BME 301A Quantitative Physiology

Physical Lab #4:

Contractile Properties of Frog Skeletal Muscle

11/1/2013

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Introduction:

Skeletal muscle is responsible for human's mobility, dexterity, finesse and motor control. Without the properties of skeletal muscle, we could not vary the force of actions as trivial as our steps or our grip. Skeletal muscle is composed of individual muscle cells called fibers. Many fibers bundle to form whole muscles. Each muscle fiber is similar in form, being composed of thin and thick filaments (made from actin and myosin respectively) that can slide past one another given enough ATP and calicium ions. Each and every fiber reacts to stimulus independent of adjacent muscle fibers. The next stepping size in the componentry of muscle is the motor unit, which is comprised of a single motor neuron and all the muscle fibers it innervates. The number of muscle fibers associated with a motor unit varies, providing a source for muscle control fidelity. When a motor neuron fires, the muscle fibers contained in the same motor unit will all contract in an all or nothing event. This single contraction is called a twitch. Contractile strength can be increased via an increase in stimulus frequency to the same motor unit, up to a fixed point where the muscle fibers cannot contract to produce more force. At this point, to increase contractile strength, recruitment is necessary. The term recruitment defines the increasing number of active motor units which sum to increase muscle contractile strength.

In this lab, isometric (fixed length) and isotonic (fixed load) conditions will be simulated to study skeletal muscle twitch behavior. After that, successive stimulus will be applied to cause twitch responses to overlap and increases developed tension in a process called summation. Without the time to relax between stimuli, a muscle's contractions will move from twitch behavior toward tetanus (a smooth and powerful contraction where all motor units are activated).

The contractile force of muscle is not dictated solely by the machinery described previously, but instead by a combination of such active forces with passive forces that result from the elastic elements of the muscle itself. The active components of muscle force require energy, primarily in the form of ATP. When the energy supply is used up, the muscle is in a temporary state of fatigue until the energy stores are replenished.

This lab includes experiments designed to test the recruitment properties of muscle under muscle and nerve stimulation, the length-tension relationship of muscle, the summation, tetanus and fatigue properties of

muscle, and finally the force-velocity relationship of muscle. This paper will outline the dissection of and experimentation on a gastrocnemius muscle (with sciatic nerve intact) from a frog. The sciatic nerve is a large nerve running down the leg along the thigh and gastrocnemius muscles toward the ankle. It sends signals for muscle contraction in the leg and is composed of many nerve fibers. The gastrocnemius muscle is the calf like muscle between the knee and ankle joint of the bullfrog. Stimulation of either the gastrocnemius muscle directly, or of the sciatic nerve that innervates it, should produce contractile force in the muscle. The recruitment properties of the muscle under nerve stimulation will be far quicker than under direct muscle stimulation as the nerve will have direct and immediate access to more motor units and muscle fibers than exterior surface stimulation. The recruitment speeds should differ.

The study of the force-velocity relationship of skeletal muscle could prove useful in understanding the cause of athletic injuries. Such an understanding would be invaluable when designing devices or apparatuses to prevent injury. Sport related injuries occur when the human body is in motion. Often muscles are asked to support weight in abnormal positions while the body is moving. Such positions might be supportable by a static muscle, but under athletic conditions where the muscle length is changing rapidly the muscle may not be able to provide enough force. Joints change angle, muscles lengthen and shorten, all while the body is in stressful motion. A good deal of injuries could be avoided if supportive devices were implemented in locations where muscle velocities are higher than the normal range a muscle is able to handle.

Materials & Methods:

Before any experiments could be performed, both sciatic nerves and gastrocnemius muscles from a bull frog (Rana Catesbiana) were moved for use. The bull frog was approximately a foot in stretched size (from toe to arm). First a guillotine was used to cut the head off the frog, and then a needle was used to destroy the spinal cord. Then, using toothed forceps and dissecting scissors, the frog's skin was removed from the mid-section down. Using bone scissors, the urostyle was removed. Then the leg muscles were pulled apart by hand to expose the sciatic nerves which were looped with thread and a glass hook, marked for their ends and stretched length and then snipped at the proximal ends. Then the gastrocnemius muscle was separated from

the rest of the lower leg and cut just proximal of the ankle. The knee joint was cut and the gastrocnemius and sciatic nerve together were placed into Ringer's solution. Throughout the entire process Ringer's solution was used to rinse everything and keep it damp.

The recording setup consisted of force transducer, bridge pot, recording cable sets, stimulator cable, weight set, femur clamp, isotonic transducer kit, Plexiglas muscle chamber and ring clamp, tape and Powerlab acquisition unit with LabScope software.

