

## Video Article

# Assessing Functional Performance in the *Mdx* Mouse Model

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## Abstract

Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder for which no cure is available. Nevertheless, several potential pharmaceutical compounds and gene therapy approaches have progressed into clinical trials. With improvement in muscle function being the most important end point in these trials, a lot of emphasis has been placed on setting up reliable, reproducible, and easy to perform functional tests to pre clinically assess muscle function, strength, condition, and coordination in the *mdx* mouse model for DMD. Both invasive and noninvasive tests are available. Tests that do not exacerbate the disease can be used to determine the natural history of the disease and the effects of therapeutic interventions (e.g. forelimb grip strength test, two different hanging tests using either a wire or a grid and rotarod running). Alternatively, forced treadmill running can be used to enhance disease progression and/or assess protective effects of therapeutic interventions on disease pathology. We here describe how to perform these most commonly used functional tests in a reliable and reproducible manner. Using these protocols based on standard operating procedures enables comparison of data between different laboratories.

## Video Link

The video component of this article can be found at <http://www.jove.com/video/51303/>

## Introduction

Duchenne muscular dystrophy (DMD) is the most common neuromuscular disorder affecting 1:5,000 newborn boys. This severe and progressive muscle wasting disease is caused by mutations in the *DMD* gene that disrupt the open reading frame and prevent the synthesis of functional dystrophin protein. Muscle fibers lacking dystrophin are vulnerable to exercise induced damage. Upon exhaustion of the muscle's regenerative capacity, and due to chronic inflammation of damaged muscle, fibers are replaced by connective tissue and fat, subsequently leading to a loss of function. Generally, DMD patients lose ambulation of the lower limbs early in the second decade. Later, also the muscles of the arms and shoulder girdle are affected and patients often develop thoracolumbar scoliosis due to asymmetric weakening of the muscles supporting the spinal cord. Assisted ventilation is generally required in the late teens or early twenties. Respiratory and heart failure lead to death in the third or fourth decade<sup>1</sup>.

Although the causative gene has been discovered over 25 years ago<sup>2</sup>, there is no cure available for DMD. However, improved health care and the use of corticosteroids have increased life expectancy in the Western world<sup>3</sup>. With the use of animal models like the *mdx* mouse, major steps forward into the discovery of potential therapeutic strategies have been made. The *mdx* mouse is the most commonly used DMD mouse model. It has a point mutation in exon 23 of the murine *Dmd* gene and consequently lacks dystrophin<sup>4</sup>. Over the last couple of years, many proposed strategies have progressed into clinical trials<sup>5-9</sup>. In these trials, improvement of muscle function is the primary endpoint, underlying the importance of testing the benefit of compounds on muscle function in mice during the pre clinical stage of testing.

Like DMD patients, also the dystrophin negative muscle fibers of *mdx* mice are vulnerable to exercise induced damage and their muscle function is impaired compared to C57BL/10ScSnJ wild type mice. This impairment can be assessed with a variety of functional tests. Some of these tests are noninvasive and do not interfere with muscle pathology (e.g. forelimb grip strength, hanging tests and rotarod running). Therefore they can be used to monitor the natural history of the disease or determine the effects of compounds on disease progression. To get an in depth picture of the influence of compounds on muscle function in *mdx* mice, a functional test regime that does not interfere with disease progression consisting of all of these tests can be used<sup>10</sup>.

Alternatively, forced treadmill running can be used to intentionally exacerbate disease progression and test the protective capacities of compounds<sup>11</sup>. The treadmill can also be used as outcome measure in which running time till exhaustion is measured<sup>12</sup>, or as a tool to fatigue *mdx* mice so that they perform less well in a subsequent functional test ensuring larger differences in performance between treatment groups<sup>13</sup>. When choosing functional tests, their effect on disease progression should be kept in mind especially when testing dystrophic mice like the *mdx* mouse<sup>14</sup>.

We here describe in detail how to perform the most commonly used functional tests in a reliable and reproducible manner based on available standard operating procedures from the TREAT-NMD network. [Click here to visit TREAT-NMD.](#)

## Protocol

The experiments described here were approved by the Animal Ethics Committee (DEC) of the Leiden University Medical Center (LUMC). Mice were bred by the animal facility of the LUMC and kept in individually ventilated cages with 12 hr light dark cycles. They had *ad libitum* access to water and standard chow.

When performing any of the functional tests described below, experimental conditions have to be strictly controlled to reduce variation. Preferably, age and gender matched mice should be used, as performance differs between age and genders. Mice belonging to the same litter should be randomized over the experimental groups. Animals should be tested by the same operator, who is blinded to the experimental groups. Tests should be performed on the same time of day and weekday, same room to equalize odors, noises, etc.<sup>14</sup> Large variation between individual mice and time points can be observed for all functional tests, therefore 6-8 mice/experimental group should be used. Functional test performance can also largely differ between different inbred wild type strains. Therefore, experimental and control wild type mice should always have corresponding backgrounds (in case of *mdx* mice use the C57BL/10ScSnJ wild type strain). All data described here have been obtained with the C57BL/10ScSnJ wild type strain, which we refer to as wild type from here on. The tests described here can be used longitudinally from at least 1-19 months of age in *mdx* and wild type mice. Tests should not be repeated more than once weekly to prevent mice from losing interest and willingness to perform the task.

