PLab 6:

Contractile Properties of Frog Skeletal Muscle at Different Temperatures

BME 301a: Quantitative Physiology

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PLab 6 Student Design

Effects of Temperature on Contractile Properties of Frog Skeletal Muscle

Lab Section: Brauer, Friday at 9am

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Scientific Question

How does temperature impact the contractile properties of frog skeletal muscle?

Hypothesized Answer

We expect that normal operating body temperature will correspond to both maximum muscle contractile force and maximum duration to fatigue for a skeletal muscle. Conversely, increasing or decreasing temperatures from the optimal value will correspond to a decreasing contractile force and a decrease in time required to fatigue.

Research based in principle off of idea from:
Edwards, R.H.T. et al. "Effect of Temperature on Muscle Energy Metabolism and Endurance During Successive Isometric Contractions, Sustained to Fatigue, of the Quadriceps Muscle in Man". J.Physiol.(1972),220,pp.335-352.

Methods Summary

- Materials Needed
  - 2 frog gastrocnemius muscles with connected sciatic muscle (1 bullfrog with two legs)
  - 2 beakers with Ringer's solution
  - Dissecting kit
  - String
  - Power Lab Acquisition Unit
  - Bridge Pod
  - Plexiglas muscle chamber
  - Force Transducer
  - Ice Bath
  - Hot Plate
  - Electrode mini-grabbers
- Dissect two gastrocnemius muscles
  - One to do experiments in room temperature and then ice bath
  - One to use in hot bath
  - We aim to reach a temperature range around 10°C, 20°C, 30°C.
- To change the temperatures,
  - The muscle will be submerged in a beaker of Ringer's solution.
  - That beaker will then be submerged in the temperature bath.

    - The muscle will be extracted from the solution and put into the conduction chamber.
- The experiments we will test are:
    - Muscle Fatigue
    - Isometric Twitch Contraction
        - To see where supramaximal voltage is for each temperature
    - Tetanus
    - Weight kit (if time permitting)

Introduction:

Obtaining and testing a functional gastrocnemius muscle from a bullfrog allows experimenters to study length-tension relationships under various thermal conditions. Skeletal muscle is responsible for human's mobility, dexterity, finesse and motor control. Without the properties of skeletal muscle, humans 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 calcium 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 that sum to increase muscle contractile strength.

This lab includes experiments testing isometric twitch contraction, tetanus, and muscle fatigue under a cold, hot, and medium temperature conditions. In Edwards et al. human muscle function was studied at various temperatures. He claims that peak muscle endurance occurred when the muscle acclimated in a 26 degrees Celsius bath. Combining

Edwards findings, along with previous studies of frog muscle, the following hypothesis was formed: It is expected that normal operating body temperature will correspond to both maximum muscle contractile force and maximum duration to fatigue for a skeletal muscle. Conversely, increasing or decreasing temperatures from the optimal value will correspond to a decreasing contractile force and a decrease in time required to fatigue.

The conclusion of this study could prove useful in therapeutic or everyday exercise, strength building and prevention of athletic injury. For example, there may be an optimal temperature environment in which to train or build muscle for both athletes and patients undergoing physical therapy. Armed with knowledge of the temperature dependence of muscle contraction, individuals could train effectively while physical therapists could advise patients appropriately to decrease rehabilitation time.

Materials and Methods:

Beginning Steps–Dissection, Muscle Preparation, and Force Transducer Calibration:

The set up procedure for this experiment greatly resembles the steps taken in Physical Lab 4 (Contractile Properties of Frog Skeletal Muscle) by Jodi Small. In order to study the gastrocnemius muscle in various temperatures, experimenters first sacrificed the bullfrog via guillotine. Then, using dissecting pins, forceps, dissecting scissors and pins, bone cutters, and a the rest of the dissecting kit, the experimenters extracted the muscle from the leg of a large bullfrog. Thread tied around the Achilles tendon allowed for easier, gentler handling. The muscle's length and width were also measured using a ruler. Ringer's solution kept the muscle and nerve moist throughout the entire experiment. Calibrating the

