Experiment AM-1: Skeletal Muscle, Weight and Work

Background

About 40% of the total body mass of a human is skeletal muscle. Skeletal muscle is intimately associated with the skeletal system and, combined, these muscles and bones are responsible for supporting and moving the body. While skeletal muscle fibers have sarcomeres and the same banded appearance, different muscles can function in different ways. For example, some are relatively weak and fatigue resistant, while others are strong but fatigue quickly. These features may be explained in terms of the biochemical properties of muscles.

The muscle fibers found in most mammalian skeletal muscles are either fast or slow twitch-types. Each type has a different myosin isoform, with different rates of ATPase activity and cross-bridge binding. Within the group of fast-twitch fibers, there are fibers that use glycolysis and oxidative phosphorylation. There are also fast-twitch fibers that just use glycolysis; this group is less reliant upon oxygen and is much stronger than the fibers using phosphorylation. However, these stronger “glycolytic” fibers breakdown glucose very inefficiently; so, that a burst of contractile activity diminishes glucose levels, causes lactic acid to accumulate, and leads to fatigue.

When a muscle tries to lift any weight, the muscle first shortens to put tension on the tendons which hold the muscle to the bones. Development of this tension before movement occurs takes time, known as the latency period, which is directly proportional to the weight attached to the muscle. Once the tension exceeds the weight of the object, any further muscle contraction produces a shortening of the muscle and a movement of the weight. The time that a muscle is in its active state (contracting) is finite; so, muscles have less time to shorten when they move heavier weights.

In this experiment, you will use a displacement transducer to determine how weight affects the shortening of a muscle. Weight influences the time that a muscle has to shorten, the speed at which the muscle shortens, the distance that the muscle shortens or moves the weight, and the amount of work the muscle completes. You will also compare the difference between: afterloading, supporting the weight before contraction; and preloading, hanging the weight on the muscle without support before contraction.

Equipment Required

PC Computer

IWX/214 data acquisition unit

USB cable

IWX/214 power supply

DT-475 Displacement transducer

A-BST-100 Stimulating electrodes

Double male banana-female BNC adapter

Ring stand and clamps

Femur clamp

Thread

6” Ruler

Set of weights and pan

Amphibian Ringer’s solution (See appendix)

IWX/214 Setup

1. Place the IWX/214 on the bench, close to the computer.
2. Check Figure T-1-1 in the Tutorial chapter for the location of the USB port and the power socket on the IWX/214.
3. Check Figure T-1-2 in the Tutorial chapter for a picture of the IWX/214 power supply.
4. Use the USB cable to connect the computer to the USB port on the rear panel of the IWX/214.
5. Plug the power supply for the IWX/214 into the electrical outlet. Insert the plug on the end of the power supply cable into the socket on the rear of the IWX/214. Use the power switch to turn on the unit. Confirm that the power light is on.

Start the Software

1. Click on the LabScribe shortcut on the computer’s desktop to open the program. If a shortcut is not available, click on the Windows **Start menu**, move the cursor to **All Programs** and then to the listing for **iWorx**. Select **LabScribe** from the **iWorx submenu**. The LabScribe **Main window** will appear as the program opens.
2. On the **Main window**, pull down the **Settings menu** and select **Load Group.**
3. Locate the folder that contains the settings group, **IPLMv4.iwxgrp**. Select this group and click **Open**.
4. Pull down the **Settings menu** again. Select the **Skeletal Muscle-Weight-Work-LS2** settings file.
5. After a short time, LabScribe will appear on the computer screen as configured by the **Skeletal Muscle-Weight-Work-LS2** settings.
6. For your information, the settings used to configure the recording channels in the LabScribe software and IWX/214 for this experiment are listed in Table AM-1-1 on page AM-1-2.