First the force transducer was calibrated by connecting the bridge pod to the power lab unit without any weight on the force transducer. The bridge pod was zeroed and for a range of weights (0-50g) recordings were made. The exact same calibration was conducted a second time, but with 2g weight tared off beforehand. Then the muscle was mounted to the holder with the knee joint fixed in the constriction and a solid knot tied at the ankle end. The sciatic nerve was draped over the stimulator leads. The apparatus was positioned so that the string was taught but no tension was in the line.

For the isometric twitch contraction, experiment began by determining the muscle resting length. Then the muscle was stimulated over a range of amplitudes with identical pulse width and frequency. The experiment was repeated by stimulating the nerve. The muscle and nerve excitation and supramaximal voltages were determined.

For the length-tension relationship experiment, the muscle was stretched 10mm in 1mm increments. At each length, after applying supramaximal stimulus voltage, the passive, active and total forces were determined.

For the muscle summation experiment, the muscle was stimulated with twin pulses at ISI's of 400ms, 200ms, 100ms, 50ms and 25ms. During the muscle tetanus experiment, the muscle was reset to its resting length and a continuous pulse train of 1 second was applied over a range of frequencies to determine tetanus inducing frequency.

For the fatigue experiment, the muscle was stimulated with and ISI of 20ms for a duration of 30 seconds. The experiment was repeated, stimulating the nerve instead of the muscle.

For the force-velocity relationship, a various weights were used to record a series of tetanic contractions. These recording were made under stimulus rate of the frequency required for fused tetanus.

Results:

Two different methods of calculating cross sectional area are shown at right in Figure 1. The method involving density resulted in a more realistic estimation, and therefore the cross sectional area used to normalize all forces into stresses was 40.3mm^2.

Figure 1: Calculations for cross sectional area of the gastrocnemius

Cross Sectional Area Calculated Using Conical Method:

Height of each cone h: 16mm; Radius a: 5mm; Radius b: 2.5mm

Volume of the dual Cone:

$$V = 2\left(\frac{\pi abh}{3}\right) = 2\left(\frac{(\pi * 5mm * 2.5mm * 16mm)}{3}\right) = 418.88mm^3$$

Cylinder: V=418.88mm^3; h=32mm

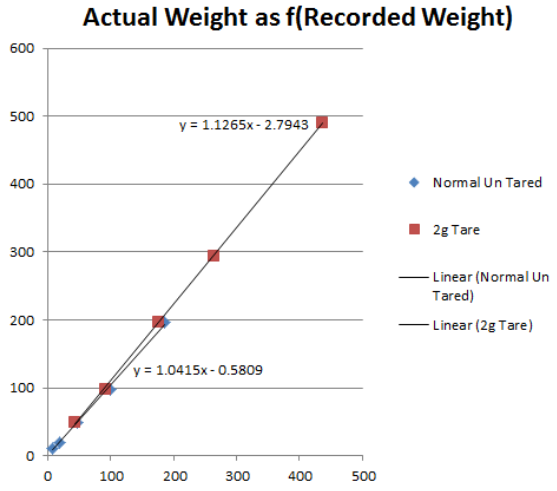
$$\boldsymbol{CSA} = \frac{V}{h} = \frac{418.88mm^3}{32mm} = 13.09mm^2$$

Cross Sectional Area calculated using Density:

Density p=1.0540g/cm^3 ; Unloaded Muscle Length L = 3.2cm; Mass m = 1.36g

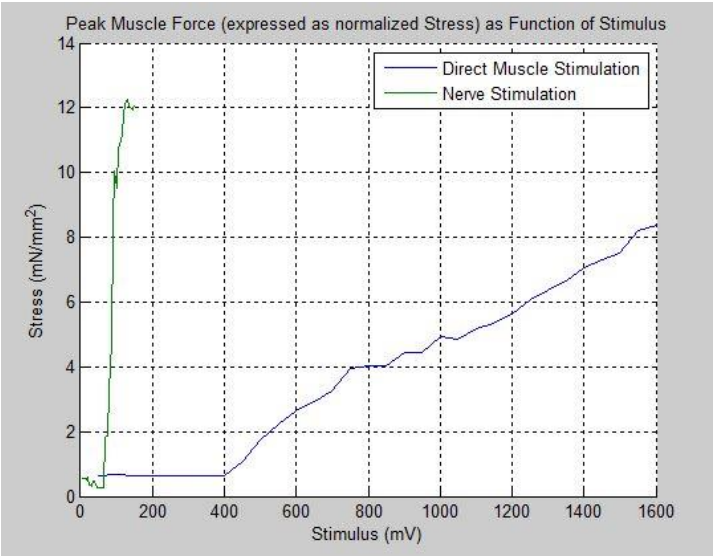
$$\boldsymbol{CSA} = \frac{m}{p * L} = \frac{1.36g}{\left(\left(\frac{1.054g}{cm^3}\right) * 3.2cm\right)} = (0.403cm^2) * \left(\frac{100mm^2}{cm^2}\right) = 40.3mm^2$$

Figure 2: Calibration curves for 0g and 2g tare



Calibration curves (for both the 0 tare and the 2g tare) were created using the data from the force transducer calibration procedure at the beginning of the lab. The curves are shown in Figure 2. They were graphed by displaying the actual weight the muscle was asked to lift as a function of the recorded weight from the transducer reading. The units are irrelevant as they are the same.