Force Transducer involved converting its raw output from millivolts to Newtons after inputting data to the PowerLab Data Acquisition Unit. After opening the Bridge Pod settings within LabChart, the knob on the physical Bridge Pod was turned to set the reading to zero. Examples of weight, such as 0g and 50g, on the transducer were inputted into the computer for reference. Finally, a 2g mass from a weight set was placed on the transducer and then "Tared." This represented the weight of the muscle so new changes in force truly reflect the experiment. In order to study the muscle and nerve, they had to be accurately inserted into the testing machinery. A ring stand supports a Plexiglas muscle chamber. Within this chamber, the knee joint is positioned snugly into the mold just below the indent. Twist-ties hold the muscle in place without constricting its movement. The string attached to the end of the Achilles tendon then wraps up to connect the muscle to the force transducer. Since experimenters did not simultaneously dissect the nerve, the mini grabber electrodes only attached to the stimulation chamber to excite the muscle.

Since these trials involved testing the muscle at different temperatures, various baths were prepared to bring the muscle to a specific temperature. Creating a cold bath involved obtaining a bowl of ice. The muscle was submerged in a beaker of Ringer's solution and then that beaker was placed in the ice bath until an analog thermometer reached 15 degrees Celsius. Placing a beaker of Ringer's solution on a hot plate created the hot bath to warm the muscle until it reached 35 degrees Celsius.

<u>Procedure 1 - Isometric Twitch Contraction:</u>

First determine the resting length by shortening the muscle so there is no force read and the strings are firm. LabChart then stimulates the muscle from 50 mV to 2.2 V in 50 mV

steps (pulse width equals 1 ms and repeat rate equals 1 Hz) allowing for the determination of the muscle maximum excitation voltage. When multiplied by 1.5, this value becomes the muscle supramaximal stimulus voltage. This procedure was executed for both the cold and warm muscle.

Procedure 2 - Muscle Tetanization:

Next, LabChart uses continuous pulses with 1 s duration stimulate the muscle at 5, 15, 25, 35, 45, and 55 Hz and induce tetanus. This procedure was tested for both a warm and cold trial.

Procedure 3 - Muscle and Nerve Fatigue:

In two different trials, LabChart stimulates the muscle and then the nerve repeatedly with an interval of 20ms for 45s, inducing fatigue. The muscle was stimulated with 2.25 V for both the cold and warm trials.

The data collected comes from two muscles, each from a different bullfrog. The medium temperature (23 degrees Celsius) data was collected from one muscle of a bullfrog. However, due to limited materials, the warm (35 degrees Celsius) and cold (15 degrees Celsius) muscle data come from the same muscle of the same frog. Procedures 1 and 2 were first ran for the muscle after sitting in a cold bath. The muscle was then heated up before running procedures 1 and 2 at the warm temperature. Lastly, Procedure 3 was run at the warm temperature, followed by cooling the muscle down and running procedure 3 at the colder temperature. The colder temperature trials ran first to avoid potentially denaturing any proteins if the muscle was heated too high.

Results:

The muscle was 3.5 cm long, weighed 1.74 g, and had an elliptical cross section with radii 4 mm and 3 mm. The density of the muscle used for the hot and cold trials was 1.0540 g/cm^3. Calculating the cross sectional area of the muscle was conducted via two different methods shown below.

Average Cross- Sectional Area: $\frac{mass}{length*density} = \frac{1.74\ g}{(3.5\ cm)(1.0540\frac{g}{cm^3})}$ = 0.472 cm^2 = 47.2 mm^2

Cone Method: $2 \cdot \left(\frac{1}{3}\pi r^2 x\right) = \pi R^2 h \Rightarrow \frac{2}{3}\pi r_1 r_2 x = \pi R^2 h \Longrightarrow \frac{2}{3}\pi \cdot 0.4 \cdot 0.3 \cdot 1.75 = \pi R^2 \cdot 3.5$

$\Longrightarrow\ R = 0.2\ \Rightarrow Cross\ Sectional\ Area =\ \pi R^2 = \pi 0.2^2 = 12.6$ mm^2

For this report, all measurements will be normalized to the first estimation for cross-sectional area. This is because there is a smaller error in measuring the mass and length than there is in measuring two lengths, as in the cone estimation method. Before any experiments were done, the force transducer was calibrated. The results of the calibration test are shown below in Figure 1. The calibration curves are for the two cases. The first case, in blue, shows the force transducer with no initial mass testing with masses from 1 to 50 g. The second case, in green, shows the force transducer with a 2 g mass initially 'tared' from the measurement to represent DC offset, tested across the same range as the first set.

