HHS Public Access

Author manuscript

Methods Mol Biol. Author manuscript; available in PMC 2018 March 01.

Published in final edited form as:

Methods Mol Biol. 2016 ; 1460: 3–18. doi:10.1007/978-1-4939-3810-0_1.

Eccentric Contraction-Induced Muscle Injury: Reproducible, Quantitative, Physiological Models to Impair Skeletal Muscle's Capacity to Generate Force

Jarrod A. Call and **Dawn A. Lowe**

Abstract

In order to investigate the molecular and cellular mechanisms of muscle regeneration an experimental injury model is required. Advantages of eccentric contraction-induced injury are that it is a controllable, reproducible, and physiologically relevant model to cause muscle injury, with injury being defined as a loss of force generating capacity. While eccentric contractions can be incorporated into conscious animal study designs such as downhill treadmill running, electrophysiological approaches to elicit eccentric contractions and examine muscle contractility, for example before and after the injurious eccentric contractions, allows researchers to circumvent common issues in determining muscle function in a conscious animal (e.g., unwillingness to participate). Herein, we describe in vitro and in vivo methods that are reliable, repeatable, and truly maximal because the muscle contractions are evoked in a controlled, quantifiable manner independent of subject motivation. Both methods can be used to initiate eccentric contraction-induced injury and are suitable for monitoring functional muscle regeneration hours to days to weeks post-injury.

Keywords

Force drop; Lengthening contraction; Muscle damage

1 Introduction

Eccentric contractions are contractions in which the external load or resistance placed on an activated muscle is greater than the force generated by that muscle, and subsequently the muscle is lengthened while it is active. There is an immediate loss of strength following the performance of eccentric contractions that is attributed to disruption of the excitation–contraction coupling process and/or frank damage to force-generating or -transmitting structures within the muscle [1–3]. Forces generated during maximal eccentric contractions exceed those produced during isometric and concentric contractions by as much as 80 %, and injury resulting from eccentric contractions has been largely attributed to these high forces [4, 5]. The extent of the strength loss can vary depending on age and disease of the subject. For example, acute strength loss can be as high as 50 % in muscles of healthy C57Bl mice, but in excess of 75 % in an *mdx* mouse (model for Duchenne muscular dystrophy).

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In vitro and in vivo methods are ideal for executing the eccentric contraction-induced injury model because the severity of muscle injury can be monitored in real time and controlled precisely by altering the number of eccentric contractions performed, the distance the muscle is lengthened, and/or the velocity at which the lengthening occurs. Additionally, these methods provide a level of consistency as far as injury at the fiber level because all motor units are activated which is in sharp contrast to fully conscious experimental models, e.g., downhill treadmill running.

1.1 In Vitro Eccentric Contractions

Live, isolated muscle preparations are utilized to assess contractile capacity of those organs. Small muscles of mice, such as the soleus and extensor digitorum longus (EDL) muscles, are suitable and most commonly used because oxygen diffusion to fibers in the core of muscle is adequate [6, 7]. These muscles also have well- defined tendons making dissection and attachment to force transducers straightforward. Interfaced to equipment as described by Sperringer and Grange in Chapter 19 of this volume [8], a host of contractile tests can be performed. The in vitro section of this chapter will focus on eccentric contractions by isolated muscles and the consequent contraction-induced injury. In the muscular dystrophy field, the term “force drop” is becoming common. This term is used to describe the outcome of eccentric contraction testing in EDL muscle lacking dystrophin as these muscles are highly susceptible to injury, as measured by the loss of force-generating capacity during and immediately following eccentric contractions. Before detailing methods for an in vitro eccentric contraction- injury protocol, a description of EDL muscle dissection is presented in this chapter because careful dissection is important in order to study contraction-induced injury to the muscle as opposed to dissection-induced injury.

1.2 In Vivo Eccentric Contractions

We have developed a modified in vivo muscle testing apparatus and protocol based on the original in vivo system reported by Ashton- Miller et al. [9], and similar to that described by Iyer et al., in Chapter 21 of this volume [10]. Briefly, peak isometric contractility of either the ankle dorsi-flexors (tibialis anterior, extensor digitorum longus, extensor hallicus longus muscles) or plantar-flexors (gastrocnemius, soleus, plantaris muscles) in anesthetized mice is determined using percutaneous electrodes to stimulate specific nerves innervating those muscle groups and specialized equipment to record the contractile output. Then an eccentric contraction-induced injury protocol is performed to cause muscle injury as immediately measurable by decrements in torque. Below we detail our eccentric contraction-induced injury model including internal controls and expected outcomes for healthy C57Bl6 mice and some examples from dystrophic *mdx* mice. Finally, a real advantage of the relatively noninvasive muscle analysis in vivo is that muscle contractile function can be measured repeatedly which can be ideal for monitoring ongoing muscle regeneration. Accordingly, we also briefly describe the recovery of muscle strength after eccentric contraction-induced injury.