A plot of peak active force as a function of stimulus intensity for both direct muscle and nerve stimulation cases is shown in figure 3. As the peak force comes about much sooner in the nerve stimulation case, an expanded graph of just the nerve stimulation is shown in figure 4.



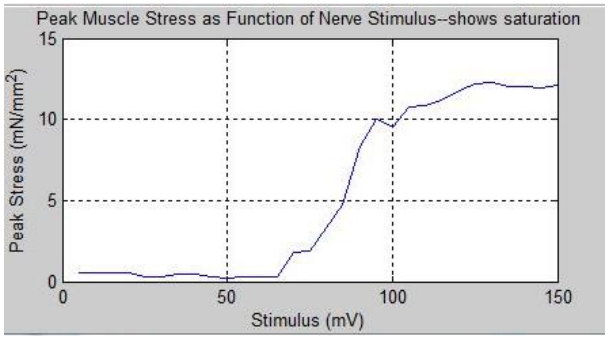


Figure 3 (left): Recruitment for increasing stimuli applied to nerve and muscle

Figure 4 (above): Close-up of nerve stimulated recruitment for increasing stimuli

Figure 5 shows 3 plots of the time course of force generation at 3 isometric lengths. The first is at the lowest muscle length studied (103.1% of resting length), the second at an intermediate (115.6%) and the third at the highest length studied (128.1%). Included on the graphs are the values of passive, active and total force along with the rise time to 50% of peak force and decay time to the same amplitude.

As figure 5 only represented 3 lengths, figure 6 is provided to show the components of force (peak total, peak active and passive) as a function of length using data acquired at 10 different lengths.