7 The settings used to configure the stimulator for this experiment are listed in Table AM-1-2 on page AM-1-2. Both groups of settings are programmed on the **Channel** and **Stimulator windows** of the **Preferences Dialog**, which can be viewed by selecting **Preferences** from the **Edit menu** on the LabScribe **Main window.**

Table AM-1-1: Settings on the Channel Window of the Preferences Dialog that Configure the iWorx System for Experiment AM-2.

Parameter	Units/Title	Setting	Mode/ Function
Acquisition Mode		Chart	
Start		User	
Stop		User	
Display Time	Sec	10	
Speed	Samples/Sec	200	
Channel A3	Muscle Twitch	√	Record
Channel S1	Stimulus	√	Record

Table AM-1-2: Settings on the Stimulator Window of the Preferences Dialog that Configure the iWorx System for Experiment AM-2.

Parameter	Units/Title	Setting
Stimulus Mode		Pulse
Stimulator Start		With Recording
Time Resolution	msec	0.01
Toolbar Step Frequency	Hz	0.5
Toolbar Step Amplitude	Volts	0.01
Toolbar Step Time	Sec	0.0001
Delay	Sec	0.050
Amplitude (Amp)	Volt	4.00
Pulses (#pulses)	Number	1
Pulse Width (W)	msec	10
Frequency (F)	Hz	0.5
Time Off Amplitude	Volts	0
Holding Potential (HP)	Volts	0

DT-475 and Stimulus Electrode Setup

1 Locate the following items in the iWorx kit: DT-475 displacement transducer (Figure AM-1-1 on page AM-1-2); A-BST-100 bipolar stimulator cable (Figure AM-1-2 on page AM-1-2); Male double banana-female BNC adapter (Figure AM-1-3 on page AM-1-2).

Figure AM-1-1: The DT-475 displacement transducer.

Figure AM-1-2: The A-BST-100 bipolar stimulating electrode.

Figure AM-1-3: The male double banana-female BNC adapter.

2 Plug the DIN8 connector of the DT-475 into the Channel 3 input (Figure AM-1-4 on page AM-1-3).

3 Plug the male double banana-female BNC adapter into the positive (red) and negative (black) banana jacks of the IWX/214 stimulator (Figure AM-1-4 on page AM-1-3). The banana plug that goes into the negative (black) stimulator output is identified by a tab, embossed with the letters **GND** (ground), on that side of the adapter (Figure AM-1-3 on page AM-1-2).

4 Attach the BNC connector of the A-BST-100 bipolar stimulator cable to the adapter on the stimulator outputs (Figure AM-1-4 on page AM-1-3).

Figure AM-1-4: The DT-475 displacement transducer and the A-BST-100 bipolar stimulating electrode connected to the IWX/214.

The Dissection

1 Place a frog in ice water for 15 minutes. Double pith the frog as soon as it is removed from the ice water.

2 Remove the skin from the legs by making an incision through the skin around the entire lower abdomen. Cut the connections between the skin and the body—especially around the base of the pelvic girdle. Use stout forceps to pull the skin off the frog in one piece (like a pair of pants).

3 Place the frog in a dissection tray with its dorsal side up.

***Note:** Moisten the exposed limbs of the frog with Ringer's solution every five minutes or so.*

4 Identify the Gastrocnemius muscle on the lower leg.

5 Use a glass hook to separate the Gastrocnemius muscle from the bone and other muscles of the lower leg.

6 Use scissors to free the Achilles tendon from the connective tissue around the heel of the foot. Double up a 24" piece of thread. Firmly tie the doubled thread around the Achilles tendon, leaving the ends of the thread long enough to attach the muscle to the displacement transducer.

***Note:** Isolate as much tendon as possible, since it will be used to attach the muscle to the transducer.*

7 Cut the Achilles tendon as close to the bottom of the foot as possible, so the thread is still attached to the Gastrocnemius muscle.

8 Move the Gastrocnemius muscle away from the rest of the lower leg. Cut the tibia just below the knee to separate the rest of the lower leg from the preparation. Rinse the preparation with Ringer's solution to moisten the tissue and rinse away any blood.

