Molecular, cellular, and muscle strip mechanics of the mdx mouse diaphragm

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Submitted 28 June 2012; accepted in final form 15 February 2013

DUCHENNE MUSCULAR DYSTROPHY (DMD) is an inherited X-linked recessive disorder affecting 1 in ~3,300 live male births (12). Dystrophin is a large cytoskeletal protein located on the inner cytoplasmic surface of skeletal and cardiac muscle cell membranes (41, 43). The dystrophin-glycoprotein complex connects the actin cytoskeleton to the extracellular matrix of the cell (43) and plays an important role in cell sarcomembrane stability during muscle contraction (32). In DMD, its absence causes a progressive loss of muscle mass (19), leaving those afflicted with the disease wheelchair bound by early adolescence. DMD has a 100% mortality rate, mostly due to respiratory or cardiac muscle failure in the second to third decade of life (12).

Mechanical damage (32) is thought to lead to the muscle fiber necrosis seen early on in DMD. The early necrosis is counterbalanced by myofiber regeneration (3, 34). However, for reasons that are still unclear, an imbalance ensues between the two processes and fiber loss gets the upper hand (15), causing muscle tissue to gradually get replaced by adipose and fibrous connective tissues (3, 11). This loss of functional muscle mass is generally considered to be the main cause of the progressive muscle weakness seen in DMD patients (8, 33).

Dystrophic mouse (C57Bl/10 mdx) diaphragm undergoes a gradual and significant loss of muscle tissue accompanied by a decrease in maximal isometric tetanic force (Pt). However, studies performed in mice from 6 wk to 2 yr of age have shown that the loss of muscle tissue does not fully account for the loss of Pt, which has led researchers to look elsewhere for the mechanism behind this excessive muscle weakness (9, 10, 28, 33). Among the possible explanations for these observations are muscle fiber structural abnormalities (deformed fibers, complex fiber branching, etc.) observed in the dystrophic mdx mice, which compromise force generation (18). Another possibility to explain the muscle weakness seen in DMD patients is that the contractile proteins themselves suffer from alterations due to the lack of dystrophin (8, 9, 33). One key feature of mdx muscle cells is elevated levels of intracellular calcium. This can lead to increased activity of calcium-dependent proteases, such as calpains, which can cleave myofibrillar proteins, directly impairing muscle motor function (1, 36, 42). Alternatively, in the absence of dystrophin, neuronal nitric oxide synthase is delocalized from the subsarclemma and floats freely, in reduced quantities, in the cytoplasm. Because myosin is highly susceptible to damage from oxidative stress (2, 35, 38), speculations have arisen regarding the potential role of damaged myosin in DMD muscle weakness. Coirault et al. (9) used an in vitro motility assay to compare the maximal velocity (vmax) of actin propulsion by myosin purified from 9-mo-old mdx and control mouse diaphragms. They found a significant decrease in vmax for the mdx myosin compared with control myosin, which was accompanied by a change in the myosin heavy chain (MHC) isofrom composition. As of yet, there are no studies that have addressed the force-generating capacity of myosin molecules from the mdx mouse diaphragm.

In the current study, we hypothesized that in muscular dystrophy, the myosin molecular motor is altered, leading to muscle weakness at the molecular, single cell, and muscle bundle levels. Thus we measured the force-generating capacity of mdx mouse diaphragm at all these levels. Experiments were performed in 3-mo-old mice, when no shifts in myosin isofrom expression are expected, and in 12-mo-old mice, to assess the myosin function at different stages of the disease.

METHODS

Animals

All experimental protocols involving the use of animals were approved by the McGill University Animal Care Committee and...
compiled with the guidelines of the Canadian Council on Animal Care. Male C57Bl/10 and C57Bl/10 mdx mice were purchased from Jackson Laboratories or obtained from our colony, originally purchased from Jackson Laboratories, and housed in a McGill University animal care facility before experimentation. All animals were killed by exsanguination at 11–13 wk or 12 mo of age following anesthesia with pentobarbital sodium (55 mg/kg).