### 1. Forelimb Grip Strength Test

Use the forelimb grip strength test to measure the strength of the forelimbs. The test is based on the tendency of a mouse to instinctively grasp a grid when suspended by the tail<sup>15</sup>, and adapted from [DMD\\_M2.2.001.pdf](#).

1. Apparatus set up: Attach a grid to a force transducer, which measures the maximum force applied by the mouse on the grid during the pull. Make sure the setting is on Peak tension mode (T-PK) for pulling. The units of force can be adjusted in either ounces-of-force, grams-of-force, pounds-of-force, kilograms-of-force, or Newtons.  
Note: We prefer to work with grams as unit of values. Multiple meters are commercially available, but only axial transducers give reliable outcomes as lever type force transducers are negatively influenced by the physical laws of the lever effect. Either a nonflexible grid or triangle can be used with bars that are 1-2 mm in diameter.
2. Prior to the test, assess the body weight of the mouse, to allow normalization for body weight.
3. Use grams as unit of values. Reset the meter at the start of each recording.
4. Remove the mouse from its cage by grabbing the tail and moving it horizontally towards the grid.
5. Check that the mouse grasps the grid tightly with both forepaws.
6. Pull the mouse away from the grid so that its grasp is broken; the highest force applied to the grid will be shown on the transducer's display, which can be either manually or automatically recorded.
7. Only take pulls into account in which the mouse shows resistance to the experimenter. Reject measures in which only one forepaw, or the hindlimbs were used and in which the mouse turned during the pull.
8. Let the mouse pull the grid three times in a row and then return it in the cage for a resting period of at least one minute. **Note:** between series of pulls a resting period is necessary for the mouse to recover and avoid habit formation.
9. Then let the mouse perform four series of pulls, each followed by a short resting period. In this way the mouse has pulled a total of 15x (3 pulls x 5 times = 15 pulls).
10. Determine the maximum grip strength and normalize for body weight by taking the average of the three highest values out of the 15 values collected.
11. Optional: Determine fatigue by calculating the decrement between the average of the first two and the last two series of pulls  $1+2+3=A$ ,  $4+5+6=B$ ,  $10+11+12=C$  and  $13+14+15=D$ . The formula:  $(C+D)/(A+B)$  gives a value of 1 for mice which are not fatigued. This can be expressed in percentages so that a mouse without fatigue has a value of 0% and a mouse which forelimbs are completely fatigued has a value of 100%.

### 2. Hanging Tests

With hanging tests, balance, coordination and muscle condition can be assessed. These tests are based on the knowledge that mice are eager to remain hanging on a wire or grid till exhaustion<sup>16</sup>. There are two distinctive hanging tests in which at the start of the test either only the two forelimbs or all four limbs are used, using a wire or grid respectively. The hanging test using the wire and the grid are the longest suspension time method adapted from [DMD\\_M.2.1.004.pdf](#) and [DMD\\_M.2.1.005.pdf](#) respectively. A fixed hanging limit is used of 600 sec. The majority of wild type mice can hang for 600 sec, while dystrophic mice cannot. To reduce time spend performing this test, a maximum hanging time was set in place. Mice that fall off the wire or grid before then are given up to two more tries. This is done to reinsure that mice are really unable to hang and do not fall due to clumsiness.

1. Hanging test with two limbs
  1. Apparatus set up: Tightly secure a 2 mm thick metal cloth hanger to a shelf with tape and maintain the hanger around 37 cm above a layer of bedding. Note: alternatively, a 55 cm wide 2 mm thick metallic wire which is tightly secured between 2 vertical stands could be used. The distance of 37 cm is sufficient to encourage mice to remain hanging, but also low enough to prevent mice from injuries when falling down. The wire should not vibrate or displace during the test as this could interfere with the performance of the mouse.
  2. Handle the mouse via the tail and bring it near the wire.
  3. Let the mouse grasp the wire with the two forepaws only, and lower the hindlimbs in such a way that the mouse only hangs with the two forepaws on the wire (**Figure 2B**).

4. Directly start the timer when the mouse is released. After release, strong mice try to catch the wire with all the four limbs and the tail, which is allowed (**Figure 2C**).
  5. When a mouse shows improper behavior (like balancing on or deliberately jumping off the wire as shown in **Figures 2D and 2E**), directly address this by replacing the mouse on the wire without stopping the timer.
  6. When a mouse falls off the wire, stop the timer and record the hanging time.
  7. When mice are able to hang for 600 sec, take them off the wire and return them to the cage. Mice that fall before this limit are given a maximum of two more tries.
  8. Record the maximum hanging time (*i.e.* the longest of the trials) and use this for further analysis.
2. Hanging test with four limbs
    1. Apparatus set up: Use either a hand made square or the lid of a big cage for a rat or rabbit for this test. Position the grid 25 cm above soft bedding to prevent mice from harming themselves upon falling, but also to discourage mice to intentionally jump off the grid. Tightly secure the grid so that the experimenter does not have to manually hold the grid during the experiment as these movements might interfere with the mouse's performance.
    2. Place the mouse on the grid so that it grasps it with its four paws.
    3. Invert the grid so that the mouse is hanging and directly start the timer.
    4. The test session ends for mice that are able to hang for a duration of 600 sec. Give mice that fall off the grid earlier a maximum of two more tries.
    5. Use the maximum hanging time (*i.e.* the longest of the trials) for further analysis.