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2 Materials

2.1 Equipment and Surgical Instruments for Delicate Muscle Dissections

In Vitro

1. Dissecting microscope (e.g., Leica S6D).
2. Fiber-Lite Dual Gooseneck Light (this is needed if there is not a light ring on dissecting scope).
3. Halstead Mosquito Forceps (~hemostat), curved, delicate 5′ length (e.g., George Tiemann #105-1107).
4. Dressing and Tissue Forceps, delicate with teeth (e.g., George Tiemann #105-205-1).
5. Extra fine Graefe Forceps, curved, finely grated tips (Fine Science #11151-10).
6. Dumont Forceps, pointed tip (e.g., Fine Science #11295-10).
7. Tissue Scissors, delicate and straight 3 3/4″ (e.g., George Tiemann #105-421).
8. Student Vannas Spring Scissors, sharp and non-serrated tips (e.g., Fine Science #91500-09).
9. McPherson Vannas Scissors, very finely serrated and delicate tips (George Tiemann #160-140).
10. Digital Caliper (e.g., World Precision Instrument).
11. 5-0 braided silk suture, non-absorbable; cut in 5 in. pieces.
12. Gel type cyanoacrylate (i.e., super glue) and 25 G needle for applying the glue.

2.2 In Vitro System for Eccentric Contractions

1. 300C-LR, Dual-Mode Lever System, Aurora Scientific.
2. S48 Stimulator with SUI5 Stimulus Isolation Unit, Grass Technologies.
3. Refrigerated/heating circulating water bath, controllable to 0.1°.
4. Organ bath, volume 1.2 ml; custom made by glass blower (Fig. 1b) or large organ baths available commercially.
5. Thermocouple and probe to measure/confirm temperature of buffer in organ bath (e.g., BAT-12 Microprobe Thermometer with IT-18 Flexible Microprobe).
6. Platinum wire electrodes (1.3 mm diameter, 99.95 pure platinum) (Fig. 1b).
7. 95% O_2, 5 % CO_2 tank with corresponding gas regulator.
8. Flow meter, 5.8 ml/min air with high resolution valve (e.g., Cole-Parmer, WZ-32044-00).
9. Computer, A/D board, and software.

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2.3 Equipment for In Vivo Eccentric Contractions

In Vivo

1. Dual-Mode Lever System (Aurora Scientific Inc., 300C-LR).
2. 16-bit National Instruments M series A/D card (Aurora, 603C).
3. Signal interface (Aurora, 604A), Grass stimulator and stimulation isolation unit (Grass s48 and SIU5) or high power follow stimulator (Aurora 701C).
4. Dynamic muscle Control/Dynamic Muscle Analysis-High Throughput Software Suite (Aurora, 615B) or similar data acquisition and analysis software (e.g., TestPoint).
5. Fuzzy logic PID temperature controller (Cole-Parmer C-89810-04).
6. Self-adhesive probe (Cole-Parmer C-08519-54).
7. Kapton heater pad (Cole-Parmer C-36060-50).
8. Extension adapter cord (Cole-Parmer C-03122-71).
9. Platinum-iridium electrodes (Grass Technologies E2-12).
10. Soldering helping hands, adhesive tape, and computer with Windows OS.

A plexiglass platform and mouse footplate were both custom manufactured by the university machine shop. An alternative platform and footplate are available through Aurora Scientific (809B testing apparatus). A heated circulating water bath will be necessary if using Aurora Scientific testing apparatus (809A).

3 Methods

3.1 EDL Muscle Dissection

In Vitro

1. Anesthetize mouse with an intraperitoneal injection of sodium pentobarbital at 100 mg/kg body mass using a 3/10 cc insulin syringe (e.g., for a 30 g mouse use 60 μL of a 50 mg/ml pentobarbital stock) (*see* Note 1).
2. Pull skin back from posterior hind limb up to the hamstrings using dressing and tissue forceps with teeth.
3. From the anterior hind limb, working on big toe side of the tibialis anterior muscle (TA) to stay away from the EDL muscle, pull the skin up past the knee and down to the ankle.
4. Pull the skin back over the heel being careful to not break the ankle.
5. From this point forward, it is highly recommended to use a dissecting microscope.

[1] For *mdx* mice, we find backing down the pentobarbital dose to 75 mg/kg body mass is preferable to keep mouse deeply anesthetized but still alive during the dissection.