**Muscle Strip Mechanics**

Diaphragms were dissected from 3-mo-old control and mdx mice and quickly transferred to oxygenated Krebs-Henseleit (K-H) solution (120 mM NaCl, 4.5 mM KCl, 2.5 mM MgSO₄, 1.2 mM KH₂PO₄, 25.5 mM NaHCO₃, 10 mM glucose, and 2.5 mM CaCl₂, pH 7.4). Thin strips were cut (3.5 × 7 mm) with intact fiber attachments to the rib cage and central tendon, and mounted in a horizontal tissue bath filled with oxygenated K-H solution (33). To determine the optimal voltage for muscle strip electric field stimulation, strips were activated by single twitches for 200 μs at successively higher voltages (from 5 to 70 V) at 1-min intervals until maximum force was achieved. This optimal voltage was maintained to determine the optimal length (L₀) for force production as follows. Muscle length was changed gradually within a range of −5 to +50% of its in situ length until a maximum force was achieved. The optimal voltage and L₀ were maintained throughout the experiments, and a force-frequency relation was determined for each muscle strip. The strips were stimulated for 1 s at 2-min intervals and frequencies ranging from 40 to 150 Hz. P₂₅ was defined as the highest force value obtained and was normalized to the muscle strip cross-sectional area (CSA), yielding specific force. To obtain the CSA, the nonmuscle tissue was removed and the muscle strip blotted dry before being weighed. Total CSA in centimeters squared was calculated as follows:

\[
CSA = m/(L_0 \cdot p)
\]

where \( m \) is the mass measured in grams, \( L_0 \) is measured in cm, and \( p \) is the muscle tissue density, i.e., 1.06 g/cm³ (7, 33).

**Cellular Mechanics**

Small diaphragm strips from 3-mo-old control and mdx mice were dissected, tied on wood sticks, and chemically permeabilized following standard procedures (22, 29). The muscle strips were incubated in rigor solution for ~4 h, after which they were transferred to a rigor-glycerol (50:50) solution for 15 h. They were subsequently placed in a fresh rigor-glycerol (50:50) solution with the addition of a cocktail of protease inhibitors (Roche Diagnostics) and stored at −20°C for at least 7 days. On the day of the experiment, a muscle strip was transferred to a fresh rigor solution and stored at 4°C for 1 h before use. A small section of the sample was cut and single fibers were carefully dissected in relaxing solution.

The fiber was gripped at each end with T-shaped foil clips, transferred to a temperature controlled chamber, and attached between a force transducer (model 403A; Aurora Scientific, Toronto, Canada; a force transducer (model 403A; Aurora Scientific, Toronto, Canada; and a length controller (model 312B; Scientific 901A, Toronto, Canada)). Images from a selected region of the fibers were used to calculate SL by fast Fourier transform analysis, based on the striation spacing produced by dark and light bands of myosin and actin, respectively. The fiber diameter and length were measured using a second CCD camera (Go-3; QImaging; pixel size: 3.2 μm), and the CSA was estimated assuming circular symmetry. Passive length adjustments were made to set the fiber at an initial SL of 2.5 μm before activation.

Before and after the shortening/stretching protocol, the fibers were subjected to a quick step stretch (0.5% L₀) for measurements of stiffness (K), calculated as Δforce/Δlength. If fibers sustained significant damage during activation, as evaluated by a decrease in force (>5%), they were discarded from the study.

Indexes of the cross-bridge cycling rate towards a strongly bound state were estimated using a previously described method (5, 6, 21, 37). Briefly, the rate constants for force development (Kact) and redevelopment after the shortening-restretch protocol (Kdes), were calculated by fitting the force transients to a single exponential equation as follows:

\[
F = a(1 - e^{-ct}) + b
\]

where \( t \) is time, \( a \) is the amplitude of the exponential force development, \( b \) is the initial force value, and \( c \) is the rate constant for force development, i.e., \( K_{act} \) or \( K_{des} \).