### 3. Rotarod Running

With the rotarod test muscle strength, coordination, balance, and condition can be determined<sup>17</sup>.

1. Apparatus set up: For this test, mice have to run on a rotating tube. Ensure that the steady speed is set at 5 rotations per min (rpm), and that the speed increases from 5-45 rpm in the first 15 sec when started. After this it has to maintain its speed.
2. Place the mice on the tube of the rotarod when it rotates at a slow steady speed of 5 rpm. Five mice can be tested simultaneously.
3. Start the run once all mice are positioned. Within the first 15 sec the speed of the tube accelerates from 5-45 rpm after which it maintains that speed.
4. Monitor the run. The running time is continuously recorded by the software. Running time stops automatically when a mouse falls off the tube as this activates the time bar positioned below the tube. Reposition mice that turn around facing the opposite direction on the tube while running without stopping the tube to rotate.
5. End the test session for mice that are able to run for a duration of 500 sec. Give mice a maximum of two more tries allowing them to improve their running time, when they fall earlier.
6. Use the maximum running time (*i.e.* the longest of the trials) for further analysis.

### 4. Treadmill Exercise

The treadmill can be used in three ways as a tool in pre clinical research. Firstly, forced treadmill running can be used to exacerbate disease pathology as described in this protocol (see also: [DMD\\_M2.1.001.pdf](#)). Secondly, the maximal running capability of mice and the effects of treatments on this can be assessed (See for the method to let mice run till exhaustion [DMD\\_M2.1.003.pdf](#)). Finally, treadmill running can be used prior to another functional test to exhaust the mouse so that it performs less well in the second test<sup>13</sup>. This is done by exercising mice twice or three times weekly as described below, directly followed by either one of the functional tests described in protocol 1-3.

1. Apparatus set up: There are several treadmills commercially available on which several mice can run simultaneously and for which elevation, duration and speed can be adjusted. Some treadmills are equipped with a grid to deliver low intensity shocks to encourage mice to run. However, *mdx* mice are sensitive to stress and can easily be motivated in a friendlier manner by a gentle push with the hand in the running direction. Therefore, it is strongly encouraged to NOT use the shock grid. Generally, stimulation with the hand is only needed during the first running session.
  2. Place the mice on the horizontal treadmill.
  3. Start the treadmill at a running speed of 12 m/min. Lower speeds (8 m/min) have to be used in old mice (>15 months), where higher speeds easily lead to exhaustion.
  4. During the first session, encourage mice to run by gently pushing them when they are near the end of the belt.
  5. When the mice have run for a duration of 30 min, place them back into their cage.
  6. Repeat this twice weekly for e.g. 12 weeks.
  7. Allow resting periods when needed. For example, some *mdx* mice have to stop running and should be allowed to rest for a few min. If this happens, turn the belt off, give all mice a resting period of two min, turn the belt on for two min at 4 m/min. After this, increase speed to 12 m/min and allow the mice to finish the protocol. It is important that all mice complete the entire running protocol.
- Note:** In case *mdx* mice need resting periods, consider a warm up before the 30 min exercise protocol. This warm up session consists of: a 2 min acclimatization period at a speed of 4 m/min, immediately followed by an 8 min warmup at 8 m/min. In our hands 4-16 week old female *mdx* mice are able to complete the 30 min exercise protocol without resting. Others have reported that in age matched male *mdx* mice 45% of the mice do need resting periods to finish the exercise. The warm up protocol reduces the amount of stops<sup>12</sup>.

## Representative Results

The forelimb grip strength of wild type and *mdx* mice increases between the age of 4-12 weeks and reduces again in older mice. Impairments in force can already be observed in young *mdx* mice. Representative data of 9 week old female mice are shown in **Figures 1A** and **1B**. Although fatigue does not differ between the strains yet at this age, *mdx* mice are weaker than wild type mice. We do not have data yet on fatigability in older *mdx* and wild type mice.

To obtain reliable and reproducible results, multiple assessments need to be done by the same experimenter. We here describe to pull 15 times/individual, however smaller numbers of pulls (as low as 5 pulls) also provide reliable data. Careful attention should be drawn to the positioning of the paws on the grid as this can largely influence outcomes. During the pull, only both forepaws should be used and they have to be placed nicely next to each other (**Figure 1C**). When the mouse is not showing resistance to the pull, the value should not be taken into account.