9 Dissect away the muscles of the upper leg and expose the femur. Use a stout pair of scissors to cut the femur close to the pelvis. Rinse the preparation with Ringer's solution to moisten the tissue and rinse away any blood.

The Preparation

1 Use the femur clamp to mount the preparation on the ringstand (Figure AM-1-5 on page AM-1-3).

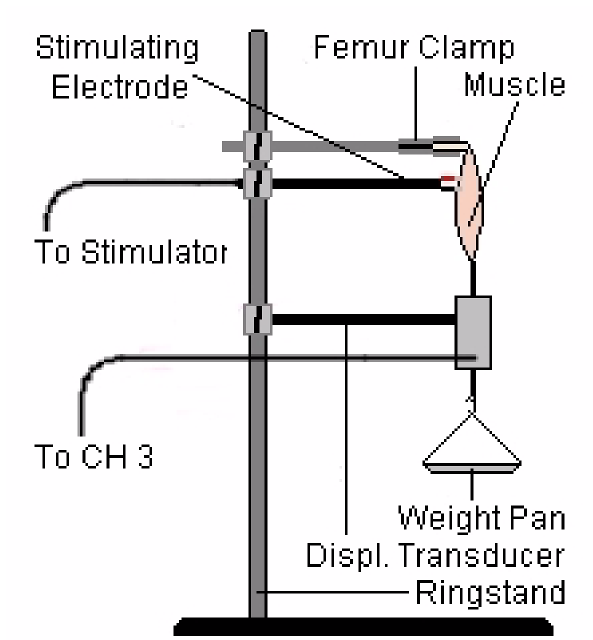

Figure AM-1-5: The arrangement of the equipment used to evoke and record contractions from the frog Gastrocnemius muscle.

2 Attach the thread on the Achilles tendon to the upper eyelet on the rod of the displacement transducer.

3 Use a paper clip to attach the weight pan to the lower eyelet on the rod.

4 Adjust the femur clamp and the displacement transducer so the thread from the Achilles tendon to the transducer rod is vertical.

5 To prevent the weight in the pan from stretching the muscle, the knob on the upper end of the rod should be resting on the bushing in the top of the transducer case.

6 Position the stimulating electrodes so they lay against the muscle about midway between the knee and the tendon. The two electrodes should not touch one another.

7 Place two nickels (10 g) in the weight pan.

Warning: The muscle preparation used in this experiment is functional for a limited period of time. If the muscle is bathed periodically in Ringer's solution, it will work for about four hours. To conserve time, complete all the exercises in the experiment before analyzing the data.

Exercise 1: Maximum Contraction

Aim: To make sure all fibers contract when the muscle is stimulated.

Procedure

1. Click the **Stimulator Preferences icon** on the LabScribe **toolbar** (Figure AM-1-6 on page AM-1-4) to open the **stimulator control panel** (Figure AM-1-7 on page AM-1-4) on the **Main window**.

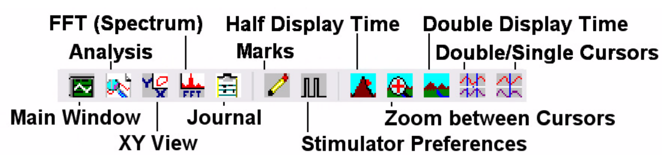

Figure AM-1-6: The LabScribe toolbar.

2. Check the values for the stimulus parameters that are listed in the **stimulator control panel** on the **Main window**: the **pulse amplitude** (**Amp**) should be set to **4.0 V**; the **number of pulses** (**#pulses**) to **1**; and, the **pulse width** (**W**) to **10ms**. To change a stimulus parameter, click on the **arrow buttons** to the right of the window that displays the value of the parameter to increase or decrease the value, or type the value of the parameter in the window next to the label of the parameter. Click the **Apply button** to finalize the change in any stimulus parameter.

