Eccentric contractions lead to myofibrillar dysfunction in muscular dystrophy

Bert Blaauw,1,2 Lisa Agatea,2 Luana Toniolo,1 Marta Canato,1 Marco Quarta,1 Kenneth A. Dyar,2 Daniela Danieli-Betto,1 Romeo Betto,3 Stefano Schiaffino,2,3 and Carlo Reggiani1,3

1Department of Human Anatomy and Physiology, University of Padua, 2Venetian Institute of Molecular Medicine, and 3Consiglio Nazionale delle Ricerche Institute of Neuroscience, Padua, Italy

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Blaauw B, Agatea L, Toniolo L, Canato M, Quarta M, Dyar KA, Danieli-Betto D, Betto R, Schiaffino S, Reggiani C. Eccentric contractions lead to myofibrillar dysfunction in muscular dystrophy. J Appl Physiol 108: 105–111, 2010. First published November 12, 2009; doi:10.1152/japplphysiol.00803.2009.—It is commonly accepted that skeletal muscles from dystrophin-deficient mdx mice are more susceptible to damage than wild-type mice. However, the downstream mechanisms involved in this enhanced force drop remain controversial. We studied the reduction of contractile force induced by eccentric contractions elicited in vivo in the gastrocnemius muscle of wild-type mice and the reduction of contractile force induced by eccentric contractions in vitro and in vivo and have measured force generation in permeabilized fibers dissected from gastrocnemius muscle that had been exposed in vivo to eccentric contractions and from the contralateral unstimulated muscle. A force loss in skinned fibers after in vivo eccentric contractions was detectable in fibers from mdx and Sgca-null, but not wild-type and Col6al-null, mice. The enhanced force reduction in mdx and Sgca-null mice was observed only when eccentric contractions were elicited in vivo, since eccentric contractions elicited in vitro had identical effects in wild-type and dystrophic skinned fibers. These results suggest that 1) the enhanced force loss is due to a myofibrillar impairment that is present in all fibers, and not to individual fiber degeneration, and 2) the mechanism causing the enhanced force reduction is active in vivo and is lost after fiber permeabilization.

mdx; α-sarcoglycan-null; collagen 6a1-null; skinned muscle fibers

ELONGATION DURING CONTRACTION, i.e., eccentric contraction, is the condition that allows muscles and muscle fibers to reach the highest levels of developed tension, well above levels reached in isometric conditions (25). Repeated eccentric contractions, however, are accompanied by muscle damage, which is characterized by pain (known as delayed-onset muscle soreness), structural and ultrastructural alterations, and decreased force development (34). Many studies have described the damage induced by eccentric contractions in vitro and in vivo and have identified two main determinants: 1) myofibrillar disorganization (Z-line streaming and sarcomere length heterogeneity) and 2) calcium homeostasis alteration, which is possibly related to t-tubule disruption (1, 31).

Dystrophic muscles are more susceptible to damage induced by eccentric contractions. This was initially demonstrated in muscles from mdx, i.e., muscles lacking dystrophin, mice (24). The loss of force, as well as the cellular damage, shown by sarcolemmal disruption and Procion orange or Evans blue penetration is more pronounced in muscles from mdx than wild-type mice, particularly fast-twitch muscles (20, 24). Subsequent studies have extended the analysis to other murine models of muscle dystrophy, such as mice with a mutation of α2-laminin (16) and γ-sarcoglycan-deficient mice (14), where the drop of force is not greater than in muscles from wild-type mice, and α-sarcoglycan-deficient mice, which show a marked susceptibility to eccentric contractions (11). The results obtained in different studies, however, are often difficult to compare because of the diversity in the experimental protocols. Stimulation time, frequency, stretch speed and amplitude, time between repetitions, and repetition number vary widely from one study to another. Most studies have been performed in ex vivo or in vitro preparations, in which stretching protocols and initial lengths might be far from the physiological optimum. Indeed, despite a general consensus on the high susceptibility of mdx muscles to eccentric contractions, contradictory results have been reported (19, 26).

