Branched fibers in dystrophic mdx muscle are associated with a loss of force following lengthening contractions

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Chan S, Head SI, Morley JW. Branched fibers in dystrophic mdx muscle are associated with a loss of force following lengthening contractions. Am J Physiol Cell Physiol 293: C985–C992, 2007. First published June 13, 2007; doi:10.1152/ajpcell.00128.2007.—We demonstrated that the susceptibility of skeletal muscle to injury from lengthening contractions in the dystrophin-deficient mdx mouse is directly linked with the extent of fiber branching within the muscles and that both parameters increase as the mdx animal ages. We subjected isolated extensor digitorum longus muscles to a lengthening contraction protocol of 15% strain and measured the resulting drop in force production (force deficit). We also examined the morphology of individual muscle fibers. In mdx mice 1–2 mo of age, 17% of muscle fibers were branched, and the force deficit of 7% was not significantly different from that of age-matched littermate controls. In mdx mice 6–7 mo of age, 89% of muscle fibers were branched, and the force deficit of 58% was significantly higher than the 25% force deficit of age-matched littermate controls. These data demonstrated an association between the extent of branching and the greater vulnerability to contraction-induced injury in the older fast-twitch dystrophic muscle. Our findings demonstrate that fiber branching may play a role in the pathogenesis of muscular dystrophy in mdx mice, and this could affect the interpretation of previous studies involving lengthening contractions in this animal.

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder characterized by progressive wasting of skeletal muscle, affecting 1 in 3,500 live male births. It is caused by the absence of dystrophin, a 427-kDa protein that, in normal muscle fibers, is found just internal to the sarcolemma. Controversy still surrounds the function of dystrophin and the mechanisms by which its absence leads to the skeletal muscle degeneration seen in DMD (4, 10). There are several hypotheses as to the role played by dystrophin in skeletal muscle. For simplicity, we have grouped them under two headings: 1) the structural hypothesis (20) and 2) the ion channel hypothesis (5).

1) In the structural hypothesis, the absence of dystrophin leaves the membrane weakened and susceptible to tearing during muscle contraction, leading to irreversible fiber damage followed by necrosis. Support for this theory comes from well-established evidence that the fast-twitch muscles of the dystrophin-deficient mdx mouse are more easily damaged by lengthening contractions (contractions with stretch) than are normal muscles (see Table 1).

2) In the ion channel hypothesis, the absence of dystrophin is suggested to lead to the pathological function of a sarcomeric ion channel, and several ion channels have been singled out as promising candidates for the primary cause of the pathology in dystrophin-deficient dystrophies (for review, see Ref. 1). The majority of these studies hypothesize that the end consequence of the proposed channelopathy is an increased intracellular flux of Ca²⁺, and this increase in intracellular Ca²⁺ concentration results in fiber damage through mediators such as proteases and reactive oxygen species (23). It is important to note that, in the ion channel hypothesis, the absence of dystrophin does not chronically weaken the sarcolemma; rather it disrupts ion channel function.

The etiology of the muscle degeneration in mdx mice is complicated by the fact that, as mdx muscle ages, the architecture of the dystrophic muscle fibers becomes grossly abnormal (13). These abnormal fibers, termed branched or split fibers, are more prone to damage during contraction, as high shear stresses occur at branch points during intense contractile activity, leading to fiber rupture at these points. Direct evidence demonstrating the increased fragility of branched fibers was presented in Ref. 12, where it was demonstrated that, when individual fibers were stimulated, fiber segments that contained branch points were more liable to rupture than fiber segments without branch points, and when whole muscles were stimulated, branched fibers were preferentially damaged over unbranched fibers within the muscle. The question arises, then, as to whether the increased susceptibility of mdx muscles to injury during lengthening contractions is due directly to the absence of dystrophin, or whether the morphological changes (branching) of fibers leads to weakened regions (branch points) that are the site of damage during contraction.

The aim of our study was to address this question by examining mdx mice in two age groups: a “younger” group ~1–2 mo old, in which fiber branching was moderate (~20%), and an “older” group ~6–7 mo old, in which fiber branching was more extensive (>80%). We hypothesized that the “older” group would experience a greater loss of force than the “younger” group following a mild protocol of lengthening contractions. By examining the association between the extent of fiber branching and the susceptibility to injury, we attempted to gain some insight into the role of branched fibers in the pathogenesis of muscular dystrophy in the mdx mouse.