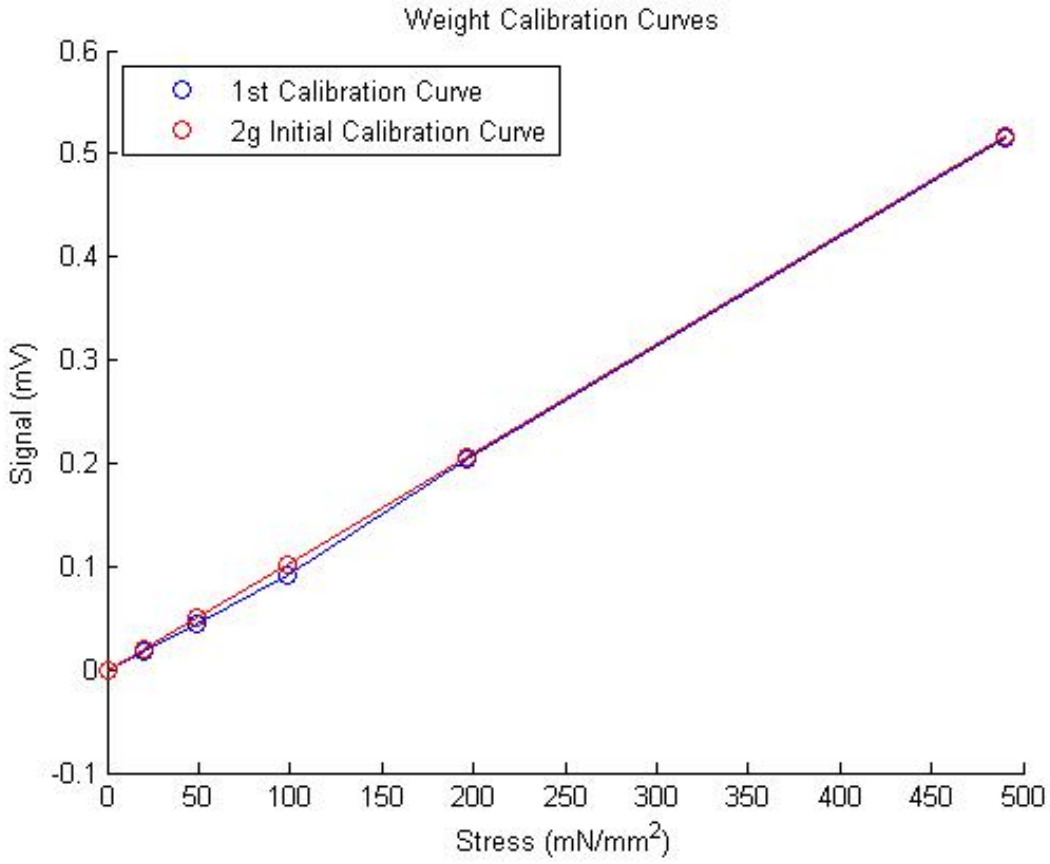

Figure 1: This plot shows the calibration curves that were used to initialize the force transducer.

The first set of data collected for the muscle was for the isometric twitches. The muscle was directly stimulated at a range of intensities to see the force of contraction. The results of this testing is shown in Figure 2.

