An active learning mammalian skeletal muscle lab demonstrating contractile and kinetic properties of fast- and slow-twitch muscle

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Head SI, Arber MB. An active learning mammalian skeletal muscle lab demonstrating contractile and kinetic properties of fast and slow-twitch muscle. Adv Physiol Educ 37: 405–414, 2013; doi:10.1152/advan.00155.2012.—The fact that humans possess fast- and slow-twitch muscle in the ratio of ~50% has profound implications for designing exercise training strategies for power and endurance activities. With the growth of exercise and sport science courses, we have seen the need to develop an undergraduate student laboratory that demonstrates the basic properties of fast- and slow-twitch mammalian skeletal muscle. This laboratory illustrates the major differences in contractile properties and fatigue profiles exhibited by the two muscle types. Students compare and contrast twitch kinetics, fused tetanus characteristics, force-frequency relationships, and fatigue properties of fast- and slow-twitch muscles. Examples of results collected by students during class are used to illustrate the type of data collected and analysis performed. During the laboratory, students are encouraged to connect factual information from their skeletal muscle lectures to their laboratory findings. This enables student learning in an active fashion; in particular, the isolated muscle preparation demonstrates that much of what makes muscle fast or slow is myogenic and not the product of the nervous or circulatory systems. This has far-reaching implications for motor control and exercise behavior and therefore is a crucial element in exercise science, with its focus on power and endurance sport activities. To measure student satisfaction with this active learning technique, a questionnaire was administered after the laboratory; 96% of the comments were positive in their support of active versus passive learning strategies.

isolated muscle; fatigue; contraction; skeletal muscle physiology laboratories; active learning

THE OBJECTIVE of this laboratory is to actively acquire an understanding of the differences in basic physiological properties between fast- and slow-twitch mammalian skeletal muscle. Using isolated rat muscles that have already been dissected for them, students collect and analyze data on important contractile properties such as the length-tension relationship, twitch and tetanus kinetics, force-frequency relationship, and response to fatigue while relating their findings to material already covered in lectures.

Background

Historically, skeletal muscle teaching laboratories have used the amphibian gastrocnemius/sciatic nerve preparation. While this preparation is adequate for illustrating basic contractile properties common to vertebrate voluntary muscle, it does not demonstrate important specializations that have evolved in mammalian skeletal muscle. Especially important is the difference in power and fatigue properties between fast- and slow-twitch muscles (10). In mammals, the slow-twitch muscles are predominantly used to maintain posture and for endurance activities. Metabolically, slow muscles are predominantly aerobic and use O₂ to generate ATP via the tricarboxylic acid cycle in mitochondria. In contrast, the fast-twitch muscles used in sprinting and power exercises are anaerobic and predominantly use the glycolytic pathway in the myoplasm to generate ATP.

It was in 1873 that the French physician and physiologist Ranvier first recognized that skeletal muscles not only differ in color, with some being dark red and others almost white, but that they also have different contractile properties (8). For example, in mammals, the soleus muscle at the back of the leg is red in appearance and slow as far as its contractile properties are concerned. In contrast, the extensor digitorum longus (EDL) muscle found at the front of the leg is a white muscle that contracts and relaxes rapidly. These two muscles are commonly used in physiology research. This laboratory class uses these two muscles as examples of an isolated intact fast- and slow-muscle preparation.

A word about muscle types and fiber types is relevant at this point. Functionally, for the purposes of this laboratory, the EDL behaves as a fast-twitch muscle, whereas the soleus behaves as a slow-twitch muscle. The fast-twitch and slow-twitch categories refer to the contractile kinetics of the muscle, with the fast-twitch muscle contracting and relaxing significantly faster than the slow muscle. However, students should be aware that within these broad categories there exists a range of fiber types. In the skeletal muscles of mammals, there are four major fiber types, which are, from slowest to fastest, 1, 2A, 2X, and 2B. This categorization is based on the type of myosin expressed, with the 2B fibers being the fastest at catalyzing the energy release from ATP and moving the myosin heads during the cross-bridge cycle (students should review their lecture notes on muscle contraction for further details). In faster fiber types, there is a dense distribution of the internal Ca²⁺ store, the sarcoplasmic reticulum, which enables the fast release and reuptake of Ca²⁺ to trigger muscle contraction and relaxation, respectively. In contrast, in slower muscle fiber types, the sarcoplasmic reticulum is sparser, resulting in a slower release and uptake of Ca²⁺, which results in slower contractile kinetics.