METHODS

Animals used. The mdx mice with littermate controls were obtained from the Animal Resources Centre (Perth, Australia). Female C57BL/10ScSn-DMD (mdx) mice were mated with male C57BL/10ScSn/H11001 mice. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
FIBER BRANCHING AND CONTRACTILE DAMAGE

Table 1. Force deficits of normal and mdx fast-twitch muscles following lengthening contractions

<table>
<thead>
<tr>
<th>Study</th>
<th>Ref. No.</th>
<th>Age of Mice, wk</th>
<th>Strain, %</th>
<th>Muscle</th>
<th>Force Deficits, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>McArdle et al. (1991)</td>
<td>17</td>
<td>5–6</td>
<td>30</td>
<td>EDL</td>
<td>Normal 93, mdx 94</td>
</tr>
<tr>
<td>Sacco et al. (1992)</td>
<td>22</td>
<td>16–26</td>
<td>In situ*</td>
<td>Tibialis anterior</td>
<td>44, 48</td>
</tr>
<tr>
<td>Head et al. (1992)</td>
<td>13</td>
<td>&gt;45</td>
<td>12</td>
<td>EDL</td>
<td>2, 66‡</td>
</tr>
<tr>
<td>Moens et al. (1993)</td>
<td>18</td>
<td>~3–70</td>
<td>~8</td>
<td>EDL</td>
<td>13, 38‡</td>
</tr>
<tr>
<td>Petrof et al. (1993)</td>
<td>20</td>
<td>12–15</td>
<td>10</td>
<td>EDL, diaphragm</td>
<td>29, 57‡</td>
</tr>
<tr>
<td>Deconinck et al. (1996)</td>
<td>9</td>
<td>16</td>
<td>7</td>
<td>Gastrocnemius</td>
<td>20, 64‡</td>
</tr>
<tr>
<td>Deconinck et al. (1998)</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>EDL</td>
<td>10, 41‡</td>
</tr>
<tr>
<td>Grange et al. (2002)</td>
<td>11</td>
<td>1–2</td>
<td>10</td>
<td>EDL</td>
<td>23, 27</td>
</tr>
<tr>
<td>Raymackers et al. (2003)</td>
<td>21</td>
<td>12</td>
<td>7</td>
<td>EDL</td>
<td>19, 69‡</td>
</tr>
<tr>
<td>Consolino and Brooks (2004)</td>
<td>6</td>
<td>20</td>
<td>18†</td>
<td>EDL</td>
<td>15, 40‡</td>
</tr>
</tbody>
</table>

Strain is the length by which the muscle is stretched, as a percentage of its original length. Higher force deficits are taken as indicators of a greater degree of muscle damage. *The length of stretch could not be measured in this study, as the muscle was not dissected out. Instead, the muscle was stretched by moving the foot. †A variety of strains was used in this study. Only the results for the middle strain are shown. ‡Significant difference in force deficit between mdx and normal.

The offspring of this first mating were then mated together. The male offspring of this second mating comprise a colony of mdx mice and littermate controls sharing a common genetic background, and it is this colony that was used in this study. Littermate control mice were distinguished from mdx mice on the basis of serum creatine kinase (CK) levels. Mice with CK < 1,000 U/l were classified as controls, while mice with CK > 1,000 U/l were classified as mdx. Western blotting for the presence of dystrophin has shown this to be an ultrareliable method for phenotyping the mice in this colony (15). Phenotype was further confirmed when muscle fibers were examined by confocal microscopy; mdx fibers have many centrally located nuclei, while almost all nuclei in control fibers are peripherally located.

In all, 13 mice were used for the experiments assessing contractile properties, contraction-induced damage, and fiber morphology. These consisted of six “younger” mice aged 6–8 wk and seven “older” mice aged 27–31 wk. Immediately before experimentation, animals were anesthetized with halothane and killed by cervical dislocation. Use of animals was approved by the University of New South Wales Animal Care and Ethics Committee.