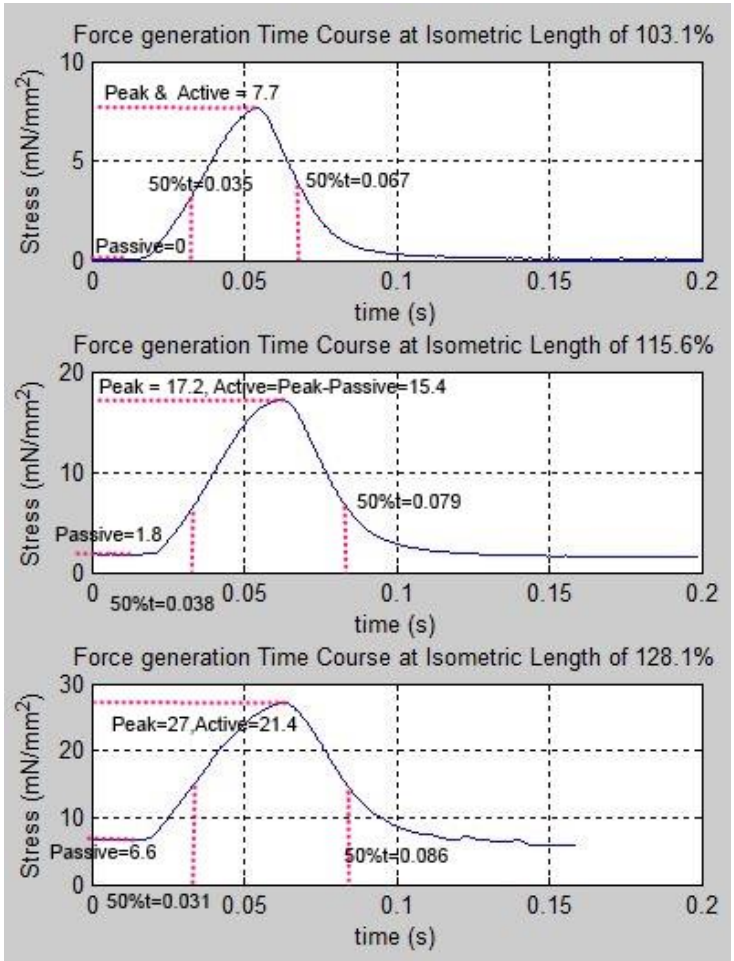


Figure 5 (above): Force Generation for 3 different muscle lengths

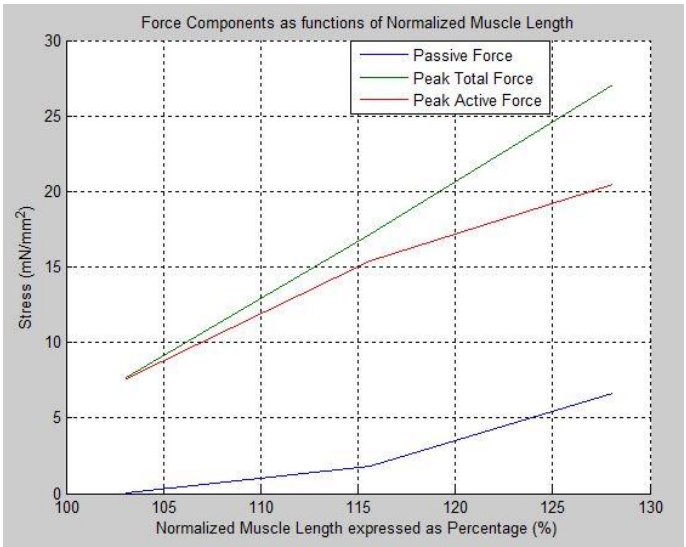


Figure 6 (left): Force Components for 10 different muscle lengths

A table of maximum contraction force of both the first and second peaks (at each pulse interval) from the summation procedure is shown in table 1.

Max Contracticle Stress from Muscle Summation at Different Pulse Intervals

Pulse Interval (ms)	Peak 1 Max Contractile Stress (mN/mm^2)	Peak 2 Max Contractile Stress (mN/mm^2)
400	13.17	14.58
200	13.60	13.94
100	12.61	14.61
50	12.50	17.10
25 (Peaks were Indistinguishable)	17.11	17.11

Table 1 (above): Contractile Stress from Muscle Summation at decreasing pulse intervals

3 plots of the force versus time recorded during the study of tetanized muscle are shown in figure 7. The 3 frequencies included are the lowest frequency studied, an intermediate frequency where tetanus was not completely fused, and finally the tetanus inducing frequency.

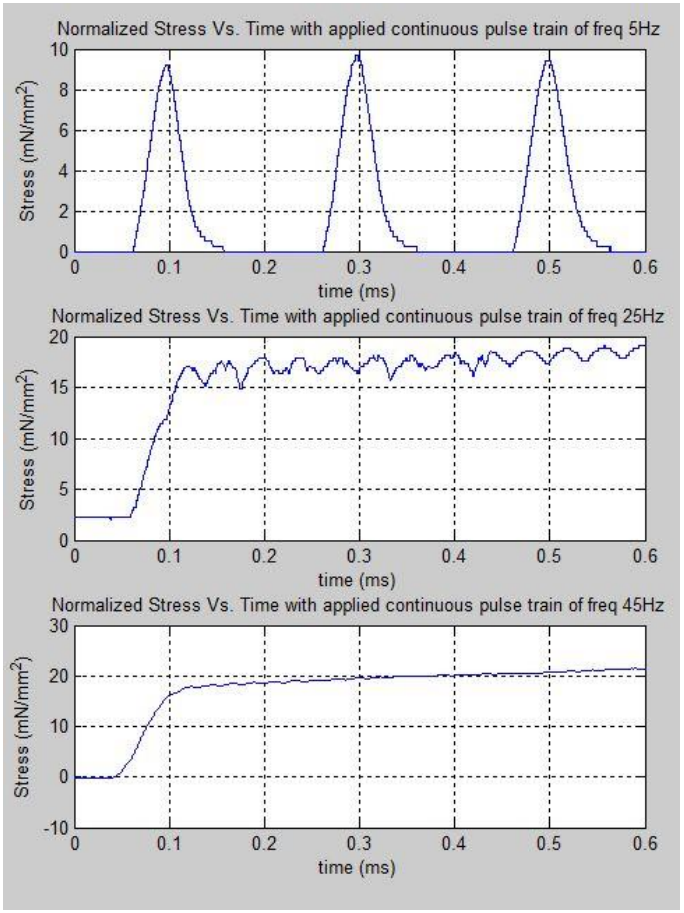


Figure 7 (above): Induction of tetanus as a result of increasing frequency of stimulus

Plots of muscle-stimulated and nerve-stimulated fatigue are shown on the same axis in figure 8.