The fast and slow fibers also differ in the types of metabolic pathway they use to generate energy (ATP) to power muscle contraction. Slow muscle fibers largely use aerobic mitochondrial pathways to produce ATP from glucose/glycogen and, to a lesser extent, fatty acids. Consequently, slow muscle fibers are well endowed with mitochondria and contain a dense concentration of the respiratory pigment myoglobin, which acts as an additional O₂ store and gives them their red appearance. In contrast, fast fibers are predominantly anaerobic in

Address for reprint requests and other correspondence: S. I. Head, School of Medical Sciences, Univ. of New South Wales, Sydney 2052, NSW, Australia (e-mail: S.Head@unsw.edu.au).
Describe and explain the shape of the length-tension relationship. Extension of the learning pathway is further allowed by enabling students to match theory acquired in lectures to skills of scientific discovery. The current laboratory fulfills this by isolating muscles without their blood and nerve supply. Students can now observe that contractile and fatigue characteristics are not contained by the animal’s blood and nerve supply. Students actively learn that a large component of an individual muscle’s contractile physiology, such as twitch kinetics and fatigue characteristics, is myogenic in origin. They are not dependent on the central nervous system or the peripheral nerve supply.

A key feature of the laboratory is that the muscles are isolated from the animal’s blood and nerve supply. Students can now observe that contractile and fatigue characteristics are a property of the muscle alone. Students actively learn that a large component of an individual muscle’s contractile physiology, such as twitch kinetics and fatigue characteristics, is myogenic in origin. They are not dependent on the central nervous system or the peripheral nerve supply.

As discussed by Sweeney et al. (16), an important goal of laboratory classes is to engage students directly in the process of scientific discovery. The current laboratory fulfills this by enabling students to match theory acquired in lectures to skills and knowledge gained through their own practical experimentation. Extension of the learning pathway is further allowed by discussion of findings against a backdrop of current and past muscle research, lectures, and, if available, followup tutorials.

**Learning Objectives**

**Content knowledge.** After completing this laboratory, students will be able to:
- Describe and explain the shape of the length-tension relationship
- Describe and explain the differences in twitch and tetanus kinetics between fast- and slow-twitch muscles
- Describe and explain the differences in the shape of the force-frequency relationship between fast- and slow-twitch muscles
- Describe and explain the differences between fast- and slow-twitch muscles in their response to fatigue

**Process skills.** After completing this lab, students will be able to:
- Measure the basic physiological properties of isolated whole skeletal muscle
- Record data using scientific chart recording software
- Analyze these data using a statistical analysis program
- Appreciate the concept of mean and variability in scientific data
- Understand the usefulness of fitting mathematical models to data
- Present data in graphic form

**Activity Level**

This laboratory has been designed for university students who have already completed a basic physiology course and therefore forms part of an advanced skeletal muscle physiology study for students in the final part of their degree.

**Prerequisite Student Knowledge or Skills**

From previous courses, or from lectures given earlier in this course, students will already have a basic understanding of muscle contraction. To perform the data analysis, students should be proficient in the use of a computer.

**Time Required**

This activity typically requires ~3 h to complete per muscle. In addition, the muscle dissection conducted before the laboratory takes ~1 h.

**METHODS**

**Class Structure and Delivery**

Students work in groups of two to six students depending on class size. Ideally, the laboratory should be conducted over two sessions, with each group working on either the soleus or EDL muscle in the first session and then on the other muscle in the second session. Each session typically lasts ~3 h. If it is not possible to run two sessions, students can either endeavor to work on both muscles in a single extended session or use one muscle and exchange results with another group. The equipment setup, solution preparation, and muscle dissection takes ~1 h before each session.

**Equipment Required by Each Group**

The muscle setup consists of the following: a PowerLab 4/20T (AD Instruments, Sydney, NSW, Australia, with Chart 5 or later software), Bridge Pod (AD Instruments), force transducer MLT 500A with a hook attachment, muscle bath and positioning manipulator (organ bath system, AD Instruments), carbogen supply to bubble Krebs solution (mixture of 95% O₂-5% CO₂, obtainable from standard gas suppliers), tubing + thin plastic tubing (end heated to create a 45° angle) for directing gas into the bath, and Grass stimulator (SD9) and stimulating electrodes (made from a thin platinum sheet of ~0.1 mm thick, 50 mm long, and 4 mm wide).

Each group is given a tray containing the following items: a 25-ml syringe with attached pipette end, two disposable pipettes, a 100-ml plastic beaker, spring dividers (>50 mm, for measuring muscle length), a 15-cm ruler, blunt forceps, a 10-g weight for calibration, and a stopwatch.

**Krebs Solution**

Five liters of solution is required for ten setups. The standard composition of Krebs solution is as follows (in mM): 4.75 KCl, 118 NaCl, 1.18 KH₂PO₄, 1.18 MgSO₄, 24.8 NaHCO₃, 2.5 CaCl₂, and 11 glucose. FCS (1 ml, Sigma) is added to every 1 liter of Krebs solution to enhance muscle viability. The solution is bubbled with carbogen to maintain the pH at 7.4. Note that the CaCl₂ should be left out until the end; the solution needs to be bubbled for 15 min before the addition of CaCl₂ to prevent precipitation.

