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Monitoring Murine Skeletal Muscle Function for Muscle Gene Therapy

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Abstract

The primary function of skeletal muscle is to generate force. Muscle force production is compromised in various forms of acquired and/or inherited muscle diseases. An important goal of muscle gene therapy is to recover muscle strength. Genetically engineered mice and spontaneous mouse mutants are readily available for preclinical muscle gene therapy studies. In this chapter, we outlined the methods commonly used for measuring murine skeletal muscle function. These include *ex vivo* and *in situ* analysis of the contractile profile of a single intact limb muscle (the extensor digitorum longus for *ex vivo* assay and the tibialis anterior muscle for *in situ* assay), grip force analysis, and downhill treadmill exercise. Force measurement in a single muscle is extremely useful for pilot testing of new gene therapy protocols by local gene transfer. Grip force and treadmill assessments offer body-wide evaluation following systemic muscle gene therapy.

Keywords

Skeletal muscle; Twitch force; Tetanic force; Eccentric contraction; Grip strength; Treadmill; Gene therapy

1. Introduction

Skeletal muscles comprise about half of the body weight. The force generated by skeletal muscle is essential to voluntary actions such as talking, lifting, and running. Skeletal muscles distribute the loads along the skeleton. A skeletal muscle consists of myofibers that are bundled together by the endomysium. Each myofiber contains numerous myofibrils. Myofibrils are the force generating apparatus and they comprise about 80% of the muscle fiber volume and extend along the length of the myofiber. The basic unit of a myofibril is the sarcomere, a structure of highly organized contractile proteins (1). Muscle force is reduced in diseases that affect sarcomere configurations and/or myofibril integrity.

The force generated in the myofibrils is transmitted either longitudinally to the bone through the muscle tendon junction (MTJ) or laterally to adjacent myofibers (2, 3). In either case, the force is transferred to the surrounding tissues. The sarcolemma plays an important role in force transfer. The integrity of the sarcolemma is maintained by various transmembrane protein complexes, such as the integrin complex and the dystrophin-associated glycoprotein complex (4, 5). Defects in any one of these transmembrane linkages may destabilize the sarcolemma, compromise force transmission, and eventually decrease muscle strength (6). In summary, force reduction is a major physiological consequence of muscle diseases.

Many pharmacological treatments have been developed to modulate signaling and other cellular events (such as inflammatory responses) in various muscle diseases. Although limited clinical benefit has been documented, it is understood that such treatments cannot fully compensate for the function of the missing structural proteins. Gene therapy offers the hope of correcting the genetic defect at the molecular level. Murine models of various muscle diseases have been developed. These models open the door for preclinical testing of different muscle gene therapy strategies. A critical aspect in evaluating muscle gene therapy efficacy is to determine muscle force changes. This can be achieved by measuring the force of a single intact muscle, force generated from the entire limb, or the exercise capacity of the animal. In this chapter we present protocols for (a) ex vivo analysis of muscle forces generated from an intact extensor digitorum longus (EDL) muscle, (b) in situ analysis of muscle forces of the tibialis anterior (TA) muscle, (c) measurement of whole limb force by a grip strength meter, and (d) treadmill performance in conscious mice (7–12).

2. Materials

Get the approval from relevant authorities (such as the institutional animal care and use committee) prior to in vivo animal studies.

2.1. Ex Vivo Analysis of Muscle Forces of an Intact EDL Muscle

1. Anesthetic cocktail: 25 mg/mL ketamine, 2.5 mg/mL xylazine, and 0.5 mg/mL acepromazine dissolved in 0.9% NaCl. Keep the cocktail away from light and store at 4°C.
2. A custom-fabricated plexiglass dissection board (Fig. 1a). The dissection board is used for positioning the animal during muscle dissection (see Note 1).
3. Heating lamp (Tensor Lighting Company, Boston, MA, USA).
4. Regular dissecting forceps and scissors (World Precision Instruments, Sarasota, FL, USA).
5. Microdissecting scissors and forceps (straight and 45° angled) (Fine Science Tools, Foster City, CA, USA).
6. Bread silk suture #4-0 (SofSilk USSC Sutures, Norwalk, CT, USA).
7. Stereo dissecting microscope (Nikon, Melville, NY, USA).
8. Ringer's Buffer: 1.2 mM NaH₂PO₄, 1 mM MgSO₄, 4.83 mM KCl, 137 mM NaCl, 24 mM NaHCO₃, 2 mM CaCl₂, and 10 mM Glucose, pH 7.4. Store at 4°C (see Note 2).
9. Circulating water bath (Fisher Scientific, Waltham, MA, USA).
10. Oxygen tank containing 95% O₂ and 5% CO₂ (Airgas National, Charlotte, NC, USA) (see Note 3).
11. Digital caliper (Fisher Scientific).