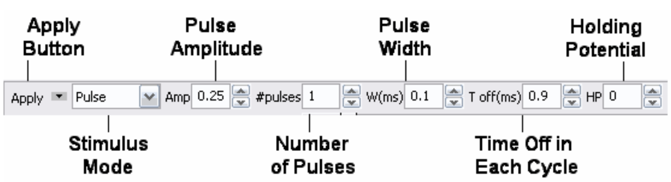

Figure AM-1-7: The stimulator control panel

3. Click **Record** to stimulate the nerve with **4.0V**. Type **4.0V** in the **Mark box** to the right of the **Mark button**. and press the **Enter key** to attach a comment to the recording.

4. Click **Stop** to halt the recording.

5. Change the stimulus amplitude (**Amp**) to **4.5V** using one of the techniques described in Step 2. Click the **Apply button** to finalize the change in the stimulus amplitude.

6. Click **Record** to stimulate the nerve with **4.5V**. Type **4.5V** in the **Mark box** and press the **Enter key** to attach a comment to the recording.

7. Click **Stop** to halt the recording.

8. Repeat Steps 4, 5, and 6 using a stimulus amplitude (**Amp**) of **5.0V**.

9. Select **Save As** in the **File menu**, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder). Designate the file type as ***.iwxdata**. Click on the **Save** button to save the data file.

10. Moisten the muscle with frog Ringer's solution.

Exercise 2: Afterloaded Weight and Contractile Strength

Aim: To measure the strength of contraction while the muscle is lifting afterloaded weights.

Procedure

1. Make sure the thread connecting the Achilles tendon to the eye of the rod is vertical, and the knob on the upper end of the sliding rod of the transducer is resting on the top of the transducer case.

2. Check the values for the stimulus parameters that are listed in the **stimulator control panel** on the **Main window**. All stimulus parameters, except the **pulse amplitude** (**Amp**), should be set to the same values as the ones used in Exercise 1. Set the **pulse amplitude** (**Amp**) to a **voltage that causes a maximal muscle response**. To change a stimulus parameter, click on the **arrow buttons** to the right of the window that displays the value of the parameter to increase or decrease the value, or type the value of the parameter in the window next to the label of the parameter. Click the **Apply button** to finalize the change in any stimulus parameter.

3. Type **10g** in the **Mark box** to the right of the **Mark button**.With the two nickels in the weight pan, click the **Record button** to record a single twitch. Press the **Enter key** on the keyboard to mark the trace. Click **Stop** to halt the recording.

4. Add another 10g, or two more nickels, to bring the weight in the pan to a total of 20g. Type **20g** in the **Mark box**. Click the **Record button** to record a single twitch. Press the **Enter key** on the keyboard to mark the trace. Click **Stop** to halt the recording.

5. Increase the weight in the pan in 10g increments. Record the muscle response for each weight. Mark each recording to indicate the total weight lifted by the muscle. Continue to increase the weight until the muscle response is very small.

6. Select **Save** in the **File** menu.

7. Moisten the muscle with frog Ringer's solution.

Exercise 3: Preloaded Weight and Contractile Strength

Aim: To measure the strength of contraction while the muscle is lifting preloaded weights.

Procedure

1. Lower the displacement transducer. The knob on the lower end of the sliding rod should just be touching the bushing on the bottom of the transducer case. In this configuration, the weight in the pan stretches the muscle before it is stimu-

lated. Make sure the thread connecting the Achilles tendon to the eye of the rod is vertical.

2 Repeat Exercise 2, applying the weights to the pan in 10g increments. Stop adding weight and recording responses when the muscle response is zero, or when the upper knob on the transducer rod contacts the bushing on the top of the transducer case.

3 Select **Save** in the **File** menu.

Data Analysis

Exercise 1: Maximum Contraction

1 Scroll through the data from Exercise 1 and find the first muscle twitch to be generated by a stimulus pulse. Click the **AutoScale button** to maximize the size of the muscle twitch on the window. Note the stimulus voltage used to generate this twitch.