For the two limb and four limb hanging tests, especially young (4-16 weeks old) wild type mice can easily reach the maximum hanging time of 600 sec. Contrastingly, performance of young *mdx* mice is impaired (they hardly ever achieve maximum hanging time) and also deteriorates with age, even though both strains put all effort in performing these hang tests at their best abilities (**Figures 2A** and **3A**). Larger differences in hanging times between *mdx* and wild type mice are obtained with the wire. Therefore, even small effect sizes of compounds on muscle function can be detected using this test. Hanging performance (or any other type of performance) differs within and between individuals over time resulting in high standard deviation bars. Nonetheless, *mdx* mice consistently perform worse than age matched wild type mice (**Figure 2A**). Performing multiple assessments can provide more detailed insight in functional improvements upon treatment than only endpoint measurements. It should be kept in mind that in the first session animals learn how to perform a functional test. This learning curve, which is present in all tests, is clearly visible between 4-6 weeks of age. However, because mice also grow rapidly in this age period, a distinction between improvement due to learning and/or growth cannot be made. Gender differences in hanging performance for the two limbs hang test have also been found. Performance of female *mdx* mice exceeds that of males by ~100 sec, and performance of treadmill challenged female *mdx* mice is almost comparable to that of the unchallenged males (compare **Figures 2A** with **4A**). This finding underlines the importance of using age and gender matched mice to avoid bias. We have preliminary data suggesting that differences in performance in both hanging tests between *mdx* and wild type mice increases in very old (18 months) mice.

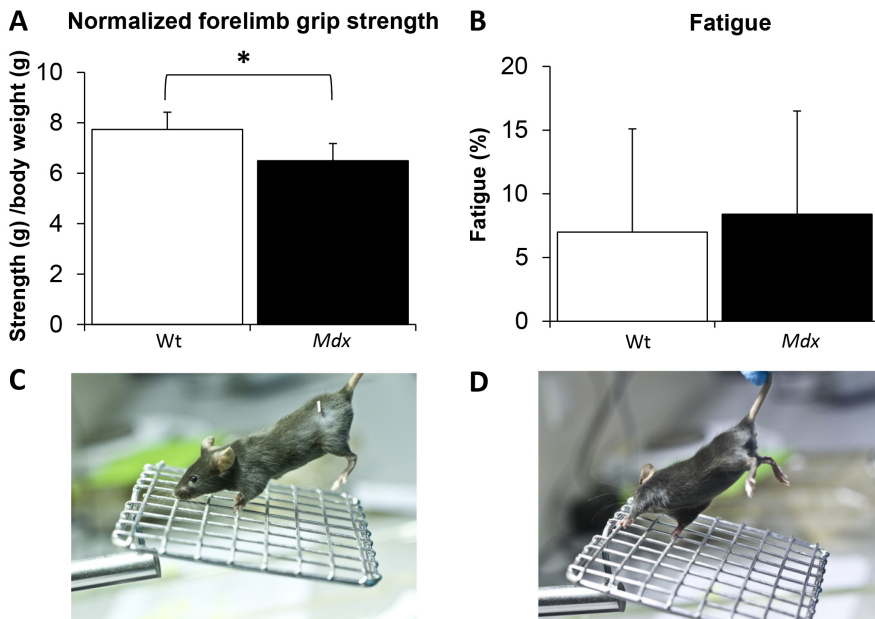
Some mice display inappropriate behavior to avoid hanging on the wire like; balancing on the wire, jumping off the wire deliberately *etc.* (**Figures 2D** and **2E**), although the majority of mice comply with the test and hang with either two or four limbs (**Figures 2B** and **2C**). Occasionally, strong mice jump off the wire intentionally. They hang prior to jumping with only the two hindlimbs and the tail on the wire and look down to estimate the distance to the ground. Inappropriate behavior that is occasionally seen on the grid during the four limb hanging test consists of deliberately jumping off the grid or climbing on the grid. All inappropriate forms of behavior can be easily distinguished and should not be allowed. Mice that avoid hanging in one of these ways should be directly placed back on the wire or grid without stopping the timer.

On the rotarod, *mdx* mice hardly ever run for the maximum running time of 500 sec, while a larger proportion of wild type mice do (**Figure 3B**). With age, running performance of both strains decreases. Some mice are able to clamp tightly on to the rotating tube and avoid running by 'cartwheeling' around. This cannot be corrected for and is a severe limitation of the test when multiple mice start doing this for prolonged periods, thereby increasing variation within the experimental groups. Especially for some mice which partly run and partly cartwheel, and during the transition from cartwheeling into running fall. Some mice turn around on the rotating tube while running. This behavior should be addressed for by directly repositioning the mice on the tube, without stopping it. Also this kind of behavior limits the usefulness of this test.