¹The dissection board (9 in. wide and 7 in. long) is made from a ½ inch thick plexiglass. At the lower portion of the board, a 5 in. (width) × 3 in. (length) × 0.25 in. (depth) reservoir is grooved to collect excess of buffer during muscle superfusion. The excess buffer is removed from the reservoir through a vacuum line. In the middle of the reservoir, a sylgard ring of a ~1.5 in. diameter and 0.5 in. height is glued for securing the hind limb (Fig. 1a).

²We strongly suggest using the freshly prepared Ringer's buffer. Discard the buffer if it is more than 2 weeks old. Some investigators have included 6–25 µM tubocurarine chloride in their Ringer's buffer (15, 16). However, we found this is not necessary.

³Visit the company website www.airgas.com to find a local supplier. Oxygen tension profoundly influences force generation (17). We recommend using Blood Gas Mix 2Pt 210 CF (catalog number 730237). It is important to not use 100% pure oxygen. 5% CO₂ is essential to maintain the pH of the Ringer's buffer at 7.4 during the assay.

12. Microbalance (Fisher Scientific).
13. In vitro muscle function assay system (Aurora Scientific, Aurora, ON, Canada). The system consists of a 300B or 305B dual mode transducer that measures/controls the muscle force and length, a 701A stimulator, a 604B signal interface, and an 805A in vitro test apparatus equipped with a vertically mounted tissue organ bath (Fig. 1b) (see Note 4).
14. A custom-made 2-in.-long stainless steel hook and a custom-made 2.5-in.-long stainless steel hook (see Note 5).
15. Dynamic muscle control (DMC) software (Aurora Scientific). The software controls the force-length transducer and the stimulator. It allows real-time acquisition of the force and length data.
16. Dynamic muscle control data analysis (DMA) software (Aurora Scientific). The software analyzes the contractile data acquired by the DMC software.

2.2. In Situ TA Muscle Force Analysis

1. Physiological saline (0.9% NaCl).
2. In situ muscle function assay system (Aurora Scientific). The system consists of a 305C-LR dual mode transducer, a 701B stimulator, a 604A signal interface, and an 809A footplate apparatus (Fig. 1c).
3. A custom-made 0.5-in.-long stainless steel hook (see Note 5).
4. Custom-made 25G platinum electrodes (see Note 6) (Fig. 1c).
5. Circulating water bath, heating lamp, sutures, and dissection instruments (same as in Subheading 2.1).
6. Gooseneck holder (SK Science Kit, Tonawanda, NY, USA).
7. Oxygen tank containing 100% oxygen (Airgas National).

2.3. Grip Force Measurement

1. Grip strength meter (Columbus Instruments, Columbus, OH, USA). The apparatus includes a pulling bar attached to a force transducer and a digital display (Fig. 2a).

2.4. Downhill Treadmill Assay

1. Exer 3/6 treadmill system (Columbus Instruments). The system includes a treadmill with an electrical stimulus and a control unit. The control unit regulates the

⁴The 300B and the 305B transducers yield the same results (10). However, the larger force range of the 305B transducer allows a full on-screen graphic view of the force profile during eccentric contraction. The tissue organ bath should be cleaned periodically. For cleaning, disassemble the glassware (including the oxygen disperser tube, Teflon valve, and Radnoti organ bath) and hand wash with the Alconox detergent. Rinse with tap water and soak in 1 N HCl for 1 min. Rinse again with excess of tap water and then rinse with deionized water. Air dry.