**Molecular Mechanics**

**Protein purifications.** Actin was purified and polymerized from chicken pectoralis acetone powder according to Pardee and Spudich (31) and labeled for fluorescence with tetramethylrhodamine isothiocyanate phalloidin (TRITC P1951; Sigma). Three- and twelve-month-old control and mdx mouse diaphragms were removed with the ribs still attached, and the latter were then cut away in ice cold saline solution, followed by a myosin purification protocol optimized for small samples according to Palmiter et al. (30). All molecular mechanics measurements were performed within 48 h of purification to ensure sample freshness and myosin functionality. To perform the in vitro motility mixture assay (see below), a reference myosin with a different in vitro velocity from that of the myosin of interest is needed. Thus chicken pectoralis (CP) myosin was purified following a large-scale purification protocol (20) with the exception that the final dilution was done in 0.6 M KCl buffer to obtain monomeric myosin. The protein concentration was determined by a standard Bradford assay (Bio-Rad, Ontario, Canada).

\( v_{max} \): Determination by in vitro motility assay. Myosin buffer consisted of 300 mM KCl, 25 mM imidazole, 1 mM EGTA, 4 mM MgCl₂, and 60 mM dithiothreitol (DTT), pH 7.4; actin buffer: 25 mM KCl, 25 mM EGTA, 4 mM MgCl₂, and 60 mM DTT, pH 7.4; and methylcellulose buffer: 0.5% methylcellulose, 2 mM ATP di Na, 25 mM KCl, 25 mM imidazole, 1 mM EGTA, 4 mM MgCl₂, and 60 mM DTT.
pH 7.4. An oxygen scavenger (0.16 mg/ml glucose oxidase, 0.045 mg/ml catalase, and 5.75 mg/ml glucose) was added to the actin and methylcellulose buffers.

The in vitro motility assay was performed as previously described (26). Briefly, nonfunctional myosin molecules, defined as noncycling and binding to actin in a rigor-like manner, were removed by ultracentrifugation (24) whereby myosin (0.5 mg/ml) was mixed with an equimolar quantity of filamentous actin and 1 mM MgATP and spun for 31 min (4°C, 42,000 rpm, 42.2Ti rotor). The supernatant (functional myosin) was collected, perfused into a flow-through chamber (40), and allowed to attach to the nitrocellulose-coated coverslip for 2 min. BSA (0.5 mg/ml) was then perfused into the chamber, followed by unlabeled monomeric actin (1.33 μM) to bind and thus inhibit any remaining nonfunctional myosin, followed by actin buffer containing 1 mM ATP to wash out the excess unlabeled actin (25). This was then followed by actin buffer washes, actin (0.03 μM) fluorescently labeled with tetramethylrhodamine isothiocyanate phalloidin (TRITC P1951; Sigma) in actin buffer that was left to incubate for 1 min, and two washes of methylcellulose buffer containing ATP (2 mM) to initiate movement.

Actin filament movement was recorded by a CCD camera (Hitachi, KP-E500), digitized at 29.94 Hz (custom-built computer by Norbec Communication, Montreal, QC, Canada; Pinnacle Studio DV/AV V.9 PCI Capture Card, AMCap software V9.20). The velocity of actin propulsion (v_\text{max}) was determined from the total path followed by the filaments divided by the elapsed time using our automated software based on the National Institutes of Health filament tracking software, coded in Matlab (R2009b).