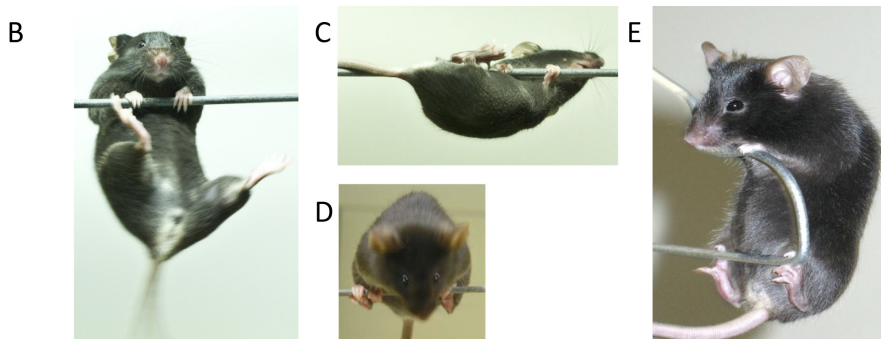
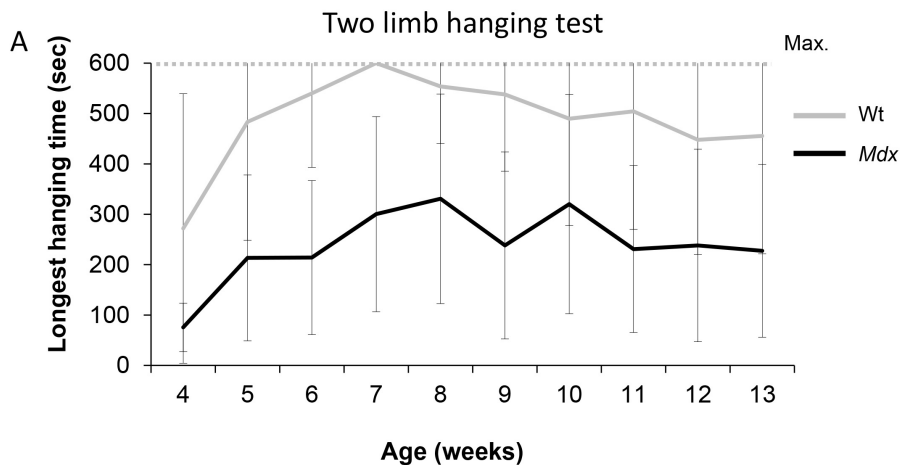
Forced treadmill running is an easy and effective exercise to exacerbate disease pathology in nontreated *mdx* mice, while wild type mice undergoing the same protocol are not affected. Generally, mice become familiar with the treadmill after an initial training session and are willing to run, especially when multiple mice are running simultaneously. Old *mdx* mice (over 15 months of age) have difficulties in running and cannot cope with the same running speed of 12 m/min for 30 min used for young mice. Therefore, a slower running speed of 8 m/min for 30 min is recommended enabling all mice to finish the entire protocol. *Mdx* mice are especially vulnerable to eccentric contractions, therefore downhill running can only be used for a short duration.

Alternatively, other functional tests like the two limb hanging wire test can be performed directly after running (**Figure 4A**). Using this study design, differences between strains or treatment arms are likely to increase as treadmill challenged untreated *mdx* mice are less capable of performing these tests than sedentary *mdx* mice<sup>13</sup>.

As mentioned earlier, when studying muscle function in *mdx* mice, the C57BL/10ScSnJ wild type strain needs to be used which is of the corresponding genetic background. We advise this as even between inbred wild type strains treadmill running performance differs<sup>18,19</sup>. But also in noninvasive functional tests, functional performance is influenced by genetic backgrounds. **Figure 5** illustrates this in three representative graphs where performance of *mdx* mice on a BL/10 background and on a mixed background consisting of BL/10, BL/6J, DBA2 and 129OLA are compared. As can be appreciated the mixed background mice perform better in the hanging wire tests and worse on the rotarod.

