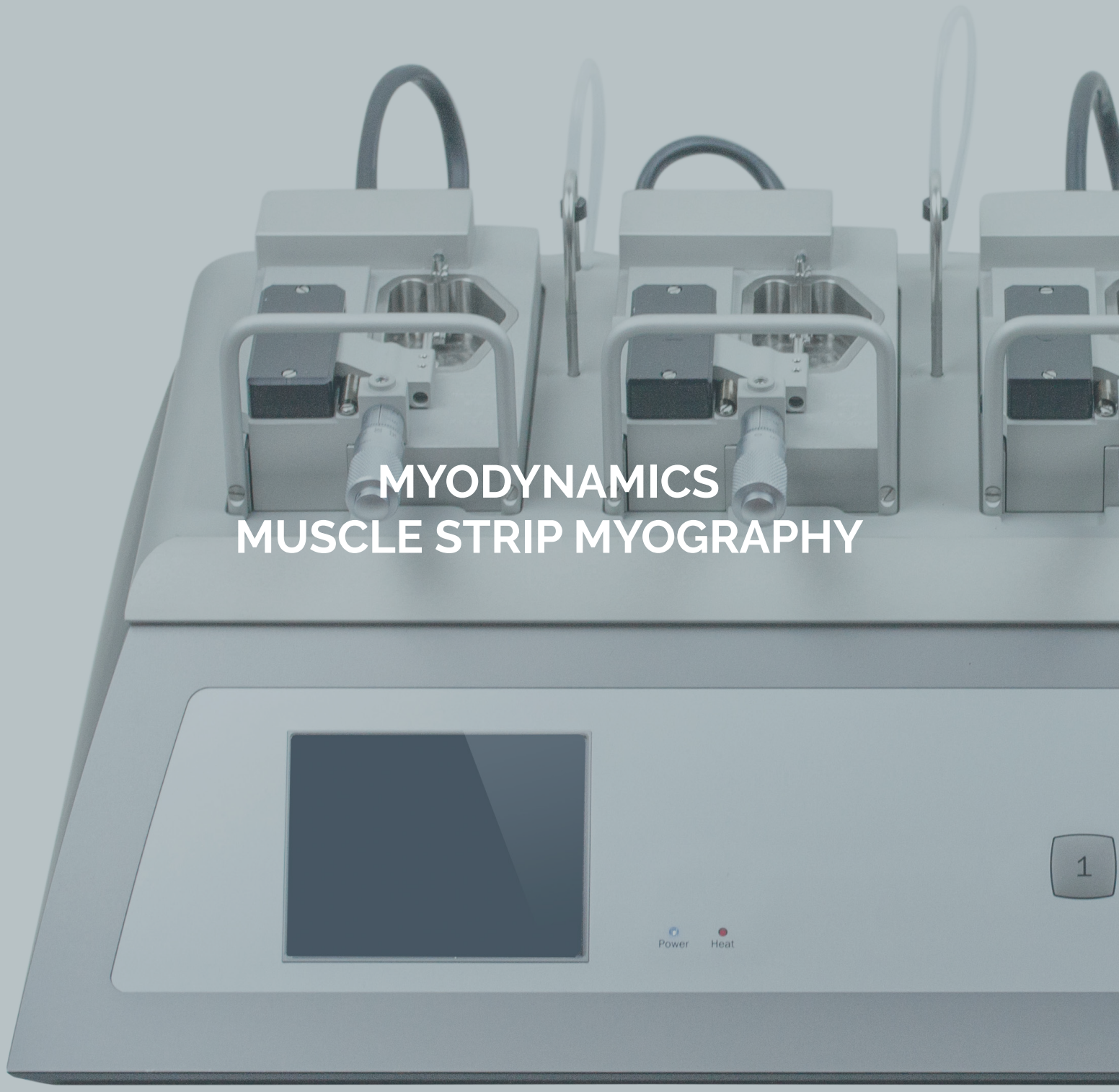
USER GUIDE - VOL.1.0

MYOGRAPH SYSTEM - 840MD

DMT



MYODYNAMICS
MUSCLE STRIP MYOGRAPHY

CONTENTS

CHAPTER 1 - MYODYNAMICS MUSLE STRIP MYOGRAPH OVERVIEW

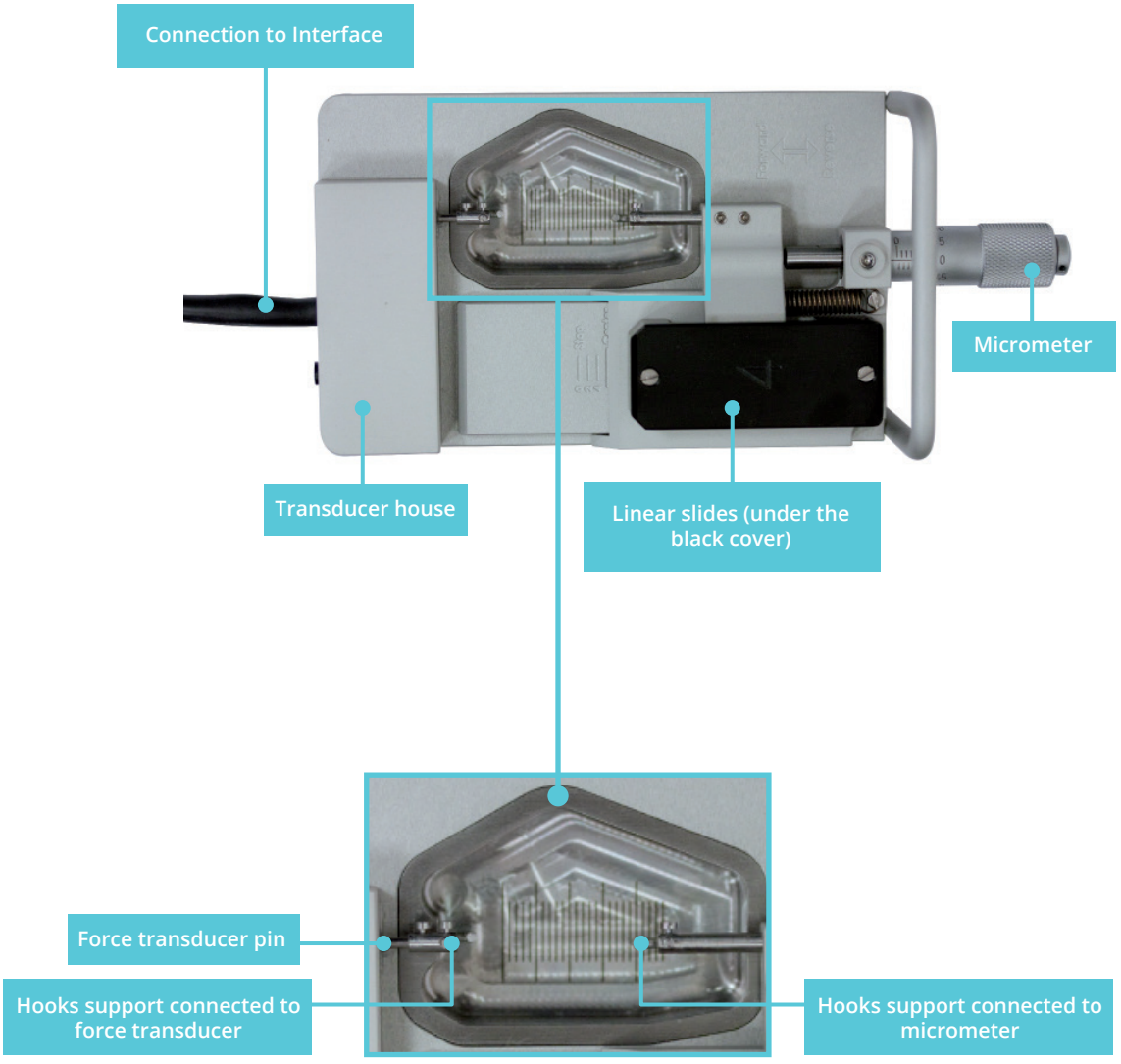


Figure 1.1 MyoDynamics Muscle Strip Myograph with close-up of

CHAPTER 2 - SETTING UP MYODYNAMICS MUSCLE STRIP MYOGRAPH

Each chamber contains supports with mounting hooks to facilitate the mounting of muscle strips with sutures. DMT is also able to deliver a large variety of different mounting supports. Please contact DMT Sales to acquire more information about available mounting supports for the MyoDynamics Muscle Strip Myograph.

IMPORTANT: Be very careful when tightening the suture on the transducer. Excessive pressure on the screw or excessive torque force will damage the force transducer.

2.1 CHANGING THE MOUNTING HOOKS (FIGURE 2.1)

1. Use the small screwdriver provided to gently loosen screw “B”.
2. Gently pull the support away from the transducer pin.
3. Loosen screw “A” on the micrometer side with the appropriate fitting Allen key.
4. Pull the support away.