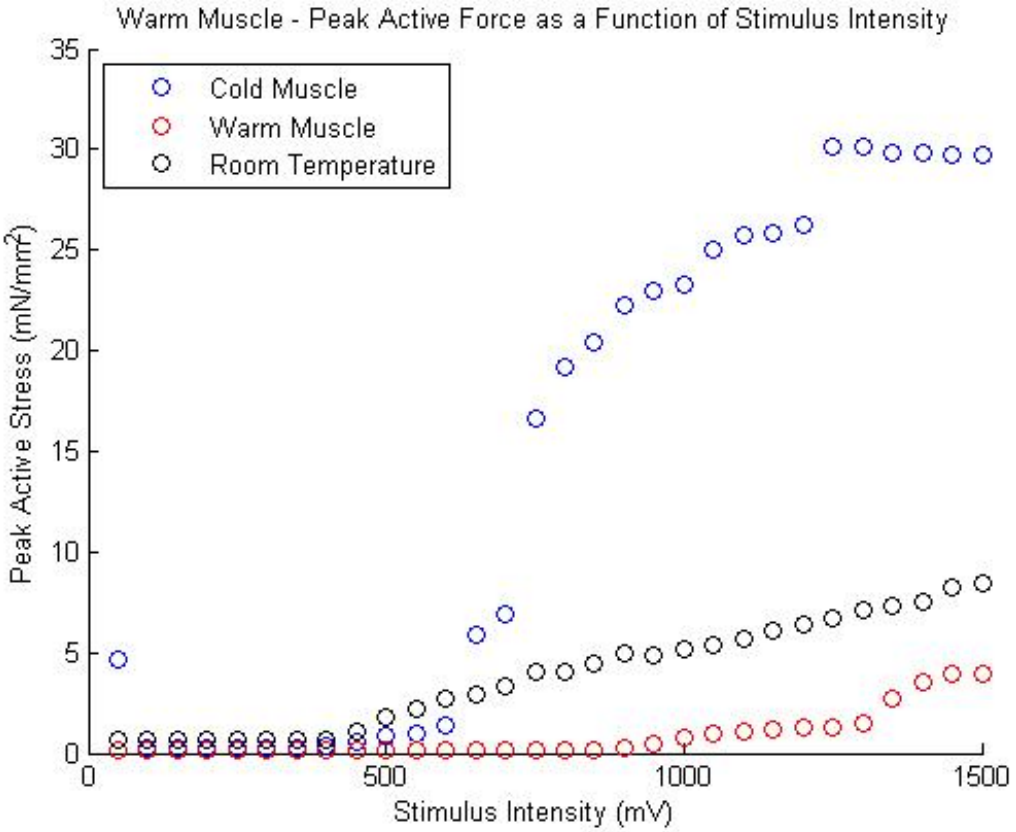

Figure 2: *Shows the peak active stress across a range of stimulus intensities for cold (blue), warm (red), and medium temperature (black) muscle.*

Changing the time between two stimulating pulses can affect the force generated by the muscle during the second contraction due to summation. To test the temperature dependence of summation as the muscle approaches tetanus, the muscles were stimulated from 5 to 55 Hz in increments of 10 Hz. Figure 3 below shows the dependence of the muscle's stress on the temperature of the muscle and the frequency of the applied stimulus.