Due to time constraints, we typically prepare this solution in advance for the students. However, if instructors wish and time permits, the solution preparation can be performed by the students as an exercise in chemistry skills and calculations.

**Animals**

The procedures described here have been approved by the Animal Care and Ethics Committee of the University of New South Wales (ACEC 10/73B). Adopters of this activity are responsible for obtaining permission for animal research from their home institution. For a summary of the “Guiding Principles for Research Involving Animals and Human Beings,” please see www.theaps.org/mm/Publications/Ethical-Policies/Animal-and-Human-Research.
Muscle Dissection

Due to the complexity of the procedure, the muscle dissection is performed by the demonstrators before the start of the laboratory. Instructors requiring additional help with this procedure can consult Hakim et al. (6), which demonstrates the dissection of the EDL muscle in the mouse.

Equipment. Each demonstrator requires the following: one cork dissecting board, two fine scissors, two forceps, one medium scissors, one medium forceps, one bone-cutting scissors, one Pasteur pipette, strong cotton thread, and a lamp.

Dissection procedures. Skin the hindlegs and cut through the femur with the strong bone-cutting scissors. Pin out the leg on a cork dissecting board. Throughout the procedure, ensure the tissue stays wet by dropping Krebs solution onto it with a Pasteur pipette. Start with the EDL muscle. The tendons on the lateral surface of the paw can clearly be seen running down to each of the four toes. Insert a pair of fine scissors underneath and cut all the tendons. Note where the tendons run into the muscle mass above the ankle and free the tendon at this end by inserting a pair of fine scissors underneath. Use the top of the tendon to pull the distal cut ends through the boney cuff at the ankle. Next, cut away the biceps femoris and tibialis anterior muscles to expose the underlying EDL muscle. Now, pick up the distal end of the EDL tendon and carefully free the EDL muscle from its bed, cutting the nerve and artery, which emerges about halfway along the muscle. At the knee, expose the proximal tendon. Using the bone-cutting scissors, cut through the knee joint and then cut through the femoral condyles to leave a small bit of bone attached to the tendon. This will facilitate tying. Using strong thread, tie a knot around the distal tendon, close to the musculotendinous junction. Tie a second knot, but before closing the knot the tendon may be looped back through to strengthen the tie and prevent slippage. Use the remaining string to tie a small loop, which will be used to fix the ends of the muscle in the water bath. Tie another double knot around the proximal tendon, keeping the knot right up against the bone to prevent slippage. Also, make a loop here for placement in the water bath. Store the preparation in a beaker of Krebs solution bubbled with carbogen.

Now dissect the soleus muscle. Turn the paw so that the medial face is uppermost. Cut through the Achilles tendon and ankle bone using bone cutters. Remove the overlying gastrocnemius and plantaris muscles. The soleus muscle is flat and red in appearance and lies flush with the surface of the tibia. Free the soleus muscle from the bone and preserve as much of the wide flat proximal tendon as possible as well as some of its bony attachment to facilitate tying. As for the EDL muscle, tie looped sutures to the tendons as before and store in bubbled Krebs solution. Muscles will remain viable for ~6 h in this condition.

Calibration of the Force Transducer

Students must perform this calibration at the very start so that the voltage recorded by the force transducer can be converted to units of force. Throughout this article, we report force as grams of weight (1 g of weight = 9.8 mN). The force transducer is placed in a horizontal position, and a 10-g weight is hung on it so that the transducer is pulled in the same direction as it would be when the muscle pulls on it in the water bath. The voltage produced by this 10 g of weight is entered into the calibration section of the PowerLab software (refer to software documentation).

Muscle Setup

Students are now ready to set up the muscle in the water bath. One loop is hung onto the hook at the end of the water bath; the other loop is hung onto the force transducer. The muscle is set to a length at which the slack is just taken up and the muscle is tight but not stretched; this is the slack length. Students should be reminded to handle the muscle only by the ties, not to touch the muscle itself, and to always ensure that the muscle is covered with Krebs solution and that the solution is always bubbled with carbogen. With these precautions, and with FCS present in the Krebs solution, the muscle should remain viable until the end of the session.

Experimental Procedures

Students are now ready to perform the following experiments on the muscle: length-tension relationship, twitch and tetanus kinetics, force-frequency relationship, and response to fatigue. With each experiment, students are asked to investigate certain physiological questions and are provided with guidelines on standard methods used to investigate such questions. The methodological guidelines provided to the students make up the remainder of METHODS. In the RESULTS, we describe the questions that the students are expected to investigate, provide sample results, and relate these results to the experimental questions.