NOTE: Number the supports with the chamber number they where removed from using a permanent marker. Store the supports in the provided plastic case. Numbering the supports will save time when the supports are replaced. It will reduce the amount of adjustments required after each change.

2.3 ADJUSTMENT OF THE GLASS CANNULAS

1. Loosen screw “A” to move the micrometer-side pin toward or away from the micrometer.

2. Loosen screw “B” to move transducer-side pin toward or away from the transducer.

NOTE: Between the back side of the transducer pin and the chamber wall there must be a small gap of 0,1 to 0.3 mm.

3. Loosen screw “C” to vertically alight the transducer pin. Screw “C” is the screw on the transducer side support that is furthest away from the transducer.

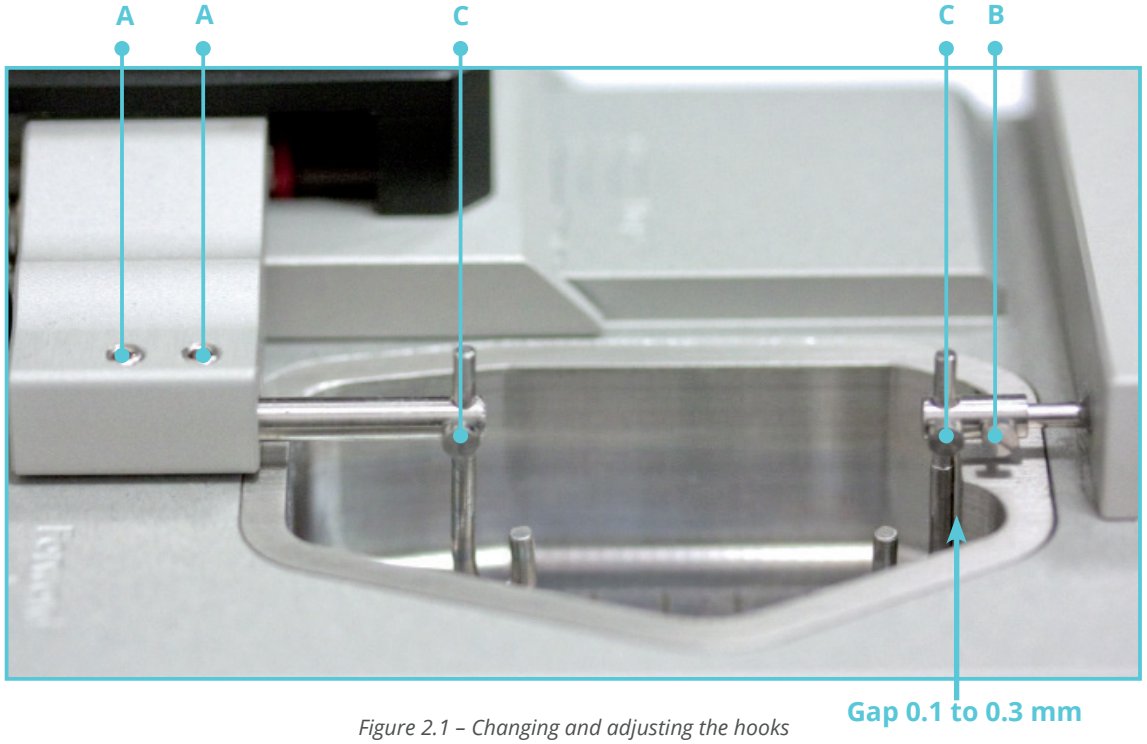


Figure 2.1 – Changing and adjusting the hooks

2.3 THE FIRST FORCE CALIBRATION

Prior to shipping the MyoDynamics Muscle Strip Myograph is subjected to two days of continuous testing, including a final force calibration. However DMT recommends that a new force calibration is performed before using the MyoDynamics Muscle Strip Myograph for the first time. The force calibration procedure is described in detail in chapter 3.6.1 Force calibration in MyoDynamics Muscle Strip Myograph System - User Manual.