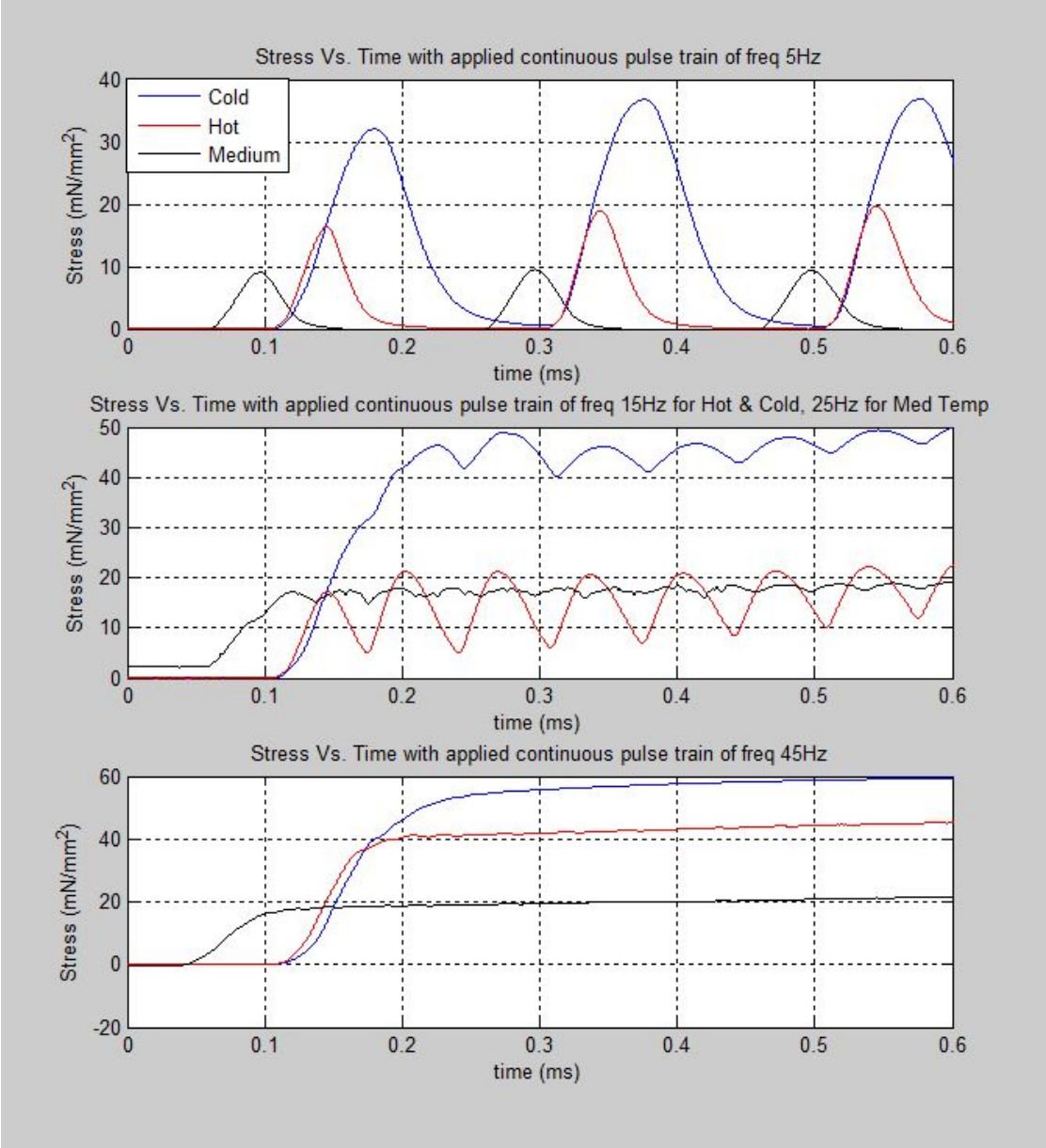

Figure 3: *Shows the stress in the muscle for low (top), medium (middle), and high (bottom) frequency stimuli for cold (blue), medium temperature (black), and warm (red) muscle.*

The last set of experiments performed was the fatigue of the muscle. After an extended period of stimulation, the muscle clearly fatigues as shown in Figure 4. There exists variation between the different temperatures for the amplitude of the fatigue as well as the time constants associated with the fatigues.