**Myosin force determination by in vitro motility mixture assay.** To determine the relative force of myosin purified from control and mdx mouse diaphragm, we used the in vitro motility mixture assay (17, 40). Unlike the regular in vitro motility assay, two different myosin types, with two different v_\text{max}, are mixed and perfused in the flow-through chamber to interact simultaneously with the actin filaments. The resulting v_\text{max} of different ratios of the two types of myosin is studied, and the results are plotted as v_\text{max} vs. myosin type ratio (17, 40). The shape of the resulting curve depends on the relative force of the two myosin types, i.e., a concave up curve means that the faster myosin is weaker whereas a concave down curve means that the faster myosin is stronger than the slow myosin (17, 40). Because the control and mdx mouse diaphragm myosins propel actin at similar velocities, they were each studied separately when mixed with CP myosin, which itself is faster than the diaphragm myosins.

**Assessment of myosin degradation using the in vitro motility assay.** To compare the level of degradation of mdx and control diaphragm myosin, the in vitro motility assay was modified to measure the length of actin filaments. That is, because degraded nonfunctional myosin binds in a rigor-like manner to actin, the pulling action of the remaining functional myosin results in broken actin filaments. The purified but noncentrifuged control and mdx myosin samples were diluted to 0.25 mg/ml in myosin buffer. Unlabeled actin was not used because that step hinders the effect of the broken myosin. The average actin filament length was quantified in the presence of control and mdx myosins, using our automated software based on the National Institutes of Health filament tracking software, coded in Matlab (R2009b).

**Myosin electrophoresis.** Because the myosin isoforms I and IIb exhibit very different v_\text{max} in the in vitro motility assay, alterations in their expression would affect the molecular mechanics. Thus, we performed electrophoresis on 7% (3 mo) and 5% (12 mo) SDS polyacrylamide minigels, which were run in a Bio-Rad Mini-Protean II Dual Slab Cell electrophoresis setup using a Laemmli buffer system. Each well was loaded with 10 μg of sample, and the gels were run for 2 h at 100 V at room temperature. Gels were stained with Coomassie blue, and the relative proportion of the type I and type IIb MHC isoforms was quantified by densitometry using a Fluorchem 8500 imaging system with AlphaEase software (Alpha Innotech).

### RESULTS

**Muscle Strip Mechanics**

As previously reported in the literature, the *mdx* mice used in this study exhibited diaphragm weakness at the muscle strip level at 3 mo of age. A lower stress was observed in the *mdx* mice (0.23 kg/cm² ± 0.01) compared with controls (0.69 kg/cm² ± 0.11; *P < 0.05*) (Fig. 1).

**Single Fiber Mechanics Results**

Typical fiber shortening/restretching traces of 3 mo-old mice are shown in Fig. 2, A and C, in which a rapid drop in force followed by force redevelopment towards a new equilibrium state can be observed. The stress produced during the full contraction, before the shortening/stretch protocol, was not different between control and mdx fibers (Table 1). The values of K_\text{act} and K_\text{tr} between the two groups either (Fig. 2, B and D; Table 1), suggesting that cross-bridge kinetics and the cycling rate in transitions from weakly- to strongly-bound states are not altered in *mdx* mice. To ascertain that the number of cross-bridges attached to actin was the same throughout the shortening/restretching period, stiffness was measured before and after the mechanical maneuver. Stiffness was not different between control and *mdx* mice, with similar changes of 4.2 ± 1.8 and 4.7 ± 1.3%, *P = 0.828*, respectively.

**Data Analysis**

All data are reported as means ± SE. Differences in P, v_\text{max}, actin filament length, and MHC composition were tested by unpaired t-tests. For molecular force measurements, a linear regression was fit with the following as predictors: categorical control, mdx, and the interaction between the two. To test the significance of the interactions, a Wald χ² test was performed. For cellular force measurements, comparisons of stress, stiffness, K_\text{act} and K_\text{tr} between the two groups before and after the shortening/restretching protocol were performed using a two-way ANOVA for repeated measurements. A significance level of *P < 0.05* was used for all analyses. At the tissue level, the value of n is the number of mice per group. At the fiber level, n is the number of fibers. At the molecular level, the value of n is the number of mice; for each mouse, several experiments were performed for each of which several filaments were analyzed (see figure legends for details).