CHAPTER 3 - EXPERIMENTAL SET-UP

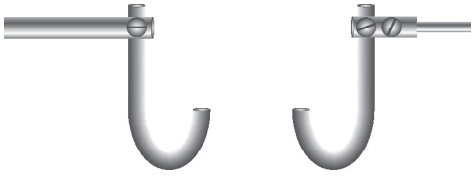
This Chapter contains experimental set-up examples and recommendations to do proper isometric force contraction studies of isolated striated muscle using the DMT 840MD Myograph.

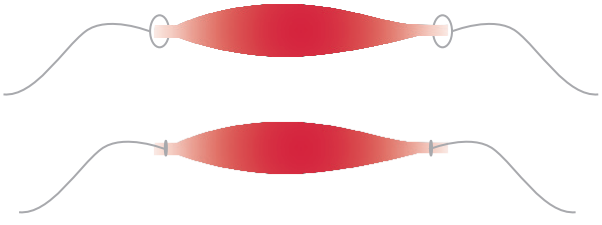
3.1 MOUNTING PROTOCOL

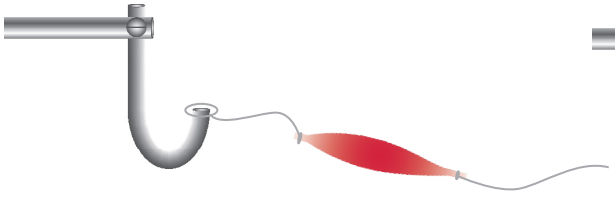
Each chamber contains supports with mounting hooks to facilitate the mounting of muscle strips in the myograph chambers.

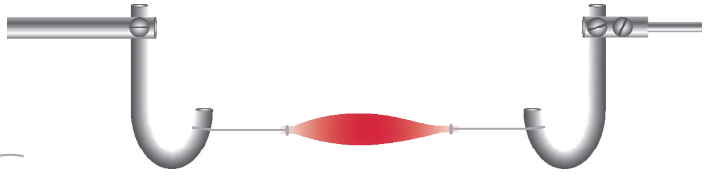
1. Make a loop with nylon suture to use for securing the muscle to the hook. Double loops are best and will prevent the loops from slipping loose once tightened.

2. Position the sutures appropriately on both hooks so that they are ready for use when the muscle is mounted.









In Appendix 2 we have examples with tips, tricks and how to do a proper ‘normalization’ of the mounted muscle before doing the real experiments. This include:

1. Establish Supramaximal Stimulation Conditions
2. Establish Optimum Length:
3. The Optimal Voltage for maximum isometric tension:
4. Establish frequency-force relationship:
5. Maximum Isometric Tetanic Force:

The above normally has to be performed before continuing with the experiments.

CHAPTER 4 - CLEANING AND MAINTENANCE

4.1 CLEANING THE MYODYNAMICS MUSCLE STRIP MYOGRAPH

NOTE: DMT strongly recommends that the MyoDynamics Muscle Strip Myograph chambers and surrounding areas are cleaned after each experiment.

At the end of each experiment, use the following procedure to clean the chambers and supports.

1. Fill the chamber to the edge with an 8% acetic acid solution and allow it to work for a few minutes to dissolve calcium deposits and other salt build-up. Use a cotton-tipped applicator to mechanically clean all chamber surfaces.

2. Remove the acetic acid and wash the chamber and supports several times with double-distilled water.

3. If any kind of hydrophobic reagents have been used which might be difficult to remove using steps 1) and 2), try incubating the chamber and supports with 96% ethanol or a weak detergent solution (i.e. 0.1% triton-100).

4. To remove more resistant or toxic chemicals, incubate the chamber and supports with 1M HCl for up to 1 hour. In exceptional cases, incubate the chamber and supports with no stronger than a 3M HNO3 solution for about 15 minutes.

5. Wash the chamber and supports several times with double-distilled water.

6. If If acids such as 1M HCl and 3M HNO_3 are used to clean the chambers, make sure ALL surfaces are thoroughly dried after repeated washes with double-distilled water. Any residual acid will cause corrosion of the hooks.

To prevent the tubing from becoming blocked with buffer salt deposits after an experiment, remove the chamber cover and turn on the vacuum and press the vacuum valve button for about 10 seconds. Turn off the vacuum and gas supply. Remove any water or buffer remaining in the chamber or on the tubing using absorbent paper.

IMPORTANT: Be very careful using HCL or HNO_3 because these acids may cause extreme damage to the stainless steel chambers and supports. DO NOT use bleach to clean the chambers. Repeated use of chlorinated solutions such as bleach and HCL will cause damage to the stainless steel parts of your Myodynamics Muscle Strip Myograph. Avoid using them if at all possible.
After cleaning, always check that the grease around the transducer pin is sufficient to keep the buffer and water from entering the transducer housing (see figure 4.1)

If red or brown discolorations appear on the chamber sides or on the supports, the following cleaning procedure will work in most cases:

7. Incubate the chamber and supports for 30 minutes with 2mM T-1210 Tetrakis- (2-pyridylmethyl)-ethylenediamine solution dissolved in double-distilled water.

8. Use a cotton-tip applicator to mechanically clean all the affected surfaces during the last 15 minutes of the incubation period.

9. Wash the chamber and supports several times with double-distilled water.

10. Incubate the chamber with 96% ethanol for 10 minutes while continuing the mechanical cleaning with a cotton- tip applicator.

11. Remove the ethanol solution and wash a few times with double-distilled water. Incubate the chamber and supports with an 8% acetic acid solution for 10 minutes and continue the mechanical cleaning with a swab-stick.

12. Wash the chamber and supports several times with double-distilled water.

13. Dry the surfaces using absorbent paper (i.e. Kim-Wipes) or cotton-tip applicators.

NOTE: In exceptional cases, the hooks supports may need to be removed from the chamber and cleaned individually to assure proper cleaning of all support surfaces. NEVER SOAK THE SUPPORTS IN ANYTHING STRONGER THAN 8% ACETIC ACID FOR EXTENDED PERIODS OF TIME (i.e. several hours or overnight)!