⁵The 2-in-long hook is made of S/S 304V (0.18 in. diameter) stainless steel wire (Small Parts, Inc. <http://www.smallparts.com>). It connects the proximal end of the EDL muscle to the level arm of the 305B transducer. The 2.5-in-long hook connects the proximal end of the EDL muscle to the level arm of the 300B transducer. It is made of S/S 304V (0.006 in. diameter) stainless steel wire (Small Parts, Inc). The 0.5-in-long hook is made of the same material as that of the 2-in-long hook. It connects the distal tendon of the TA muscle to the level arm of the 305C-LR transducer.

⁶To make the electrode, solder two 0.016-in.-thick platinum wires to two 24G electric wires (Chalgren Enterprises, Gilroy, CA), respectively. Bend the tip of the wire into a hook shape. Carefully pack the wire with the electric heat shrink wrap into the empty barrel of a 1 mL syringe. Attach the assembled electrode to the gooseneck holder. Connect the other ends of the 24G electric wire to the stimulator via a pair of binding posts and a BNC connector.

treadmill speed and the intensity of the electrical shock. The system allows simultaneous analysis up to six animals in separate compartments (Fig. 2b).

2. 9-in. Cooling fan (Lasko, West Chester, PA, USA).
3. Jiffy jack (Cole Parmer, Vernon Hills, IL, USA).

3. Methods

3.1. Ex Vivo Analysis of Muscle Forces of an Intact EDL Muscle

1. Fill the organ bath with the Ringer's buffer. Turn on the circulating water bath to 30°C and start equilibrating the Ringer's buffer with 95% O₂ and 5% CO₂ (see Notes 3 and 7).
2. Load the DMC program and set the parameters at the dynamic muscle control and data acquisition panel as following: test duration, 3 s; update frequency, 500 Hz; sampling frequency, 1,000 Hz; input style, rise/fall; first stimulation, 1 s; last stimulation, 2 s; stimulation delay, 6 ms; pulse width, 200 μ s. Turn on the dual mode level system. Turn on the simulator and set the stimulator parameters as following: trigger mode, follow; pulse phase, biphasic; current multiplier, $\times 100$; current adjust, 8.5 (see Note 8).
3. Record mouse and experimental information (body weight, date of birth, gender, strain, ear tag number, project title).
4. Anesthetize the mouse with intraperitoneal injection of 2.5 μ L/g body weight of the anesthetic cocktail.
5. Set the heating lamp at the low intensity and adjust the lamp position to ~6" above the mouse body. Position the mouse face up on the dissection board and carefully peel off the skin to expose the hind limb muscles. Secure the hind limb on the sylagard ring using regular dressmaker pins. Place the first pin between the metatarsal bones and the second pin in the distal end of the gracilis muscle. Constantly superfuse the exposed muscles with the Ringer's buffer (see Note 9).
6. Dissect the remaining skin towards the metatarsal bones to expose the distal tendon of the TA muscle. Gently peel off the fascia on the surface of the TA muscle. Cut off the extensor ligament that covers the distal TA tendon. Lift up the distal TA tendon with a pair of 45° microdissection forces and separate it from the distal EDL tendon. Cut off the TA tendon and slowly peel off the TA muscle towards the knee to expose the EDL muscle. Cut out the TA muscle from its proximal attachment near the knee (see Note 10) (Fig. 1).

⁷The contractility and fatigability of isolated mouse muscles are affected by temperature (18, 19). We found that 30°C yielded consistent force output without inducing apparent fatigue. The Ringer's buffer should be equilibrated with 95% O₂ and 5% CO₂ for at least 20 min before each use. During force measurement, one should adjust oxygen valve to allow for a steady flow of gas without generating large bubbles.

⁸A single DMC panel can be used to simultaneously control more than one muscle assay systems. Define each system with a different bath number. Make sure to select the correct bath number. If you are using an earlier version of the DMC software (such as version 3.1.2), you will also need to set the parameter for "delay after the protocol" as 10 s. However, this is not necessary for the newer version of the software. There are several other parameters on the stimulator (including pulse width, PPS rate, and PPS multiplier). Under the "follow" trigger mode, the setting of these parameters on the stimulator is not functioning. Instead, the DMC software will control these parameters as defined by the investigator.