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6. Remove fascia from TA using Dumont pointed forceps to puncture fascia at the mid-belly of TA.
7. Tear back fascia towards proximal end of the muscle and then towards distal end, again working on big toe side to avoid poking the EDL especially at the distal end.
8. Separate the distal EDL and TA tendons by inserting Dumont pointed forceps between the two tendons (that is, forceps should be under the TA tendon and over the EDL tendon; Fig. 2a).
9. Slide the forceps (still under the TA) up to the knee to completely separate the TA and EDL muscles. This is only possible when fascia is completely removed.
10. Insert one blade of the McPherson Vannas scissors under the distal TA tendon and cut as close to the foot as possible.
11. Stabilize the foot with the hemostat and then pull back TA towards the knee using Graefe forceps.
12. Pull the TA to the side and get one blade of the McPherson Vannas scissors under the TA fascia, parallel to EDL fibers, and cut a small slit to expose the proximal EDL tendon at the knee (Fig. 2b); a hamstring muscle inserting at the knee also lies perpendicular, over the proximal EDL tendon, and will be severed with this cut.
13. Use tissue scissors to cut off the TA at the knee.
14. Locate and pull out the extensor hallucis longus muscle (the big toe muscle).
15. Carefully remove any connective tissue or fat around the distal tendon of the EDL (*see* Note 2).
16. Insert Dumont pointed forceps under the distal tendon of the EDL muscle and pull suture through, under the tendon. Position suture precisely at the myotendinous junction (MTJ) making sure there is no connective tissue or fat between the suture and the tendon before tying knots (Fig. 2c). Also, be certain that the suture is around all four slips of the tendon, which are often distinguishable in atrophied EDL muscles.
17. Tie three knots, with the first being perpendicular to the fibers and the final parallel to fibers so that the transmission of force to the transducer is in series with fibers.
18. Using Student Vannas scissors, cut retinaculum of the foot parallel to the EDL tendon to expose about 2 mm of the EDL tendon distal to the MTJ and then cut the tendon (*see* Notes 3 and 4).

[2]We find this critical when doing eccentric contractions so that the suture does not slip when muscle is lengthened.
[3]This is a second critical point of dissection when doing eccentric contractions, again to avoid the tendon from slipping.
[4]Do not use delicate and expensive McPherson Vannas scissors to cut through the retinaculum or else you will ruin them!

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19. Using Graefe forceps to hold the tendon, gently pull back the EDL muscle toward the knee; if “slit” at the knee (**step 12**) was done correctly when removing the TA, the proximal EDL tendon will be exposed and easily pops out.

20. Place the second suture under proximal tendon, precisely at the MTJ, and tie three knots as described for the distal tendon; before doing this make sure the muscle is not twisted (you should be able to see the distal tendon on the top surface of the muscle; Fig. 2d).

21. Make small cut in knee capsule to get longer tendon by cutting parallel to tendon; the length of the proximal tendon is shorter than the distal tendon but strive to get 0.5 mm.

22. Place excised EDL muscle in a petri dish with chilled, oxygenated Krebs buffer (for example recipe *see* Sperringer and Grange in this volume [8]); use a hemostat on each suture to keep slight tension on the muscle (*see* Note 5).

23. Raise muscle out of buffer, blot off excess buffer on tendons and knots, and carefully apply super glue with a needle to each knot with the tendon positioned parallel with the suture (Fig. 2e) (*see* Note 6).

24. Move muscle to the organ bath of the in vitro system and attach suture to lever arm, add Krebs buffer and apply O_2; occasionally confirm that temperature of the buffer in the organ bath is precisely as desired using the microprobe thermometer.

25. Let the muscle equilibrate at least 5 min, set the muscle to its anatomical optimal muscle length (L_o), and then measure that length, from MTJ to MTJ, using the calipers (*see* Note 7).

3.2 Eccentric Contraction Protocol: In Vitro

Determine maximal isometric tetanic force (P_o) to establish pre- injury force generating capacity

1 With our system, maximal tetanic contraction of EDL muscle is elicited with 200 ms of 0.5-ms pulses at 180 Hz with stimulator set to maximal 150 V (*see* Note 8).

2 Repeat tetanic contractions with 3 min of rest in between (to avoid fatigue) until minimal increase in force occurs between two consecutive contractions (<0.5 g); this typically occurs at three contractions for EDL muscle and 5–8 contractions for soleus muscle with our set up at 25 °C (*see* Note 9).

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[5]We add insulin and branch chained amino acids to the Krebs buffer to improve viability of the preparation [6].

[6]The third critical point to avoid tendon slipping out of suture when doing eccentric contractions is applying super glue to cover knots and tendons (but avoiding muscle fibers!).

[7]We prefer to set anatomical instead of physiological L_o for reasons delineated in [16, 17]; in practice this is done by adjusting the length of the muscle such that resting tension is 0.4 g for EDL (0.5 g for soleus). Others report resting tensions near 1.0 g (*see* Chapter 19 by Sperringer and Grange in this volume [8]); this should be optimized in each individual lab set up.

[8]The pulse duration should be optimized for each set up as not all systems will be optimal at 0.5-ms pulses. Alternatively, a series of twitches are used to optimize contraction parameters (*see* Sperringer and Grange [8]).