Muscle preparation. The extensor digitorum longus muscle was dissected from the hindlimb and tied by its tendons to a force transducer (World Precision Instruments, Fort 10) at one end and a linear tissue puller (University of New South Wales) at the other, using silk suture (Deknatel 6.0). The muscle was placed in a bath continuously superfused with Krebs solution, with composition as follows (in mM): 4.75 KCl, 118 NaCl, 1.18 KH2PO4, 1.18 MgSO4, 24.8 NaHCO3, 2.5 CaCl2, and 10 glucose, with 0.1% fetal calf serum, and continuously bubbled with 95% O2–5% CO2 to maintain pH at 7.4. The muscle was stimulated by delivering an electrical current between two parallel platinum electrodes, using an electrical stimulator (A-M Systems). At the start of the experiment, the muscle was set to its optimum length (L0) by finding the length that produced maximum twitch force. All experiments were conducted at room temperature (~22–24°C).

In total, 23 muscles were used, 11 in the “younger” group (5 controls and 6 mdx) and 12 in the “older” group (4 controls and 8 mdx).

Lengthening contraction protocol. The lengthening contraction protocol is illustrated in Fig. 1. At time = 0 ms, the muscle was stimulated by supramaximal pulses of 1-ms duration and 100-Hz frequency. At time = 750 ms, after it had attained its maximum isometric force, the muscle was stretched at a speed of 1 mm/s until it was 15% longer than its L0, held at this length for 2 s, then returned at the same speed to its original position. The electrical stimulus was stopped at time = 5,000 ms. This lengthening contraction was performed three times, at intervals of 5 min. The strain of 15% of muscle length was equivalent to a strain of 33% of fiber length, assuming that fiber length is 45% of muscle length (2).

A mild strain of 15% was chosen because preliminary experiments indicated that this strain did not damage control muscles of younger mice (unpublished data). This would allow us to determine whether muscles from mdx mice were more susceptible to damage. An excessively severe strain would substantially damage all muscles, thus obscuring differences in fragility between mdx and control muscles. The lengthening contraction protocol was initiated at a similar iso-
metric force plateau in all groups tested to ensure a similar absolute force was experienced by all the muscles.

**Force measurement.** Muscle force was measured using a force-frequency curve, an example of which is shown in Fig. 2. The muscle was stimulated for 500 ms at different frequencies (5, 15, 25, 37.5, 50, 62.5, 75, 87.5, and 100 Hz), and maximum force was recorded at each frequency of stimulation. A curve relating the muscle force $P$ to the stimulation frequency $f$ was then fitted to these data. The curve had the following equation (19):

$$P = P_{\text{min}} + \frac{P_{\text{max}} - P_{\text{min}}}{1 + \left(\frac{K_f}{f}\right)^h}$$

The values of the parameters $P_{\text{min}}, P_{\text{max}}, K_f$, and $h$ were outputs of the fitting procedure, and their meaning in relation to the force-frequency curve is illustrated in Fig. 2. $P_{\text{max}}$ is the force developed at minimum stimulation frequency; $P_{\text{max}}$ is the force developed at maximum stimulation frequency; $K_f$ is the frequency at which the force developed is halfway between $P_{\text{min}}$ and $P_{\text{max}}$; and $h$ is known as the Hill coefficient. In this study, the values of $r^2$ for the fitting procedure were not lower than 99.6%.

Three contractile properties of the muscle were determined from the fitted parameters of the force-frequency curve: the maximum tetanic force ($P_{\text{max}}$), the twitch-to-tetanus ratio ($P_{\text{min}}/P_{\text{max}}$), and the half-frequency ($K_f$).

One force-frequency curve was obtained immediately before the lengthening contraction protocol. Twenty minutes after the final lengthening contraction, the setting of the $L_o$ was repeated, and then a second force-frequency curve was obtained. Muscle damage was assessed functionally, by comparing the above-mentioned contractile properties before and after the contraction protocol. Indicators of damage were the percentage fall in $P_{\text{max}}$, the percentage change in $K_f$, and the percentage change in $P_{\text{min}}/P_{\text{max}}$. The primary indicator of damage was the percentage fall in $P_{\text{max}}$, which will be referred to as the force deficit.