⁹To preserve the contractility of the contralateral EDL muscle, it is critical to keep the core body temperature of an anesthetized mouse at 37°C. The investigator may need to empirically test the position of the heating lamp while monitoring the core temperature with a rectal thermometer (Thermocouple thermometer, Fisher Scientific). We usually obtain comparable muscle forces from the left and right EDL muscles. During the dissection procedure, there will be minor bleedings from small surface vessels, constantly superfuse with the Ringer's buffer to keep the dissection area clean.

7. Identify the EDL tendons and clear them from the surrounding connective tissue and fat. Tie a double square knot with a bread silk suture around the distal EDL tendon. Tie a double square knot around the proximal EDL tendon and tie another double square knot using the same suture to secure the proximal EDL tendon to the distal end of the steel hook. Cut the proximal EDL tendon superior to the suture knot. Gently pull off the EDL muscle from the proximal end. Use a pair of microdissection scissors to separate the EDL muscle from connective tissue and vessels beneath the muscle. Cut the distal EDL tendon inferior to the suture knot and remove the intact EDL muscle. Attach the proximal end of the steel hook to the level arm of the transducer. Vertically position the EDL muscle between two platinum electrodes before securing the distal EDL tendon to a fixed clamp (see Note 11) (Fig. 1b).
8. Submerge the EDL muscle in the Ringer's buffer and adjust the muscle length to generate a 1 g resting tension. Allow the muscle to equilibrate for 10 min.
9. Adjust the muscle length to generate a resting tension of 0.5 g. Stimulate the muscle at 1 Hz (twitch stimulation). Record muscle force. Rest for ~15 s. Repeat twitch stimulation at muscle lengths that generate 0.8, 1.0, 1.2, 1.5, 1.8, and 2.0 g of resting tension. The length that generates the maximal twitch force is defined as the optimal muscle length (L_o). Record L_o with a digital caliper (see Note 12).
10. Rest muscle for 3 min. Set the muscle length to L_o and stimulate muscle with a single pulse. Record the force as the absolute twitch force (see Note 13).
11. Rest muscle for 3 min. Set the muscle length to L_o and stimulate muscle at 50, 80, 120, and 150 Hz. Allow 1 min rest between each contraction. Record the forces as the absolute tetanic forces under each stimulation frequency.
12. Rest muscle for 10 min. Set the muscle length to L_o and apply 10 cycles of eccentric contraction. For each cycle, stimulate muscle at 150 Hz for 700 ms. During the last 200 ms of stimulation, stretch the muscle for 10% L_o (0.5 L_o /s). After each stimulus, restore the muscle length to L_o at a speed of 0.5 L_o /s. Allow a 2 min rest between each cycle of eccentric contraction. Record the tetanic force of the first 500 ms stimulation for each cycle. The tetanic force of the first cycle is arbitrarily designated as 100%. This force equals the tetanic force obtained at the 150 Hz stimulation frequency in step 11.
13. Detach the EDL muscle and the steel hook from the force transducer. Cut the proximal and distal EDL tendons at the MTJ. Blot the muscle with Kimwipes twice and record the muscle weight (see Note 14).
14. Calculate the EDL muscle cross-sectional area using the equation of cross-sectional area = (muscle mass, in gram)/[1.06 g/cm³ × (optimal fiber length, in cm)]. 1.06 g/

¹⁰Take extreme care to avoid damaging the EDL muscle during dissection. The rupture of the vessels at the proximal end of the TA muscle may cause bleeding. This can be stopped with a thin piece of Ringer's buffer-soaked cotton.

¹¹To clearly expose the proximal EDL tendon, one may need to cut open the distal biceps femoris muscle. While taking the EDL muscle out, there will be bleeding from the vessels beneath the muscle. This can be stopped with a thin piece of Ringer's buffer-soaked cotton. Tie the knot as close to the MTJ as possible. When mounting the EDL muscle, make sure it is positioned right in the middle of two electrodes.

¹²We usually obtain the optimal muscle length at a resting tension of 1 g.