The mechanism responsible for the increased susceptibility to the damage caused by eccentric contractions in some dystrophies, but not in others, remains elusive. A potential explanation could be that the lack of dystrophin and the lack of the dystrophin-associated complex in mdx mice lead to a reduced mechanical resistance and, subsequently, sarcolemmal ruptures on exposure to the high stresses developed during eccentric contractions (24). These sarcolemmal ruptures would lead to an increased number of dysfunctional fibers, which would correspond to the increased number of Evans blue-positive fibers in these muscles after eccentric contractions (8). Indeed, when Lynch et al. (18) elicited eccentric contractions in single permeabilized fibers from mdx and wild-type animals, they found no intrinsic difference in myofibrillar resistance to stress, which led the authors to suggest that dystrophic symptoms do not arise from factors within the myofibrils. On the other hand, various other studies showed no correlation between the number of Evans blue-positive fibers and the force drop after eccentric contractions (3, 27), suggesting a more subtle mechanism.

In the present study, we comparatively analyze four groups of mice: wild-type mice; mice of two dystrophic strains, mdx and α-sarcoglycan-null mice, in which increased susceptibility to eccentric contractions has been reported; and mice with null mutation of the gene coding for a subunit of collagen 6, which were not previously studied for this specific property (17). The aim of this study was to identify the contribution of the myofibrillar damage to the loss of force due to eccentric contractions. We used a well-defined protocol to elicit eccentric contractions in the gastrocnemius muscle in vivo, taking care that eccentric contractions were elicited on the ascending limb of the force-length curve to ensure that the damage was...
caused by the eccentric contractions, and not by excessive elongation of the muscles (1). To identify whether the reduction in force was due to an impairment of myofibrillar function, we determined isometric force in skinned single fibers from the gastrocnemius muscle exposed to eccentric contractions compared with fibers from the contralateral, unstimulated, leg. Furthermore, we compared the effects of eccentric contractions applied directly on permeabilized single fibers.

METHODS

Animals. Mice of four different strains were compared: C57BL/6J, used as the wild-type strain, and mdx, Sgca-null, and Col6a1-null, with defective expression of dystrophin, α-sarcoglycan, and collagen 6α1, respectively. C57BL6/J and mdx mice were purchased from Charles River, Col6a1-null mice were a gift of P. Bonaldo (University of Padua), and Sgca-null mice were a gift of E. Engvall (Burnham Institute, San Diego, CA). The age, sex, and body size of the mice studied are reported in Table 1. All mice were between 3 and 6 mo of age. At the beginning of the experiments, mice were anesthetized by 1:1 zolazepam-tiletamine (10 mg/kg; Zoetelit 100, Laboratoire Virbac) and 2% xylazine (0.06 ml/kg; Rompum, Bayer). Once responsiveness was completely absent, a small incision was made from the knee to the hip to expose the sciatic nerve, and electrodes for electrical stimulation were implanted. Eccentric contractions were induced according to the protocol described below. At the end of the experiment, mice were killed by cervical dislocation, and gastrocnemius muscles were carefully removed, weighed, and frozen in isopentane cooled with liquid nitrogen for further analysis. The study was approved by the competent authority of the University of Padua and authorized by the Italian Ministry of Health.

In vivo gastrocnemius mechanics. The contractile performance of the gastrocnemius muscle was measured in vivo using a muscle-lever system (model 305B, Aurora Scientific) in mice anesthetized as described above. Mice were placed on a thermostatically controlled table, the knee was kept stationary, and the foot was firmly fixed to a footplate, which was connected to the shaft of the motor.

Contraction was elicited by electrical stimulation of the sciatic nerve. Teflon-coated seven-stranded steel wires (AS 632, Cooner Sales, Chatsworth, CA) were implanted with sutures on either side of the sciatic nerve proximal to the knee before its branching. At the distal ends of the two wires, the insulation was removed, and the proximal ends were connected to a stimulator (model S88, Grass). To avoid recruitment of the dorsal flexor muscles, we cut the common peroneal nerve.

The torque developed during isometric contractions was measured at stepwise increasing stimulation frequency, with ≥20-s pauses between stimuli to avoid effects due to fatigue. Duration of the trains never exceeded 600 ms. Force was calculated from the torque, by measurement of the distance between the Achilles tendon insertion and the ankle, which was carefully aligned with the motor axis. Force-length curves were determined by rotation of the foot stepwise by 7°, which equals 1 mm, in the interval between isometric contractions. An ankle angle of 75° was taken as a reference position (3), and a range of 45–90° was explored. As shown by Gruner et al. (13), the working range of the rodent ankle during locomotion is 36–120°.

