1%	-18.5 ± 7.1	0.027

Fractal Dimension

The fractal dimension was also calculated for each muscle. Example waveforms are shown in Fig. 5.5. The fractal dimension decreased for every subject with an average change shown in Fig. 5.6 and Table 5.2.

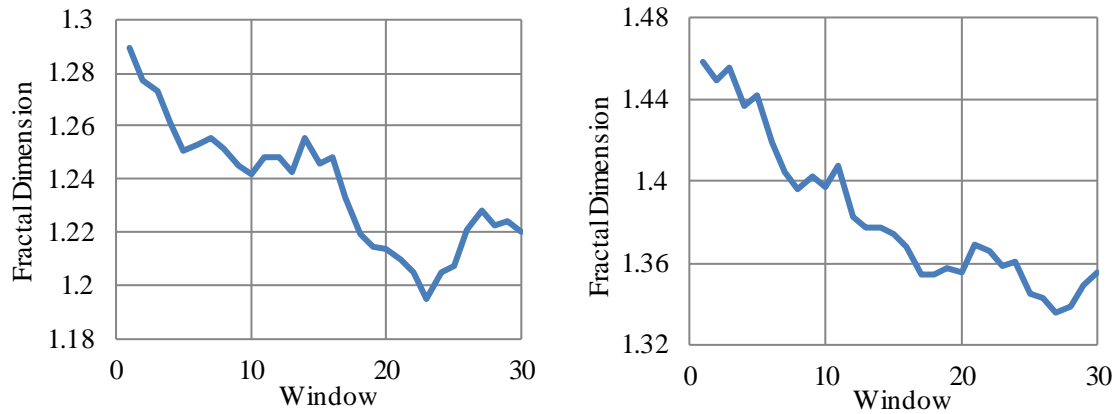


Fig. 5.5. Exemplary fractal dimension data from subject 1 for fractal dimension of the left) right gastrocnemius medialis and the right) right gastrocnemius lateralis

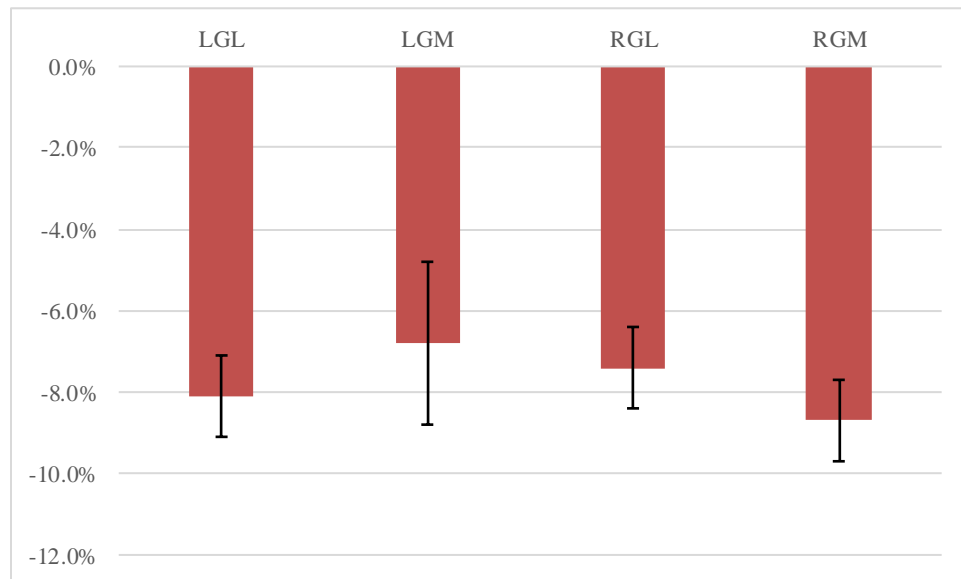


Fig 5.6. Average fractal dimension percent change in each leg muscle during calf raise exercise with standard error. Key takeaway: all muscles decreased in fractal dimension across all subjects.

Table 5.2. Average percent change of the fractal dimension for each muscle in each subject. Averages are shown with standard error. P-values are shown in the last column.

Muscles	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	AVG	P-Value
LGL	-3.2%	-6.8%	-6.4%	-8.3%	-8.8%	-9.2%	-	-13.8%	-8.1 ± 1%	0.00045
LGM	-4.6%	-0.6%	-9.0%	-2.2%	-10.2%	-8.5%	-	-12.7%	-6.8 ± 2%	0.00929
RGL	-6.9%	-5.4%	-3.6%	-3.8%	-10.3%	-13.9%	-6.7%	-8.5%	-7.4 ± 1%	0.00048
RGM	-10.3%	-3.0%	-5.2%	-10.7%	-9.0%	-12.4%	-6.8%	-12.2%	-8.7 ± 1%	0.00048

The change in mean frequency over the course of the calf raise trial trended down for every muscle in every subject, except for subject 2's left leg. The p-value for mean frequency for each leg muscle was statistically significant, with a value less than 0.05. The fractal had a similar development, decreasing for every subject and having an overall average of $-8.1 \pm 1\%$ and $-6.8 \pm 2\%$ for the left leg's gastrocnemius lateralis and medialis, respectively. The right leg's average was $-7.4 \pm 1\%$ and $-8.7 \pm 1\%$ for the right leg's gastrocnemius lateralis and medialis, respectively. The p-value for fractal dimension for each leg muscle was statistically significant, with a value less than 0.05. The trends were consistent and repeatable between legs and subjects.

Running Trials

The heart rate and Borg rating of the subjects increased at a fairly similar rate, with an average increase of $19.43 \pm 1.8\%$ and $62.86 \pm 5.3\%$, respectively. This is shown for each subject in Table 5.3. The running trials measured parameters: mean frequency, RMS amplitude, 2nd-order Dimitrov spectral index, and fractal dimension. Eleven subjects completed the full trial successfully. These trials included the fatiguing five kilometer run portion, as well as the pre- and post – trial calf raise isometric exercise in the Biodex 3 system.

Table 5.3. Percent change of the heart rate and Borg for each subject. Averages are shown with standard error.

Parameters	Subject1	Subject 2	Subject 3	Subject5	Subject6	Subject7	Subject8	Subject10	Subject11	Subject12	AVG
Heart Rate	-	21.0%	27.4%	22.6%	20.4%	11.6%	20.4%	18.6%	22.1%	10.7%	$19.43 \pm 1.8\%$
Borg Scale	57.1%	50.0%	100.0%	64.3%	71.4%	50.0%	71.4%	50.0%	42.9%	71.4%	$62.86 \pm 5.3\%$

Torque

The torque parameter was a measurement taken from the Biodex 3 system before and after the fatiguing run. The subject performed a calf raise while lying face-down in the chair. The changes in torque after the run are reported for each subject in Fig. 5.7.

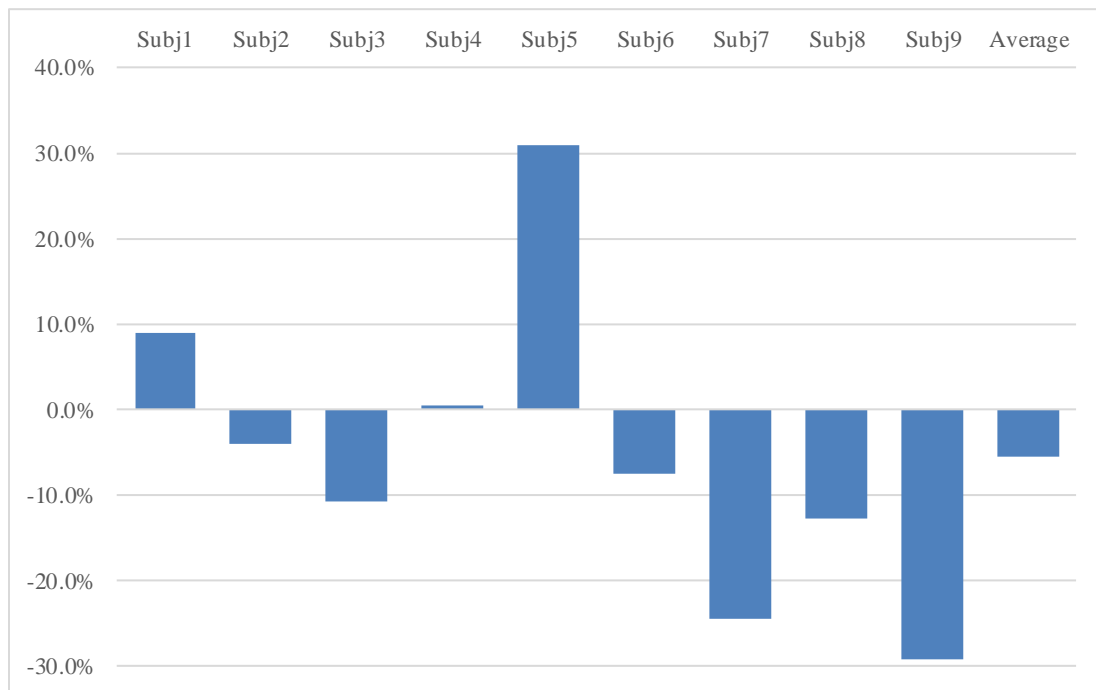


Fig 5.7. Torque change after a fatiguing run for each subject. The data of two subjects was deleted due to corruption of the recording. Key takeaway: torque consistently decreased, showing that the subjects fatigued during endurance run.

After the torque data of all subjects were averaged together, the average maximal pre-run torque was overall lower than the average maximal post-run torque. The average maximum difference between the pre-run and post-run torque was 5.4%. However, the outlier of 30.9% is removed, since it is unrealistic that the torque after a fatiguing run is 30.9% higher than before the run. This value was likely caused by equipment failure or electrode misplacement. Removing this value makes the average maximum difference 9.9%. Note that this value was slightly impacted by the longer-than-desired recovery time

between the end of the run and the post-run biodex trial, with a maximum of about five minutes.

Mean Frequency

During the running trials, every muscle pulse, from the savgol filter signal, was found and processed for mean frequency. After the mean frequency was found for every pulse, it was graphed versus time. Examples of the mean frequency graphs are shown in Fig. 5.8.