4.2 MAINTENANCE OF THE FORCE TRANSDUCER

The force transducer is the most delicate and fragile component of the MyoDynamics Muscle Strip Myograph. Extreme care must be used when handling or touching the force transducers. As a part of daily maintenance, inspect the grease around the transducer pin extending from the transducer housing pinhole (see figure 4.1) before starting any experiment. Insufficient grease in this area will allow buffer and water to enter the transducer housing and cause damage to the force transducer.

IMPORTANT: DMT recommends that the high vacuum grease sealing the transducer pinhole is checked and sealed at least once a week, especially if the myograph is used frequently.
DMT takes no responsibilities for the use of any other kinds of high vacuum grease other than the one supplied by DMT.
DMT takes no responsibilities for any kind of damage applied to the force transducers.

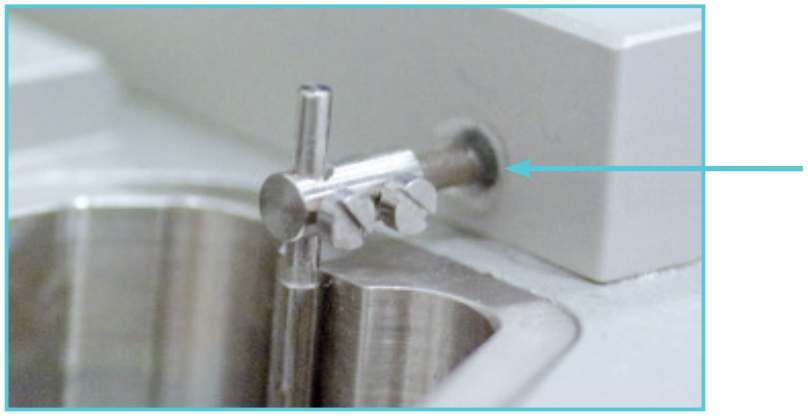


Figure 4.1 - Close-up of transducer pin extending from the transducer housing pinhole. The arrow indicates the place that the grease needs to be applied to prevent water and buffer from damaging the transducer

4.2.1 CHECKING THE FORCE TRANSDUCER

The force transducer is a strain gauge connected to a Wheatstone bridge. The force transducers for each chamber are housed in a separate, protective compartment (transducer house). While the protective cover offers some mechanical protection for the force transducers, they are still very vulnerable to applied forces exceeding 2 Newton (200 grams) or fluid running into the transducer compartment due to insufficient greasing of the transducer pinhole. If the force readings on the Interface appear unstable or noisy, then first check that the MyoDynamics Muscle Strip Myographs are connected properly to the Interface and that the MyoDynamics Muscle Strip Myographs are plugged all the way into the Interface. If the force reading(s) are still unstable or noisy, then perform a new calibration of the force transducer. During the new calibration, monitor the relative force reading values on the Interface as described in chapter 3.6.1 Force calibration (step 4 of the calibration procedure) in MyoDynamics Muscle Strip Myograph System - User Manual. The normal operating values for the force transducer during calibration should be between 3000 and 3500.

- If the value is 0, a single digit, or a three digit number, the force transducer is broken and needs to be replaced.

- If the value is less than 2000 or greater than 4500, the force transducer is broken and needs to be replaced.

- If the message “OFF” is displayed on the main page of the Interface, even though the MyoDynamics Muscle Strip Myograph is plugged in at the rear of the Interface, the force transducer is broken and needs to be replaced. In addition, if the force reading(s) appear yellow in color, cannot be reset to zero, AND the transducer cannot be recalibrated, the force transducer is broken and needs to be replaced.

If any other problems related to the force transducer are encountered, please contact DMT for advice or further instructions.

4.2.2 FORCE TRANSDUCER REPLACEMENT

If the force transducer breaks and needs to be replaced, follow this step-by-step replacement procedure carefully:

1. Remove the pin from the transducer pin coming out of the transducer housing.

2. Disconnect the MyoDynamics Muscle Strip Myograph from the Interface.

3. Turn the MyoDynamics Muscle Strip Myograph upside down and remove the bottom plate by loosening and removing the screws as illustrated in figure 4.2.