Eccentric contractions were induced to study muscle damage, as described elsewhere (3). Muscle lengthening was achieved by movement of the foot at a velocity of 40 mm/s during stimulation of the gastrocnemius muscle with a frequency sufficient to induce full tetanic fusion (100 Hz). The elongation was started 200 ms after initiation of the stimulation train; thus eccentric pull occurred during the isometric plateau of the tetanus. The amplitude of the rotation during the pull was 30°, clearly inside physiological limits of movement for the foot. Total duration of tetanic stimulation was limited to 600 ms, ensuring no sag of force. This protocol was repeated 20 times with 20-s pauses, and the decrease in the isometric force plateau (measured before initiation of the stretch) was taken as an indication of muscle damage. At the end of the protocol, the mouse was killed, and the gastrocnemius muscle was quickly dissected.

Histology. Muscle structure was examined using hematoxylin and eosin staining. Cryostat sections are fixed in 4% paraformaldehyde, placed in Harris hematoxylin, dipped briefly in alcoholic acid, and incubated for 1 min in eosin. Hematoxylin stains the acidic regions (nuclei), and eosin stains the basic regions (cytoplasm).

In vitro mechanics on gastrocnemius skinned fibers. From the gastrocnemius muscle exposed to eccentric contractions and from the contralateral unstimulated muscle, a fragment of the superficial layer was dissected and immersed in ice-cold skinning solution. Single fibers were manually dissected from fiber bundles under a stereomicroscope (×10–20 magnification). At the end of the dissection, fibers were bathed for 30 min in skinning solution containing 1% Triton X-100 to ensure complete membrane solubilization. Segments (1–2 mm long) were then cut from the fibers, and light aluminum clips were applied at both ends. In each fiber segment, isometric tension was measured during maximal activations at 20°C, pCa 4.8, at an initial sarcomere length of 2.75 μm. This sarcomere length was chosen to ensure that, in the presence of an elongation of the series compliance of ~5% (30), sarcomere length during contraction reached ~2.6 μm, which is at the upper edge of the plateau of the tension-length relationship (10, 28). Digitized images of each fiber were obtained with a camera connected to the microscope at ×360 magnification. Cross-sectional area was calculated from the average of three diameters, spaced at equal intervals along the length of the fiber segment, with the assumption that the segment was circular. The compositions of the solutions (skinning, relaxing, preactivating, and activating) are described elsewhere (23).

We adopted the following protocol to study the effect of eccentric contraction applied directly to skinned fibers in vitro. In each fiber segment, isometric tension was measured during two maximal activations (pCa 4.8) at 20°C with an initial sarcomere length of 2.75 μm. Two eccentric contractions were induced by stretching the fiber segment by 10% at 0.15 l/s for 0.7 s during maximal activation. With the internal shortening during the isometric phase taken into account, sarcomere length reached 2.85 μm at the end of the stretch phase. After the two eccentric contractions, two maximal activations were induced in isometric conditions. To determine the damage to the fiber, isometric tension generated in two activations after the two stretches was expressed in relation to isometric tension before the two stretches.

Statistical analysis. Values are means ± SE. Comparison between data from wild-type and dystrophic mice was carried out with Student’s t-test; ANOVA followed by Newman-Keuls test was used to compare dystrophic strains among each other. Statistical significance was set at P < 0.05.

RESULTS

Muscle weight and force in different animal models. Data from mice from three distinct murine models of muscular

Table 1. Body and muscle weight

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>mdx</th>
<th>Sgca-null</th>
<th>Col6a1-null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Male</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>26 ± 2</td>
<td>25 ± 1</td>
<td>33 ± 2</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Gastrocnemius muscle wt, mg</td>
<td>136 ± 7</td>
<td>106 ± 9</td>
<td>191 ± 7</td>
<td>156 ± 7</td>
</tr>
<tr>
<td>Muscle wt-to-body wt ratio, mg/g</td>
<td>5.2 ± 0.6</td>
<td>6.6 ± 0.6*</td>
<td>5.9 ± 0.7</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Age, mo</td>
<td>3–6</td>
<td>3–6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Wt, wild-type mice; mdx, dystrophin-lacking mice. Sgca-null, α-sarcoglycan-null mice; Col6a1, collagen 6α1-null mice. *Significantly different from wild-type.
dystrophy (mdx, Col6a1-null, and Sgca-null) were compared with results from wild-type mice, which were used as a reference. Age and body size of the animals are reported in Table 1, together with the weight of the gastrocnemius muscles, which were utilized in all functional analyses. Muscles from all dystrophic mice were slightly larger than those from wild-type mice, but when gastrocnemius muscle weight was normalized to body weight, only the muscle-to-body weight ratio of mdx mice was significantly greater than that of wild-type mice. This represents a pathological hypertrophy, which has been described in previous studies (7). The age of the animals was chosen such that all studies were performed in adult animals in which the pathological phenotype was well established. No differences were observed between wild-type and mdx mice at 3–6 mo of age.