Throughout the laboratory, students record data using the PowerLab chart recording program and analyze it using the GraphPad Prism statistical package. If instructors wish to see an example of the recordings collected and analyses performed during the laboratory, sample files and free viewers are available via the following link: http://medicalsciences.med.unsw.edu.au/research/groups/cellular-and-systems-physiology/advances-physiology-education. We have also provided, as Supplemental Material, an Excel spreadsheet containing the data tables from the Prism file.

Length-Tension Relationship

Students measure the twitch force produced at different muscle lengths to determine the optimum length ($L_0$) of the muscle.

1. Measure the slack length of the muscle using the dividers and ruler.
2. Produce three twitches by giving the muscle three single pulses with the Grass stimulator (voltage should be set to maximum to ensure that a supramaximal stimulus is given).
3. From the LabChart recording, measure the force of each twitch. Enter the length and forces into the Prism file to produce a plot of force versus length.
4. Using the manipulator attached to the force transducer, increase the muscle length by 1 mm. Repeat steps 2 and 3.
5. Keep increasing the muscle length by 1 mm until maximum force is reached. This is the muscle’s $L_0$. However, if a maximum has still not been reached after the muscle has been stretched by 25% of its slack length, do not stretch the muscle any further as it may become damaged.

Twitch Kinetics

Here, the students measure parameters relating to the speed of contraction and relaxation.

1. Stimulate the muscle with three single pulses, as described above in Length-Tension Relationship.
2. For each twitch, measure the time to peak (time taken to reach maximum force) and half-relaxation time (time taken for force to fall to half of the maximum).

1Supplemental Material for this article is available at the Advances in Physiology Education website.
3. Enter these values into the Prism file, which will automatically calculate the mean and SE.

**Tetanus Kinetics**

Here, the students measure the relaxation kinetics after a fused tetanic contraction.

1. Set the frequency on the stimulator to 40 Hz.
2. Stimulate the EDL muscle for 1 s and the soleus muscle for 2 s by holding the stimulator switch on “Repeat” for the required duration. Record three tetani, leaving an ~20-s rest between each tetanus.
3. Measure the half-relaxation time of each tetanus.
4. Enter these values into the Prism file, which will automatically calculate the mean and SE.

**Force-Frequency Relationship**

Here, the students measure the force produced at different frequencies of stimulation.

1. The muscle should be stimulated at the following frequencies: 5, 10, 15, 20, 25, 30, and 40 Hz.
2. At each frequency of stimulation, keep the stimulator switch on “Repeat” until a stable level of force has been reached and then release it. Leave an ~20-s rest before moving on to the next frequency.
3. Measure the force produced at each frequency and enter it into the Prism file to produce a plot of force versus frequency.
4. Use Prism to fit a sigmoidal curve to the data points plotted in step 3, using the following equation:

\[ P = P_{\text{min}} + \left( \frac{P_{\text{max}} - P_{\text{min}}}{1 + \left( \frac{f}{f_{\text{hf}}} \right)^h} \right) \]

where \( P \) is force, \( f \) is frequency, \( P_{\text{min}} \) is minimum force, \( P_{\text{max}} \) is maximum force, \( f_{\text{hf}} \) is half-frequency, and \( h \) is the Hill coefficient. Students should record the values of \( P_{\text{max}}, P_{\text{max}}, f_{\text{hf}}, \) and \( h \) produced by the fitting procedure and also calculate the twitch-to-tetanus ratio (\( P_{\text{min}}/P_{\text{max}} \)).

**Fatigue**

Here, the students examine the decline in force during fatiguing stimulation.

1. Stimulate the muscle at 40 Hz (1 s on, 1 s off for the EDL muscle and 2 s on, 1 s off for the soleus muscle) until the force has declined to ~50% of the original force or until 10 min have elapsed, whichever occurs first.
2. Plot the decline in force over the course of the fatigue run.

**Troubleshooting**

The following are problems sometimes encountered and their likely causes:

1. The muscle shows erratic tetani. Check that the muscle is completely covered by solution and that the solution is bubbling with carbogen.
2. The muscle is producing no force and is not twitching. Check that the Krebs solution has been made up properly and that glucose and Ca²⁺ have been added.
3. The muscle appears to be losing force. Check that the muscle length has not changed and that the muscle tendons are not slipping through the knots.

**Safety Considerations**

Because the Krebs solution is a normal physiological saline solution, no special precautions need to be taken by the students, apart from wearing the usual laboratory dress (laboratory gown and closed shoes).