¹³Prior to the twitch and tetanic force measurement, we usually apply three rounds of 0.5 s tetanic stimulation at 150 Hz to warm up the muscle. Rest the muscle for 1 min between each warm-up stimulus. This treatment stabilizes muscle and allows consistent muscle force output during subsequent measurements (20). Make sure to adjust the resting tension to 1 g after each contraction.

¹⁴When measuring the muscle weight, make sure to remove the tendons and sutures. We recommend cut right at the position of the MTJ.

cm^3 is the muscle density. The optimal fiber length is calculated as $0.44 \times L_o$. 0.44 represents the ratio of the fiber length to the L_o of the EDL muscle (13).

15. Load the DMA program and open the file containing the force data. Analyze the data using the software. Export the data to an Excel file. The force value obtained from the DMA program represents the absolute force. Calculate the specific muscle force by dividing the absolute force with the cross-sectional area. Calculate the force drop after eccentric contraction with the formula of Force drop % = $(F_1 - F_n)/F_1$. F_1 is the tetanic force obtained during the first cycle. F_n is the tetanic force obtained during the n th cycle.

3.2. In Situ TA Muscle Force Analysis

1. Load the DMC program and set the parameters at the dynamic muscle control and data acquisition panel as following: test duration, 3 s; update frequency, 1,000 Hz; sampling frequency, 10,000 Hz; input style, rise/fall; first stimulation, 0.5 s; last stimulation, 0.8 s; stimulation delay, 4 ms; pulse width, 200 μs . Turn on the dual mode level system and the simulator. Set the circulating water bath to 37°C.
2. Record mouse/experimental information and anesthetize the mouse as described in Subheading 3.1.
3. Position the mouse face up under the heating lamp. Carefully remove the hind limb skin to expose the TA muscle. Identify the patella tendon at the knee. Tie two double square knots around the patella tendon leaving a ~2 mm loop between two knots (Fig. 1c). Tie two double square knots around the distal TA tendon leaving a ~10 mm loop between two knots (Fig. 1c). Make an incision on the lateral side of the thigh. Expose the biceps femoris muscle. Cut open the muscle to reveal the sciatic nerve. Tie a double square knot around the nerve and cut the nerve superior to the knot (Fig. 1c). Constantly superfuse exposed muscles with saline.
4. Gently transfer the mouse to the thermo-controlled platform of the footplate apparatus. Supply oxygen to the mouse by placing the oxygen cone to the mouse nose. Secure the knee position by running the metal pin through the patella tendon suture loop. Attach the distal TA tendon suture loop to the level arm of the force transducer. Position the gooseneck holder to secure the sciatic nerve to the electrodes (Fig. 1c). Set the muscle length at a resting tension of 5 g for 5 min. Periodically apply prewarmed (37°C) physiological saline to the exposed tendon and muscle to keep moist. Stimulate the TA muscle by applying electric pulses to the sciatic nerve. Determine L_o as the muscle length at which the maximal twitch force is elicited. Record L_o with a digital caliper (see Note 15).
5. Rest muscle for 2 min. Set the muscle length to L_o and stimulate muscle with a single pulse. Record the force as the absolute twitch force.
6. Rest muscle for 3 min. Set the muscle length to L_o and stimulate muscle at 50, 100, 150, and 200 Hz. Allow 1 min rest between each contraction. Record the forces as the absolute tetanic forces under each stimulation frequency.
7. Rest muscle for 10 min. Set the muscle length to L_o and apply 10 cycles of eccentric contraction. At each cycle of eccentric contraction, stimulate the muscle for a total of 350 ms at 200 Hz. During the last 200 ms, stretch the muscle by 10%

¹⁵The TA muscle is innervated by the peroneal nerve, one of the two branches of the sciatic nerve. Alternatively, the TA muscle can be stimulated by applying electric pulse directly to the peroneal nerve. We typically get the optimal TA muscle length at a resting tension of 5 g. After measuring the L_o , we usually apply three preliminary tetanic stimuli (150 Hz, 300 ms duration, 1-min rest between contractions) to warm up the muscle. Adjust the resting muscle tension to 5 g after each round of stimulus.

of L_0 at the speed of 0.5 L_0 /s. At the end of each cycle, readjust the muscle length to L_0 . The maximal isometric tetanic force developed during the first 150 ms of stimulation of the first cycle was designated as 100%.