**Fig. 1. Stress [maximum isometric tetanic force (P_t) normalized to cross-sectional area], of control and dystrophic mouse diaphragm. *P = 0.014; n = 3 mice/group.**
**Molecular Mechanics**

The in vitro motility assay was used to assess $v_{\text{max}}$ of the control and mdx mouse diaphragm myosin. For the 3-mo-old mice, $v_{\text{max}}$ of the mdx myosin (1.14 ± 0.08 μm/s) was not different from the control myosin (1.38 ± 0.12 μm/s; $P = 0.118$; Fig. 3A). Similarly for the 12-mo-old mice, $v_{\text{max}}$ was not different between the control myosin (0.591 ± 0.09 μm/s) and mdx myosin (0.482 ± 0.02 μm/s; $P = 0.29$; Fig. 3B).

**Relative force generation of control and mdx myosins.** The relative force of control and mdx myosin was assessed using the in vitro motility mixture assay. The shape of each curve indicated that both control and mdx myosins generated more force than CP myosin for the 3-mo-old (Fig. 4A) and the 12-mo-old (Fig. 4B) mice. Regression analysis of the $v_{\text{max}}$ vs. percentage of CP myosin curves demonstrated that there was no difference between control and mdx myosin at 3 mo ($P = 0.447$; Fig. 4A) nor between control and mdx myosin at 12 mo ($P = 0.212$; Fig. 4B). These results indicate that at 3 mo the control and dystrophic myosins generate the same force; this is also the case for the 12-mo control and dystrophic myosins.

**Assessment of myosin degradation using the in vitro motility assay.** To ascertain that the myosin from mdx mouse diaphragm was not degraded, we measured the actin filament length in the in vitro motility assay (actin gets broken down in the motility assay in the presence of degraded myosin). No differences were observed between actin filament length quantified in the presence of control (2.13 ± 0.05 μm) and mdx (2.0 ± 0.03 μm; $P = 0.107$; Fig. 5) myosin. These data suggest that our molecular mechanics measurements were not affected by excessive degradation of mdx myosin and its removal by centrifugation.

**Myosin electrophoresis.** The myosin type I and IIB composition of mdx and control diaphragms from 3- and 12-mo-old animals was assessed by SDS-PAGE gels and densitometry. The diaphragm samples consisted mainly of MHC types IIA and IIX, with lesser amounts of types I and IIB. Thus to resolve the type I and IIB bands properly, more sample had to be loaded so that the IIA and IIX bands became saturated. They were thus left out of the quantification. At 3 mo (Fig. 6, A and B), no difference was observed in the relative proportion of type I (51.7 ± 0.6% for mdx and 52.3 ± 0.9% for control) or type IIB (48.3 ± 0.6% for mdx and 47.7 ± 0.9% for control; $P = 0.568$). At 12 mo (Fig. 6, C and D), no difference was observed in the relative proportion of type I (55.5 ± 2.1% for

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**Table 1. Single fiber mechanics**

<table>
<thead>
<tr>
<th></th>
<th>Stress, mN/mm²</th>
<th>$K_{\text{v}}$</th>
<th>$K_{\text{r}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 24 muscle fibers)</td>
<td>25.9 ± 2.0</td>
<td>3.0 ± 0.4</td>
<td>14.6 ± 1.4</td>
</tr>
<tr>
<td>mdx (n = 22 muscle fibers)</td>
<td>21.1 ± 1.6</td>
<td>3.0 ± 0.5</td>
<td>13.4 ± 1.6</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.242</td>
<td>0.991</td>
<td>0.562</td>
</tr>
</tbody>
</table>

Values are means ± SE. Stress, rate of force development ($K_{\text{v}}$), and rate of force redevelopment ($K_{\text{r}}$) in muscle fibers isolated from control and mdx mouse diaphragms.
DISCUSSION