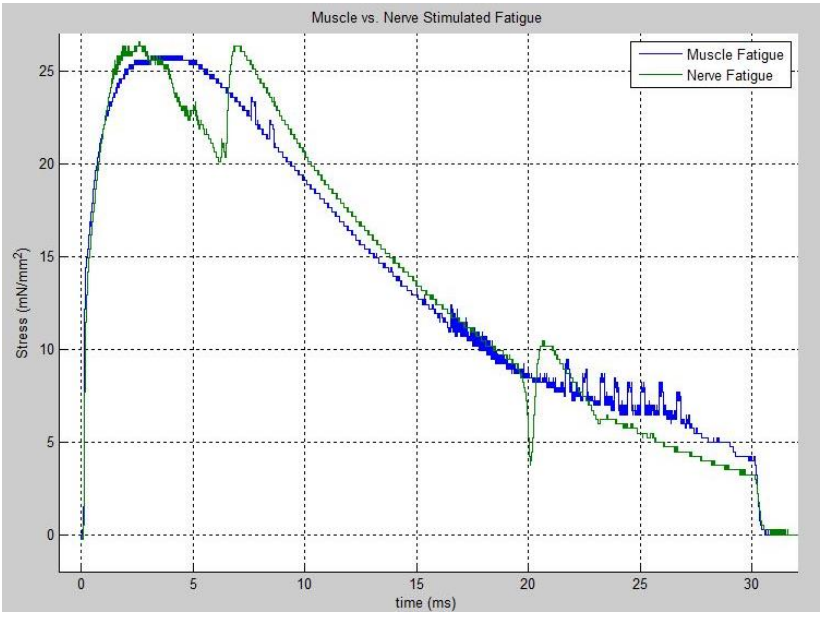


Figure 8 (left): Muscle fatigue for direct muscle stimulation vs. nerve stimulation

Plots of length as a function of time can be seen in figure 9 for the lowest load used, an arbitrary mid-range load, and the highest load used. On each plot, the average velocity of shortening expressed as a percentage of the resting length per second is shown. A lengthening corresponds to a "+" change in length and a "+" velocity, a shortening corresponds to a "-" change in length and a "-" velocity. Velocities of the muscle contraction at each load the muscle was being asked to generate are shown in table 2.

Velocity as Function of Load the Muscle was Asked to Generate

Load (g)	Length 2 (%)	Delta Time	Delta Length (%)	Velocity (% length/s)
20.00	97.29	0.02	-1.71	-112.50
40.00	97.42	0.02	-1.46	-82.02
60.00	98.88	0.01	-0.57	-41.61
80.00	100.00	0.01	-0.10	-9.09
100.00	100.09	0.02	-0.01	-0.56
120.00	100.10	0.02	0.00	0.00

Table 2 (above): Muscle Contraction Velocity at varying isotonic loads.

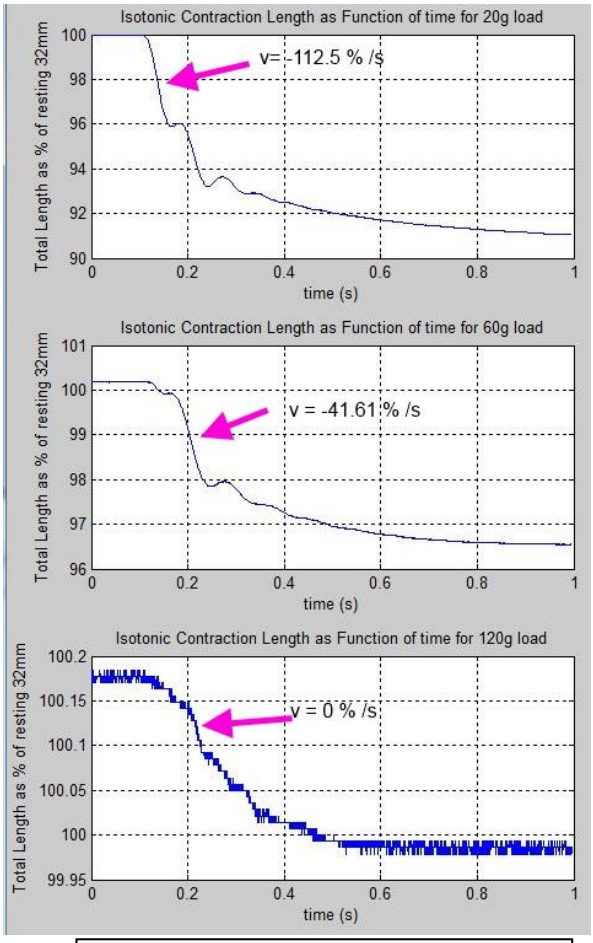


Figure 9 (above): Isotonic contraction length as a function of time for varying loads

A force-velocity curve is shown in figure 10. The plot includes all observations from table 2.