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3 In between these "warm up" tetanic contractions, optimize L_o; we do this by adjusting resting tension to 0.4 g as it will drift down (refer to Note 7 for justification).

Eccentric contractions

4 Calculate 20 % of the L_o measured with calipers; this will be the length change during the eccentric contraction (e.g., 2.4 mm is 20 % of an EDL muscle with a L_o of 12 mm).

5 Passively shorten the muscle 10 % of L_o (e.g., 1.2 mm) (*see* Note 10 and Fig. 3a).

6 Initiate a 133 ms contraction at 180 Hz while simultaneously lengthening the muscle 20 % of L_o (e.g., 2.4 mm) at a rate of 1.5 L_o/s (*see* Notes 11 and 12).

7 Passively move the muscle back to the starting position, i.e., L_o.

8 Repeat eccentric contractions every 3 min up to 19 times.

Measure post-injury P_o to determine extent of injury, i.e., loss of force generating capacity

9 Upon completion of the final eccentric contraction, reset L_o.

10 Three minutes after the final eccentric contraction, re-measure P_o.

11 Calculate force loss (aka force drop)

$$\%\text{eccentric force loss} = [(\text{ECC}\mathit{last} \quad \text{ECC}\mathit{first})/\text{ECC}\mathit{first}] \times 100$$
$$\%\text{isometric force loss} = [(\text{post}P_o \quad \text{pre}P_o)/\text{pre}P_o] \times 100$$

Expected outcomes:

12 Re-injury P_o of an 8 mg EDL muscle from a wild type mouse should generate about 35 mg of isometric force (which should be converted to SI unit of force = 343 mN for reporting).

13 To calculate specific force, fiber to muscle length must be known; *see* Table 1.

14 The first eccentric contraction should generate force that is >150% of P_o; this is critical because it is the high force generation that causes injury to the muscle [4, 5] (Fig. 3b and d).

15 A fewer number of eccentric contractions are typically performed on *mdx* muscles due to their higher susceptibility to injury (Fig. 3c).

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[9] 25 °C is the most common temperature used because (a) it facilitates lowered muscle metabolism ensuring adequate O_2 diffusion and ATP production keeping muscle viable [6] and (b) extent of injury to muscle in terms of force loss is highly temperature sensitive [17].
[10] Our software is programmed to split the total length change around L_o with the goal to keep the length excursion within anatomical range, whereas beginning at L_o and lengthening a full 20 % may reach physiological and anatomical in vivo range for a muscle.
[11] In vitro eccentric contractions by other groups are commonly started with the muscle contracting isometrically and then a length change is imposed during the final ~200 ms of the stimulation; eccentric force generation and subsequent force loss are similar for two approaches (e.g., [18]).
[12] We use a length change of 10 % and a rate of change of 0.75 L_o/s for *mdx* EDL muscle (as opposed to 20 % and 1.5 L_o/s to induce injury to wild type muscle).

16. The % of eccentric force loss should be similar in extent to that calculated for % isometric force loss.

3.3 In Vivo System for Eccentric Contractions

In Vivo—The in vivo system is very similar to that described by Iyer et al. in Chapter 21 of this volume [10], with only slight modifications (Fig. 4a).

1. The in vivo apparatus is setup so that the animal lies on its side with the left hind limb secured to the footplate and force transducer (Fig. 4b). This provides best access to the common peroneal and sciatic nerve for percutaneous stimulation with the platinum-iridium electrode needles.
2. In lieu of a platform connected to a circulating water bath, place a Kapton heater pad on the testing platform and the self-adhesive probe is positioned so that it will rest beneath the animal during testing. The probe provides animal body temperature feedback to the temperature controller which can adjust the temperature of the heating pad appropriately (*see* Note 13).
3. Two soldering helping hands will grasp the needle electrodes and hold them in place during testing (Fig. 4c). A third soldering helping hand will serve as a knee clamp and maintain proper knee-ankle joint alignment and resting torque during testing.

3.4 Animal Preparation for In Vivo Eccentric Contractions

1. Mice anesthetized as described by Iyer et al. in this volume [10] (*see* Note 13).
2. The left hind limb from ankle to hip is shaved, hair removed with depilatory cream, and disinfected with repeated washes of betadine and 70 % ethanol.
3. The animal is secured to the pre-warmed (37 °C) in vivo platform (*see* Note 14), and knee secured with the knee clamp so that the ankle and knee joints are at 90° (Fig. 4c).
4. Pure platinum-iridium (Pt-Ir) electrodes are placed on either side of the peroneal nerve that innervates the dorsi-flexors (Fig. 4c) (*see* Note 15).
5. To injure the plantar-flexors, the peroneal nerve is severed and then Pt-Ir electrodes are placed on either side of the sciatic nerve (Fig. 4d). The sciatic nerve branches into the tibial nerve that innervates the plantar-flexors and the peroneal nerve that innervates the dorsi-flexors. Direct stimulation of the tibial branch nerve is not possible, and that is why the sciatic nerve is stimulated instead. The peroneal nerve must be severed as to not co-contract the antagonist dorsi-flexors (*see* Notes 16 and 17).