To facilitate comparison between different muscles, forces are expressed as force per cross-sectional area (units mN/mm²). Cross-sectional area was calculated by dividing the muscle’s mass by the product of its $L_o$ and the density of mammalian muscle (1.06 mg/mm³).

**Muscle stiffness.** To assess differences in stiffness between muscles, we analyzed the change in force during the ramp phase of the first lengthening contraction in each muscle. Some muscles reached a force that exceeded the capacity of the force transducer during the ramp phase, so we limited our analysis to the first part of the ramp (until the muscle reached 108% of $L_o$). Stiffness was assessed by dividing the percent change in force over this time by the percent change in length.

**Statistical analyses.** Analyses were conducted using two-way ANOVA. The null hypothesis was that the effect of dystrophin deficiency is the same in both “younger” and “older” mice; that is, the effect of dystrophin deficiency is independent of age. Posttests, comparing $mdx$ with control within each age group, were performed.
Muscle fiber morphology. Immediately following experimentation, the muscles were digested to yield individual fibers. The solution used for the digestion was Krebs solution containing 3 mg/ml collagenase Type I (Sigma) and 1 mg/ml trypsin inhibitor (Sigma), continuously bubbled with 95% O2–5% CO2 and maintained at 37°C. After ~30 min, the muscles were removed from this solution, rinsed in Krebs solution, and placed in a relaxing solution with the following composition (concentrations in mM): 117 K+, 36 Na+, 1 Mg2+, 60 HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), 8 ATP, 50 EGTA2− (ethylene glycol-bis(β-aminoethyl ether)-N,N′,N′,N′-tetraacetic acid), and free Ca2+ of 10−7 M. The free calcium was determined after titrating the solution with CaCl2 to determine the excess EGTA. The muscle was then gently agitated using pipette suction, releasing some individual fibers from the muscle mass.

Individual fibers were examined either with a light microscope (Olympus BX60) or a laser-scanning confocal microscope (Leica TCS SP). The extent of fiber branching was assessed by counting the number of fibers that were branched and, for those fibers that were branched, counting the number of branches.

RESULTS

Length, mass, and cross-sectional area. Muscles from older mice (~6–7 mo old) were longer than muscles from younger mice (~1–2 mo old), but there were no differences in length between mdx and controls in each age group (Fig. 3A). In younger mice, mdx muscles were similar to control muscles in both mass and cross-sectional area, while in older mice, the mass and cross-sectional area of mdx muscles were ~60% higher than those of control muscles (Fig. 3, B and C). Over the approximate 5-mo period separating the two age groups of mice used in this study, the mass and cross-sectional area of littersmate control muscles increased by ~5%/mo, whereas those of mdx muscles increased by ~15%/mo. Hence, in our new colony of dystrophin-deficient mdx mice with littersmate controls, dystrophin deficiency is associated with a higher rate of growth in the physical bulk of muscles. Similar findings were reported for the original colony of mdx mice compared with age-matched wild-type controls from a separate colony (16).

Contractile properties before lengthening contractions. The contractile properties of the muscles before lengthening contractions are shown in Fig. 4. Figure 4A shows the Pmax expressed as an absolute force (not corrected for cross-sectional area). The absolute forces of control and mdx muscles were similar in each age group. However, when corrected for cross-sectional area, the specific force produced by mdx muscles was significantly lower than that for controls in each age group (Fig. 4B). In younger mice, the specific force produced by mdx muscles was ~15% lower than that for controls, and in older mice ~30% lower than that for controls. In both age groups, the difference was statistically significant. These results show that, as the mdx animal ages, the impairment of specific force generation becomes more marked, although the animal is able to compensate for this by muscle hypertrophy, thus maintaining absolute forces at normal levels. The values of specific force obtained here are comparable to those obtained in other studies (for example, in study from Ref. 6: 235 mN/mm² for control, 173 mN/mm² for mdx, in extensor digitorum longus muscles of ~20-wk-old mice).

The twitch-to-tetanus ratios were similar across all of the muscles studied (Fig. 4C) and were comparable to the value of ~0.2 normally found in mammalian muscle (14).