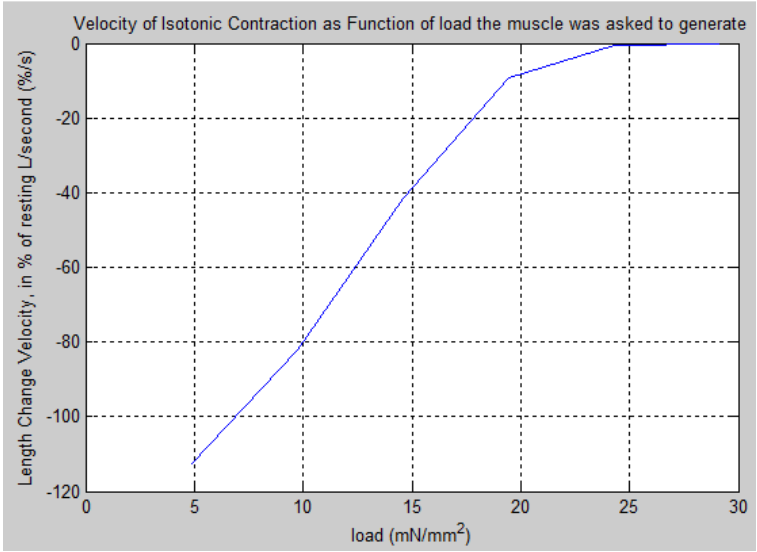


Figure 10 (right): Velocity of Isotonic Contraction as a function of load, including all observations from table 2.

Discussion:

The calibration curves were obtained by applying linear trend lines to five data points for both the 0g and 2g tare calibrations. Each point was a pair: the actual weight which the muscle was to support, and a recorded weight from the force transducer (both in mN). Units here were irrelevant because the function was relative between the two. The curves remained linear for the entire set of actual weights tested, and although they were extremely similar, the curves deviated in slope by 8%. I would not expect the two curves to be identical because the starting applied force was different. The ratio of actual force to recorded force could be read off the graphs as the slope from the resulting trend line equation. For the normal calibration, without a tare, the slope was 1.04. For the 2g tare the slope was 1.12. I believe the 2g tare is the appropriate calibration curve to use, as it takes into account the mass of the muscle itself (which, at 1.36g, was closer to 2g than to 0g) as well as the possibility that the initial slack in the calibrator setup was not exactly 0 for the normal calibration, but instead some small starting value.

A common trend in physiological systems is a dynamic or variable function resulting from the actions or interactions of subcomponents. In the case of muscle, the skeletal muscles we consider (such as the frog gastrocnemius) to carry out functions involving graded response are made up of countless skeletal muscle cells or fibers. Each fiber is capable of generating force independently. Although individual fibers may only be able to activate in an all-or-none event, the variable number of muscle fibers (which sum to form the total muscle force) permit a force-gradient of responses. In figure 3, as stimulus intensity increases gradually, the number of muscle fibers recruited (being activated) increases gradually which in turn increases twitch force gradually.

Rising stress should occur faster than decaying stress. This makes sense based off the basic biochemistry accomplished behind the scenes. When a muscle fiber relaxes, time is required for the cross bridge to finish its power stroke and for actin and myosin to break apart. This can be broken down further. When stress rises, as muscles fibers contract, the cross bridge formation requires the release of calcium ions from the sarcoplasmic reticulum (the calcium helps remove the tropomyosin from blocking the myosin head binding site on the actin filament). This process occurs very rapidly as the ions flow out. However when the muscle fiber wishes to

relax, actin and myosin cannot break apart until the calcium concentration inside the cell is reduced from its concentrated state. The primary way that resting calcium concentration is restored is by pumping calcium ions back into the sarcoplasmic reticulum at the cost of energy (ATP). This process of restoring the concentration of calcium (to relax the muscle fiber) takes significantly longer than does the disruption of the concentration (contraction of the muscle).

From the peak muscle stress as function of stimuli plot (figure 3), the nerve vs. muscle stimulus scenarios can be compared. Stimulation of the nerve produced an almost instantaneous response (relative to the direct muscle stimulation) which shot to peak muscle stress. In the other case, stress build up under direct muscle stimulation remained constant for some amplitude of stimulus (implying stimulation of even a few muscle fibers had a higher threshold than the nerve) until it began to ramp up gradually in a linear fashion. I would expect that if stimulus had been increased another 25% or so, the graph would show the direct muscle stimulation curve reaching the same maximum value as the nerve stimulation. This is because the true maximum force the muscle can generate is intrinsic to the muscle, not the type of stimulation. To achieve the same maximum force, the direct muscle stimulus must be greater than the nerve stimulus. All these behaviors make sense. The motor neuron has a relatively miniature threshold value for activating all the muscle fibers. This is because the nerve innervates nearly all the muscle fibers, so stimulation of the nerve is stimulation of nearly every muscle fiber simultaneously. When stimulating the muscle directly, increasing voltage is required to stimulate more muscle fibers which combine to form the total peak muscle stress. Therefore the slope of the direct muscle stimulus curve should be significantly less steep than the nerve stimulation.

The passive and active length-tension curves in class differ from those produced in this lab, however they share some very basic properties. They should be similar, as the passive and active elements of the graph during tetanus and twitch should be relatively similar, just as different amplitudes. This is because the cause of the contractile force is the same in each individual fiber, so at small numbers of summated fibers (during twitch producing frequencies) the amplitude is different than in maximum numbers of summated fibers (during tetanus inducing frequencies).