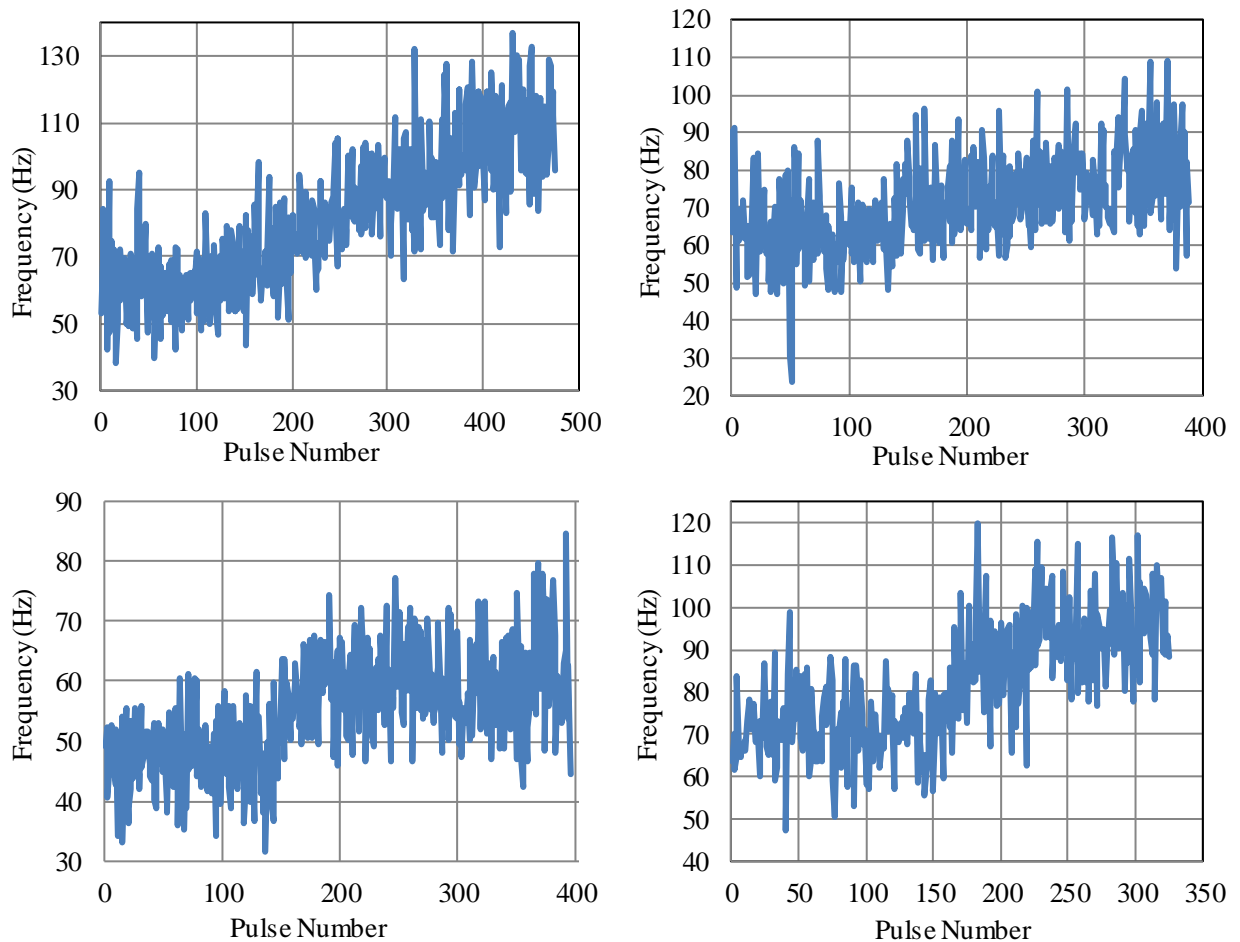


Fig. 5.8. Example of mean frequency waveforms: subject 1's gastrocnemius lateralis (top left), subject 6's gastrocnemius lateralis (top right), subject 7's gastrocnemius medialis (bottom left), and subject 6's vastus lateralis (bottom right). Multiple subjects are displayed to show the change is repeatable over differing subjects and muscles.

The general behavior of the mean frequency was an upward trend for both gastrocnemius muscles and a steady trend for the vastus lateralis. The mean frequency for the gastrocnemius medialis went up for seven subjects and went down for two subjects. The gastrocnemius lateralis had a similar trend, with seven subjects increasing and three subjects decreasing. The vastus lateralis had five subjects trend upwards and four go down. The average increase was $14.6 \pm 4.6\%$, $12.0 \pm 7.5\%$, and $2.7 \pm 9.8\%$ for the gastrocnemius medialis, gastrocnemius lateralis, and vastus lateralis, respectively. The p-values show that the gastrocnemius lateralis changes were statistically significant, while the other two muscles were not. These values and trends are shown in Fig. 5.9 and Table 5.4.

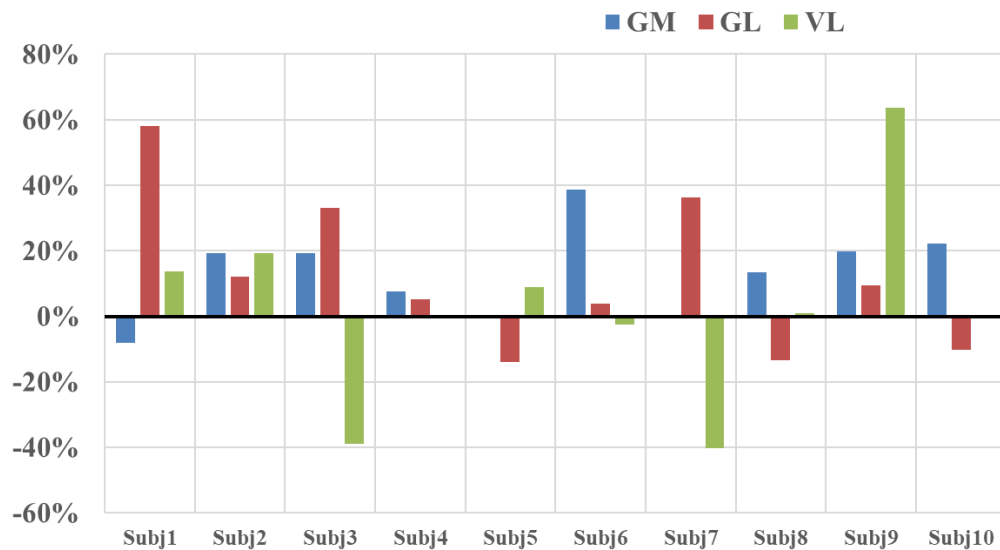


Fig. 5.9. Mean frequency trends by percentage for each muscle in each subject.

Table 5.4. Mean frequency change in each muscle in each subject. Averages are shown with standard error. P-values are shown in the last column. Key takeaway: consistent increase in mean frequency and a statistically significant change in the gastrocnemius lateralis.

Muscles	Subject1	Subject 2	Subject 3	Subject5	Subject6	Subject7	Subject8	Subject10	Subject11	Subject12	AVG	P-Value
Gastrocnemius Medialis	-8.1%	19.2%	19.3%	7.5%	-0.2%	38.6%	—	13.4%	19.9%	22.2%	14.6 ± 4.6%	0.1004
Gastrocnemius Lateralis	58.0%	12.0%	33.1%	5.1%	-14.0%	3.8%	36.2%	-13.5%	9.5%	-10.4%	12.0 ± 7.5%	0.0131
Vastus Lateralis	13.6%	19.2%	-38.9%	—	9.0%	-2.4%	-40.2%	1.0%	63.7%	-0.5%	2.7 ± 9.8%	0.8700

Root Mean Square

The average power from every pulse was also calculated and processed as the root mean square. Every subject and every muscle decreased in RMS amplitude, except for subject 8's vastus lateralis. The average percent change was a decrease of $-51.6 \pm 7.2\%$, $-55.0 \pm 10.9\%$, and $-40.4 \pm 12.1\%$ for the gastrocnemius medialis, gastrocnemius lateralis, and the vastus lateralis, respectively. The p-values showed that the changes for both gastrocnemius muscles were statistically significant, but the changes for the vastus lateralis were not. These values and trends are shown in Table 5.5 and Fig 5.11. Example waveforms of RMS are shown in Fig. 5.10.

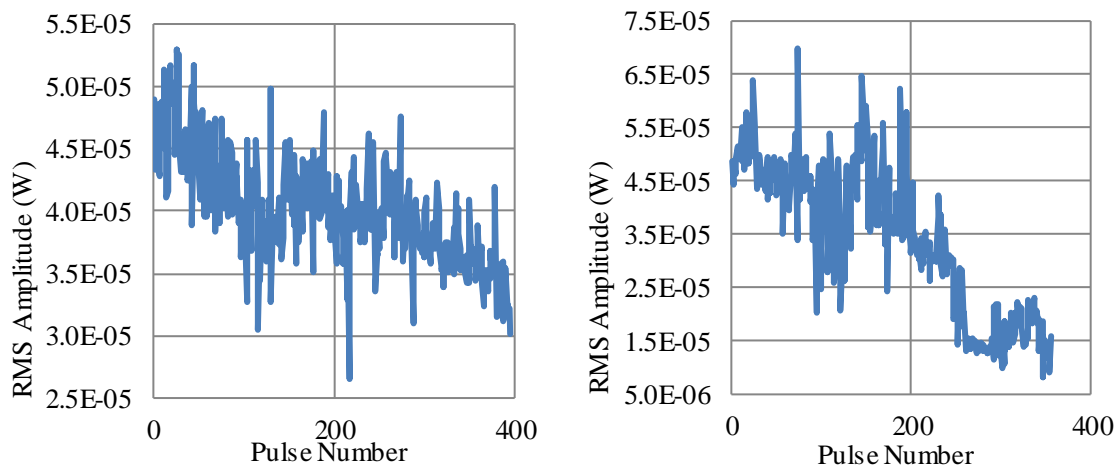


Fig. 5.10. Example of RMS amplitude from subject 10's leg muscles in the gastrocnemius medialis (left) and gastrocnemius lateralis (right).

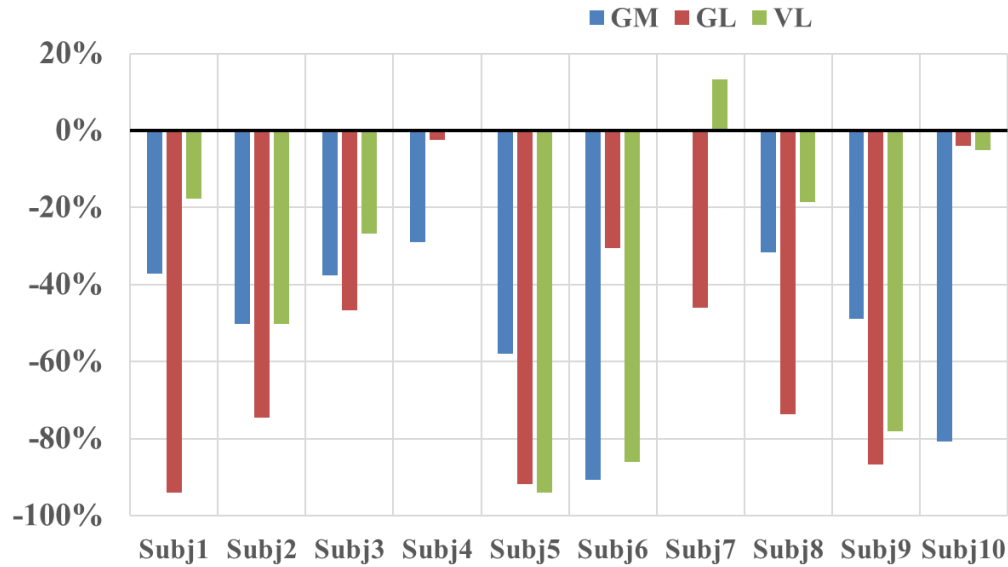


Fig. 5.11. RMS amplitude trends by percentage for each muscle in each subject.