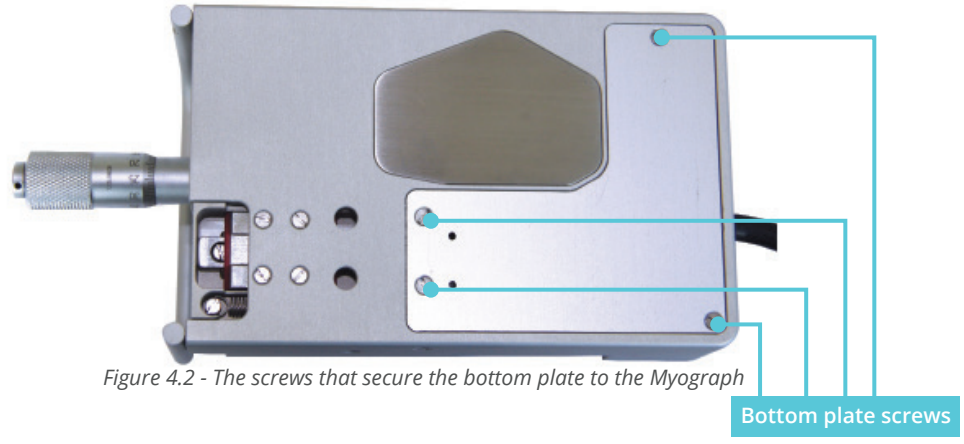


Figure 4.2 - The screws that secure the bottom plate to the Myograph

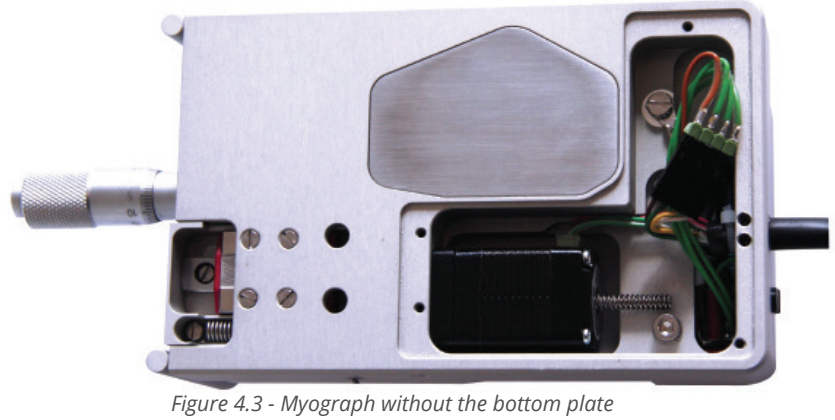


Figure 4.3 - Myograph without the bottom plate

4. Disconnect the connector to the transducer.

5. Remove the two transducer screws that hold the transducer housing, see figure 4.4.

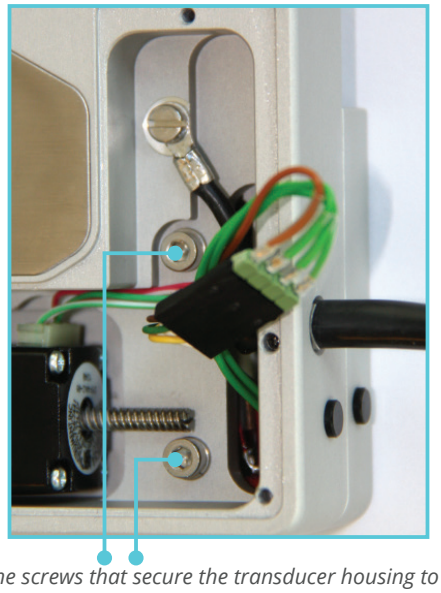


Figure 4.4 - The screws that secure the transducer housing to the chamber

6. The replacement transducer will be shipped with the new transducer inside a new transducer housing.

7. Place a small amount of vacuum grease (clear or whitish grease) around the bottom of the transducer housing to seal when put back in place.

8. Carefully realign the transducer housing with the new transducer on the MyoDynamics Muscle Strip Myograph and reinsert the Allen screws through the bottom of the MyoDynamics Muscle Strip Myograph.

9. Tighten the screws and place some vacuum grease around the transducer pin that protrudes from the transducer housing. Make sure that the hole is completely sealed to prevent buffer solution or water from entering the transducer housing and damaging the new force transducer (see figure 4.1).

10. Reconnect the new transducer connector.

11. Place some vacuum grease at the edge of the bottom plate. Place and tighten the bottom plates.

IMPORTANT: Calibrate the new force transducer before performing a new experiment.

4.3 MAINTENANCE OF THE LINEAR SLIDES

Check the linear slides (under the black covers) for grease at least once a week. In case of insufficient lubrication, grease the slides with the “Grease for Linear Slides” included with your system.

APPENDIX 1 - READING A MILLIMETRE MICROMETER

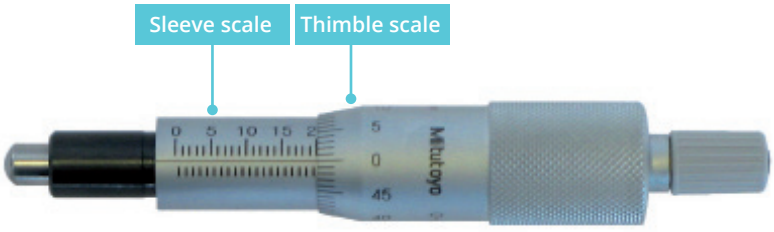


Figure A2.1 Overview of the micrometer parts (actual reading 20000 µm = 20 mm)

SLEEVE SCALE

The micrometer sleeve scale has a total length of 25 mm divided into 50 equal parts. Each part of a division above the horizontal line represents 1 mm, where each 5th line is marked by a longer line and a number designating the length in mm. Each division below the horizontal line is placed between each 1 mm mark (scale above the horizontal line) and represents 0.5 mm.

THIMBLE SCALE

The thimble is divided into 50 equal parts, and one complete rotation of the thimble is indicated by the smallest division on the sleeve, which equals 0.5 mm. Each division on the thimble scale is 10 µm. If the thimble scale falls between two lines, then a number between 0 and 10 µm must be approximated.

EXAMPLE 1

1. Note that the thimble has stopped at a point beyond "10" on the sleeve indicating 10000 µm (10 mm).

2. Note that there is no mark completely visible between the 10 mm mark and the thimble.

3. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

A.	Reading on sleeve:	10000 µm
B.	No additional mark visible:	0 µm
C.	Thimble reading:	380 µm
	Total reading:	10380 µm

Figure A2.2 Example 1: reading = 10380 µm

Example 2:

Note that the thimble has stopped at a point beyond “16” on the sleeve indicating 16000 µm (16 mm).

Note that this time a mark is visible between the 16 mm mark and the thimble indication 500 µm.

Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

A.	Reading on sleeve:	16000 µm
B.	One additional mark visible:	500 µm
C.	Thimble reading:	280 µm
	Total reading:	16780 µm

Figure A2.3 Example 2: reading = 16780 µm

APPENDIX 2 - HOW TO MEASURE ISOMETRIC FORCE OF STRIATED MUSCLES IN VITRO IN A 820MS OR 840MD MYOGRAPH

1. INTRODUCTION

This document is an example on how to perform isolated muscle function measurements using DMT Muscle Strip Myograph Systems; the 820MS Muscle Strip Myograph and 840MD MyoDYNAMICS Myograph system.

A description on the methodology performing function measurements for mouse extensor digitorum longus (EDL), soleus, and diaphragm is provided in this document as examples. Furthermore the document provides reference values for isometric contractile properties of muscles from mdx and C57 mice.

Many factors influence the accurate measurement of maximum muscle force-producing capacity (and power output) in vitro including surgical dissection, the use of accurate force recording equipment, and adequate muscle perfusion to prevent any part of the muscle becoming anoxic. Field stimulation with an electrode to contract muscles and muscle length can be maintained (isometric contractions). This in vitro analysis also facilitates the assessment of physiological parameters of the diaphragm muscle, the most severely affected muscle in the mdx mouse, as well as limb muscles (the EDL and soleus) which possess an ideal geometry for isolated muscle functional testing.

This document is an example on how to perform isolated muscle function and it will provide a general description of dissection methods that have been utilized successfully in several labs for functional measurements, and a discussion of the potential pitfalls that can prevent optimal measurements.

These descriptions are limited to the evaluation of isometric forces of isolated mouse muscles in vitro.