**RESULTS**

**Length-Tension Relationship**

In this part of this laboratory, students investigate the following questions: “How does muscle force vary with muscle length? What is the importance of this for a muscle in the body?” Figure 1 shows a LabChart recording of muscle force taken during the length-tension relationship experiment on an EDL muscle, taken from the sample data file. As the students gradually increase the length of the muscle, they are asked to consider: “Why is the baseline rising? Why is the height of each twitch increasing?” In Fig. 2A, the heights of the twitches shown in Fig. 1 have been measured and plotted in Prism to produce a graph of active force versus length. Figure 2B is a plot for a soleus muscle, also taken from the sample data. Note that in these particular cases, a maximum tension has not been reached as the students have been instructed not to stretch the muscle by >25% past slack length.

From this exercise, students can see first hand that stretching a muscle increases the tension of its elastic elements, thus causing the baseline passive tension to increase, and that stretching a muscle increases the force it can actively produce.
due to the cross-bridge interactions between the myosin heads and binding sites on the actin filaments. At a muscle’s $L_0$, this active force is at a maximum because the number of cross-bridges is at a maximum. The importance of this is that many muscles in the body are at $L_0$ when they are at rest, thus preparing them to develop maximum tension when they contract (2).

**Twitch Kinetics**

In this part of this laboratory, students investigate the following question: “Why are muscles classified as fast twitch or slow twitch?” Figure 3 shows sample LabChart recordings for three twitches in an EDL muscle (A) and a soleus muscle (B). Table 1 contains the measurements of time to peak and half-relaxation time made with these recordings. These have been entered into Prism to determine the mean and SE shown. Students are asked to consider the following questions: “Why does the fast-twitch muscle reach peak force more quickly than the slow-twitch muscle? Why does the fast-twitch muscle relax more quickly than slow-twitch muscle?”

This exercise provides the students with a striking illustration, both visually (Fig. 3) and quantitatively (Table 1), of why muscles are designated as “fast twitch” or “slow twitch.”

**Tetanus Kinetics**

Here, students find a further illustration of why are muscles classified as fast twitch or slow twitch. Figure 4 shows sample LabChart recordings for three tetani in an EDL muscle (A) and a soleus muscle (B). Table 2 shows the measurements of half-relaxation time made on these tetani. Means and SEs were calculated by Prism. It is clear that the distinction between fast-twitch muscle and slow-twitch muscle applies to a tetanus as well as a twitch.

**Force-Frequency Relationship**

In this experiment, students investigate the following questions: “How does the frequency of stimulation affect muscle force? Is this relationship different between fast-twitch and slow-twitch muscles?” Figure 5 shows sample LabChart recordings for force-frequency runs conducted on an EDL muscle (A) and a soleus muscle (B). Recordings at 5 and 10 Hz are shown on an expanded timescale in Fig. 5, C and D. The forces produced at each frequency were measured and entered into Prism to produce the plot of force versus frequency shown in Fig. 6. Each data point on this graph represents the force measured at a particular frequency of stimulation. The solid lines on this graph are the sigmoidal curves fitted to these data points using the equation shown in METHODS. Table 3 shows the values of the parameters that define the fitted curves.

This exercise gives students an opportunity to generate their own force-frequency curves and then consider the following...
questions: “How do the force-frequency curves of fast-twitch and slow-twitch muscles differ? How do these differences in shape arise? What is the physiological advantage of these differences?”

It is clear from Fig. 6 that in the fast-twitch EDL muscle, the force-frequency curve stays flat at low frequencies before rising rapidly from ∼15 Hz onward, whereas in the slow-twitch soleus muscle, the force-frequency curve is already rising at very low frequencies. Hence, the curve for the EDL muscle appears to be shifted to the right compared with that for the soleus muscle. The expanded recordings shown in Fig. 5, C and D, show why this is so. At low frequencies in the EDL muscle, the tension “spike” produced by each stimulus remains separate from each preceding spike, a consequence of the rapid relaxation of fast-twitch muscle. Hence, the force produced by a train of stimuli is not noticeably different from that of a single twitch. However, in the soleus muscle at low frequencies, each tension spike builds on the previous spike, because with its slower relaxation, the force has not yet returned to zero before the next stimulus comes along. This is called mechanical summation, and because this occurs at lower frequencies in the soleus muscle than in the EDL muscle, the force-frequency curve of the soleus muscle starts to rise earlier than that of the EDL muscle. The physiological benefit of this is that slow-twitch muscles, which are mainly used for maintenance of posture, can produce sustained contractions of moderate force at low frequencies, whereas fast-twitch muscles produce powerful contractions at high frequencies for activities such as sprinting (9).