In this study, we demonstrated that the molecular motor myosin is not functionally impaired in the mdx mouse diaphragm at 3 and 12 mo of age and, therefore, does not appear to play an important role in diaphragm muscle weakness. No shift in MHC isoform composition was found in mdx animals, and $v_{\text{max}}$ was not significantly different between the two groups. More importantly, there was no difference in force between the control and mdx diaphragm myosins at either 3 or 12 mo of age. Furthermore, we showed that in the 3-mo-old mice, at the single permeabilized fiber level, there is no reduction in force in the mdx diaphragm compared with control. Only at the intact muscle strip level did we find a significant reduction in specific force of the mdx diaphragm compared with control, in accordance with the results of others (10, 28, 33).

A shift in the MHC isoform composition of mdx mouse diaphragm compared with control has previously been reported by Coirault et al. in 6 and 9 mo-old animals (8, 9). They found a shift from fast type IIx to type IIa. However, because the maximum velocity of shortening of these two fiber types does not differ significantly at the whole muscle level, this shift is not expected to affect $v_{\text{max}}$ (4, 13, 23). Furthermore, measurements performed at the sarcomeric level suggest that these two isoforms also generate the same force (14). Based on these assumptions, we focused our efforts on the less prominent isoforms that would be most likely to affect $v_{\text{max}}$ significantly, types I and IIb. Unlike Coirault et al. (9), we loaded large quantities of myosin onto our gels, effectively saturating the IIa and IIx bands, in an effort to make visible the other two isoforms. Thus we could not analyze the IIa and IIx bands and chose to omit them in our relative quantification of the type I and IIb, i.e., we quantified them with respect to their sum and not to the total myosin isoforms.

Coirault et al. (9) also reported a lower $v_{\text{max}}$ in the motility assay of mdx compared with control myosin. We found no such difference at either 3 or 12 mo of age. However, the difference that they reported ($1.9 \pm 0.1$ vs. $1.2 \pm 0.1\ \mu\text{m/s}$, for control and mdx myosins, respectively) was relatively small (30%) and such a difference at the molecular level has not yet been shown to translate into a difference at the whole muscle level.
level, these differences are not observed at the single perme-

gle muscle fibers did not yield any difference in stress or rate

with 3-mo-old control and older mice.

force as myosin from control diaphragm in young as well as

that myosin from the dystrophic diaphragm generates the same

with our direct measurements. Hence we show for the first time

change in force-generating capacity is largely in agreement

phragm at the molecular level. This prediction of a minimal

Coirault et al. (8) have also previously used Huxley’s model

and inferred indirectly from muscle strip level measurements

there were no differences in isoform composition.

an index of cross-bridge cycling rate (5, 6, 21, 37). Thus, while mechanical differences are observed

between mdx and control diaphragms at the intact muscle strip

level, these differences are not observed at the single perme-

abilized cell or molecular levels. Note that our fiber level

contractility studies obviously selected for the nonnecrotic

fibers because the noncontractile ones would have been elimi-

ated as potentially damaged during the dissection procedure.

This allows us to conclude that nonnecrotic fibers in mdx

diaphragms are mechanically behaving normally. Furthermore,

whereas no other cellular mechanics measurements have yet

been performed with the diaphragm, a study looking at the

contractility of single extensor digitorum longus muscle fibers

also reported no difference in force between control and mdx at

21 days (27).

The control and mdx velocity values were lower at 12 than

at 3 mo. Whereas it has previously been demonstrated that the

isoform composition of the mouse diaphragm shifts towards

the slower type I isoform when the animals have reached 2 yr

of age (33), we did not observe such a shift at 1 yr to explain

the slower velocities that we found in our study. The in vitro

motility assay is sensitive to the buffer conditions and slight

changes have enormous effects on $v_{\text{max}}$. Thus our 3- and

12-mo-old mouse data were not compared with each other

because they were not collected simultaneously, and so, there

was a possibility that the buffers used were not identical and

differences in $v_{\text{max}}$ would have been misinterpreted. Neverthe-

less, there were no differences in $v_{\text{max}}$ and in molecular force

between mdx and control mice at 3 mo or at 12 mo of age, nor

was there any difference in isoform composition.