NOTE: Evaluation of muscle function in vitro requires careful surgical tendon-tendon excision of muscles from anesthetizes animals, and these techniques take time to perfect. The slightest damage to muscle fiber integrity during surgery compromises the muscle's force-producing capacity. This includes excessive pulling on the muscle, touching it directly, or allowing it to dry out.

2. MATERIALS

DMT Systems required for force measurement in striated muscles:

Muscle Strip Myograph System - 820MS

The Muscle Strip Myograph System - 820MS represents a state-of-the-art 4-channel myograph system for muscle strips of up to 19 mm in length. The system was originally developed to give the skeletal muscle physiologist a highly sophisticated, easy-to-use, robust, high-throughput muscle myograph. The rectangular design of the chamber, however, gives this system the flexibility to mount.

In the 820MS chamber the muscles can be clamped directly to the mounting clips or by replacing the mounting clips with mounting hooks the muscle can be tied via suture to the immovable hook and the force transducer. Several types of mounting supports exist, depending on the user's preferences.

Customized mounting supports can be made upon request. In addition the mygraph can also be used for the study of larger smooth muscle strips, because of the somewhat rectangular chamber.

MyoDynamics Muscle Strip Myograph System - 840MD

The MyoDynamics Muscle Strip Myograph System - 840MD represents a state-of-the-art 4-channel myograph system for muscle strips of up to 30 mm in length. The system was originally developed to give the skeletal muscle physiologist a precise, easy-to-use, high-throughput muscle myograph with the capacity to stretch and retract the muscle under a range of conditions including electric field stimulation. The 840MD system is optimal for fatigue, eccentric and concentric contraction studies using the build-in motors. The special design of the chamber, however, gives this system the flexibility to mount larger muscle strips of various organs and striated muscle up to 30mm.

In the 840MD chamber the muscles can be tied via suture to the immovable hook and the force transducer. Several types of mounting supports exist, depending on the user's preferences.

DMT Stimulator – CS4/ CS8

The CS4 and CS8 are 4-channel and 8-channel stimulators, respectively. The CS4 and CS8 stimulators combine a user-friendly interface with advanced electrical stimulation features required in electrophysiological experiments. Both stimulators are controlled by the MyoPULSE PC software where the stimulation protocol are made and transferred to the stimulators. The CS4 and CS8 have built-in trigger function making them able to trigger e.g. the motors on the 840MD system to start (Eccentric and concentric contractions) and they also initiate stimulation protocols be receiving external triggers e.g. from a data acquisition system as a Powerlab.

The CS4 and CS8 are modular, highly versatile voltage stimulators suitable for use with all DMT Myograph Systems.

Electrodes

A pair of platinum plate electrodes is needed to flank the isolated muscle on either side. The electrodes run the length of the preparation and are positioned a sufficient distance (~0.8 cm) apart to ensure that the muscles are field stimulated and not by direct stimulation.

Stimulation electrodes are available built into the chamber cover and can be used to activate the muscle via field stimulation with a stimulator.

Data

DMT recommends the Powerlab/LabChart platform as the data acquisition system of choice for DMT Muscle Strip Myograph Systems.

BUFFER RECIPE

Table 1 lists the components normally used to bathe isolated muscles for functional testing. The primary goal is to maintain physiological conditions to support muscle stability for the duration of the testing regimen, including ionic and osmotic strength, metabolites, pH, and there is variability in all of these conditions across a number of labs. Two differences deserve further explanation.

Table 1. Buffer conditions for isolated muscle functional testing

Component	Consensus values (mM)
Ions	
NaCl	118-140
KCl	4.7-5.9
$CaCl_2$	1.5 - 2.5
KH_2PO_4	1 - 1.2
$MgSO_4$	0.5 - 1.2
$MgCl_2$	0 - 1.2
Buffers	
HEPES	0 - 25
$NaHCO_3$	0 - 25
gas equilibration	0-5%CO2,95-100% O2
Energy	
Glucose	0 - 11
Pyruvate	0 - 1
Inhibitors	
D-Tubocurarine	0 - 0.3
Solution Conditions	
Temperature	20 - 30 ^{O}C
pH	7.3 - 7.6
Osmotic Strength	270 - 290 mOsm/L

First, some investigators add D-tubocurarine chloride to the buffer in order to prevent muscle stimulation via the neuromuscular junction. Its use is dependent upon the intent of the specific study. However, a recent publication demonstrated that D-tubocurarine chloride did not affect force output at high voltage stimulation (Cairns, et al, 2007). Thus, for evaluation of maximal force production in mdx muscle, such blocking agents are not necessary.

It is important to keep the temperature of the bath solution constant throughout the experiment to avoid instability of the muscle preparation. Large muscles (~20mg), in particular, are susceptible to diminished force production and shortened stability when muscle superfusion is no longer sufficient to support increased metabolic demand caused by high temperatures.

2. METHODS

Muscle Dissection

For all procedures, mice are euthanized or anesthetized deeply such that there is no response to tactile stimulation. A commonly used anesthesia is a mixture of 10mg/mL ketamine + 1mg/mL xylazine, injected intraperitoneally at 10 mg/Kg body weight. In general, the muscle of choice (e.g. EDL, soleus, etc.) is carefully dissected with tendons intact on both ends. The excised muscle is placed immediately into oxygenated Ringer buffer (maintained at 24-25°C) and equilibrated for 5-10 min. Upon placing the muscle in a dish, it is gently stretched (not over stretched) by tying a Vicryl suture (USP 6/0 FS-3 45 cm V387H, DMT product #100373) to the proximal and distal tendons during dissection and used as anchors to maintain resting length in the dish. (DMT Petri dish, Product No. 100103: 90 mm Glass Petri dish coated with 5 mm black Sylgard)

EDL muscle:

The EDL proximal tendon can be seen lateral to the knee under the distal end of the rectus femoris, and cut at this point to maximize the length of the tendon stump. The distal tendons are under the tibialis anterior (TA) tendon – ensure that all tendons leading to the toes are cut before removing the muscle.

Soleus muscle:

The most straightforward dissection of the soleus requires cutting the Achilles tendon (which includes the distal soleus tendon) and carefully peeling all posterior muscles away from the rest of the limb. The proximal tendon is evident on the interior surface of the muscles close to the knee. Cut as close as possible to the back of the knee to maximize the length of the tendon stump.