This exercise also encourages the students to consider the following questions: “What is the advantage of fitting a curve to the data? How do the fitted parameters relate functionally to the muscle?” A mathematical model is a concise way of describing the relationship between a series of data points and provides parameters by which to describe this relationship quantitatively. The sigmoidal dose-response curve is a highly appropriate model for the force-frequency relationship, and, in our experience, the r² for the fitting procedure is almost always >95%. The equation of this curve is provided in METHODS. The process of curve fitting involves trying different values of $P_{\text{min}}$, $P_{\text{max}}$, $K_f$, and $h$, producing different force-frequency curves, and the one that passes closest to the data points is chosen as the best-fit curve.

The values of $P_{\text{min}}$, $P_{\text{max}}$, $K_f$, and $h$ that define this best-fit curve, shown in Table 3, provide a way of quantitatively describing the features that we can see by eye in Fig. 6. $P_{\text{min}}$ is the lower limit of the force-frequency curve; it is the force we would reach if the curve were to be continued infinitely to the left and, hence, corresponds functionally to the twitch force. $P_{\text{max}}$ is the upper limit of the force-frequency curve; it is the force we would reach if the curve were to be continued infinitely to the right and, hence, corresponds functionally to the maximum tetanic force. $K_f$ is the half-frequency, or the frequency at which force is halfway between minimum and maximum, and provides a measure of the location of the force-frequency curve. The $K_f$ for the EDL muscle is greater than that for the soleus muscle; this is because, as one can see from Fig. 6, the curve for the EDL muscle is located to the right of that for the soleus muscle, a consequence of the difference in frequencies at which mechanical summation occurs, as described above. $h$ is the Hill coefficient and indicates the steepness of the curve. It is apparent from Fig. 6 that the curve for the EDL muscle, once it starts to rise, is steeper than that for the soleus muscle, and this is reflected in a higher $h$ value for

Table 1. Measurements of twitch kinetics made on the sample recordings shown in Fig. 3

<table>
<thead>
<tr>
<th></th>
<th>EDL Muscle</th>
<th>Soleus Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TTP, ms</td>
<td>HRT, ms</td>
</tr>
<tr>
<td>Twitch 1</td>
<td>37.5</td>
<td>33</td>
</tr>
<tr>
<td>Twitch 2</td>
<td>38</td>
<td>31</td>
</tr>
<tr>
<td>Twitch 3</td>
<td>36.5</td>
<td>29.5</td>
</tr>
<tr>
<td>Mean</td>
<td>37.3</td>
<td>31.2</td>
</tr>
<tr>
<td>SE</td>
<td>0.44</td>
<td>1.01</td>
</tr>
</tbody>
</table>

EDL, extensor digitorum longus; TTP, time to peak; HRT, half-relaxation time. Means and SEs were calculated by Prism.

Table 2. Measurements of tetanus kinetics made on sample recordings

<table>
<thead>
<tr>
<th></th>
<th>EDL Muscle HRT, ms</th>
<th>Soleus Muscle HRT, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus 1</td>
<td>55.5</td>
<td>156</td>
</tr>
<tr>
<td>Tetanus 2</td>
<td>47.5</td>
<td>134</td>
</tr>
<tr>
<td>Tetanus 3</td>
<td>53</td>
<td>136</td>
</tr>
<tr>
<td>Mean</td>
<td>52.0</td>
<td>142</td>
</tr>
<tr>
<td>SE</td>
<td>2.36</td>
<td>7.02</td>
</tr>
</tbody>
</table>

Means and SEs were calculated by Prism.

Fig. 4. Sample LabChart recordings for three tetani in an EDL muscle (A) and a soleus muscle (B). Note that the stimulation period for the second tetanus in the soleus muscle is for ∼1 s, for comparison with the EDL tetani.
the EDL muscle. Students also calculate the twitch-to-tetanus ratio, a commonly measured contractile property that is usually ~1:4 in mammalian muscle (2).

Due to time constraints, we have the sigmoidal dose-response equation already entered into the customized Prism file provided to the students. The curve fitting is then automatically performed by the Prism software once the students enter their force-frequency data. In other courses aimed to emphasize the importance of curve fitting further, students can be encouraged to enter the equation themselves and review the extensive notes on curve fitting provided within the Prism help files.

### Fatigue

In this experiment, students investigate the following questions: “What is muscle fatigue? Is it something intrinsic to the muscle, or is it due to external factors such as the nervous system? Do fast-twitch and slow-twitch muscles differ in their responses to fatiguing stimulation? What causes muscle fatigue?” Figure 7 shows the LabChart recordings of this experiment in the EDL (A) and soleus (B) muscles, illustrating the reduction in tetanic force that occurs with repetitive stimulation. This is the definition of muscle fatigue. As the muscle is totally isolated from the donor rat, the development of fatigue in this preparation demonstrates to the students that a major component of skeletal muscle fatigue is intrinsic to the muscle.