8. Carefully dissect out the TA muscle. Blot the muscle with Kimwipes twice and record the muscle weight (see Note 14).
9. Calculate the TA muscle cross-sectional area as described in step 14 of Subheading 3.1 except using a TA muscle-specific fiber length to the L_0 ratio of 0.6 (14).
10. Load the DMA program and analyze the data. Calculate specific muscle force and force drop after eccentric contraction as described in step 15 of Subheading 3.1.

3.3. Grip Force Measurement

1. Record mouse/experimental information as described in Subheading 3.1. Check to make sure there is no visible sore in the forelimbs or toes. Turn on the grip meter and set it to measure the peak tension.
2. Grab the mouse by tip of the tail and gently lower the mouse to allow the forepaws to grab the metal grip bar (see Note 16) (Fig. 2).
3. Swiftly pull the mouse away from the grip bar by the tail. Record the reading when the mouse can no longer grasp the bar (see Note 17).
4. Rest the mouse for 30 s. Repeat the procedure five times and record each reading. Take the mean of the three highest readings as the absolute grip force. Divide the absolute grip force with the body weight to get the normalized grip force.

3.4. Downhill Treadmill Assay

1. Record mouse/experimental information as described in Subheading 3.1. Check to make sure there is no visible injury in the limbs and toes. Record room temperature. Set up the electric shocker at the intensity of 7 and the repetition rate of 9. Adjust the inclination of the treadmill platform with the Jiffy jack. Turn on the fan to the low setting and have the air blow in the same direction as the mouse is running (see Note 18) (Fig. 2).
2. Acclimate the mouse to the treadmill for 5 days. On day 1, place the mouse on an unmoving flat treadmill for 2 min, an unmoving 15° downhill treadmill for 5 min, a 15° downhill treadmill at the speed of 5 m/min for 15 min, and a 15° downhill treadmill at the speed of 10 m/min for 5 min. On day 2, place the mouse on an unmoving 15° downhill treadmill for 2 min, a 15° downhill treadmill at the speed of 5 m/min for 5 min, a 15° downhill treadmill at the speed of 10 m/min for 15 min, and a 15° downhill treadmill at the speed of 12 m/min for 5 min. On day 3, perform

¹⁶We usually let the mouse acclimate to the test apparatus for ~5 min. When positioning the mouse, make sure it has a firm hold on the grip bar. Both forepaws should face forward and space evenly apart on the grip bar. Mice that have undergone toe clipping have difficulties to grasp. We recommend not using these mice.

¹⁷The mouse must be in the horizontal plane and perpendicular to the grip bar during the pulling procedure (Fig. 2). It is imperative that the mouse has a good grip on the bar and is willing to pull. Sometimes the mouse will merely let go of the bar without pulling. If this happens, let the mouse adjust its grip on the bar and then pull. Occasionally, the mouse may squirm, bump, and even flip over the grip bar. This may yield an abnormally high reading. In this case, let the mouse rest for 5–10 min and then repeat the assay. In general, the difference between three highest readings should be less than 20 g.

¹⁸To test skeletal muscle function, we recommend using 15° downhill treadmill running. The strain, age, and sex influence treadmill performance (21). In the C57BL/6 strain, the body weight normalized running distance is shorter in male than that in female (12). The ideal room temperature for treadmill running is ~70°F. If higher than 80°F, mouse performance will be affected. A mild electric shock is used as a negative stimulus to discourage the mouse from standing/sitting at the end of the belt rather than running on the track. If the electric shock is too strong, it may damage the mouse. Since toe clipping may affect the gait, we recommend not using mice that have undergone toe clipping.

the same type of training as on day 2 except extend the last step from 5 to 10 min. On day 4, perform the same type of training as on day 3 except add an additional session on a 15° downhill treadmill at the speed of 15 m/min for 5 min. On day 5, perform the same type of training as on day 4 (see Note 19).

3. On day 6 (the test day), subject the mouse to a single bout of 15° downhill running starting at the speed of 10 m/min. Twenty min later, increase the treadmill speed 1 m/min every 2 min until the mouse is exhausted. Continuously nudge the mouse to keep it stay on the track. Exhaustion is defined as the point at which the mouse spends more than 10 s on the shocker without attempting to resume running when nudged (see Note 20).
4. Record the running distance.