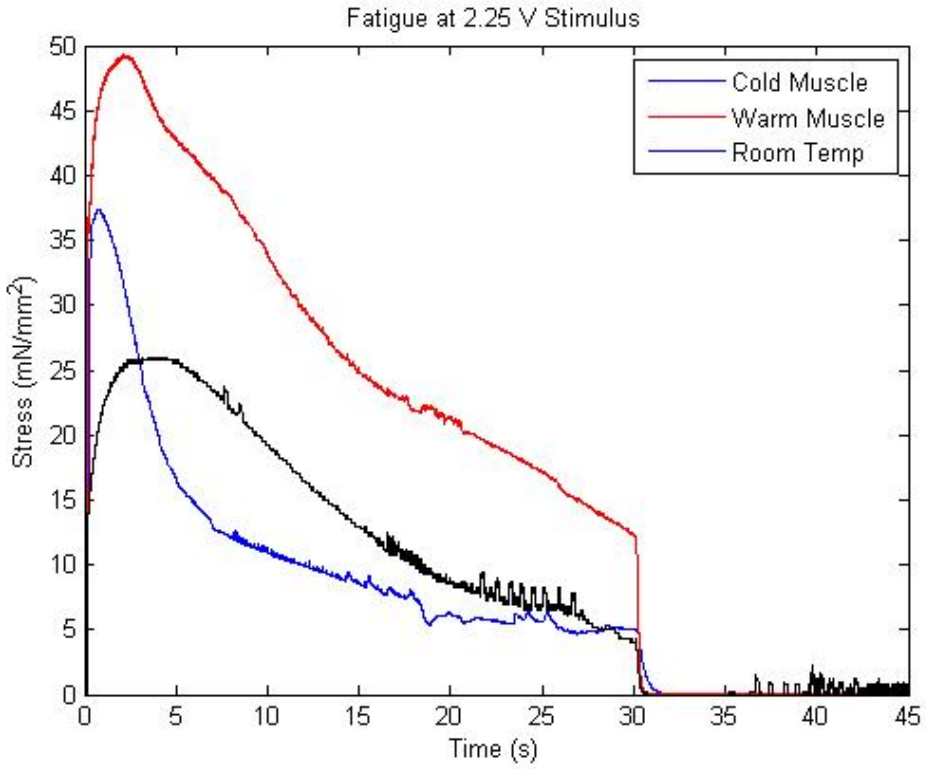

Figure 4: *Shows the fatigue testing of cold (blue), medium temperature (black), and warm (red) muscle.*

Discussion:

Overall the results of this experiment did not coincide with our hypothesis. We will discuss the trends shown in the data, but we are still confident in our original hypothesis. The difference between our predicted and actual results may originate from a few large sources of error.

First, the medium temperature results were produced by a muscle taken from a completely different frog on a completely different test day and setup. Even though all data is reported as stress normalized by specific muscle cross sectional areas, the properties of different muscles will vary slightly.

Second, the same muscle was used for both the hot and the cold tests, meaning one test was run after the other. The hot tests were run after our cold tests. The freezing and heating cycles likely took a toll on the health and strength of the muscle. This handicap likely contributes to the hot muscle's performance deficit. Ideally two muscles would have been taken from the same frog, adjusted in temperature and then tested at the same time.

Third, the range of temperatures used did not overlap enough with the internal body temperature of a bullfrog to indicate the performance declines on either side of an ideal temperature peak that was predicted. The muscle was heated in a bath at 35 degrees Celsius, meaning the muscle probably ended up somewhere between 30-35 degrees Celsius. Normal body temperature of a bullfrog is between 29 and 33 degrees Celsius. Our hottest test was right around the ideal body temperature that was hypothesized to coincide with the strongest muscle performance. The muscle temperatures in the medium and cold tests were both significantly below the normal operating body temperature of a bullfrog, and therefore both resided on the colder side of the optimal temperature we hypothesized.

Lastly, experimenters did not have a sample size greater than 1 for each temperature tested, and we did not test more than 3 temperatures. Statistically any trend in the data is not a valid base from which to extrapolate general muscle behavior as a function of temperature. Because of this, one cannot make a conclusion without further testing. Ideally, further testing would involve a larger quantity of muscles and a broader range of temperatures with smaller temperature testing increments.

In addition, the original hypothesis was based primarily off of experiments conducted on muscles in vivo. In such a scenario, blood flow and oxygen's temperature dependent affinity to hemoglobin play a significant role in muscle contraction for varying temperatures. When considering a muscle in vivo, at cold temperatures oxygen binds tighter to hemoglobin and

therefore the muscle receives less oxygen than at higher temperatures (Hlastala, Woodson & Wranne). Because ATP production, during aerobic respiration, requires oxygen, as oxygen supply decreases less ATP will be produced. Since muscles only contract in the presence of ATP, it is logical that muscles in vivo would have weaker contractions and fatigue quicker at colder temperatures.

Both of the calibration curves seen in Figure 1 are linear over the range tested, however they are not completely identical. There is a slight fluctuation between 50 and 200 mN/mm^2 on the Stress axis. They should be very close because they are essentially representing the same value. They are not exactly the same because there could be slight fluctuations in the weight added or deviations due to experimental error, even though the value was zeroed with the bridge pod. It's better to use the 2g-calibration curve because that curve most accurately depicts the added weight of the muscle during testing.