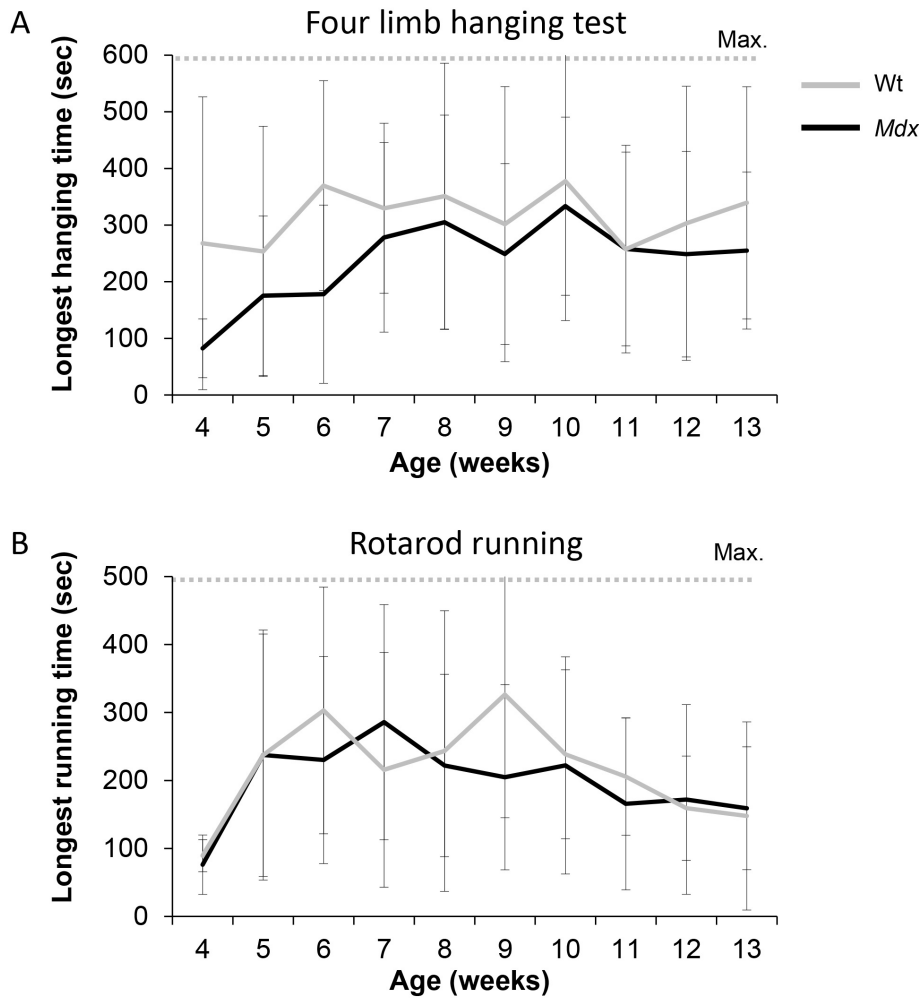


**Figure 1. Forelimb grip strength, representative results and correct positioning of the paws.** **A.** Forelimb grip strength normalized for body weight of 9 weeks old female *mdx* (n=5) and wild type (n=4) mice. Grip strength is already impaired in young *mdx* mice. Asterisks indicate  $p < 0.05$  and data are presented as mean  $\pm$  st.dev. **B.** Fatigue of the same individuals as shown in A, was on average less than 10% and did not vary between strains. **C.** To obtain reliable data, attention should be paid to the positioning of the paws during forelimb grip strength analysis. Correct positioning of the mouse; two forepaws are next to each other, hindlimbs are not touching the grid and the mouse is pulling in a straight line. **D.** Incorrect positioning of the forepaws; the mouse is not pulling in a straight line. When this happens, or when only one forepaw or also the hindlimbs are used, the mouse turns around during pulling or lacks to show resistance, data should be discarded. [Please click here to view a larger version of this figure.](#)