An important step in the molecular mechanics assays is the

centrifugation of myosin before its use. This ensures that most

nonfunctional myosins are removed so that they do not form

rigor-like bonds with actin and shred the filaments apart when

other functional myosin molecules pull on the same filaments.

If the mdx myosin is altered or damaged and rendered non-

functional, as defined by rigor-like bonds, due to the absence of

dystrophin, it might be removed from the sample along with

the nonfunctional myosin during this centrifugation step. Thus

Fig. 5. Actin filament length measured in the presence of noncentrifuged control and mdx diaphragm myosin to compare the level of nonfunctional myosin between the 2 samples; $n = 3$ mice per group (1,222 filaments were analyzed for control and 1,170 for mdx); $P = 0.107$. See METHODS for details.

Fig. 6. Electrophoresis and densitometry of myosin heavy chain (MHC) isoform composition of 3- and 12-mo-old control and dystrophic mouse diaphragms. A: electrophoresis of 3-mo-old control and mdx myosin. CP myosin was used as a positive control. B: quantification of the MHC isoforms present in 3-mo control (closed bars) and mdx (open bars) diaphragm; $n = 8$ mice/group; $P = 0.568$. C: electrophoresis of 12-mo control and mdx myosin purifications. CP myosin was used as a positive control. D: quantification of the MHC isoforms present in 12-mo control (closed bars) and mdx (open bars) diaphragm; $n = 5$ control mice and $n = 4$ mdx mice; $P = 0.905$. 

AJP-Cell Physiol • doi:10.1152/ajpcell.00220.2012 • www.ajpcell.org
the mechanical effect of the mdx myosin could remain silent because it would have been eliminated in the spin-down. Furthermore, whereas velocity is not dependent on myosin concentration above a certain threshold (16, 39), within which the above experiments were performed, force is and could be greatly affected if the mdx myosin was being removed from our sample. Thus it was important to check these possibilities. In addition, although we hypothesized that mdx myosin would be damaged and thus weaker, others (35) have shown that myosin exposed to peroxyrinite, a strong oxidizing agent, was slower in the in vitro motility assay yet stronger in the mixture assay than control myosin. Such a mechanical effect of peroxynrinite would also go undetected if damaged or dysfunctional mdx myosin was eliminated during the ultracentrifugation step. Therefore, additional in vitro motility measurements were made with noncentrifuged control and mdx myosin samples to assess whether actin filament breakage was occurring more in the presence of mdx than control myosin; shorter actin filaments would indicate a greater quantity of dysfunctional/nonfunctional myosin. The average actin filament length was not found to differ significantly between control and mdx myosin samples. Taken together, these results demonstrate that the control and mdx myosins have similar amounts of nonfunctional myosin and that potential alterations due to conditions inside the mdx cells do not alter myosin structure and function.

Conclusion

In this study, we reported the first molecular and single cell force measurements performed in mdx mouse diaphragm. We demonstrated that while muscle weakness in the mdx mouse diaphragm manifests at the intact muscle strip level, the function of the molecular myosin and the single permeabilized muscle cell mechanics are not affected. Thus our data do not support the hypothesis that dysfunctional myosin or single muscle fiber mechanics underlie the muscle weakness observed in DMD.

GRANTS

This work was supported by grants from the Natural Science and Engineering Council of Canada and the Canadian Institutes of Health Research. The Meakins-Christie Laboratories are supported in part by a center grant from Le Fonds de la Recherche en Santé du Québec and by the J. T. Costello Memorial Research Fund.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.B., S.S., L.K., N.B.Z., D.R., and A.-M.L. concep-

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