Table 2 shows the force developed after single (twitch) or repeated (fused tetanus) stimulations normalized to the muscle weight. A typical force-frequency curve is shown in Fig. 1A; it extends from the twitch, at the lower left end, to the fused tetanus, in the upper right region. All dystrophic muscles showed a similar normalized force significantly lower than muscles from wild-type mice, except Col6a1-null mice, which developed significantly higher tetanic tension (29.3 ± 1.4 kN/kg) than mdx or Sgca-null mice (25.9 ± 1.0 and 23.6 ± 1.0 kN/kg, respectively).

Drop of force after in vivo eccentric contractions. The relationship between the length changes imposed on the gastrocnemius muscle before the onset of stimulation and the isometric force, i.e., the force-length curve, is shown in Fig. 1B–D, whereas the impact of length changes during contraction (eccentric contraction) is shown in Fig. 2. Elongation of ~4 mm, corresponding to ~30° dorsal rotation of the foot on the ankle joint, determined a ±5% change in isometric force, with no differences between wild-type and dystrophic muscles (Fig. 1B–D). The same elongation applied during the contraction, i.e., in an eccentric contraction, caused force to increase by ~30% (Fig. 2A). The potentiation effect produced by the eccentric contraction was, however, followed by a decrease of the isometric force in the next isometric tetanus. The progressive decline of isometric tension during the sequence of 20 eccentric contractions is shown in Fig. 2B–D. At the end of the sequence (Fig. 2E), the decline reached 14% in gastrocnemius muscles from wild-type mice, whereas it was much greater in muscles from mdx (38%) and Sgca-null (35%) mice. In Col6a1-null mice, the decline was not significantly different from that in wild-type mice (18%).

Eccentric contractions lead to reduced myofibrillar force production in skinned fibers. To better understand the mechanism underlying the greater force drop in some muscular dystrophies, we measured the isometric force generation of single skinned fibers from the gastrocnemius muscle. Since it has been reported that skinned fibers from extensor digitorum longus muscles from mdx and wild-type mice show no difference in their susceptibility to eccentric contractions (18), we first determined the force drop of skinned fibers exposed to elongation during contraction in vitro. As shown in Fig. 3A, the force drop after two eccentric contractions in vitro was 10–15% in all strains, with no significant differences between wild-type and dystrophic mice, confirming the previous report.

We recently reported that the force drop in vivo after eccentric stimulation of the gastrocnemius muscle of mdx mice was accompanied by a reduction of the tension generated in vitro by the fibers dissected from the same muscle (3). As shown in Fig. 3B, no significant difference in the isometric tension generated during maximal activations was observed between skinned fibers dissected from the gastrocnemius muscle exposed to eccentric contraction in vivo and those dissected from the contralateral, unstimulated, gastrocnemius muscle in wild-type mice. In mdx mice, on the other hand, isometric tension was significantly (28 ± 13%) reduced in fibers from the previously eccentrically stimulated muscle compared with fibers from the contralateral gastrocnemius muscle. As shown in Fig. 3C, also in Sgca-null mice, isometric tension was decreased 31 ± 15% in fibers from eccentrically stimulated muscle compared with fibers from contralateral muscle. When the same comparison was repeated for Col6a1-null mice, no difference was found in isometric tension production by stimulated and unstimulated fibers (Fig. 3D).

Finally, we compared isometric tension generation in skinned fibers dissected from gastrocnemius muscle not ex-
posed to eccentric contractions among the four murine strains. Although no significant difference in tension production was detectable among wild-type, Sgca-null, and mdx muscles, fibers dissected from Col6a1-null mice were significantly weaker (Fig. 3E). This is particularly interesting in light of the fact that, when measured in vivo, the isometric tension produced in the gastrocnemius muscle was significantly higher in Col6a1-null than in Sgca-null and mdx mice (Table 2).

DISCUSSION

In this study, we designed a protocol of eccentric contractions in vivo, and we used it to compare the susceptibility to stretch-induced damage of the gastrocnemius muscle from three different models of muscular dystrophy: mdx, Sgca-null, and Col6a1-null mice. The results confirm that a series of eccentric contractions cause a greater drop in isometric force (35–38%) in muscles from mdx and Sgca-null than wild-type mice. Interestingly, in the newly studied Col6a1-null mice, which are also affected by a severe dystrophy (17), the loss of muscle force was not greater than in wild-type controls (14–18%).