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[13]There are alternatives to isoflurane for anesthesia during in vivo testing, and attention should always be paid to the effect of depth and duration of anesthesia on muscle strength [19].

[14]Maintaining the body temperature at 37 °C is critical for the eccentric contraction induced injury. If body temperature is not regulated, the injury will not be as severe.

[15]For dorsiflexion, the tibialis anterior muscle contributes approximately 89 % of the torque [20].

[16]For plantarflexion, the gastrocnemius muscle makes up ~78 % of the cross-sectional area of the plantar-flexors [21] but contributions to torque have not been thoroughly assessed.

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3.5 Eccentric Contraction Protocol: In Vivo

1 We recommend that isometric contractions are used to optimize current or voltage applied to the nerve (in lieu of twitches).

2 A starting current of 0.5 Amps (A) or voltage (V) of 2 V is recommended. Perform a 150 ms isometric contraction (250 Hz) every 45 s adjusting the stimulation until a peak-isometric torque is achieved. Increments of 0.5 A or 0.2 V are typical.

3 Resting torque values should be accounted for when calculating pre-injury peak-isometric torque.

4 Peak-isometric torques for healthy C57Bl mice are 95–105 N mm per kg body mass (N mm/kg) prior to injury. Peak-isometric torques for dystrophic *mdx* mice can range from 60 to 85 N mm/kg depending on the age of the *mdx* mouse, as disease severity tends to be more severe between 3–8 weeks (*see* Notes 18 and 19).

5 After optimizing the stimulation parameters, proceed to the eccentric contraction-induced injury protocol described below:

Eccentric contraction protocol (dorsi-flexors) in vivo

6 The ankle joint should be at a starting position of 90°.

7 Passively (no stimulation) rotate the foot 20° toward dorsi-flexion (~1 s).

8 Initiate a 150 ms contraction at maximal stimulation frequency. The first 100 ms will be an isometric contraction at the dorsi-flexion position. During the final 50 ms, the ankle joint should move 40° toward plantarflexion at an angular velocity of 800°/s (*see* Note 20).

9 Passively (no stimulation) rotate the foot 20° toward dorsiflex-ion and back to the original starting position (~1 s).

10 Repeat eccentric contractions every 10 s up to 149 times.

Expected outcomes

11 Peak-eccentric torque should be 50–100 % greater than peak- isometric torque pre-injury (Fig. 5). This is a good internal control to ensure proper eccentric contraction protocol.

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[17] Alternative models of eccentric contraction-induced injury have been reported, e.g., stimulating the sciatic nerve before it branches out to the common peroneal and tibial nerves in the absence of a dual-mode lever system. Due to the greater mass of the collective plantar-flexors relative to the dorsi-flexors, a co-contraction would result in a concentric contraction by the plantar-flexors and an eccentric contraction by the dorsi- flexors. These co-contractions have previously been used to injure the dorsi-flexors [22]. This is not a precise, quantifiable model for eccentric contraction-induced injury as initial torque or the subsequent loss of torque generating capacity is not measured or controlled.

[18] The common cause of low torque values is improper electrode placement. Muscle contractions should be observed especially for the dorsi-flexors to ensure that the antagonistic plantar- flexors are not also being stimulated.

[19] The common cause of extremely high torque values is improper system calibration.

[20] The 40° rotation during the eccentric contraction protocol is the excursion limit for the Aurora 300C-LR motor, but the company can accommodate for investigators interested in a larger excursion limit (e.g., 90° rotation). With a greater excursion, muscle lengthening will be greater and fewer eccentric contractions are required to elicit maximal strength loss (*see* Iyer et al., Chapter 21 in this volume [10], as well as [14]).

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12 To execute an eccentric contraction-induced injury of the plantar-flexors the above protocol is the same with the exception that the foot is first passively moved toward plantarflexion and then rotated toward dorsiflexion during the eccentric contraction.

13 Figure 6a and b demonstrates the effectiveness of the eccentric contraction-induced injury protocol for initiating muscle damage and strength loss of the dorsi- and plantar-flexors of wild type (WT) and dystrophic mice (*mdx*, het, Fiona).

14 Real time monitoring of injury can be done by analyzing the isometric portion (first 100 ms) of the eccentric contraction and/or the decrease in peak eccentric torque. Figure 6c demonstrates how live monitoring of the strength loss can be used to reach equal injuries in different animal models (wild type vs. *mdx*).