The passive model is an elastic element in parallel with a damping element. Looking at such a model, where the elastic element is represented by a spring, a dashpot (damping element) will determine the majority of the behavior of the system. If the passive force represents the spring, this makes sense compared to my data. Changing the passive force did not change the shape or curve (ie. behavior) of the active and total force, simply it changed the vertical shift. The active component is independent of the passive component. In a physiological explanation, this makes sense. The passive component of stress resulting from the muscle being elastic and wanting to return naturally to its upstretched state, does not affect the chemically based active component of the muscle force accomplished by stimulation and cross bridge formation.

In short, increasing the stimulation frequency will increase the contractile force. The contractile force will oscillate until the frequency has reached a minimum value required to induce tetanus. During tetanus, myosin heads and actin filaments are permanently bound together as increased stimulus frequency is keeping the net flux of ions out of the sarcoplasmic reticulum as opposed to pumped back in. When stimulus frequency increases, the frequency of propagation of depolarization along the muscle fiber exterior increases as well. As the increased propagations reach down into the transverse tubules and stimulate the DHP receptor, which activates the Ryanodine receptor which stimulates release of calcium from the sarcoplasmic reticulum, ATP driven pumps in the sarcoplasmic reticulum cannot compensate fast enough for the flux of calcium out of the reticulum. The calcium keeps tropomyosin off of the myosin binding site on actin. Hence the muscle remains in tetanus until the stimulus slows and calcium concentration can re-stabilize.

Summation is a useful function of skeletal muscle. Skeletal muscles vary in force generation to match the needed force for any given physical task. If the force of skeletal muscles could not be varied via summation, normal movements like walking would be nearly impossible. In cardiac muscle however, where the required task for the muscle is constant and cycles for the life of the body, there should be no summation. The presence of summation would be harmful. Cardiac muscle has a longer refractory period relative to skeletal muscle (Reference #2). In parts of the heart, instances of pumping actions require rhythmic contractions that occur evenly and simultaneously throughout the muscle. This behavior requires simultaneous stimulation and not

summation. If the cardiac muscle were to behave with summation, our hearts would struggle to pump blood through our bodies.

As the rest period between stimuli decreases, the individual peaks of stress grow closer. When the duration of rest decreases to a certain point the peaks will overlap and begin to sum without allowing the stress to drop to 0. As the duration of rest between pulses decreases, the repetitive release of calcium from the sarcoplasmic reticulum, in response to each stimulus, overpowers the calcium being pumped back into the sarcoplasmic reticulum. Because of this, the stress increases until the muscle is tetanized. The frequencies of stimuli, that produce responses between individual peaks and complete tetanus, produce stresses that oscillate around a relatively constant amplitude of stress that grows as a function of the frequency. As the muscle moves from single twitch to tetanus, the behavior can be seen as a summation of twitches. As the frequency increases, the amplitude value around which the stress oscillates increases toward max at tetanus, while the amplitude of the actual oscillations decreases.

My data for increasing frequencies show the tension rising for increasing frequency. As the frequency increases, the muscle becomes fused in tetanus. The maximum force a muscle can generate occurs when the muscle is in tetanus. My data supports this, as the plots where the muscle was tetanized coincided with the highest generation of stress.

The steady state response in the lowest frequency scenario was 0. This was the case of individual twitches, where a stimulus resulted in a twitch. For the intermediate frequency, the initial response was similar to the beginning of a twitch where the stress increases rapidly. However the steady state response of the intermediate frequency was an oscillation around a constant amplitude. The initial response of our tetanus case was again like the beginning of the twitch, but the steady state response was constant rather than oscillatory and at a higher amplitude than the intermediate.

During direct muscle stimulation, fatigue onset occurred after reaching 25.9 (mN/mm^2) at 4.7 seconds. The nerve stimulated muscle began to fatigue after reaching a maximum of 26.4 (mN/mm^2) at 7.25 seconds.

The percent decrease in contractile force between the maximum and the end of the experiment was 84.6% for the direct muscle stimulation and 87.7% for the nerve stimulation.

The overall fatigue curves were fairly similar; however there were a few differences. Primarily, the nerve stimulation produced a curve of the same overall shape but with spikes and decreases that occurred sporadically. A possible explanation for this would be the increased sensitivity of the nerve compared to the muscle stimulation where a small change in stimulus can cause powerful changes in stress. Additionally the muscle in the nerve stimulation scenario seems to fatigue slightly after the muscle in the direct muscle stimulation scenario. A possible explanation for this behavior is that the required stimulus to penetrate into the deeper tissue to activate every muscle fiber, during direct muscle stimulation, is significantly greater than the stimulus required to activate every muscle fiber when stimulating the nerve. Because of this disparity in required stimulus, the direct muscle stimulation scenario is exhausting the muscle faster by over stimulating the tissue, compared to the nerve stimulation, to produce the same response.