Table 5.5. Root mean square change in each muscle in each subject. Averages are shown with standard error. P-values are shown in the last column. Key takeaway: consistent decrease in RMS amplitude and a statistically significant change in the gastrocnemius medialis and gastrocnemius lateralis.

Muscles	Subject1	Subject 2	Subject 3	Subject5	Subject6	Subject7	Subject8	Subject10	Subject11	Subject12	AVG	P-Value
Gastrocnemius Medialis	-37.1%	-50.3%	-37.7%	-29.0%	-57.9%	-90.8%	—	-31.7%	-48.9%	-80.7%	-51.6 ± 7.2%	0.0026
Gastrocnemius Lateralis	-94.0%	-74.5%	-46.6%	-2.5%	-91.8%	-30.4%	-46.0%	-73.8%	-86.6%	-4.0%	-55.0 ± 10.9%	0.0058
Vastus Lateralis	-17.7%	-50.3%	-26.8%	—	-93.9%	-86.1%	13.2%	-18.6%	-78.0%	-5.2%	-40.4 ± 12.1%	0.3326

Spectral Index

Dimitrov's spectral index is found by dividing the spectral moment of order zero by the spectral moment of a chosen order. In this situation, the 2nd order spectral index was chosen. The gastrocnemius medialis for all subjects trended downward. The gastrocnemius lateralis had seven subjects increase, and three subjects decrease. The vastus lateralis had five subjects go down, and four subjects go up. The average change was a decrease of $-28.0 \pm 4.3\%$ and $-16.2 \pm 9.0\%$ for the gastrocnemius medialis and gastrocnemius lateralis, respectively, and a $9.1 \pm 15.6\%$ increase for the vastus lateralis.

The p-values showed a weak statistical significance for the gastrocnemius medialis, but there was no statistical significance for the gastrocnemius lateralis or the vastus lateralis. These values and their trends for each subject are shown in Table 5.6 and Fig. 5.13. Fig. 5.12 shows some example spectral index waveforms.

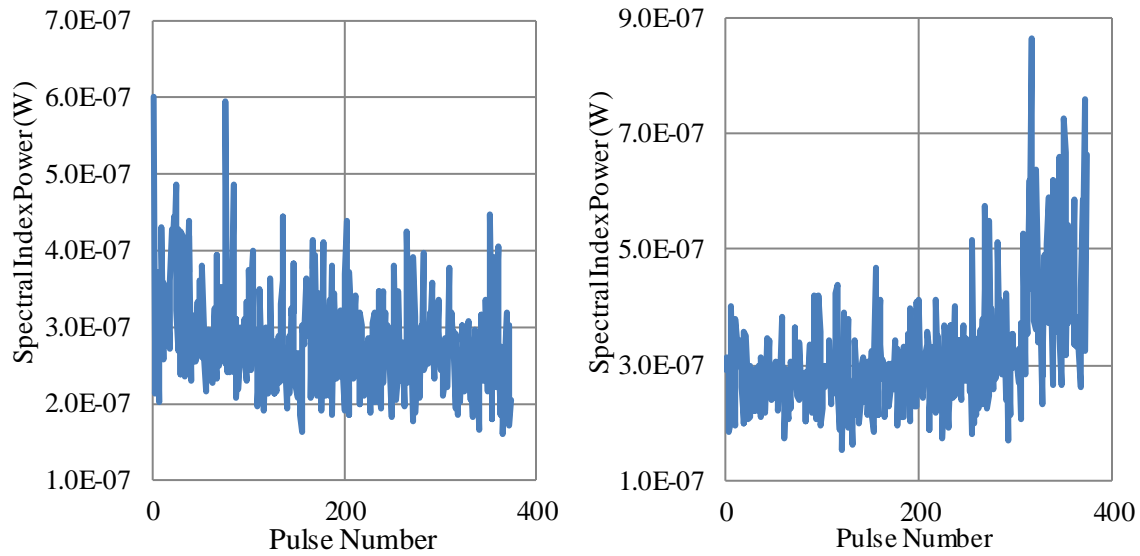


Fig. 5.12. Example of 2nd order spectral index from subject 3's leg muscles in the gastrocnemius lateralis (left) and vastus lateralis (right).

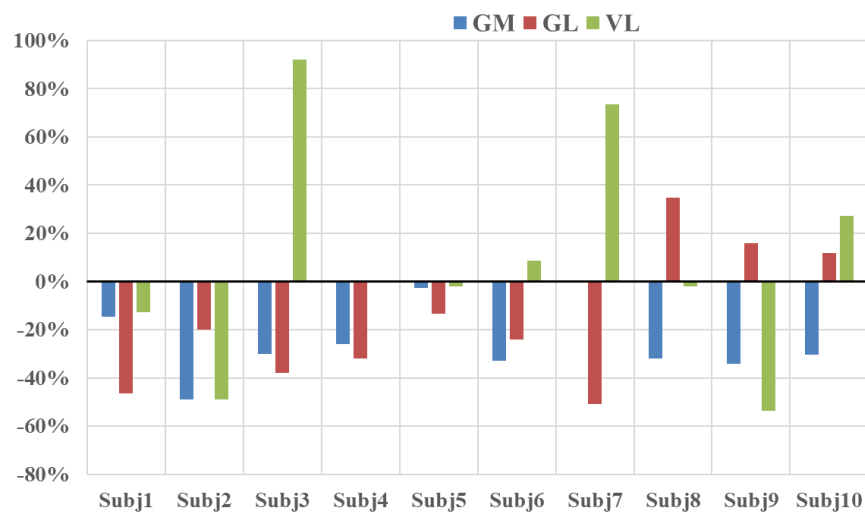


Fig. 5.13. Spectral index trends by percentage for each muscle in each subject.

Table 5.6. 2nd order spectral index change for each muscle in each subject. Averages are shown with standard error. P-values are shown in the last column. Key takeaway: consistent decrease in spectral index, but no statistically significant changes in any muscle.

Muscles	Subject1	Subject 2	Subject 3	Subject5	Subject6	Subject7	Subject8	Subject10	Subject11	Subject12	AVG	P-Value
Gastrocnemius Medialis	-14.8%	-49.1%	-30.1%	-26.0%	-2.7%	-33.0%	—	-31.8%	-34.1%	-30.4%	-28.0 ± 4.3%	0.0545
Gastrocnemius Lateralis	-46.4%	-19.9%	-37.9%	-32.0%	-13.3%	-24.1%	-50.7%	34.7%	15.8%	11.9%	-16.2 ± 9.0%	0.8936
Vastus Lateralis	-12.9%	-49.1%	92.2%	—	-2.2%	8.6%	73.5%	-1.9%	-53.8%	27.1%	9.1 ± 15.6%	0.4825

Fractal Dimension

The fractal dimension looks at the change in the partial dimension that the wave exists. Unlike the other parameters, the fractal dimension is calculated over the entire waveform, as it looks for the self-similarity of the wave. The algorithm used to find the fractal dimension was Higuchi's algorithm. Every muscle for every subject trended upwards, except for subject 3's vastus lateralis and subject 5's gastrocnemius lateralis. The average percent change of the fractal dimension was an increase of 8.7%, 11.6%, and 9.5% for the gastrocnemius medialis, gastrocnemius lateralis, and vastus lateralis, respectively. The p-values for the muscles showed a statistical significance for all three. These values and their trends are shown in Table 5.7 and Fig. 5.15. Example waveforms for fractal dimension are displayed in Fig. 5.14.

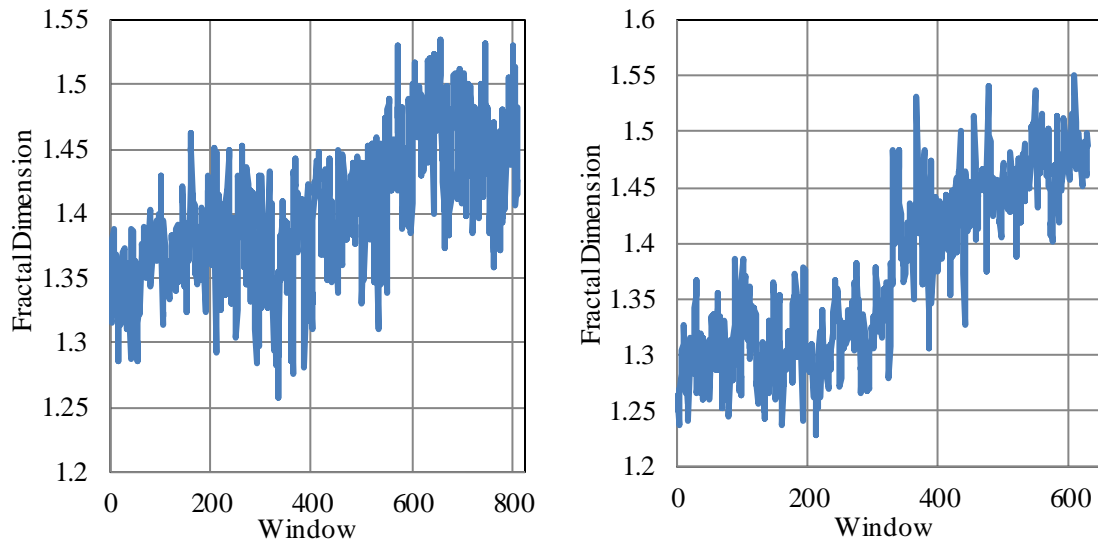


Fig. 5.14. Example of fractal dimension in subject 10's gastrocnemius lateralis (left) and subject 7's gastrocnemius medialis (right).