Figure 4D shows the Kf, which is the stimulation frequency at which the force generated was halfway between the twitch force and the Pmax. This is an indicator of the muscle’s responsiveness to increases in frequency. A higher Kf means that the force-frequency curve has shifted to the right (see Fig. 2), so that the muscle needs higher stimulation frequencies to produce the same amount of force. The Kf of mdx muscles was similar to that of controls in each age group. However, older muscles had higher Kf than younger muscles, suggesting that, as the animal grows older, its muscles become less responsive to increases in frequency.

Damage following lengthening contractions. The muscles were subjected to a lengthening contraction protocol of 15% strain. A mild strain was used so that any differences in fragility between normal and dystrophic muscles could be determined. Various contractile properties were measured before and after the contractions, and the changes in these
properties were used as functional indicators of the extent of damage. The results are shown in Fig. 5.

The force deficit, or the reduction in $P_{\text{max}}$ following lengthening contractions, was the primary indicator of muscle damage in this study (Fig. 5A). The force deficit is the most reliable and reproducible indicator of damage following lengthening contractions (3). In younger mice, the force deficit for $mdx$ muscles ($7.3 \pm 4.8\%$, $n = 6$) was not significantly different from the force deficit for control muscles ($-1.2 \pm 3.5\%$, $n = 5$), suggesting that dystrophin deficiency did not increase the susceptibility of younger muscles to contraction-induced damage. However, in older mice, the force deficit for $mdx$ muscles ($58.0 \pm 5.0\%$, $n = 8$) was significantly higher ($P < 0.001$) than the force deficit for controls ($24.8 \pm 5.3\%$, $n = 4$), suggesting that dystrophin deficiency did increase the susceptibility of older muscles to contraction-induced injury. Hence the effect of dystrophin deficiency on a muscle’s vulnerability to damage was dependent on age, with the effect being more pronounced at older ages.

Our secondary indicators of muscle damage were the change in twitch-to-tetanus ratio and the change in $K_f$ following lengthening contractions. For the twitch-to-tetanus ratio, the change in ratio for older $mdx$ muscles was opposite in direction to the change in ratio for all of the other muscles (Fig. 5B). However, none of the comparisons made between muscles was statistically significant. For the $K_f$, the change in older $mdx$ muscles was opposite in direction from the changes in all other muscles (Fig. 5C). The $K_f$ decreased in older $mdx$ muscles, whereas in all other muscles it increased. In this case, the comparison between $mdx$ and control in the older age group was statistically significant ($P < 0.001$). These secondary measures of muscle damage suggest that the older $mdx$ muscles have been affected differently from all of the other muscles and add further to the suggestion from the force deficit results that older $mdx$ muscles are more susceptible to injury.

Muscle fiber morphology. No branched fibers were found among the 151 fibers (63 younger, 88 older) examined from littermate control muscles. The results for $mdx$ muscles are shown in Fig. 6. Each of the fibers was categorized according to the number of branch points it contained (none, 1, 2, 3, or 4+). Figure 6A shows the proportion of fibers in each category, whereas Fig. 6B shows the absolute numbers in each category. Of the 176 $mdx$ fibers (106 younger, 70 older) examined, only 17% of the younger $mdx$ fibers contained branch points, whereas 89% of the older $mdx$ fibers were branched (Fig. 6A). Not only did older $mdx$ muscles contain more branched fibers than younger $mdx$ muscles, they also had more branch points on these fibers. The branched fibers in young $mdx$ muscle usually had just one branch point on a fiber, while the branched fibers in older $mdx$ muscle usually had multiple branch points on a fiber.

In addition to having more branched fibers and more branches per fiber, the branching patterns in older $mdx$ fibers were also more complex than those in younger $mdx$ fibers, as is evident from comparing Fig. 7 with Fig. 8. There appeared to be three basic patterns of branching: 1) a small branch leaving the main fiber, as in Fig. 7C; 2) the main fiber dividing into two similarly sized branches, as in Fig. 8A; and 3) the two branches rejoining into one fiber again, as in Fig. 7D. The branches in younger $mdx$ fibers are shorter and smaller than in the older $mdx$ fibers. In addition, some older $mdx$ fibers

![Fig. 5. Damage following lengthening contractions.](http://ajpcell.physiology.org/)
displayed branching so complex that it was difficult to classify them into any of the above patterns.