15 At the conclusion of the eccentric contraction-induced injury protocol, wait 5–10 min before assessing peak-isometric torque for the immediate post-injury time point. Figure 6d demonstrates the typical recovery of strength during the first hour post-injury. Measurements made within the first 5 min may be variable due to a small fatigue component of the eccentric contraction induced injury protocol.

16 The in vivo system also represents an excellent approach for monitoring muscle regeneration after injury, specifically the recovery of strength. Figure 6e and f show the recovery of torque at different stimulation frequencies for muscles that were maximally injured (performed 150 eccentric contractions) compared to muscles that were injured to 25 % loss of eccentric torque.

Acknowledgments

This work was supported by the NIH National Institute on Aging (R01 AG031743).

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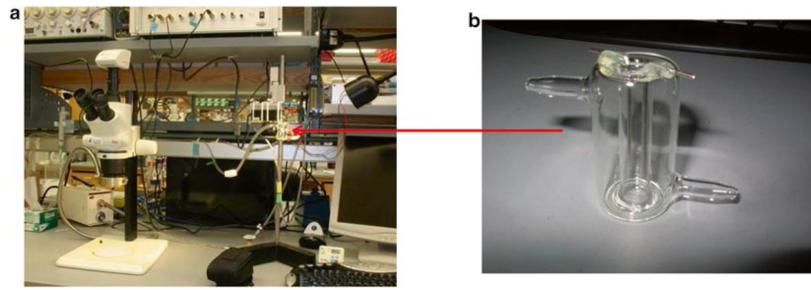

Fig. 1.

(**a**) In vitro muscle physiology system and (**b**) custom, 1.2 ml organ bath with electrodes sized for mouse soleus or EDL muscle. The in vitro system described here is similar to that described by Sperringer and Grange in Chapter 19 of this volume [8], particularly in regard to the lever system

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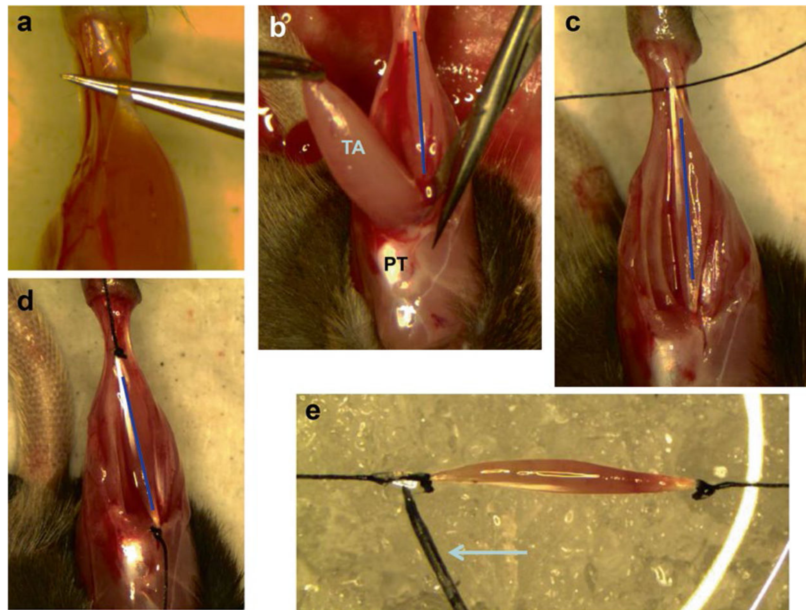

Fig. 2.

Dissection of mouse EDL muscle. Orientation is such that in (**a**)–(**d**) the foot of the mouse is at the top of the picture. (**a**) Distal tendon of the tibialis anterior (TA) muscle is separated from the distal EDL tendon by forceps. (**b**) Distal TA tendon is cut exposing EDL muscle. Blade of Vannas scissors is shown cutting proximal TA muscle to expose medial EDL tendon. *Blue line* demarks midline of the EDL muscle. PT = patellar tendon. (**c**) Suture placed under distal EDL tendon, precisely at myotendinous junction. Note that tendon has been cleared of connective tissue and fat. (**d**) Both distal tendon (*top*) and medial tendon (*bottom*) of the EDL muscle are secured by suture. We prefer to tie sutures on the muscle in vivo to keep muscle at its anatomical length as long as possible during the dissection and to minimize handling of the muscle ex vivo. (**e**) Once completely excised, gel type cyanoacrylate adhesive is carefully applied with a needle (*arrow*) to cover tendons and knots of the suture to avoid slippage during eccentric contractions