In Fig. 8, the force produced in each tetanus was measured and plotted as a function of time. It is clear from the data shown in Fig. 8 that slow-twitch muscles are more resistant to fatigue than fast-twitch muscles.

Muscle fatigue is a multifactorial phenomenon that depends on the type of activity being undertaken. A common misconception is that acidosis of the muscle as a result of lactate production during repetitive activity is responsible for produc-

### Table 3. Best-fit parameters of the force-frequency curves shown in Fig. 6

<table>
<thead>
<tr>
<th></th>
<th>EDL Muscle</th>
<th>Soleus Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{min}, g}$</td>
<td>16.8</td>
<td>21.0</td>
</tr>
<tr>
<td>$P_{\text{max}, g}$</td>
<td>71.4</td>
<td>67.8</td>
</tr>
<tr>
<td>$K_F$, Hz</td>
<td>18.5</td>
<td>10.5</td>
</tr>
<tr>
<td>$h$</td>
<td>9.51</td>
<td>3.63</td>
</tr>
<tr>
<td>Twitch-to-tetanus ratio</td>
<td>1:4.24 (or 0.236)</td>
<td>1:3.23 (or 0.310)</td>
</tr>
<tr>
<td>$P_{\text{max}}$</td>
<td>minimum force; $P_{\text{max}}$</td>
<td>maximum force; $K_F$, half-frequency; $h$, Hill coefficient.</td>
</tr>
</tbody>
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ing the force loss that occurs during fatigue. Research findings from many independent laboratories have now shown that acidosis does not produce fatigue, and, in fact, the lactate produced during activity may even help to maintain force output. Individuals suffering from McArdle’s disease (myo-phosphorylase deficiency) cannot produce lactate so they never experience acidosis, but their muscles still fatigue. Students interested in the cellular mechanisms of fatigue in skeletal muscle should refer to the major review by Allen et al. (1), with special reference to the debunking of the lactate-induced fatigue theory. The following is a brief description of the causes of fatigue during different types of activities.

The 100-m sprint. Relatively little lactic acid is formed during such a short activity. Instead, most of the energy comes from breakdown of phosphocreatine. Breakdown of phosphocreatine consumes H+, which is why the net effect is that myoplasmic pH is not significantly altered during the sprint. One product of phosphocreatine breakdown is phosphate ions, and these have been shown to depress muscle function. They reduce both the Ca2+ sensitivity of the contractile proteins and the ability of the contractile proteins to produce force. Thus, phosphate ion accumulation is probably an important contributor to fatigue during a 100-m sprint.

A continuous maximal contraction, such as occurs when lifting something very heavy, like a piano. Everyone will be aware of how rapidly fatigue can set in during such activities. In this situation, the muscle machinery is going at full speed and energy is consumed at a rapid rate. In addition, the blood flow to the active muscle(s) is stopped during maximal contractions so that no delivery of O2 to support muscle contraction or removal of metabolites or ions will occur. In this case, severe fatigue develops within seconds and the muscle becomes rapidly weaker. Each action potential is associated with entry of Na+ into the cell and exit of K+ from the cell; consequently, K+ tends to accumulate outside of the fibers, and this results in depolarization and impaired electrical activation of muscle cells. This extracellular accumulation of K+ is likely to be larger in the narrow lumen of the t-tubules, which is a restricted space for diffusion, so this will depolarize the tubular system, causing a failure of electrical excitability in the muscle cell. Due to the fact that the blood supply is cut off by the contracting muscle, the excess K+ is not removed by the circulation; this accounts for the large local build up of K+ at the contracting muscle. Students should refer to their course note or textbook on the Nernst potential to understand why an increase in extracellular K+ will depolarize the muscle cell.

The 5-km run. Events of this sort last 10 min or so and are performed at quite close to the maximum capacity of the muscles and involve both the aerobic and anaerobic ATP pathways. The main cause of the force drop during fatigue here is a failure of Ca2+ release from the sarcoplasmic reticulum as a consequence of the metabolic byproduct of ATP breakdown, phosphate, entering into the sarcoplasmic reticulum and reacting with Ca2+ to produce calcium phosphate.

Marathon-like distances of >42 km. This type of activity causes near-final depletion of glycogen in muscles. Muscle biopsies show that at the end of a marathon glycogen is depleted; in contrast, ATP is only marginally reduced, lactic acid accumulation is minimal, and myoplasmic phosphate accumulation is also only moderate. The main factor involved in fatigue here seems to be failure of Ca2+ release from the sarcoplasmic reticulum, which is associated with the glycogen depletion.