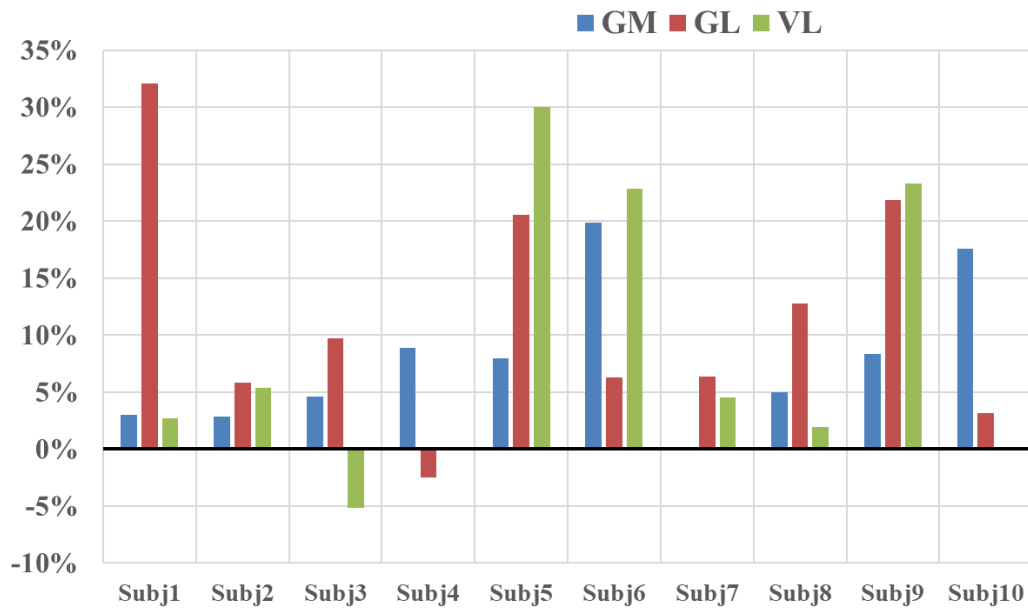


Fig. 5.15. Fractal dimension trends by percentage for each muscle in each subject.

Table 5.7. Fractal dimension change for each muscle in each subject. Averages are shown with standard error. P-values are shown in the last column. Key takeaway: consistent increase in fractal dimension and a statistically significant change all three muscles.

Muscles	Subject1	Subject 2	Subject 3	Subject5	Subject6	Subject7	Subject8	Subject10	Subject11	Subject12	AVG	P-Value
Gastrocnemius Medialis	3.0%	2.8%	4.6%	8.9%	7.9%	19.9%	—	4.9%	8.3%	17.5%	$8.7 \pm 2.0\%$	0.0056
Gastrocnemius Lateralis	32.1%	5.8%	9.7%	-2.5%	20.6%	6.3%	6.3%	12.8%	21.8%	3.2%	$11.6 \pm 3.3\%$	0.0063
Vastus Lateralis	2.7%	5.4%	-5.2%	—	30.0%	22.8%	4.5%	1.9%	23.3%	0.0%	$9.5 \pm 3.9\%$	0.0324

Comparison

For the gastrocnemius medialis, when the mean frequency increased $14.6 \pm 4.6\%$, the root mean squared amplitude and the spectral index decreased $-51.6 \pm 7.2\%$ and $-28.0 \pm 4.3\%$, respectively. For the gastrocnemius lateralis, when the mean frequency increased $12.0 \pm 7.5\%$, the root mean squared amplitude decreased $-55.0 \pm 10.9\%$, and the spectral index decreased $-16.2 \pm 9.0\%$. For the vastus lateralis, when the mean frequency increased $2.7 \pm 9.8\%$, the root mean squared amplitude decreased $-40.4 \pm 12.1\%$, and the spectral index increased $9.1 \pm 15.6\%$. The fractal dimension increased $8.7 \pm 2.0\%$, $11.6 \pm 3.3\%$, and $9.5 \pm 3.9\%$ for all three, respectively.

In terms of statistical significance, the p-values shows that, for mean frequency, only one muscle, the gastrocnemius lateralis, was less than 0.05. In RMS, two muscles, both the gastrocnemius muscles, showed statistical significance as well. However, the spectral index did not have any p-values under 0.05, but the gastrocnemius medialis came close with a value of 0.0545. The fractal dimension showed very low p-values for every muscle, with p-values for the gastrocnemius muscles lower than 0.01 and the vastus lateralis lower than 0.05. A comparison of changes is shown in Table 5.8.

Table 5.8. Summary table of average changes in each parameter across subjects for each muscle. Green box denotes statistical significance.

Muscles	Mean Frequency	RMS	Spectral Index	Fractal Dimension
Gastrocnemius Medialis	-14.6 ± 4.6%	-51.6 ± 7.2%	-28.0 ± 4.3%	8.7 ± 2.0%
Gastrocnemius Lateralis	-12.0 ± 7.5%	-55.0 ± 10.9%	-16.2 ± 9.0%	11.6 ± 3.3%
Vastus Lateralis	-2.7 ± 9.8%	-40.4 ± 12.1%	9.1 ± 15.6%	9.5 ± 3.9%

CHAPTER SIX

Discussion

Purpose

This research attempts to show that muscular fatigue exhibits noticeable changes in key parameters of electromyographic waveforms. It is imperative to gain an understanding of these parameters in order to aid injury prevention.

Validation – Calf Raise Trial

The first step in this research is to verify that the data recording and processing methods were accurate. This was accomplished by completing a controlled dynamic lower limb exercise to exhaustion and comparing the results to current literature, as well as to the results for the less-controlled running task.

Miyamoto et. al. found that when subjects performed one-legged calf raises for 15 sets of 10 repetitions wearing minimally compressive sport stockings, their mean frequency dropped 18.7 Hz in the medial gastrocnemius [70]. Österberg et. al. performed a similar calf raise exercise and divided their calf raise into an eccentric and concentric portion. Each portion had a mean frequency decrease of 32% and 16% for the eccentric phase and the concentric phase of the gastrocnemius medialis, respectively [71]. In a follow-up study, Österberg et. al. found that the frequency decreased 9% for the concentric phase and 20% for the eccentric phase [72].

In another one-legged calf raise study, the medial gastrocnemius had a “trend towards decreased mean power frequency... with increasing number of heel-rises” [73].

Finally, after forty contractions in a one-legged calf raise study comparing high-heel users to flat-heel users, Gefen et. al. found that, for the control group of flat-heel users, the median frequency decrease for the lateral gastrocnemius was 15% , and the decrease for the medial gastrocnemius was approximately 20% [74].

The results of these articles are comparable to the research conducted in this thesis. For the research presented in this thesis, the calf raise portion showed significant decrease of the mean frequency parameter, declining between 12.6-23.4%, or 11.42 – 23.33 Hz, for the lateral gastrocnemius, which is statistically significant, and 13-20.3%, or 15.01 – 24.44 Hz, for the medial gastrocnemius. Comparatively, this puts these values in a similar range to those found in the articles above, providing support for the methods used are adequate to detect and quantify muscular fatigue in a calf raise trial.

Consequently, the results from the controlled calf raise exercise supports the use of similar frequency-based measures for less controlled dynamic lower body exercises. A comparative study between these papers and the research presented in this thesis is shown in Table 6.1.

Table 6.1. Comparison of previous literature and the results obtained in the calf raise portion of this thesis. Key takeaway: the results found during the calf raise portion of this thesis is in a similar range to that of three other calf raise papers.

Muscles	Miyamoto et al	Osterberg et al	Gefen et al	This Research
GM	-18.7 Hz	-32 % (Ecc) -16% (Conc)	-20%	-15.0 - 24.4 Hz -13.0 - 20.3%
GL	—	—	-15%	-11.4 - 23.3 Hz -12.6 - 23.4%

The fractal dimension was an experimental parameter measured in these trials. The fractal dimension decreased by at least 6.8% on average for every subject’s calf raise

trial. In comparison, Beretta-Piccoli et. al. showed that a fractal dimension decrease is evident with a decrease in conduction velocity, which follows a decrease in median and mean frequency. They showed a 2-5% fractal dimension decline in the vastii muscles in a 60% MVC endurance knee extension exercise. It was also theorized that fractal dimension decreases with motor unit synchronization [26]. Motor unit synchronization is when the neurons firings to the muscles synchronize to aid force development. This synchronization increases with fatigue, particularly in strength-based exercises. It is also accompanied by a decrease in mean frequency and an increase in amplitude [23], [24]. This thesis' results also showed a decrease in mean frequency with the fractal decrease. Since the results for the fractal portion seen in this thesis are similar results and trends to those presented and theorized in other papers, it would suggest that fractal dimension can also be used to measure muscular fatigue.

Overall, the preliminary results from the calf raise trials were supported by existing literature and demonstrated the feasibility and potential capability of these measures to identify muscle fatigue during uncontrolled dynamic motions, such as running.

Experimental – Running Trial

For the running trials, each subject was fatigued as evidenced by their heart rate and subjective borg rating. When the heart rate reached 85% of the subject's maximum heart rate, or their Borg rating reached 17, the subject was considered fatigue [66],[67]. Ten of the eleven subjects reached these markers by the end of the treadmill run. The one subject who did not reach this threshold had a Borg of 16 and a maximal heart rate percentage of 80.4%.

Mean Frequency

In the running trials, the same data processing methods were used as those used in the calf raise trials. Mean frequency was one of the four components obtained from the EMG data, along with RMS amplitude, the 2nd order Dimitrov spectral index, and the fractal dimension.

While the mean frequency decreased during the calf raise trials, the running trials had the opposite reaction. In the gastrocnemius muscles, the mean frequency increased. Hägg and Ojok had a similar result when their subjects performed shoulder holds, seeing an approximately 12% rise [75]. Petrofsky also found that, during cycling at low force loads, the mean frequency of the vastus lateralis increased for the first twenty minutes of the endurance exercise [38].

However, this is contrary to most papers. The usual conclusion is that when fatigued, a muscle has a mean frequency reduction [19]. For example, Nicol et. al. had subjects perform isometric knee extensions before and after running a marathon, and they showed a 16% drop in the mean frequency of their vastus medialis [48]. During a handgrip exercise, Petrofsky and Lind saw a lower mean frequency at the end of the trial [39]. In the triceps surae, a set of two hundred maximum effort plantar flexions lowered the mean frequency after the first seventy repetitions and then stabilized for the remaining repetitions, as found by Gerdle et. al [76].

While these results seem to be contradictory, these exercises tend to be two different types of exercises. Strength-based exercises, like maximal effort movements and those with high workloads, see a decline in mean frequency movements and an increase in motor unit synchronicity. Motor unit synchronization is when the body matches motor

unit firing times in order to aid the muscle to create force quickly during rapid contractions [77]. Conversely, endurance and low workload exercises, generally see a mean frequency increase. This seems to imply that fast twitch muscles are needed for power in the strength exercises, but if high force is not needed to complete a movement, slow twitch muscles are relied upon initially.

Therefore, it would seem that mean frequency is a decent predictor of fatigue in running when considered to be a low workload endurance activity. When combining the three muscles and ten subjects, after removing the corrupted data, there were nineteen increases and nine decreases. The frequency increased by at least 10% for thirteen of the nineteen increases, showing that, in the majority of cases, the increases was large when it increased.