Diaphragm:

For examination of the contractile function of the diaphragm, muscle strips composed of longitudinally arranged full length muscle fibers (2-4 mm wide) can be cut from the central region of the lateral costal hemidiaphragm and tied firmly with braided surgical silk at the central tendon at one end, and sutured through a portion of rib attached to the distal end of the strip at the other end (Figure 1A).

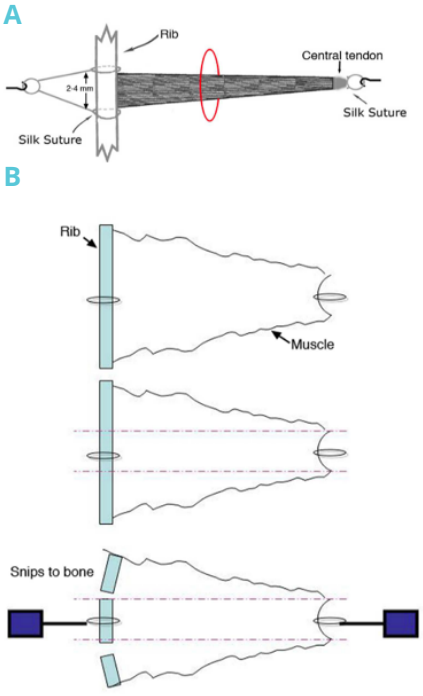


Figure 1. Diagram of diaphragm muscle dis-section. A. Final muscle strip should be no more than 4 mm wide, and tied firmly at the central tendon and the rib. Red Circle indi-cates the region of muscle cross section taken for measuring damaged fibers in Fig 3. B. Alternate protocol for isolating functional strip of diaphragm without damaging edge of preparation

Since damage always occurs on the fibers located at the sides of the strip, an alternative approach is to prepare a slightly triangular strip, and then giving two tiny incisions perpendicular to the rib (avoiding the muscle), in order to be sure that the force is only generated from sane muscle fibers restricted in the so determined quadrilateral portion (Figure 1B).

The nerve and blood supply to each muscle are severed just prior to excision to ensure optimum condition of muscles. Tendon sutures should be as close to the myotendinous junction as possible, but not in contact with the muscle fibers. Optimum placement of the sutures ensures less contribution of the tendon to compliance, and minimizes failure of this attachment during stimulation. It is essential that all attachments are secured very tightly since the muscles produce considerable forces. If the attachments to the muscle or to the equipment are not secured, the contracting muscle can tear away from these points of weakness.

4. FORCE MEASUREMENTS

Establish Supramaximal Stimulation Conditions:

Measurements of maximum isometric tension require that all muscle fibers in a muscle are stimulated. For example, bath size or the type of stimulator can affect the intensity of stimulation. Twitch stimulation is a reasonable way to determine supramaximal stimulation conditions.

Establish Optimum Length:

Muscles are adjusted to the optimum length (Lo) for the development of isometric twitch force. To this end, the muscle is stimulated with a single electrical pulse to produce a twitch response. Stimulation voltage is that which produces a maximal twitch response. A thumb of rule is applying 20volt/cm (measure the distance in cm between the two electrodes on the coverlid and multiply it with 20volts). An example if the distance between the two electrodes is 0.8cm the muscle should have 0.8x20 volts = 16 volts. Muscle length is adjusted very carefully and in small increments using the micrometer positioner on the 820MS or 840MD chamber (or decrements) to longer (or shorter) lengths. Rest the muscle for at least 30 s between twitch responses. Optimal muscle length (Lo) is achieved when twitch force is maximal. Record muscle length (the length between the myotendinous junctions) using Vernier calipers and monitor Lo prior to and after the muscle is stimulated to ensure Lo is maintained.

The Optimal Voltage for maximum isometric tension:

After the Optimal Length (Lo) have been determined as described in the above section the optimal voltage can be determined by doing twitch stimulation of the mounted muscle at the Optimal Length (Lo) for the given muscle. Start twitch stimulation on the mounted muscle at e.g. 10 volts and then increase the voltage by 1-3 volts per twitch and remember to have at least a 30 second pause between each twitch. The Optimal Voltage for maximum isometric tension is that which produce maximal twitch force.

NOTE: Do not increase the voltage after reaching a plateau. To high voltage will destroy the muscle permanently.

A B

Figure 2. DMT Cover lids with or without electrodes. A) 820MS NFS Cover lid (DMT #100066) B) 840MD NFS Coverlid (DMT #100238)

The 820MS and 840MD NFS Cover lids have electrodes that can slide along the mounted muscle. Move the electrodes along the muscle and do a twitch stimulation at the different positions of the NFS electrodes to find the optimal position of the electrodes resulting the maximal force contraction.

Establish frequency-force relationship:

Once Lo (Optimal length) has been achieved, the frequency- force relationship can be established. The muscle is stimulated at increasing frequencies, typically 10, 30, 50, 80, 100, 120, 150, 180, 200, and 250 Hz. Stimulation is delivered for a period of 500-900 msec. The muscle is rested for 3-5 min between successive stimuli. Maximum absolute isometric tetanic force (Po) is determined from the plateau of the frequency-force relationship. The plateau for EDL muscles is typically achieved with 150 Hz, and for soleus muscles with 100 Hz. This forms the basis for determining maximum isometric force. For any given study, once the frequency-force relationship is established, it is not required to perform the range of frequencies on all muscles. However, this relationship can be extremely useful to evaluating therapies that alter muscle fiber properties or calcium handling (refs here), which are reflected in shifts in the frequency-force relationship.

Maximum Isometric Tetanic Force:

One is now ready to determine optimum force generation of a given muscle. Muscles should be stimulated at supramaximal voltage at Lo at a plateau stimulation frequency. Muscles are typically stimulated 2-3 times with rest periods of 3-5 minutes between stimulation bouts. Muscles can then be removed from the apparatus for further processing. Muscle mass is needed to determine the cross-sectional area and

specific force calculations.

Absolute maximum force of isolated mouse muscles in vitro will vary depending on which muscle is being investigated and on the size of the muscle. Typical values for normal and dystrophic mouse muscles are shown in the Table 2 below.