To better understand the mechanism of force reduction, we analyzed the direct effect of elongation during contraction in single skinned muscle fibers. If skinned fibers were elongated during contraction, isometric force in subsequent activations decreased. Such a decrease in the ability to develop tension was similar in muscles from the wild-type mice and the three dystrophic strains of mice. This suggests that the mechanism underlying the contractile impairment in skinned fibers is the same in healthy and dystrophic mice and very likely is related to myofibrillar cytoskeletal damage and subsequent sarcomere instability (18, 21). In contrast, when skinned fibers were dissected from muscles that had undergone eccentric contractions in vivo, isometric force was lower in mdx and Sgca-null mice, i.e., the strains that show in vivo a higher susceptibility to damage caused by elongation during contraction, than in
Col6a1-null and wild-type mice. This outcome can be interpreted by distinguishing two components of the loss of force caused by elongation during contraction in vivo: 1) a component, present in all four strains and tentatively quantified as 15–20% of initial isometric force, that disappears without leaving any trace, i.e., without causing impaired contractile performance, in skinned fibers, and 2) a component, present only in mdx and Sgca-null mice, that can be estimated at another 15–20% (which implies a total force loss of 35–40% in vivo) and is detectable in skinned fibers in the form of a reduction of the contractile force. Component 1, which was present in dystrophic, as well as healthy, fibers and disappears in skinned fibers, might be localized at the level of the intracellular calcium signaling, inasmuch as it is completely ruled out by the direct activation with calcium employed in skinned fibers. Evidence of an involvement of t tubules or other components of the calcium-controlling system is available in the literature (2). Component 2 was limited to some specific dystrophic strains, mdx and Sgca-null, although it was not present in Col6a1-null mice. The fact that component 2 was detectable in skinned fibers gives a clue about its identification as a component that results in structural damage at the myofibrillar level that is observed in vivo and remains detectable in vitro.

In Sgca-null mice, the dystrophin complex is perturbed, leading to an even more severe pathology than in mdx mice (9). The pathological picture includes reduced membrane integrity, increased muscle mass, and impaired muscle function (6). Membrane preparations of Sgca-null mice show an increased activity of calcium-activated proteases, such as calpains (9), suggesting an increase in intracellular calcium concentration, most likely due to the reduced membrane integrity, which leads to their translocation from the cytoplasm to the membrane (29). Calpains are mainly responsible for the initial cleavage of myofibrillar proteins, after which the actual degradation occurs through another system, most likely the proteasome (15).

Furthermore, eccentric contractions can activate calpains in healthy muscle (22), suggesting a possible mechanism through which stretch during contraction in vivo leads to reduced tension production in skinned fibers by modifications of the myofibrils. In mdx mice, oxidative stress has been shown to play a role in the force drop after eccentric contractions, inasmuch as treatment with an antioxidant reduced the susceptibility to eccentric damage (32). In addition, in a transgenic model overexpressing Akt in the mdx mouse, we previously showed that force drop after eccentric contractions is reduced (3), and we provided evidence that protection from force loss is associated with an increased expression of antioxidants together with an upregulation of proteins involved in the Z disk and costameres. Taken together with the fact that blockers of stretch-activated calcium channels during eccentric contractions reduce the force drop (33), it becomes tempting to assume that, in mdx mice as well, an excessive entry of calcium leads to a cascade of events leading to increased oxidative stress and myofibrillar dysfunction. Thus the high susceptibility to damage caused by eccentric contractions, in muscles from Sgca-null and mdx mice, is likely due to increased calcium ion entry and reactive oxygen species production and not an intrinsic weakness of the myofibrils, because, in agreement with a previous report (18), skinned fibers from mdx mice are not more susceptible to eccentric contractions performed in vitro.

The finding that eccentric contractions in vivo in mdx and Sgca-null mice cause long-lasting structural damage at the myofibrillar level that is detectable in single skinned fibers has an interesting implication. The tension drop observed in whole muscles in vivo (present study) or ex vivo (24) is likely not due to the sudden loss of a small number of fibers that no longer produce tension but, more likely, is due to a general decrease in tension of the majority of fibers. This finding can help explain the discrepancy in the literature between the number of Evans blue-positive fibers after eccentric contractions in mdx mice and the decrease in muscle force (8, 24). The decrease in force after eccentric contractions is two- to threefold higher than expected on the basis of the number of Evans blue-positive fibers, and in some studies no increase in the number of Evans blue-positive fibers was found (4, 5). This would suggest that mechanical stress increases in most, if not all, fibers, leading to a general decrease in myofibrillar function, whereas the negative cascade of events leads to fiber permeabilization and dye penetration in only a few fibers.