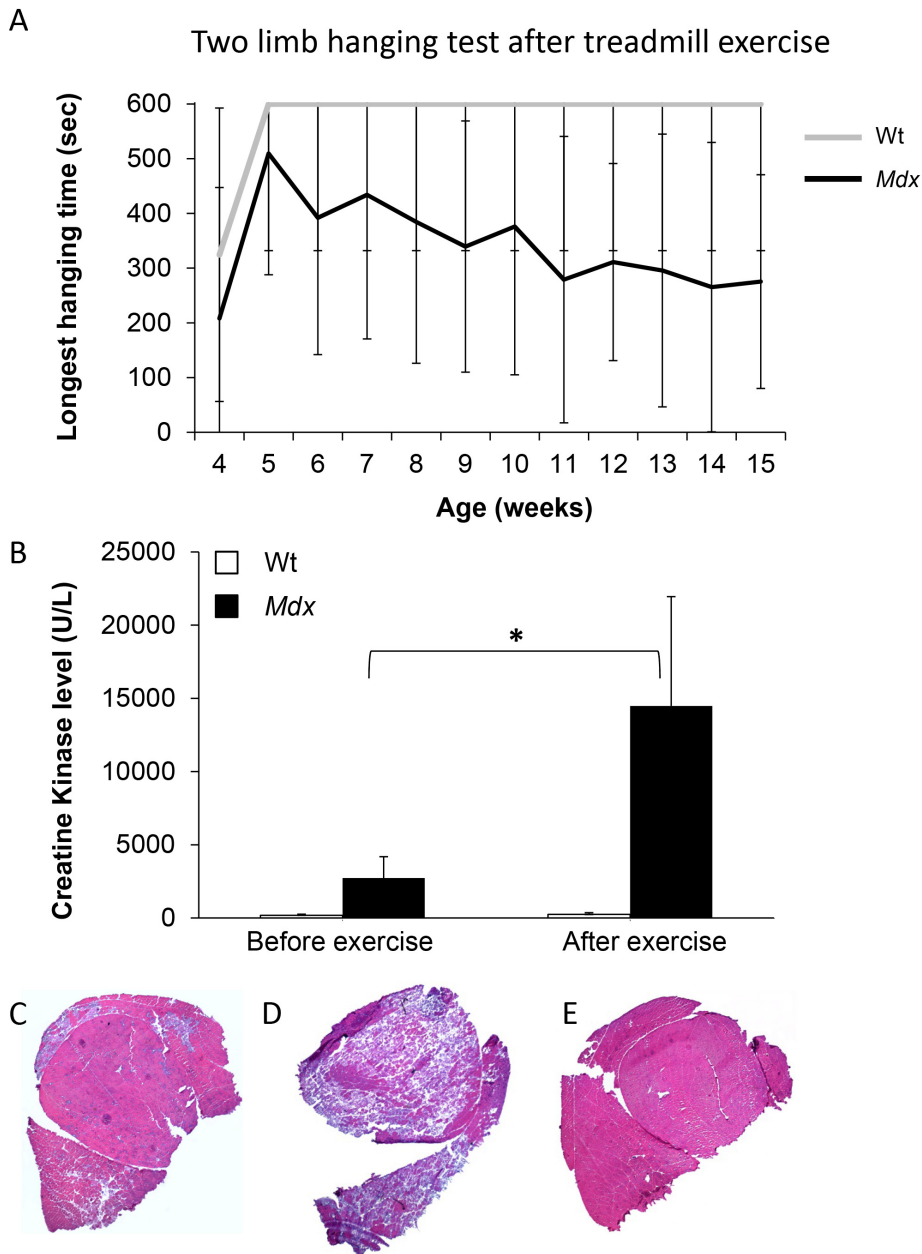


**Figure 2. Two limb hanging tests, representative results and appropriate and inappropriate hanging behavior.** **A.** A representative example of the two limb hanging test performed once weekly in male *mdx* (n=18, 4-10 weeks, n=13, 11 and 12 weeks, n=10, 13 weeks) and age and gender matched wild type mice (n=6). A learning curve is visible for both strains in the first few weeks of testing. Performance of *mdx* mice was worse compared to that of wild type mice. Data presented as mean±st.dev. Maximum hanging time allowed is indicated by the dotted line. **B.** The correct starting position of this test is with the two forepaws. **C.** Depending on the functional ability of the mouse it can also use the hindlimbs and tail. **D** and **E.** A small subset of mice, especially strong wild type mice, can occasionally avoid hanging by climbing on the side bars or balancing on the wire. Some mice intentionally jump off the wire. [Please click here to view a larger version of this figure.](#)



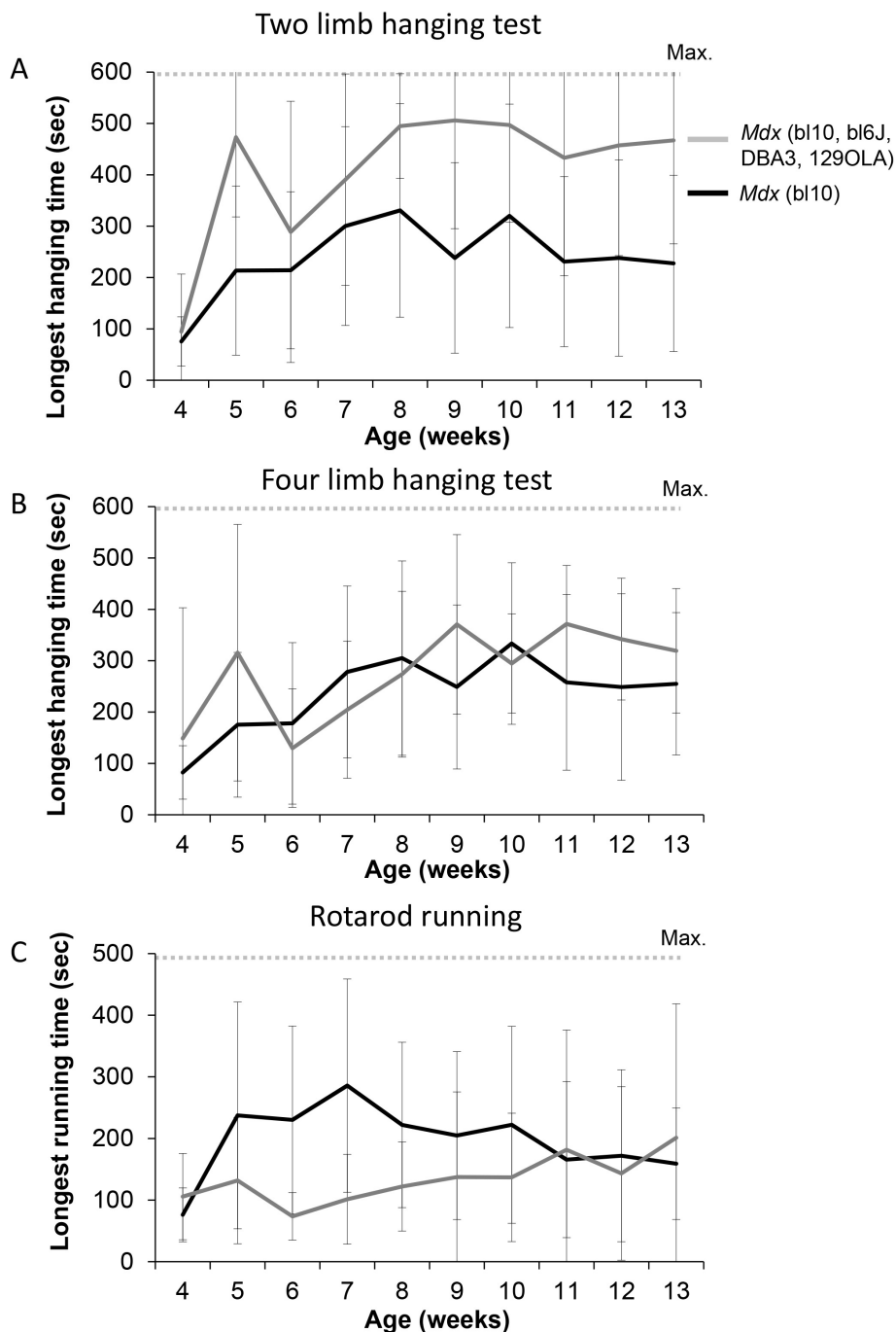


**Figure 3. Four limb hanging and rotarod running test.** **A.** Four limb hanging performance assessed once weekly in male *mdx* (n=18, 4-10 weeks, n=13, 11 and 12 weeks, n=8, 13 weeks) and wild type (n=6) mice. Over time, *mdx* mice hang less long than wild type mice. **B.** Rotarod running times did not differ between young male *mdx* (n=18, 4-10 weeks, n=13, 11 and 12 weeks, n=10, 13 weeks) and wild type mice (n=6). Data are represented as mean  $\pm$  st.dev.