Root Mean Square

The root mean square amplitude is very closely related to mean frequency. Because of the low-pass filter effect of skin, when the mean frequency is high, the amplitude dips. If the mean frequency drops, the amplitude is less affected by the low-pass filter effect, which increases the amplitude.

In cycling-based exercises, Castronovo et. al., Petrofsky, and Takaishi et. al. found that, in the vastus lateralis, the amplitude of the waveform increased as fatigue was induced [32],[38],[43]. The vastus lateralis is the power producer of the legs during cycling, so as fatigue sets in, power must increase for this muscle to maintain a constant output [40]. Horita and Ishiko had their subjects perform isokinetic knee extensions and observed that the amplitude in their vastus lateralis increased as they fatigue [37].

In the research presented in this paper, the average final amplitude is half of the original for the gastrocnemius muscles and 60% of the original for the vastus lateralis. This decline is linear in nature and is observed over the whole waveform.

Several papers show similar results to those found by this paper. Returning to Nicol et. al.'s research, after running a marathon, the amplitude dropped in the vastii muscles during knee extensions [48]. Komi and Tesch also showed a similar drop during knee extensions [46]. A cycling exercise showed that the tibialis anterior and gastrocnemius medialis muscles of the leg had an amplitude decrease when fatigue, as well as a change in the pattern of muscle activation [42]. The plantar flexions exercise mentioned earlier also had an amplitude decrease in the middle of the trial [76].

The difference seems to be based on the objective of the paper. If the article tried to keep the output constant, the amplitude increased while the output was constant. If the article tried to observe the muscle's reaction to fatigue while trying to perform an action, the amplitude would decline as the subject becomes too tired to adequately perform the action, thus putting less power into the movement.

When looking at the trends for all ten subjects and three muscles combined, after removing two corrupted data sets, there were 27 out of 28 decreases for RMS. This would seem to indicate that RMS is an excellent marker of fatigue. However, the interpretation of the amplitude changes still seems especially tied to mean frequency changes. Since mean frequency changes indicate the type of muscle fiber being recruited, the decrease in the muscle signal amplitude could mean that the fast twitch muscles are being recruited to take over for fatigued slow twitch fibers or that the muscle force output is decreasing.

Spectral Index

The 2nd order Dimitrov spectral index changes presented in this thesis showed a decrease for both gastrocnemius muscles and an increase for the vastus lateralis. Literature, however, is pretty consistent, showing significant increases with fatigue. During CPR chest compressions, Lee et. al. discovered a 65-72% increase of the 5th order Dimitrov spectral index with fatigue in the biceps brachii and triceps brachii. Gorostiaga et. al. found that fatigue increased the 5th order spectral index for the vastii muscles during leg press exercises [52].

The decrease seen in this paper could be explained by the decreases seen in both the mean frequency and the RMS amplitude. Since Dimitrov's spectral index is found with the product of the spectral power and the frequency, it can be seen how decreases in both values would cause a decrease in the spectral index.

Spectral index was fairly consistent with 21 out of 28 of all subjects' muscle trends decreasing. However, spectral index seems to be greatly affected by noise, as the amplitude is exponentiated. If any amount of noise is present in the EMG signal, it will be exponentiated by the order number of the spectral index. Since sEMG can have noise introduced by dynamic motion and sweat, both of which are present in a fatiguing run, this is a limited factor in the use of spectral index. Therefore, spectral index seems to be a poor indicator of fatigue, especially when noise is present.

Fractal Dimension

Fractal dimension is the most experimental parameter utilized in this research. Few muscle fatigue articles use this parameter in their own research, let alone mention it. Since fractal is looking at the change in complexity of the waveform, it can have many

uses and applications. Many papers have used fractal dimension to examine electroencephalograms, or brain signals.

The papers that have examined muscle signals with fractal dimension have applicable results. Several papers have used handgrip exercises to show that the fractal dimension increases linearly with workload [62],[78]. Three papers that used simulated muscle signals observed that fractal dimension increases with an increased conduction velocity and an increased muscle fiber recruitment number but decreases with an increased motor unit synchronization level [20], [79], [80]. Gupta et. al. found during a bicep curls exercise that the fractal dimension of the signal increases when the weight being curled was increased [81]. Similarly, Gitter and Czerniecki observed a comparable increase when their subjects performed isometric bicep contractions with an increasing workload [63].

The research found in this paper showed an increase of 8.66-11.60% of the fractal dimension. This increase could be explained by the increase in conduction velocity, which, as mentioned above, has an accompanying increase in the mean frequency of the muscle signal. The workload for running is theoretically low, so this would also explain the low starting fractal, which was 1.32, 1.33, and 1.27 for the gastrocnemius medialis, the gastrocnemius lateralis, and the vastus lateralis, respectively [81].

Fractal dimension showed consistent trends across muscles and subjects. Out of 28 trends, 26 of them increased, and two of them decreased. The waveforms for fractal dimension was steady and did not vary as much as the other parameters' waveforms. Considering the consistency and the statistical significance for every muscle group, the fractal dimension seemed to be a good estimator of fatigue, and, when combined with the

traditional methods of mean frequency and RMS amplitude, was able to tell a full story of what was happening to the muscles during fatigue.

Comparison

The only two parameters used in both the calf raises and running was mean frequency and fractal dimension. Both of these parameters had changes in the calf raises that opposed those seen in the running portion. For the mean frequency, there was an increase in the calf raise, while there was a decrease in the running portion. This could be understood by remembering that calf raises are a strength-based exercise, needing to recruit fast twitch muscles for power, while running is an endurance exercise that only needs slow twitch.

The fractal dimension also differed between the two exercises. The calf raise trial had a decrease in the fractal dimension, whereas the running trial had an increase in fractal dimension. The calf raise trial could be explained by the increase in the synchronization of the motor unit activation and a decrease in mean frequency. On the other hand, the increase in the fractal dimension for the running exercises would seem to be from an increased motor unit recruitment and conduction velocity.

Based on these results, there is now an ability to measure fatigue progression during an activity, instead of only before and after. Also, there is now an ability to measure and interpret low workload, endurance exercises, as well as their impact on muscle fiber activity and recruitment. This can eventually be used to allow a runner to observe their fatigue progression during a run, even the fatigue level of individual muscles. The runner then will be able to determine if their form needs to be adjusted or if they need to stop altogether, in order to prevent overuse injury brought on by fatigue.

Importance and Future Work

This research shows that muscle recruitment and fatigue are differently manifested during running than in most strength-based exercises proposed in most electromyography papers. This can inspire more papers that observe muscle activity during different activities and work demands. For example, testing can be performed to study in-depth the fatigue response difference between endurance activities and strength activities on the muscular level. This study could, for instance, look at weighted and unweighted knee extensions and see how muscle fatigue and recruitment develop over the exercise.

Another test could replicate the methods presented in this thesis alongside a comparative study with runners wearing ankle weights. This format would allow researchers to see the difference in fatigue response in a strength-based version of running versus a traditional endurance run. Furthermore, future tests can compare different forms of running, such as sprinting, 5K, and marathon distance, to see how muscles respond in each format.

Another important factor presented in this thesis is showing the usefulness of the fractal dimension parameter. The fractal parameter reflected the changes shown in the other parameters, but with more consistency. Of the four parameters presented, fractal dimension changed with the most statistical significance of any parameter.

Future studies are needed to replicate the results of this paper with a much larger test base. If the results are comparative to those presented in this paper, opportunities would be created to produce diagnostic equipment or techniques to detect fatigue in the leg muscles over the course of running exercises. For example, a pair of exercise pants,

such as a pair of leggings or spandex, could be embedded with an sEMG system measuring specific leg muscles during a fatiguing dynamic motion activity, such as running. The sEMGs send their data to the user's phone, which would run post-processing methods suggested in this thesis. The phone app then could give an alert to the user, informing them of their current fatigue level. The user is then able to make an informed decision on whether they should change form, slow down, or rest, based on their current muscular fatigue level.

Other future work would be to use fractals in other exercises. Since fractals are experimental, their use in various circumstances are not well known. More research showing their use in different exercises and setups could help establish its importance in the electromyographic research field.

Limitations

The research in this paper, while as complete as could be, had a few limitations. Firstly, only eleven subjects could be obtained to perform the test protocol. While this is a comparable amount to most papers in the muscle fatigue field, a larger sample size could help confirm the results.

Next, the surface EMG system that was used had sporadic adhesive issues. While proper skin preparation techniques were used, the impact force of running on the skin cause the EMGs to intermittently disconnect. While this occurred rarely, it still was something that had to be handled properly. Disconnects were easily noticed and accounted for when processing the data.

Contributions

- Developed methodology for measuring dynamic lower limb uncontrolled movements and obtaining EMG metrics
- Provided early support for EMG measures of fatigue within uncontrolled lower limb dynamic fatiguing activities. Prior to this study, there was not previous literature identified that addressed the specific nature of the study.
- Measured multiple parameters of fatigue at once, including the experimental parameter of fractal dimension
- Took current documented methodologies of EMG measures of fatigue from controlled, primarily lab-limited activities and adopted them to uncontrolled dynamic lower limb activities that mirror daily recreation actions

APPENDICES

APPENDIX A

Parameter Table for Each Subject

Subject Data		Subject 1	Subject 2	Subject 3	Subject 5	Subject 6	Subject 7	Subject 8	Subject 10	Subject 11	Subject 12	AVG
	HR		21.02%	27.39%	22.63%	20.41%	11.56%	20.41%	18.60%	22.14%	10.71%	19.43%
	Borg	57.14%	50.00%	100.00%	64.29%	71.43%	50.00%	71.43%	50.00%	42.86%	71.43%	62.86%
Gastrocnemius Medialis												
	Mean Frequency	-8.12%	19.16%	19.26%	7.47%	-0.23%	38.57%	–	13.40%	19.90%	22.18%	14.62%
	RMS	-37.09%	-50.28%	-37.70%	-28.99%	-57.89%	-90.81%	–	-31.68%	-48.87%	-80.70%	-51.56%
	Flism2	-14.75%	-49.12%	-30.08%	-26.01%	-2.71%	-32.97%	–	-31.84%	-34.10%	-30.44%	-28.00%
	Fractal	2.99%	2.84%	4.63%	8.85%	7.94%	19.87%	–	4.95%	8.30%	17.54%	8.66%
Gastrocnemius Lateralis												
	Mean Frequency	58.04%	12.04%	33.11%	5.11%	-14.01%	3.78%	36.18%	-13.49%	9.50%	-10.36%	11.99%
	RMS	-93.95%	-74.51%	-46.63%	-2.50%	-91.85%	-30.44%	-46.05%	-73.76%	-86.63%	-4.03%	-55.04%
	Flism2	-46.38%	-19.93%	-37.88%	-31.99%	-13.29%	-24.05%	-50.71%	34.74%	15.78%	11.88%	-16.18%
	Fractal	32.07%	5.85%	9.73%	-2.51%	20.57%	6.27%	6.32%	12.75%	21.81%	3.17%	11.60%
Vastus Lateralis												
	Mean Frequency	13.58%	19.16%	-38.92%	–	8.96%	-2.41%	-40.24%	1.04%	63.74%	-0.45%	2.72%
	RMS	-17.70%	-50.28%	-26.75%	–	-93.91%	-86.11%	13.24%	-18.57%	-78.01%	-5.15%	-40.36%
	Flism2	-12.88%	-49.12%	92.17%	–	-2.18%	8.58%	73.47%	-1.91%	-53.75%	27.09%	9.05%
	Fractal	2.70%	5.39%	-5.17%	–	30.01%	22.81%	4.50%	1.94%	23.31%	-0.04%	9.49%