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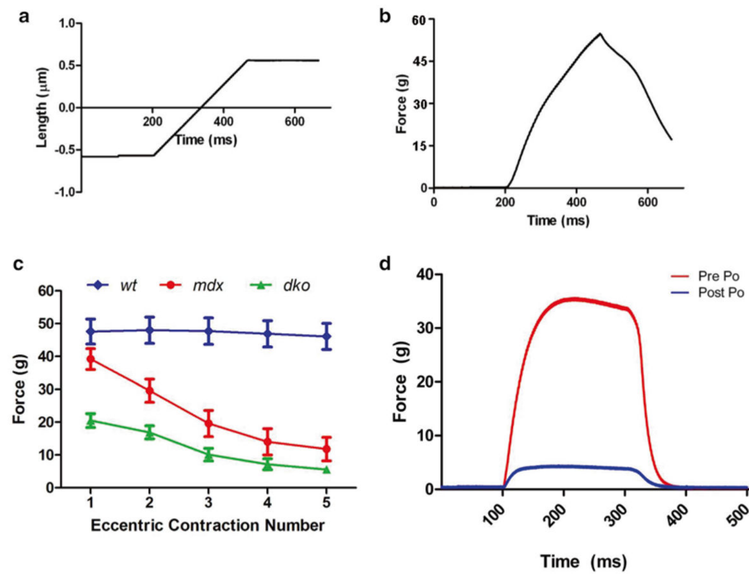

Fig. 3.

(**a**) Representative length-time and (**b**) force-time tracings of a single in vitro eccentric contraction by a mouse EDL muscle. (**c**) A series of five eccentric contractions can be titered by using only 10 % L_o length change at a slow velocity of 0.75 L_o/s to result in no force loss in healthy muscle (*wt*) but substantial force drop in muscle lacking dystrophin (*mdx*) and muscle lacking both dystrophin and utrophin (*dko*). This establishes an optimal situation to detect therapeutic improvement in the disease models. (d) In vitro contraction-induced injury shown as a loss in maximal isometric tetanic force (Po) for *mdx* mouse muscle following eccentric contractions (Post) as compared to before those injurious contractions (Pre). Note, though the force is typically measured in grams (g), it should be converted to newton (N), which is the SI unit of force for reporting. For example, an 8 mg EDL mouse muscle should generate ~35 g, i.e., 343 mN, of force. Notice that the peak-eccentric force (in **b**) is 150–175 % of peak-isometric force (Pre in **d**)

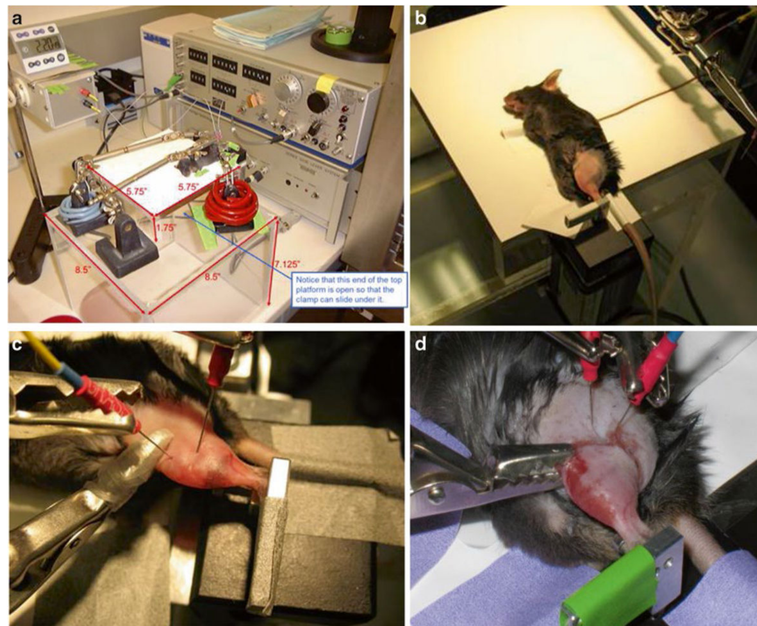

Fig. 4.

(**a**) The custom in vivo platform with dimensions. (**b**) Optimal placement of the mouse on the platform. The mouse position is secured with adhesive tape, and the self-adhesive temperature probe lies underneath the animal. (**c**) Appropriate electrode placement on either side of the peroneal nerve for dorsiflexion. (**d**) Appropriate electrode placement on either side of the sciatic nerve for plantarflexion

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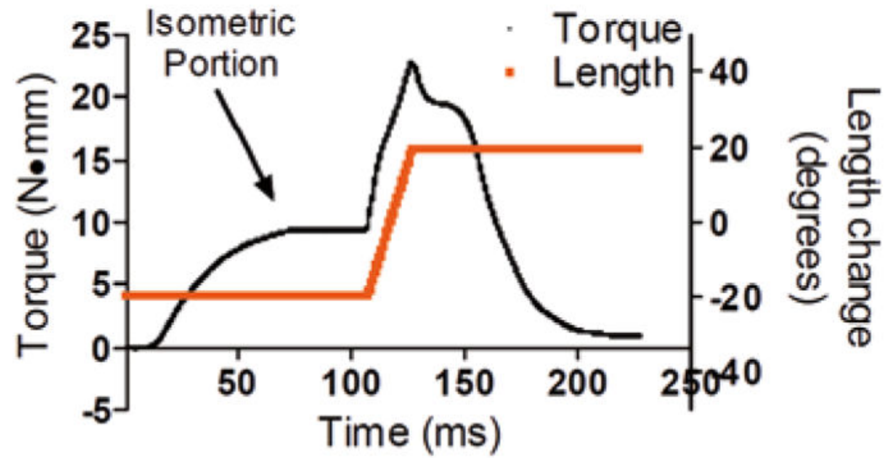

Fig. 5.