As observed in Figure 2, the muscle from the cold bath has the most rapid increase to the largest peak active force. This is in contrast to the warm and medium temperature tests, in which the muscle rose less rapidly to a lower peak active stress. Although the results of the cold temperature trial do not support the hypothesis, the medium temperature trial had a stronger overall response compared to the warm bath. This comparison between hot and medium temperature trials does support the originally stated hypothesis. Although this leaves the results inconclusive, a factor that may have led to the inconsistency with the cold temperature is that the warm and medium temperature muscles both seem to be increasing at the end of the tests. This may indicate that the warm and medium temperature muscles may not have reached their supramaximal voltage before ending the procedure. Further testing would be required before making a more definitive conclusion.

By increasing the stimulation frequency, as in Figure 3, a larger contractile force is seen. This is a result of summation, which is the concept that if a second stimulus arrives before the muscle relaxes from the first stimulus, a greater peak tension is developed. Thus, as frequency is increased, the time between stimuli is decreased, and a greater peak tension is seen because the muscle is unable to return to its resting force. Generally, the cold temperature muscle had the largest response to the stimuli at different frequencies as well as the largest amplitude of tetanus. This is in line with the previous data acquired from the peak active force, which also showed that the cold temperature muscle has the largest response. This was not expected. As stated previously, there are a large potential sources of error. Our original hypothesis presumed that temperatures congruent with the internal body temperature of the frog would produce the largest responses. Temperatures well above or well below the internal body temperature were expected to have smaller peak active force because of muscle stiffness, denaturing of proteins or possibly even presence of oxygen to the internal portions of the muscle. Instead of an optimal middle temperature for performance, the data actually shows the coldest temperature tested (which was well below internal body temperature of the frog) coincided with the largest peak active force.

Figure 4 demonstrates the fatigue and shows variations in the time constants and amplitudes of the fatigues of muscles of different temperatures. The warm muscle has the largest overall response as well as the longest time constant. On the other hand, the cold temperature muscle response had the shortest time constant and the lowest amplitude for the majority of the test. It is worth noting that the warm fatigue test took place before the cold fatigue test. The order in which the procedures were performed may have impacted the observed stress, possibly explaining why the warm muscle fatigue is slower and has a larger amplitude than the cold fatigue test. Despite this, the results are congruent with our

hypothesis if we consider the hot temperature to be indicative of the frogs internal body temperature and the cold temperature to be a large deviation from that optimal. It is possible that the colder temperature prevented continued ATP production at the same rate as in the hotter temperature trial, by the same limitations described previously. With reduced production of ATP, the muscle in the colder trial likely exhausted its energy reserves (required for contraction) quicker than the warmer muscle.

To reiterate, the results of this experiment did not coincide with our hypothesis as a whole. The data made clear a few trends that did support the existence of an optimal temperature for maximum muscle contractile force and postponed fatigue, but there is also substantial contradictory data. As outlined, there are many large sources of error that disrupt the formation of a definitive conclusion. Further, and more extensive, testing would be required to make any final statement on the temperature dependence of contractile properties of skeletal frog muscle.

References:

Barbour, Dennis, and Patricia Widder. BME 301A Lab Manual. Department of Biomedical Engineering at Washington University in St. Louis, 11 Sept. 2013. Web.

Edwards, R. H. T., et al. "Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps muscle in man." *The Journal of physiology* 220.2 (1972): 335-352.

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Lillywhite, Harvey B. "Behavioral temperature regulation in the bullfrog, Rana catesbeiana." *Copeia* (1970): 158-168.

Ranatunga, K. W., and S. R. Wylie. "Temperature-dependent transitions in isometric contractions of rat muscle." *The Journal of physiology* 339.1 (1983): 87-95.

Unofficial Sources:

1. Quantitative Physiology Lecture Slides (Module 4)

2. Collaborative Lab Group for Actual Procedure (Hu-Park-Yoo)