2 Use the **Display Time** icons to adjust the **Display Time** of the **Main window** to show the stimulus pulse used to generate the twitch and the complete twitch on the **Main window**. The stimulus pulse and the twitch can be selected by:

- Placing a cursor before the stimulus pulse, and a cursor after the muscle has completely relaxed; and
- Clicking the **Zoom between Cursors** button on the LabScribe toolbar to expand the display of the stimulus pulse and the twitch to the width of the **Main window**.

3 Click on the **Analysis window** icon in the toolbar (Figure AM-1-6 on page AM-1-4) or select **Analysis** from the **Windows menu** to transfer the data displayed in the **Main window** to the **Analysis window**.

4 Look at the **Function Table** that is above the uppermost channel displayed in the **Analysis** window. The mathematical functions, **V2-V1** and **T2-T1** should appear in this table. Values for **V2-V1** and **T2-T1** on each channel are seen in the table across the top margin of each channel.

5 Maximize the height of the trace on the **Muscle Twitch Channel** by clicking on the arrow to the left of the channel's title to open the **channel menu**. Select **Scale** from the menu and **AutoScale** from the **Scale submenu** to increase the height of the data on that channel.

6 Once the cursors are placed in the correct positions for determining the amplitudes and times for each muscle twitch, the values of the parameters in the **Function Table** can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the **Journal**, or on a separate data table.

7 The functions in the **channel pull-down menus** of the **Analysis window** can also be used to enter the names and values of the parameters from the recording to the **Journal**. To use these functions:

- Place the cursors at the locations used to measure the amplitude and times of each muscle twitch.
- Transfer the names of the mathematical functions used to determine the amplitude and times to the **Journal** using the **Add Title to Journal** function in the **Muscle Twitch Channel pull-down menu**.
- Transfer the values for the amplitude and times to the **Journal** using the **Add Ch. Data to Journal** function in the **Muscle Twitch Channel pull-down menu**.

8 On the **Muscle Twitch Channel**, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:

- **Muscle Twitch Amplitude**, which is the difference between the baseline tension of the muscle and the tension at the peak of the twitch. To measure this parameter, place one cursor at the beginning of the twitch, and the second cursor on the peak of the twitch. The value for the **V2-V1** function on the **Muscle Twitch Channel** is the muscle twitch amplitude.
- **Contraction Time**, which is the time between the beginning and the peak of the twitch. To measure this parameter, keep the cursors in the same positions used to measure the muscle twitch amplitude. The value for the **T2-T1** function on the **Muscle Twitch Channel** is the contraction time of the twitch.
- **Relaxation Time**, which is the time between the peak of the twitch and the return of the muscle tension to the baseline level. To measure this parameter, keep the cursor on the peak of the twitch and place the other cursor at the end of the twitch. The value for the **T2-T1** function on the **Muscle Twitch Channel** is the relaxation time of the twitch.
- **Latency**, which is the time it takes the muscle to start responding to a stimulus. Place one cursor at the beginning of the stimulus pulse, and the other cursor at the beginning of the muscle twitch. The value for the **T2-T1** function on the **Muscle Twitch Channel** is the latency of the muscle response

9 Record the values in the **Journal** using the one of the techniques described in Steps 7 or 8, and on Table AM-1-3 on page AM-1-6.

10 Repeat Steps 2 through 9 to find the muscle twitch amplitude, contraction time, relaxation time, and latency of the other muscle twitches recorded in this exercise. Record the values in the **Journal** and on Table AM-1-3 on page AM-1-6.