The angular displacement of the lever arm (in degrees) can be converted to a metric change in muscle length with the aid of some simple geometry. To express the total length as a percent of resting length, use the following equation where: Length = total length expressed as a % of resting length, L = length of the lever arm, L_r = the length of the muscle at rest, Θ = the angular displacement.

$$Length = 100\% - \left(100 * \left(\frac{L}{L_r}\right)\right) * \sin(\Theta)$$

Equation 1.3 from the McMahon reading is shown below. (Reference #3)

Eqn 1.3: $(T + a)(v + b) = (To + a)b$

As v increases, T must decrease. This coincides with my data which showed a decreasing stress as the velocity increased.

Finding a fit to the data from this lab, values for F_{max}, V_{max} and the "Hill model" constants were determined to be the following. Again note that a negative velocity was used to represent contraction.

F_{max} = 29.182 (mN/mm^2); V_{max} = -112.5%/s; a= -3.738; b= -19.28

The hill model matched the data fairly well. A side by side comparison of the data shows the overall trend is similar. (figure 11)

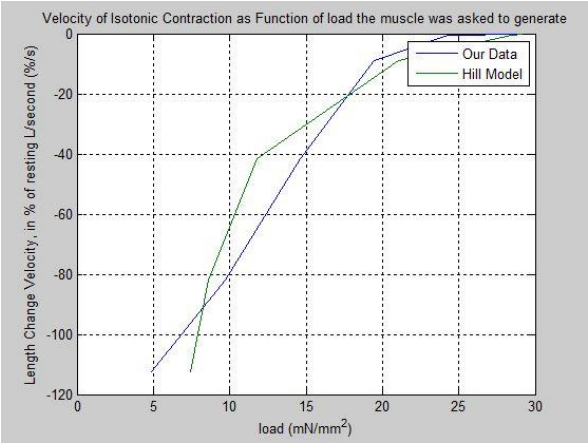


Figure 11 (left): Comparison of Hill model and personal data for Isotonic Contraction Velocity

A plot of power as a function of velocity is shown below (Figure 12). I would expect the plot to look as it does (an upside down parabola). This is because the power should be maximum at an optimal velocity that is not equal to Vmax. As the muscle contraction velocity increases, the micro scale interactions of forming cross bridges and completing power strokes (that require precise binding of myosin heads to sites on actin) cannot be accomplished as the thick and thin filaments rush past each other too fast. For this reason the maximum force should not be at the minimum (because the formation of cross bridges is possible during velocities not equal to 0) nor at maximum (because the formation of cross bridges and completion of power strokes is dependent upon the relative movement between thin and thick filaments). This is exactly what we see.

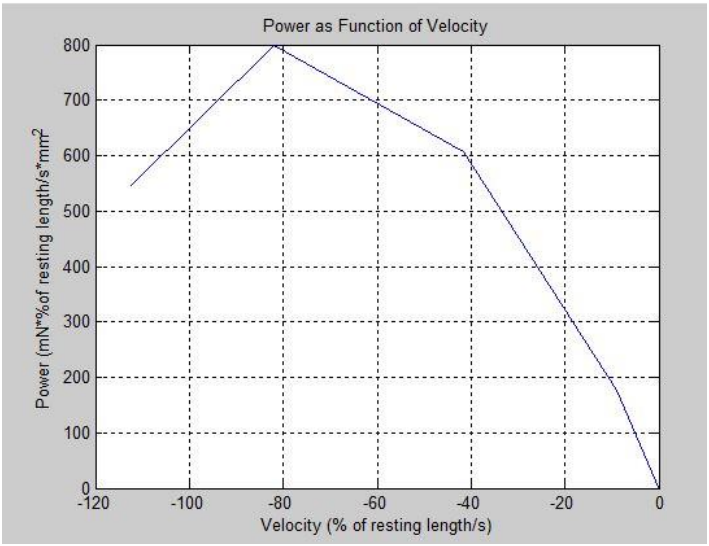


Figure 12 (left): Power as a function of velocity.

References:

1. Enderle, John D., and Joseph D. Bronzino. *Introduction to Biomedical Engineering*. Amsterdam: Elsvier/ Academic, 2012. Print.
2. Cardiovascular Physiology physic EX exercise 6 http://www.cuyamaca.edu/kimdudzik/Physiology%20labs/PhysioEx6.pdf
3. McMahon Chapter 1 https://bb.wustl.edu/bbcswebdav/pid-1280497-dt-content-rid-3122397_1/courses/FL2013.E62.BME.301A.01/4.03_McMahon%20Ch1__xid-80638_1.pdf

Unofficial Sources:

- Physical Lab Manual for Quantitative Physiology
- Computational Lab Manual for Quantitative Physiology
- Muscle Module Lecture Slides
- Blackboard Discussions
- Discussants: Lauren Bedell, Maeve Woeltje, Paras Vora, Matt Everett