4. EVALUATION AND INTERPRETATION OF RESULTS

Typical values for maximum tetanic force for mouse are included in Table 2. Values are for 10-12 week old C57BL/10 and mdx mice (see Gregorevic et al. Muscle Nerve 30: 295–304, 2004). Experiments performed in vitro at 25 C. N.B. There will be variation in the size/mass of muscles depending on the age of the mice and so absolute forces will vary accordingly. Differences in forces are expected depending in the equipment and protocols employed but it is important that experiments on muscles from control and mdx mice are done together to ensure accurate comparisons.

Table 2. Typical values for isometric contractile properties of EDL and soleus muscles from wild-type and mdx mice

	EDL		Soleus	
	Control (n = 6)	mdx (n = 7)	Control (n = 6)	mdx (n = 7)
Muscle mass (mg)	11.4 ± 0.3	18.0 ± 1.0a	7.9 ± 0.2	12.8 ± 0.5a
Optimum muscle length (mm)	11.7 ± 0.3	12.3 ± 0.3	10.9 ± 0.2	11.6 ± 0.3
Time to peak twitch (ms)	16.0 ± 0.3	15.9 ± 0.3	32.0 ± 1.5	34.1 ± 0.9
Half relaxation time (ms)	18.3 ± 0.4	18.3 ± 0.6	41.0 ± 3.0	44.0 ± 1.0
Max. isometric force (mN)	507.1 ± 14.0	520.9 ± 38.8	211.1 ± 6.4	264.9 ± 15.9a
Specific force (kN/m2)	243.4 ± 4.5	165.0 ± 7.7a	219.5 ± 9.6	180.1 ± 7.9a

It is very important to check the accuracy and validity of values for absolute and specific force since this impacts the evaluation of the efficacy of any treatment for ameliorating functional deficits in muscular dystrophy. If the accuracy of functional data is questionable, so too is the validity of the evaluation of compound efficacy.

SPECIFIC FORCE CALCULATIONS

Total muscle cross-sectional area is either calculated based on morphometric measurements made after the experiments or mathematically approximated by dividing the muscle mass by the product of optimum fiber length (Lf) and 1.06 mg/mm3, the density of mammalian muscle. Lf is determined by multiplying Lo by previously determined muscle length to fiber length ratios, 0.44 for the EDL and 0.71 for the soleus (Brooks & Faulkner Contractile properties of skeletal muscles from young, adult and aged mice. J. Physiol. 404:71-82, 1988). If morphometric measurements are used to measure CSA, it is useful to ensure that sections are cut perpendicular to the long axis and that sections are made at the mid-belly of the muscle. If mathematical approximations are used to measure CSA, it is useful to ensure, that neither the density or pennation angle changed due to the therapeutic intervention, as the method relies on these factors being constant.

Since absolute Po is dependent upon muscle size, Po values are normalized for cross- sectional area (Po is divided by the calculated total muscle cross-sectional area) and expressed as specific force (sPo; kN/m2), where sPo = Po x (muscle mass/Lf x 1.06).

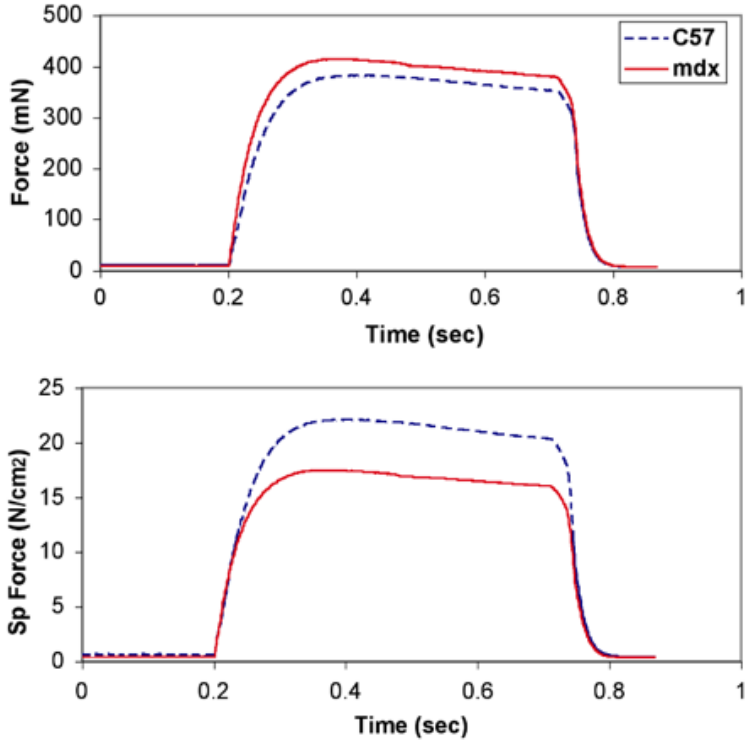


Figure 3. Comparison of mdx and C57 EDL muscles with absolute tetanic force (upper panel) and specific force (lower panel) demonstrates the need for normalizing force generating capacity by muscle cross-sectional area.

This can be very important in the assessment of mdx muscle function, particularly because mdx muscles tend to be larger than those in age-matched control mice (Table 2). An example of this is shown in Figure 3. In the top panel, absolute muscle force is shown for a representative EDL muscles from an mdx and C57 mouse. Note that tetanic force production in the mdx EDL is higher than that in the C57 muscle. However, when absolute force is normalized for muscle cross- sectional area, specific force ins lower in mdx than C57 EDL muscle (lower panel), highlighting the intrinsic weakness of dystrophic muscle.
For diaphragm muscle strips, the width and thickness and consequently mass, vary unpredictably among animals at the discretion of the individual excising them. Therefore, absolute forces developed by diaphragm muscle strips have no physiological meaning, and comparisons can only be made after the forces have been normalized for total fiber cross- sectional area (kN/m2)

Advantages:
The assessment of isolated muscle function in vitro offers the advantage of investigating contractile properties of dystrophic and normal muscles in the absence of any influences from the nerve or blood supply.

Disadvantages:
The size of the muscles to be analyzed by in vitro methods is limited. Muscles larger than 25 mg cannot be adequately supported by superfusion for long periods of time, and tend to develop an anoxic core diminishing functional output. In these cases, alternate methods of functional assessment should be utilized.
The absence of the nerve and blood supply, despite being advantageous for some investigations, also raises questions as to whether functional deficits could be attributed to compromised innervation, failures at the neuromuscular junction, or issues related to altered metabolism or circulation. This is especially relevant when interpreting the effects of muscle fatigue.

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