11 Select **Save** in the **File menu**.

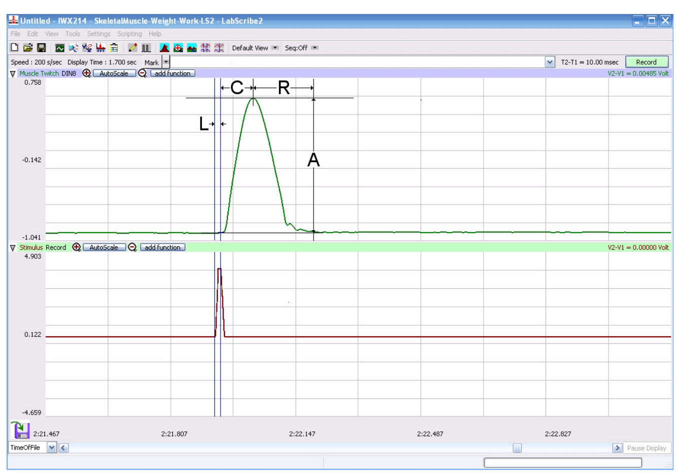

*Figure AM-1-8: A single muscle twitch and stimulus pulse displayed in the **Main** window. The labels indicate: latency (L); contraction time (C); relaxation tine (R); and twitch amplitude (A).*

Table AM-1-3: Amplitudes and Times of Maximal Muscle Contractions.

Stimulus Amplitude (V)	Muscle Twitch			
	Amplitude (mV)	Contract Time (msec)	Relax Time (msec)	Latency (msec)
4.00				
4.50				
5.00				

Exercise 2: Afterloaded Weight and Contractile Strength.

1 Scroll through the data from Exercise 2 and find the muscle twitch generated while the muscle lifted the lightest weight.

2 Use the same techniques to measure the data from this exercise as used to measure the data from Exercise 1.

3 Record the values for the amplitudes and times from the twitches in this exercise in the **Journal** and on Table AM-1-4 on page AM-1-6 using the same techniques used in the analysis of the data from Exercise 1.

Table AM-1-4: Amplitudes and Times of Contractions from an Afterloaded Muscle.

Weight (g)	Muscle Twitch			
	Amplitude (mV)	Contract Time (msec)	Relax Time (msec)	Latency (msec)
10				
20				
30				
40				
50				
60				
70				

4 Calculate the work performed and the rate of contraction for each twitch:

• Work equals weight multiplied by the amplitude of muscle response.

• Rate of contraction equals this amplitude divided by contraction time (C).

5 Repeat the measurements for all twitches.

6 Present your data in tables and graphs that relate the amplitude of the muscle response, the work performed, and the speed of contraction to weight.

Questions

1 Why did the amount of work initially increase with increased weight?

2 Why did the amount of work decrease when heavier weights were used?

3 Did any of the other parameters measured differ with weight? Why?

Exercise 3: Preloaded Weight and Contractile Strength.

1 Scroll through the data from Exercise 3 and find the muscle twitch generated while the muscle was holding the lightest weight.

2 Use the same techniques to measure the data from this exercise as used to measure the data from Exercise 1.

3 Record the values for the amplitudes and times from the twitches in this exercise in the **Journal** and on Table AM-1-5 on page AM-1-6 using the same techniques used in the analysis of the data from Exercise 1.

Table AM-1-5: Amplitudes and Times of Contractions from a Preloaded Muscle.

Weight (g)	Muscle Twitch			
	Amplitude (mV)	Contract Time (msec)	Relax Time (msec)	Latency (msec)
10				
20				
30				
40				
50				
60				
70				

Question

How do the muscle response parameters of a preloaded muscle compare to those of an afterloaded muscle?

Appendix

Table AM-1-6: Amphibian Ringer's Solution

Concentration (mMolar)	Salt	Grams/Liter DI H_20
111.0	Sodium Chloride	6.49
1.9	Potassium Chloride	0.142
1.06	Calcium Chloride*$2H_2O$	0.156
1.0	Tris	0.121
5.55	Glucose	1.00
Adjust pH to 7.6 with 6N HCl		