Representative torque-time tracing of a single in vivo eccentric contraction performed by the plantar-flexors. The length change reflects the foot being moved from 20° plantarflexion to 20° dorsiflexion during the electrical stimulus for contraction. The torque tracing demonstrates an ~100 % increase in torque production during the eccentric portion of the contraction compared to the isometric portion

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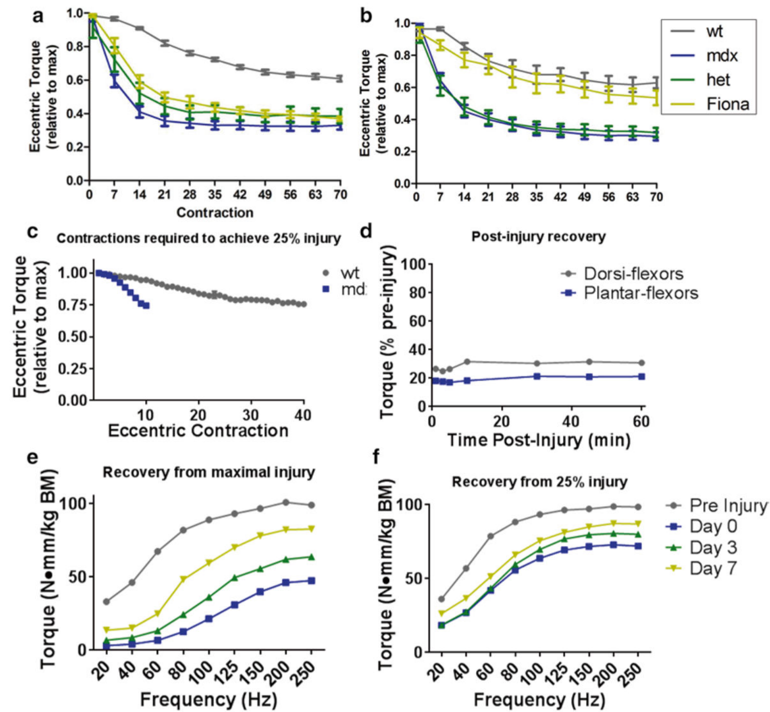

Fig. 6.

In vivo contraction-induced injury by the dorsi-flexors (**a**) and plantar-flexors (**b**) among C57Bl controls (*wt*), dystrophin deficient (*mdx*), dystrophin deficient and utrophin heterozygous (*het*), and dystrophin deficient and utrophin overexpression (*Fiona*) mice [15]. (**c**) In vivo contraction-induced injury controlled to a 25 % loss of torque in *wt* and *mdx* muscles. (**d**) Lack of recovery of dorsiflexion and plantarflexion torque during the hour immediate post-injury demonstrates injury as opposed to fatigue. (**e**) Longitudinal recovery of dorsiflexion torque as a function of stimulation frequency after a severe eccentric contraction-induced injury. (**f**) Longitudinal recovery of dorsiflexion torque as a function of stimulation frequency after a relatively mild eccentric contraction-induced injury

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Table 1

Reported fiber length to muscle length ratios across mouse background and age

Fiber length–muscle length ratio					Animal model			
TA	**EDL**	**Soleus**	**L Gastroc**	**M Gastroc**	**Species**	**Sex**	**Age**	**Ref.**
0.61	0.51	0.72	0.46	0.45	HSD mice	Female	Adult	[11]
	0.45	0.71			C57Bl/6 mice	Male	3–4 months	[12]
	0.44	0.69			C57Bl/6 mice	Male	9–10 month	
	0.45	0.69			C57Bl/6 mice	Male	26–27 months	
	0.45*	0.71*			C57Bl/6 mice	Male	3–4 months	
	0.47*	0.68*			C57Bl/6 mice	Male	9–10 month	
	0.44*	0.70*			C57Bl/6 mice	Male	26–27 months	
	0.44				Albino mice	Female	4–6 weeks	[13]

Ratios are fiber length over muscle length. Harlan Sprague Dawley (HSD). *Asterisk* indicates fiber length was determined after using nitric acid digestion (otherwise determined by microdissection)