APPENDIX B

Processing Program GUI

The screenshot displays the EMGProc application window. The title bar shows the application name 'EMGProc' and standard window controls. The menu bar includes 'File', 'Help', and 'Info'. The main interface is divided into several sections by dashed lines:

- File Management:** Contains a 'Browse for File (csv only)' button, a 'Chosen File:' label, a 'Desired Output Filename:' text input field with an 'Enter' button, and radio buttons for 'Overwrite File' (selected) and 'Add to File'.
- Output Filename:** A text input field for specifying the output filename.
- Active EMGs:** A grid of checkboxes for selecting active EMG channels, labeled EMG 1 through EMG 16.
- Save Active EMGs:** A button to save the current selection of active EMGs.
- Pulse Definitions:** Contains three input fields for 'Pulse Relative Height' (0.8), 'Pulse Min Width(in Samples)' (500), and 'Pulse Max Width(in Samples)' (2000), along with a 'Save Properties' button.
- Generate Output:** A button at the bottom to generate the final output.

APPENDIX C

Python Code

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#Final Version of Python Code Created by Adam Lewis

#GUI library package import
from tkinter import *
from tkinter import filedialog
import tkinter

#Initialization of GUI window
window = Tk()
menu = Menu(window)
d= {}

#EMG variable name creation
for i in range(1,17):
    d['v{}'.format(i)] = tkinter.IntVar()

emg options = [( 'EMG 1',1),( 'EMG 2',2),( 'EMG 3',3),( 'EMG 4',4),
                ( 'EMG 5',5),( 'EMG 6',6),( 'EMG 7',7),( 'EMG 8',8),
                ( 'EMG 9',9),( 'EMG 10',10),( 'EMG 11',11),( 'EMG 12',12),
                ( 'EMG 13',13),( 'EMG 14',14),( 'EMG 15',15),( 'EMG 16',16)]

filename = ''
window.title('EMGProc')

#various variable initialization
csv_checker = 0
color iter = 0
filenamesplit = []
emg choice = []
overwrite choice = 0
relative height = 0.8
pulse_width_min = 500
pulse width max = 2000

#function for changing the color of text background
def change_color():
    global color iter
    global bg_color
    current_color = file.txt.cget("background")
    next_color = bg_color if current_color == "red" else "red"
    file.txt.config(background=next_color)
    if color_iter < 4:
        window.after(500, change_color)
        color_iter += 1
    else:
        color_iter = 0
    return

#main data processing function, called when the user clicks "Generate" button
def dataproc():
    #grabbing global variables for use in the function
    global csv_checker
    global filename
    global filenamesplit
    global emg choice
    global overwrite choice
    global output filename choice
    global relative height
    global pulse width min
    global pulse width max
    if csv_checker == 0:
        #packages being imported
        from scipy import fftpack, signal, integrate
        from matplotlib import pyplot as plt
        import math
        import numpy as np
        import pandas as pd
        import csv
        import scipy.signal as sps
        from scipy.signal import sgolinit

        from math import floor, log
        #variable initialization
        temp = []
        samplerate = 1500
        nyq = samplerate * 0.5
        row buff val = 26
        row_buff_val2 = 15
        #function for finding linear regression, used for finding fractal
```

```

def linear_regression(x, y):
    """Fast linear regression using Numba.
    Parameters
    -----
    x, y : ndarray, shape (n times,)
    Variables
    Returns
    -----
    slope : float
        Slope of 1D least-square regression.
    intercept : float
        Intercept
    """
    n_times = x.size
    sx2 = 0
    sx = 0
    sy = 0
    sxy = 0
    for j in range(n_times):
        sx2 += x[j] ** 2
        sx += x[j]
        sxy += x[j] * y[j]
        sy += y[j]
    den = n_times * sx2 - (sx ** 2)
    num = n_times * sxy - sx * sy
    slope = num / den
    intercept = np.mean(y) - slope * np.mean(x)
    return slope, intercept
#function for finding Higuchi's fractal dimension
def higuchi_fd(x, kmax):
    n_times = x.size
    lk = np.empty(kmax)
    x_reg = np.empty(kmax)
    y_reg = np.empty(kmax)
    for k in range(1, kmax + 1):
        lm = np.empty(k)
        for m in range(k):
            ll = 0
            n_max = floor((n_times - m - 1) / k)
            n_max = int(n_max)
            for j in range(1, n_max):
                ll += abs(x[m + j * k] - x[m + (j - 1) * k])
            ll /= k
            ll *= (n_times - 1) / (k * n_max)
            lm[m] = ll
        # Mean of lm
        m_lm = 0
        for m in range(k):
            m_lm += lm[m]
        m_lm /= k
        lk[k - 1] = m_lm
        x_reg[k - 1] = log(1. / k)
        y_reg[k - 1] = log(m_lm)
    higuchi, _ = linear_regression(x_reg, y_reg)
    return higuchi

#package for making excel plot
from openpyxl import Workbook
from openpyxl.chart import (ScatterChart, Reference, Series)
import openpyxl

#filename output creation
if len(output_filename_choice.get())==0:
    dest_filename = filename + '.xlsx'
else:
    dest_filename = output_filename_choice.get() + '.xlsx'

#choosing whether to overwrite excel file or add to existing
if overwrite choice == 0:
    book = Workbook()
else:
    book = openpyxl.load_workbook(dest_filename)
sheet = book.active
sheet.title = 'Data'

sheet2 = book.create_sheet()
sheet2.title = 'Graphs'

#loop for each file chosen by the user
for h in range(len(filename)):
    temp = []
    print(filename[h])

    #reads the EMG data for the file
    with open(filename[h], 'r') as csvfile:
        csvreader = csv.reader(csvfile, delimiter=',')
        for row in csvreader:
            if csvreader.line_num == 3:
                temp.append(row)
            if csvreader.line_num >= 6:
                if row:
                    temp.append(row)
                else:
                    break

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#converts read data into a dataframe
df = pd.DataFrame(temp) # turns the array into a dataframe
df.columns = df.iloc[0] # sets the column names as the first row
df = df.drop(0) # drops the first row since it is now a duplicate of the column names
df.reindex(df.index.drop(1))
df.reset_index(drop=True, inplace=True)
df.columns = ['frames', 'subframes', 'blank', 'emg1', 'emg2', 'emg3', 'emg4', 'emg5', 'emg6', 'emg7',
'emg8',
                'emg9', 'emg10', 'emg11', 'emg12', 'emg13', 'emg14', 'emg15', 'emg16', 'unused7', 'unused8',
                'blank2']
df2 = df.drop(['frames', 'subframes', 'blank', 'unused7', 'unused8', 'blank2'], axis=1)
df2 = df2.astype(np.float)
print(len(df))
hor = np.arange(0, (len(df) - 0.5) / samplerate, 1 / samplerate) # getting the time domain in seconds

emg = []
for i in range(0, len(emg_choice)):
    j = emg_choice[i]
    print(j)
    emg.append(df2['emg{}'.format(j)])

#filters each active emg for 20 Hz high pass and 400 Hz low pass
cutoff_freq = 20 # ~20 Hz for movement according to emg book "Electromyography: Physiology, Engineering,
and Noninvasive Apps"
cut = cutoff_freq / nyq
b, a = signal.butter(5, cut, btype='highpass', analog=False)
emg_high = []
for i in range(0, len(emg)):
    temp = signal.filtfilt(b, a, emg[i])
    emg_high.append(temp)
cutoff_freq = 400 # ~500 Hz according to the emg book
cut = cutoff_freq / nyq
b, a = signal.butter(5, cut, btype='lowpass', analog=False)
emg_filt = []
for i in range(0, len(emg_high)):
    temp = signal.filtfilt(b, a, emg_high[i])
    emg_filt.append(temp)

#rectified the emg data
emg_rec = []
for i in range(0, len(emg_filt)):
    temp = abs(emg_filt[i])
    emg_rec.append(temp)

#puts emg data through a savgol filter
ynew = []
for i in range(0, len(emg_rec)):
    temp = signal.savgol_filter(emg_rec[i], 1501, 2)
    ynew.append(temp)

cutoff_freq = 10 # ~20 Hz for movement according to emg book "Electromyography: Physiology, Engineering,
and Noninvasive Apps"
cut = cutoff_freq / nyq
b, a = signal.butter(5, cut, btype='lowpass', analog=False)

ynew2 = []
for i in range(0, len(ynew)):
    temp = signal.filtfilt(b, a, ynew[i])
    ynew2.append(temp)

ynew3 = []
for i in range(0, len(ynew2)):
    temp = signal.savgol_filter(ynew2[i], 1501, 2)
    ynew3.append(temp)

# finding each pulse start and end
peaks = []
props = []
for i in range(0, len(ynew3)):
    temp1, temp2 = signal.find_peaks(ynew3[i], width=(pulse_width_min, pulse_width_max),
rel height=relative height)
    peaks.append(temp1)
    props.append(temp2)

#finding pulse times and widths
pulses_beginT = [[] for _ in range(len(peaks))]
pulses_endT = [[] for _ in range(len(peaks))]
pulses_begin = [[] for _ in range(len(peaks))]
pulses_end = [[] for _ in range(len(peaks))]
pulses_begin_ind = [[] for _ in range(len(peaks))]
pulses_end_ind = [[] for _ in range(len(peaks))]
print(len(peaks))
print('blank')
for i in range(len(peaks)):
    for j, k in zip(peaks[i], props[i]['widths']):
        pulse_sample_start = j - (math.floor(k / 2))
        pulse_sample_end = j + (math.floor(k / 2))
        pulses_begin_ind[i].append(pulse_sample_start)
        pulses_end_ind[i].append(pulse_sample_end)

pulses_beginT[i].append(pulse_sample_start / 1500)
pulses_endT[i].append(pulse_sample_end / 1500)
pulses_begin[i].append(ynew3[i][pulse_sample_start])
pulses_end[i].append(ynew3[i][pulse_sample_end])