The susceptibility to stretch-induced damage of Col6a1-null mice was examined here for the first time and found to be similar to that of wild-type mice. The pathological phenotype of Col6a1-null mice is due to the mitochondrial impairment, which follows the lack of an extracellular matrix component, a subunit of type VI collagen (17). In this pathology the function and structure of dystrophin and its related proteins are unaltered, suggesting that disruption of the dystrophin complex is required to increase the susceptibility to eccentric contraction. This view is confirmed by the fact dyj2 mice, which lack another extracellular matrix component, laminin, are also resistant to eccentric contraction (16). In addition, study of the gastrocnemius muscle of the Col6a1-null mice revealed another interesting aspect of this dystrophy. The maximal isometric tension generated by skinned fibers under control conditions was not different among the various models studied, with the exception of Col6a1-null mice, which were characterized by fibers significantly weaker than those from wild-type, mdx, and Sgca-null mice (Fig. 3E). This was expected in view of previously published work (17), but it was somewhat surprising, because force produced by the gastrocnemius muscle in vivo was slightly, but significantly, higher in Col6a1-null than in Sgca-null and mdx mice (Table 2). This can be explained by the fact that, despite the increased apoptosis and ultrastructural defects (17), fiber turnover is not as frequent in Col6a1-null as in Sgca-null and mdx mice, as indicated by the number of central nuclei, a sign of regeneration (see supplemental Fig. 1 in the online version of this article). This implies that even though most fibers have a reduced tension-generating capacity, they remain viable and contribute to muscle force. In muscles from Sgca-null and mdx mice, on the other hand, fiber turnover is rapid and fibers with a reduced contractile function are rapidly removed; thus the number of fibers contributing to muscle force is reduced.

It is worthwhile to emphasize the importance of the design of an appropriate experimental protocol. Descriptions of the effects of stretch applied during contraction on force development in skeletal muscles of different dystrophic models are abundant in the literature. Differences in train duration, stimulation frequency, stretching protocol, time between repetitions, and the number of repetitions complicate the interpretation of the results. Most experiments have been performed in...
ex vivo conditions, in which stretching protocols and optimum length are not always comparable to physiological conditions, making the relevance of the damage induced by stretch for dystrophic pathogenesis questionable. Indeed, contradictory results have been reported, even regarding the susceptibility to eccentric contractions of the mdx mouse, which is definitely the most studied model (19, 20, 24). In view of these discrepancies, we designed a standardized protocol to be applied to different models of muscular dystrophy. The protocol is based on a sequence of 20 eccentric contractions in vivo, sufficiently spaced to avoid fatigue. The contractions were elicited by nerve stimulation, and care was taken not to exceed the physiological range of movement. In rodents, the range of rotation of the ankle is 36–120° (13). The range explored in our protocol, 45–90°, was inside the ascending limb of the tension-length curve (Fig. 1), in agreement with gastrocnemius sarcomere length measurements in situ, which show a working range of 1.8–2.4 μm (12). The protocol, therefore, allows us to separate the effects of elongation from the generation of sarcomere instability, which occurs along the descending limb of the tension-length curve (25) and likely does not show diversity between dystrophic and healthy muscle fibers. Next, analysis of contractile performance of skinned fibers immediately prepared from the muscles exposed to the elongations in vivo provides a tool to verify whether eccentric contractions had long-lasting or structural effects. Importantly, the protocol could be used not only to compare wild-type with dystrophic mouse, but also to achieve a novel quantitative comparison between different dystrophic models.

In conclusion, in the present study, a newly designed experimental protocol based on eccentric contractions in vivo, in combination with in vitro force measurements on skinned fibers, was used to evaluate quantitatively the amount of functional damage induced by stretch. The great force drop after eccentric contractions seen in two murine models of muscle dystrophy, mdx and Sgea-null mice, was partly due to a reduced contractile function of the myofibrils. Importantly, this impaired myofibrillar function was not directly attributable to exceedingly high mechanical stress but was caused by structural alterations that occurred in vivo.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