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¹⁹Each training session takes about 30–40 min. We recommend performing training once per day. It is important to always perform the training at the same time of the day (e.g., always at 9 am in the morning). This should also be the time of the test. The mouse should be continuously monitored throughout the entire session. When speed reaches 10 m/min, some mice may stop running. This usually does not mean these mice are exhausted. Coax the mice to run by gently brushing the tail. Occasionally, a mouse may run towards the direction of the electric shocker. If this happens, stop the treadmill, manually place the mouse to the right direction, and restart. It may take several rounds for some mice to learn. At the end of each session, check the mouse for feet or toe damage. Exclude the mice that are either injured or reluctant to run. Up to 20% mice may fail to acclimate.

²⁰Caution should be taken when using the time spent on the electric shocker as an indicator for exhaustion (22, 23). Individual animal may display different running styles. Mice that do not run willingly or do not show a consistent running style should be excluded from the study. For aged mice (≥ 15 -m-old), instead of starting with a 20 min session of 10 m/min downhill running, we recommend starting the test with the speed of 5 m/min for 5 min, then 10 m/min for 15 min, then increase the speed 1 m/min every 5 min. It is important to point out that different laboratories may use different settings (e.g., 10 or 20° downhill instead of 15° downhill). To demonstrate therapeutic efficacy, one should always include age- and gender-matched untreated controls and normal mouse controls. We recommend starting with at least 7 mice for each group.

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Fig. 1.

Evaluating the EDL and TA muscle function. **(a)** The EDL muscle dissection system. **(a)** The dissection setup (heating lamp, dissection board, and vacuum line are marked). **(b)** The hind limb secured on the sylgard ring. **(c)** The TA muscle. **(d)** The TA muscle is partially lifted showing the underlying EDL muscle. **(e)** The EDL muscle. Note the distal EDL tendon is composed of four independent tendons while the proximal EDL tendon is not (see *insert*). **(f)** The EDL tendons are tied to the sutures and the muscle is ready to be taken out. The *dotted arrows* in **(e, f)** mark the peroneus brevis tendon. **(b)** The EDL muscle force measurement system. The EDL muscle is mounted on the system. The *left panel* shows the entire system. **(a)** The transducer. **(b)** The tubing connecting the circulating water bath and the tissue bath. **(c)** The 95% oxygen supply tubing. **(d)** The draining tubing. **(e)** The level arm. *Arrowhead*, the clamp. The *top right panel* shows a closer view of the level arm and the stainless steel hook (*asterisk*). The *bottom right panel* shows a closer view of the EDL muscle inside the organ bath. *Asterisk*, the stainless steel hook; *arrow*, the platinum electrode; *arrowhead*, the suture connecting the distal EDL MTJ and the clamp. **(c)** The TA muscle force measurement system. **(a)** An image of an experimental mouse mounted on the footplate apparatus. The gooseneck holder and the thermo-controlled platform are marked. *Arrow*, the oxygen cone; *arrowhead*, the circulating water bath inlet; *asterisk*, the electrode connecting cable. **(b)** a closer view of the dotted boxed region of the *panel a*. *Top inserts*, electrodes with (*right panel*) and without (*left panel*) the attached sciatic nerve (*arrowhead*); *bottom insert*, a closer view showing the knee secured to the metal pin. **(c)** The suture loop on the patella tendon. **(d)** The suture loop on the distal TA muscle tendon. **(e)** The hind limb with skin removed. *Arrow*, the location of the sciatic nerve. **(f)** The sciatic nerve (*asterisk*). **(g)** Isolation of the sciatic nerve.

Fig. 2.

Grip force study and treadmill exercise. **(a)** A side view image of a mouse undergoing grip force assay. *Dashed squares* mark the horizontal planes. The *insert* shows the position of the mouse perpendicular to the grip bar as marked by the *dotted lines*. **(b)** The downhill treadmill setup. *Arrow* marks the mouse running direction. *(a)* Control unit; *(b)* jack; *(c)* cooling fan; *(d)* running track.