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amplitude array = []
sectionpoints = []
sectionpoints array = []
89initial = []
lowcut = 10
highcut = 400
#Processing for each pulse
for t in range(len(ynew3)):
    M = 0
    N = 1
    cellcheck = 0

    for r,s in zip(pulses begin ind[t],pulses end ind[t]):
        q = emg_rec[t]
        sectionpoints = []
        89initial = []
        sectionpoints.extend(q[r:s])
        89initial.extend(hor[r:s])

        sectionpoints_array = np.asarray(sectionpoints)
        rectified_secarray = np.abs(sectionpoints_array)
        freq, power_spec = signal.periodogram(sectionpoints_array, samplerate)

        rms_amplitude = (np.mean(rectified_secarray))/np.sqrt(2)

        lowflag = 0
        breakflag = 0
        for c in range(len(freq)):
            if abs(freq[c]) > 20 and lowflag == 0:
                fullpulse_lowfreqbound = c
                lowflag = 1
            if abs(freq[c]) > 400:
                fullpulse_uppfreqbound = c
                break

        #finding spectral moments
        spec_mom0 = 0
        spec_mom2 = 0
        spec_mom5 = 0
        for k in range(fullpulse_lowfreqbound, fullpulse_uppfreqbound):
            spec_mom0 = spec_mom0 + (math.pow(freq[k], -1) * power_spec[k])
            spec_mom2 = spec_mom2 + (math.pow(freq[k], 2) * power_spec[k])
            spec_mom5 = spec_mom5 + (math.pow(freq[k], 5) * power_spec[k])

        #finding Dimitrov spectral index
        f2 = spec_mom0 / spec_mom2
        f5 = spec_mom0 / spec_mom5

        powsum = 0
        powarray = []

        #finding median frequency
        for l in range(len(freq)):
            powsum = integrate.simps(power_spec[:l + 1], freq[:l + 1])
            powarray.append(powsum)

        mednum = powsum / 2

        meansumcombo = 0
        meansumpow = 0

        for p in range(len(freq)):
            meansumcombo = meansumcombo + (freq[p] * power_spec[p])
            meansumpow = meansumpow + (power_spec[p])

        #finding mean frequency
        mean = meansumcombo / meansumpow
        total_int = integrate.simps(power_spec[fullpulse_lowfreqbound:], freq[fullpulse_lowfreqbound:])
        row = 2 + t*row_buff_val
        col = 1
        cellcheck = 0

        #writes median frequency to output excel file
        for u in powarray:
            if u > (0.5 * total_int):
                median = freq[powarray.index(u)-1]
                while cellcheck == 0:
                    if sheet.cell(row=row, column=col).value != None:
                        col = col + 1
                    else:
                        cellcheck = 1
                sheet.cell(row=row, column=col).value = median
                break

        cellcheck = 0
        row = row + 2
        col = 1

        #writes mean frequency to output excel file
        while cellcheck == 0:
            if sheet.cell(row=row, column=col).value != None:
                col = col + 1
            else:
                cellcheck = 1
        sheet.cell(row=row, column=col).value = mean

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cellcheck = 0
row = row + 2
col = 1
#writes 2nd order spectral index to output excel file
while cellcheck == 0:
    if sheet.cell(row=row, column=col).value != None:
        col = col + 1
    else:
        cellcheck = 1
sheet.cell(row=row, column=col).value = f2

cellcheck = 0
row = row + 2
col = 1
#writes RMS amplitude to output excel file
while cellcheck == 0:
    if sheet.cell(row=row, column=col).value != None:
        col = col + 1
    else:
        cellcheck = 1
sheet.cell(row=row, column=col).value = rms_amplitude

row = row + 2
col = 1
cellcheck = 0
samp_len = len(emg_filt[t])
ql_2 = floor(0.1 * samp_len)
overlap = floor(0.3 * ql_2)
print((len(emg_filt[t])-ql_2) // (overlap))
#finds fractal dimension and writes to an output excel
for v in range((len(emg_filt[t])-ql_2) // overlap):
    q2 = emg_filt[t]
    emg_sec = q2[(0 + (overlap * v)):(ql_2 + (overlap * v))]
    frac1 = higuchi_fd(emg_sec, 6)
    while cellcheck == 0:
        if sheet.cell(row=row, column=col).value != None:
            col = col + 1
        else:
            cellcheck = 1
    sheet.cell(row=row, column=col).value = frac1
    col = col + 1
cellcheck = 0

coll = []
col2 = []
#creates a row of index values
for e in range(len(ynew3)):
    row = 2 + e*row_buff_val
    coll.append(1)
    cellcheck = 0
    while cellcheck == 0:
        if sheet.cell(row=row, column=coll[e]).value != None:
            sheet.cell(row=(row-1), column=coll[e]).value = coll[e]
            coll[e] = coll[e] + 1
        else:
            cellcheck = 1

    row = 10 + e*row_buff_val
    col2.append(1)
    cellcheck = 0
    while cellcheck == 0:
        if sheet.cell(row=row, column=col2[e]).value != None:
            sheet.cell(row=(row-1), column=col2[e]).value = col2[e]
            col2[e] = col2[e] + 1
        else:
            cellcheck = 1

#creates chart in the output excel file for each output parameter
for b in range(len(emg_choice)):
    for c in range(1,6):
        chart = ScatterChart()
        if c == 1:
            chart.title = "EMG " + str(emg_choice[b]) + " Median Frequency"
        elif c == 2:
            chart.title = "EMG " + str(emg_choice[b]) + " Mean Frequency"
        elif c == 3:
            chart.title = "EMG " + str(emg_choice[b]) + " Spectral Index Order 2"
        elif c == 4:
            chart.title = "EMG " + str(emg_choice[b]) + " RMS Amplitude"
        elif c == 5:
            chart.title = "EMG " + str(emg_choice[b]) + " Fractal Dimension"

        if c == 5:
            chart.x_axis.title = 'Window'
        else:
            chart.x_axis.title = 'Pulse'

        if c == 1 or c == 2:
            chart.y_axis.title = 'Frequency'
        elif c == 3 or c == 4:
            chart.y_axis.title = 'Spectral Index Power'
        else:
            chart.y_axis.title = 'Fractal Dimension'

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        if c == 5:
            xvalues = Reference(sheet, min_col=1, max_col = (col2[b]-1), min_row = 9+b*row_buff_val)
        else:
            xvalues = Reference(sheet, min_col=1, max_col = (col1[b]-1), min_row = 1+b*row_buff_val)

        if c == 5:
            values = Reference(sheet, min_col=1, max_col = (col2[b]-1), min_row = (10 + b*row_buff_val))
        else:
            values = Reference(sheet, min_col=1, max_col=(col1[b]-1), min_row=((2*c)+(b*row_buff_val)))

        series = Series(values, xvalues)
        chart.series.append(series)
        chartloc = ''
        if c == 1:
            chartloc = 'A'
        elif c == 2:
            chartloc = 'J'
        elif c == 3:
            chartloc = 'S'
        elif c == 4:
            chartloc = 'AB'
        elif c == 5:
            chartloc = 'AK'
        sheet.add_chart(chart, chartloc + str(11+b*row_buff_val))

    for b in range(len(emg_choice)):
        for c in range(1,6):
            chart = ScatterChart()
            if c == 1:
                chart.title = "EMG " + str(emg_choice[b]) + " Median Frequency"
            elif c == 2:
                chart.title = "EMG " + str(emg_choice[b]) + " Mean Frequency"
            elif c == 3:
                chart.title = "EMG " + str(emg_choice[b]) + " Spectral Index Order 2"
            elif c == 4:
                chart.title = "EMG " + str(emg_choice[b]) + " RMS Amplitude"
            elif c == 5:
                chart.title = "EMG " + str(emg_choice[b]) + " Fractal Dimension"

            if c == 5:
                chart.x_axis.title = 'Window'
            else:
                chart.x_axis.title = 'Pulse'

            if c == 1 or c == 2:
                chart.y_axis.title = 'Frequency'
            elif c == 3 or c == 4:
                chart.y_axis.title = 'Spectral Index Power'
            else:
                chart.y_axis.title = 'Fractal Dimension'

            if c == 5:
                xvalues = Reference(sheet, min_col=1, max_col = (col2[b]-1), min_row = 9+b*row_buff_val)
            else:
                xvalues = Reference(sheet, min_col=1, max_col = (col1[b]-1), min_row = 1+b*row_buff_val)

            if c == 5:
                values = Reference(sheet, min_col=1, max_col = (col2[b]-1), min_row = (10 + b*row_buff_val))
            else:
                values = Reference(sheet, min_col=1, max_col=(col1[b]-1), min_row=((2*c)+(b*row_buff_val)))

            series = Series(values, xvalues)
            chart.series.append(series)
            chartloc = ''
            if c == 1:
                chartloc = 'A'
            elif c == 2:
                chartloc = 'J'
            elif c == 3:
                chartloc = 'S'
            elif c == 4:
                chartloc = 'AB'
            elif c == 5:
                chartloc = 'AK'
            sheet2.add_chart(chart, chartloc + str(11+b*row_buff_val2))
            #saves the excel file as the chosen name
            print(dest_filename)
            book.save(filename=dest_filename)
            #gives a confirmation of code completion
            gen_txt.configure(text='Generation Complete!', background = 'green')
        else:
            change_color()

#function for browsing files
def filebrowse():
    global csv_checker
    global filename
    global filenamesplit
    gen_txt.configure(text='', background=bg_color)
    output_filename_choice.config(state='normal')
    PulseRelHeight.config(state='normal')
    PulseWidthMin.config(state='normal')
    PulseWidthMax.config(state='normal')
    filename = filedialog.askopenfilenames(filetypes = (("Comma Separated Values", "*.csv"), ("all files", "*.*")))
    for w in range(len(filename)):

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        if filename[w].endswith('.csv'):
            csv_checker = 0
        else:
            file_txt.configure(text='Please choose a csv file!')
            csv_checker = 1
            break
    #gives text confirmation of files chosen
    if csv_checker == 0:
        filenamesplitfirst = filename[0].split('/')
        filenamesplitlast = filename[-1].split('/')
        filenamesplit1 = filenamesplitfirst[-1].split('.')
        filenamesplit2 = filenamesplitlast[-1].split('.')
        trial_numberfirst = filenamesplit1[-1].split('.')
        trial_numberlast = filenamesplit2[-1].split('.')
        file_txt.configure(text='Chosen Subject: ' + filenamesplit2[1] + '\nChosen TR #s: ' + trial_numberfirst[0] + ' - ' + trial_numberlast[0])
        file_txt.config(background=bg_color)