In conclusion, the answer to the question “What causes muscle fatigue?” is “It depends on the type of activity, but it’s not due to lactate accumulation.” As for the difference in fatiguability between the EDL and soleus muscles, the main factor here is the difference in the density of mitochondria and the capacity to use oxidative metabolism to generate ATP.
Slow type 1 fibers have greater oxidative capacity than fast type 2 fibers. In addition, phosphate accumulation, which inhibits force generation, occurs more rapidly in type 2 fibers because the myosin heavy chain isoform is faster and consumes ATP more rapidly (1).

Inquiry Applications

The laboratory, as it is described here, is conducted mainly as a “facilitated inquiry.” The teacher is mainly responsible for providing the research questions and methods, although students are encouraged to modify the experimental protocols and presentation of data if they wish. Methodological guidelines are given to the students because these are standard procedures for assessing muscle function. One of the most valuable aspects of this laboratory is that it allows students to use these standard procedures themselves on an actual isolated whole mammalian muscle, thus generating their own data and graphs and facilitating their understanding of muscle function.

Once students have been equipped with these basic tools, further classes may be conducted that encourage students to design their own experiments to investigate questions, such as the following: “How do fast- and slow-twitch muscles differ in their response to various drugs, such as caffeine? What would happen if glucose was removed from the bathing solution? What effect does temperature have, and does this effect differ between the two muscle types? How do warmups affect muscle function, and does this differ between the two muscle types? How do the properties of mammalian muscle differ from amphibian muscle?” Students can be encouraged to look up their own literature references to help them answer these questions. For example, Fryer et al. (5) is a good source on the effects of caffeine, and Helander et al. (7) is a good source on the effects of zero glucose.

Finally, we appreciate that many institutions may not have the necessary resources and access to laboratory animals required to carry out the experiments we have described. In this case, instructors are encouraged to use the sample data sets we have provided (please see Experimental Procedures in METHODS for details on how to access these files). Students will still gain valuable experience in analyzing these data and will still learn much about muscle function by relating the results of their analyses to the questions we have provided throughout this section. To gain a brief overview of the experimental procedures, students may view the video produced by Oishi et al. (12).

Additional Resources

For additional information pertaining to general muscle physiology, please see Refs. 3 and 4.

DISCUSSION

This practical enables students to carry out muscle contractile experiments with current research equipment and data recording and analysis software to investigate the main characteristics of fast- and slow-twitch mammalian muscles. This allows a core component of the skeletal muscle curriculum to be delivered in an active, student-centered fashion. Hence, the skeletal muscle theory of the course is accompanied by a considerable active learning component. It is a consistent finding that students using a practical student-centered approach rather than a didactic teacher-centered learning system have significantly higher exam scores (11). Active learning, that is student-centered pedagogical approaches, puts the focus on the learner. The practical described here uses this teaching technique to facilitate effective learning of not just basic skeletal muscle mammalian physiology but also an array of phys-
iological techniques, including analysis and statistical comparisons of data collected, which students can review in a follow-up tutorial. It is a particularly effective practical for sports physiology courses due to the many and interesting avenues it opens for further research in the exercise field.

It has been established that active learning has several advantages over a traditional didactic lecture. These include increased student engagement in class activities through peer learning and the development of critical-thinking skills and the enhancement of student interest through motivation responsiveness and enjoyment. The use of live animal tissue in combination with classroom lectures has several advantages over a lecture alone or a lecture in combination with computer simulations (13, 14). Some of these advantages are that students feel a sense of responsibility in their learning and gain immediate feedback from the experiment. They also have an increased sense of confidence and the chance to observe the physiological concepts of muscle contraction and fatigue. In addition to learning from textbooks and lectures, this method of teaching gives students a concrete example of science-based experimental exercise physiology. Students were required to learn general experimental skills, including the ability to generalize findings. They were also asked to solve scientific problems by observing from experimental results. Questions such as “Where is the major site of fatigue?” and “Does it reside in the central nervous system or is it a function of skeletal muscle itself?” were asked. Through active experimentation, students measure the kinetics of the twitch, generate a force-frequency curve, and look at the effects of fatigue on the skeletal muscle. Therefore, they can solve the problems and, at the same time, acquire the skills that are currently being used to solve scientific problems in active research physiology laboratories.

After the completion of the laboratory in 2011, an anonymous questionnaire comprising 10 questions was given, and 84 of 92 students responded. The 10 questions were designed to gauge student satisfaction and their response to the active learning paradigm. The questions and student responses are shown in Fig. 9. It is clear that students believed that the laboratory had enhanced their learning of basic skeletal muscle contractile physiology and aided their understanding of the lecture series that accompanied the class.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: S.I.H. conception and design of research; S.I.H. performed experiments; S.I.H. and M.A. analyzed data; S.I.H. and M.A. interpreted results of experiments; S.I.H. prepared figures; S.I.H. and M.A. drafted manuscript; S.I.H. and M.A. edited and revised manuscript; S.I.H. and M.A. approved final version of manuscript.

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