**Figure 4. The effect of forced treadmill running exercise protocol on functional performance and skeletal muscle pathology in female mice.** Muscle pathology was deliberately exacerbated by letting mice run on a horizontal treadmill three times a week at 12 m/min for 30 min for a duration of 12 weeks. Directly after running, mice had to participate in the two limb hanging test. While all wild type mice ( $n=5$ ) remain hanging till the maximum allowed, all *mdx* mice ( $n=6$ ) fall off the wire earlier ( $p<0.001$ , data presented as mean $\pm$  st.dev.). **B.** The presence of membrane damage was determined by assessing plasma Creatine Kinase (CK) levels that leak out of muscle fibers through tears in the membrane. CK levels were elevated in *mdx* mice compared to wild type mice before exercise. Treadmill exercise immediately increased levels ( $p<0.01$  indicated by asterisk, data presented as mean $\pm$  st.dev.) in *mdx* mice, while they remained low in wild type mice. **C-D.** Muscles of *mdx* mice are very vulnerable to treadmill exercise, worsening disease pathology extensively after a few weeks of running. These Haematoxylin and Eosin stainings of the quadriceps of a 16 week old nonexercised (**C**) and treadmill exercised (**D**) *mdx* mouse show that extensive fibrosis and necrosis are developed. **E.** Muscles of wild type mice undergoing the same running protocol are not affected. [Please click here to view a larger version of this figure.](#)





**Figure 5. Effect of a mixed background on functional performance in *mdx* mice.** Differences in genetic background influence functional performance. To illustrate this, performance of male *mdx* (BL/10 background, n=18, 4-10 weeks, n=13, 11 and 12 weeks, n=10, 13 weeks) and *mdx* (mixed BL/10, BL/6J, DBA2 and 129OLA background, n=5) mice was compared over time. **A.** Two limb hanging test performance significantly differed between the two strains. **B.** Four limb hanging test results were slightly higher in the mixed background *mdx* mice. **C.** Rotarod running times also slightly differed between the strains. Data presented as mean± st.dev.

## Discussion

The functional tests presented here are reproducible, easy to perform and applicable to wild type and dystrophic mice independent of their age. The tests provide useful tools to pre clinically assess muscle function, strength, condition, and coordination. When testing the effects of a compound on the natural history of the disease, the noninvasive tests described here (forelimb grip strength, both hanging tests and the rotarod test) can be nicely combined in a functional test regime where these tests are performed on consecutive days. These protocols are not detrimental to *mdx* mice and can be used in a longitudinal manner<sup>10</sup>. It should be kept in mind that outcomes of each of these tests are generated by different or partly overlapping muscle groups instead of an individual muscle. Therefore, using a combination of multiple tests

is recommended to obtain a more complete picture and thereby better insight in the functionality of the experimental groups. Alternatively, functional improvements of a sole muscle can be assessed using muscle physiology measurements<sup>20</sup>.

Like behavioral tests, also functional tests can show extensive variation between different mice, or within a mouse between different assessments. To reduce variation, all tests should be performed by the same experimenter who is familiar with the mice. External variables like smells and sounds in the room, time of the day and the day of the week on which the test is performed should be kept as constant as possible. Mice should be gender and age matched. When using treadmill running to exacerbate disease progression, it is essential to use a standardized protocol in which all running parameters (running time, speed and slope) are kept constant over time for all experimental groups, so that all mice are equally treated. Although the majority of mice are keen to participate in the functional tests and most animals show high levels of willingness, some mice (primarily strong wild type mice) occasionally avoid performing the test and show avoidance behavior. When this behavior is not corrected for, false conclusions could be drawn<sup>21</sup>. Fortunately, these types of behavior are only observed occasionally and can be corrected for by placing the mouse back on the wire, grid or rotarod, or pulling another time on the grip strength meter.

Improvements in one functional test (e.g. hanging test assessing muscle function) does not necessarily have to co occur with improvements in another test (forelimb grip strength assessing sole muscle strength). In *mdx* mice, improvements in muscle function can be distinguished earlier than in muscle strength. This is also seen in DMD patients participating in clinical trials where clinically meaningful improvements in the 6 min walk test do not cooccur with improvements in muscle strength<sup>6,7</sup>. However, this may in part depend on the working mechanism of the compound tested and it is possible that other compounds improve strength and not function. Therefore, the results of the tests should be interpreted with the mechanism of action of the compound in mind.

## Disclosures

The authors have nothing to disclose.

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