#function for choosing active EMGs
def get_emg_vals():
    global emg_choice
    emg_choice=[]
    for i in range(1,17):
        if d['v{}'.format(i)].get() == 1:
            emg_choice.append(i)
    print(emg_choice)

#initialization of GUI function
def NewFile():
    global emg_choice
    global CB1
    file_txt.configure(text='Chosen File: ')
    for i in range(1,17):
        d['v{}'.format(i)].set(0)
    gen_txt.configure(text='', background=bg_color)

#allows for keyboard shortcut
def NewFileKeyboard(self):
    global emg_choice
    global CB1
    file_txt.configure(text='Chosen File: ')
    for i in range(1, 17):
        d['v{}'.format(i)].set(0)
    gen_txt.configure(text='', background=bg_color)

#keyboard shortcut for quitting
def ExitKeyboard(self):
    window.quit()

#tutorial creation
def Tutorial():
    top = Toplevel()
    top.title("Tutorial")
    msg1 = Label(top, text = 'Hello!'
                '\n Welcome to EMGProc!'
                '\n This software was designed to work with a 16 count EMG system, as well as'
                '\n the csv file format created by Vicon Nexus (specifically v2.5 but possibly could'
                '\n work for other versions)'
                '\n'
                '\n'
                '\n How to use EMGProc:')
    msg1.grid(row=1,column = 1,pady = (20,0),padx = 30)

    msg2 = Label(top, text = 'Step 1: Choose the csv file needed to process'
                '\n Step 2: Choose the output filename, as well as if a new output file should be created'
                '\n (overwriting any file with that name) or add on to an existing file created by EMGProc'
                '\n Step 3: Choose what EMGs were active and save them'
                '\n Step 4: Click Generate Output'
                '\n The output file will be placed in the same location as the chosen file, unless you'
                '\n choose,'
                '\n your own filename, in which case it will be placed in the location of this program.'
                '\n', justify = LEFT)
    msg2.grid(row=2,column = 1,padx = 30)
    msg3 = Label(top, text = 'Keyboard Shortcuts:')
    msg3.grid(row=3,column = 1,padx = 30)
    msg4 = Label(top, text = 'Ctrl+Q: New File'
                '\n Ctrl+X: Exit', justify = LEFT)
    msg4.grid(row=4,column = 1,pady = (0,20))

    button1 = Button(top, text = 'Dismiss', command = top.destroy)
    button1.grid(row=5,column = 1,pady = (0,20))

#creation of "About" tab
def About():
    top = Toplevel()
    top.title("About")
    msg1 = Label(top, text = 'EMGProc was created by Adam Lewis as part of a Master\'s Thesis Project'
                '\n Created with PyCharm IDE and Python 3.6'
                '\n Contact email: AdamLew73@gmail.com'
                '\n'
                '\n Please do not copy or use without permission')
    msg1.grid(row=1,column = 1,pady = (20,0),padx = 30)

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button1 = Button(top, text='Dismiss', command=top.destroy)
button1.grid(row=2, column=1, pady=(0, 20))

#gui 93initialization for output filename
def focus_out_entry(event):
    output_filename.config(text='\nOutput Filename: ' + output_filename_choice.get() + '.xlsx')
def output_filename_save():
    output_filename.config(text='\nOutput Filename: ' + output_filename_choice.get() + '.xlsx')

#defining of save choice radio button (overwrite or add to)
def save_choice1():
    global overwrite_choice
    overwrite_choice = 0
    print(overwrite_choice)
def save_choice2():
    global overwrite_choice
    overwrite_choice = 1
    print(overwrite_choice)

#function for choosing pulse parameters
def Pulse Prop():
    global relative_height
    global pulse_width_min
    global pulse_width_max
    relative_height = float(PulseRelHeight.get())
    pulse_width_min = int(PulseWidthMin.get())
    pulse_width_max = int(PulseWidthMax.get())

#### GUI CREATION ####
window.config(menu = menu)

introtext = Label(window, text = '-----'
                    '\nFile Management')
introtext.grid(row=1,column = 2, columnspan = 3)

btn1 = Button(window, text = 'Browse for File (csv only)', command = filebrowse)
btn1.grid(row=2,column = 3,pady = 20)

file_txt = Label(window, text = 'Chosen File: ' + filename)
file_txt.grid(row=3,column = 2, columnspan = 3, ipadx = 100)
bg_color = file_txt.cget("background")
out_text = ''
output_filename_txt = Label(window, text = 'Desired Output Filename:')
output_filename_txt.grid(row=4,column = 2, columnspan = 2,padx = (25,100), sticky = W)
output_filename_choice = Entry(window, textvariable = out_text, state = 'disabled', width = 50)
output_filename_choice.bind("<Return>",focus_out_entry)
output_filename_choice.bind("<FocusOut>",focus_out_entry)
output_filename_choice.grid(row=4,column = 2, columnspan = 2, sticky = W, padx = (175,0))
output_file_save = Button(window, text = 'Enter', command = output_filename_save)
output_file_save.grid(row=4,column = 4, padx = (10,0), sticky = W)
file_save_choicevar = IntVar()
file_save_choice1 = Radiobutton(window, text = 'Overwrite File', variable = file_save_choicevar, value = 0, command =
save_choice1)
file_save_choice1.grid(row=3,column = 4, padx = (50,25), pady = (10,30), rowspan = 3, sticky = NE)
file_save_choice2 = Radiobutton(window, text = 'Add to File', variable = file_save_choicevar, value = 1, command =
save_choice2)
file_save_choice2.grid(row=3,column = 4, padx = (0,40), pady = (35,0), rowspan = 3, sticky = NE)

output_filename = Label(window, text = '\nOutput Filename: ')
output_filename.grid(row=5,column = 3)
septext = Label(window, text = '-----'
                    '\nActive EMGs'
                    '\n')
septext.grid(row=6,column = 2, columnspan = 3)

CB1 =Checkbutton(window, text = 'EMG 1', variable = d['v1'])
CB1.grid(row=7, column = 2, ipadx = 50)
CB2 =Checkbutton(window, text = 'EMG 2', variable = d['v2'])
CB2.grid(row=8, column = 2)
CB3 =Checkbutton(window, text = 'EMG 3', variable = d['v3'])
CB3.grid(row=9, column = 2)
CB4 =Checkbutton(window, text = 'EMG 4', variable = d['v4'])
CB4.grid(row=10, column = 2)
CB5 =Checkbutton(window, text = 'EMG 5', variable = d['v5'])
CB5.grid(row=11, column = 2)
CB6 =Checkbutton(window, text = 'EMG 6', variable = d['v6'])
CB6.grid(row=12, column = 2)
CB7 =Checkbutton(window, text = 'EMG 7', variable = d['v7'])
CB7.grid(row=7, column = 3)
CB8 =Checkbutton(window, text = 'EMG 8', variable = d['v8'])
CB8.grid(row=8, column = 3)
CB9 =Checkbutton(window, text = 'EMG 9', variable = d['v9'])
CB9.grid(row=9, column = 3)
CB10 =Checkbutton(window, text = 'EMG 10', variable = d['v10'])
CB10.grid(row=10, column = 3)
CB11 =Checkbutton(window, text = 'EMG 11', variable = d['v11'])
CB11.grid(row=11, column = 3)
CB12 =Checkbutton(window, text = 'EMG 12', variable = d['v12'])
CB12.grid(row=7, column = 4, ipadx = 50)
CB13 =Checkbutton(window, text = 'EMG 13', variable = d['v13'])
CB13.grid(row=8, column = 4)
CB14 =Checkbutton(window, text = 'EMG 14', variable = d['v14'])
CB14.grid(row=9, column = 4)
CB15 =Checkbutton(window, text = 'EMG 15', variable = d['v15'])

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CB15.grid(row=10, column = 4)
CB16 = Checkbutton(window, text = 'EMG 16', variable = d['v16'])
CB16.grid(row=11, column = 4)

btn2 = Button(window, text = 'Save Active EMGs', command = get_emg_vals)
btn2.grid(row=13, column = 3, pady = 10)

septext2 = Label(window, text = '-----'
                    '\nPulse Definitions'
                    '\n')
septext2.grid(row=14, column = 2, columnspan = 3)

v1 = StringVar(window, value='0.8')
v2 = StringVar(window, value='500')
v3 = StringVar(window, value='2000')

PulseRelHeight_Text = Label(window, text = 'Pulse Relative Height')
PulseRelHeight_Text.grid(row=15, column = 2, columnspan = 2, padx = (75,100), sticky = W)
PulseRelHeight = Entry(window, state = 'disabled', textvariable = v1, width = 10)
PulseRelHeight.grid(row=16, column = 2, columnspan = 2, padx = (100,100), sticky = W)

PulseWidthMin_Text = Label(window, text = 'Pulse Min Width(in Samples)')
PulseWidthMin_Text.grid(row=15, column = 2, columnspan = 3, padx = (50,50))
PulseWidthMin = Entry(window, state = 'disabled', textvariable = v2, width = 10)
PulseWidthMin.grid(row=16, column = 2, columnspan = 3, padx = (45,50))

PulseWidthMax_Text = Label(window, text = 'Pulse Max Width(in Samples)')
PulseWidthMax_Text.grid(row=15, column = 3, columnspan = 2, padx = (50,50), sticky = E)
PulseWidthMax = Entry(window, state = 'disabled', textvariable = v3, width = 10)
PulseWidthMax.grid(row=16, column = 3, columnspan = 2, padx = (25,100), sticky = E)

Pulse_Button = Button(window, text = 'Save Properties', command = Pulse_Prop)
Pulse_Button.grid(row=17, column = 3, pady = 20)

septext3 = Label(window, text = '-----'
                    '\n')
septext3.grid(row=18, column = 2, columnspan = 3)

btn3 = Button(window, text = 'Generate Output', command = dataproc)
btn3.grid(row=19, column = 3)
gen_txt = Label(window, text = '')
gen_txt.grid(row=20, column = 3, ipadx = 150, pady = (10,20))

filemenu = Menu(menu, tearoff = False)
filemenu2 = Menu(menu, tearoff = False)
filemenu3 = Menu(menu, tearoff = False)

menu.add_cascade(label='File', menu = filemenu)
filemenu.add_command(label='New', command=NewFile, accelerator = "Ctrl+Q")
window.bind_all("<Control-Q", NewFileKeyboard)
filemenu.add_separator()
filemenu.add_command(label='Exit', command=window.quit)
window.bind_all("<Control-x>", ExitKeyboard)
menu.add_cascade(label='Help', menu = filemenu2)
filemenu2.add_command(label='Tutorial', command=Tutorial)

menu.add_cascade(label='Info', menu = filemenu3)
filemenu3.add_command(label='About', command=About)

window.mainloop()

```

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