Muscle stiffness. To ascertain whether the differences between muscles in their susceptibility to damage might be due to differences in the stiffness of the sarcomeres, we analyzed the ramp phase of the lengthening contractions, measuring the change in force as the muscle was stretched from 100 to 108% of its $L_o$. The results are displayed in Fig. 9, which shows the percent increase in force for each 1% increase in length. No significant differences were found between $mdx$ and controls in either age group. However, older muscles showed significantly larger increases in force than younger muscles, in both $mdx$ and controls, suggesting that the sarcomeres of older muscles have more stiffness than those of younger muscles.

DISCUSSION

We have demonstrated that a lengthening contraction protocol of 15% strain causes more damage to muscles of $mdx$ mice aged 6–7 mo than to muscles of $mdx$ mice aged 1–2 mo, relative to age-matched littermate controls. Our results demonstrate that dystrophin deficiency has little effect on the vulnerability of the younger muscles to injury; however, it significantly increases the vulnerability of the older muscles to injury.

Why should dystrophin deficiency have a greater effect on skeletal muscle’s vulnerability to damage as age increases? One possible reason, suggested by our fiber morphology results, is that the fiber branching associated with the dystrophic process becomes more extensive as the dystrophic animal ages. It has been demonstrated previously that fibers containing branches are more liable to be damaged during contraction than fibers without branches (12). Compared with younger $mdx$ muscles, older $mdx$ muscles had more branched fibers, more branch points on each fiber, and greater complexity of branching patterns. These morphological changes mean that the potential number of “weak” branch points is substantially greater in older $mdx$ animals, rendering them susceptible to damage by contractions that would not damage either normal (nonbranched) dystrophin-positive skeletal muscle or skeletal

Fig. 6. Fiber branching in younger and older $mdx$ muscles. Fibers were categorized according to the number of branch points they displayed (0, 1, 2, 3, or 4+). A: proportion of fibers in each category. B: absolute number of fibers in each category. Compared with younger $mdx$ muscles, older $mdx$ muscles had a greater number of branched fibers and a greater number of fibers with multiple branches.

Fig. 7. Low-power images of enzymatically dispersed single-muscle fibers from younger $mdx$ mice (1–2 mo of age). A: an example of an unbranched extensor digitorum longus fiber. B: an image from a confocal laser scanning microscope with the fiber stained with ethidium bromide to label the nuclei, which are predominantly in the center of the fiber; this fiber has one small central branch. C: a fiber with two small sprouts coming off the middle and a larger fissure lower left. D: a fiber with a split which reconnects. Scale bar measurements are in μm. The insets are diagrammatic representations of the main deformity displayed by the fiber.
muscle from younger (nonbranched) dystrophin-negative mdx animals.

The observed association between the degree of damage and the extent of branching has implications for the interpretation of past studies involving lengthening contractions in the mdx mouse. The studies in Table 1 that found larger force deficits for mdx muscles all used mice that were older than the 6- to 8-wk-old mice of our study. Given that, in our study, 17% of fibers were already branched at 6 – 8 wk, and 89% were branched by 27–31 wk, the muscles in these other studies may have contained a significant proportion of branched fibers. This means that at least part of the force deficits observed in mdx muscle may have been associated with the presence of the branched fibers, rather than the absence of dystrophin.

To remove the potential confounding effect of branched fibers, and to gain a clearer understanding of what is the primary effect of the lack of dystrophin, we can look at muscles in which fiber branching is minimal. One study by Grange et al. (11) used dystrophic mouse pups 9 – 12 days old. This is much younger than any of the studies listed in Table 1, and hence the effect of fiber branching would be minimized. They found that the extent of membrane damage (as measured by dye uptake) was no different between dystrophic and control animals following lengthening contractions. This suggests that a lack of dystrophin does not in itself weaken the sarcolemma. In contrast to the present study, Yeung et al. (24) used unbranched single fibers from the flexor digitorum brevis muscle and found that unbranched mdx fibers had slightly higher force deficits than controls following lengthening contractions. Interestingly, however, this difference was eliminated when specific blockers of stretch-activated ion channels were added to the unbranched single fibers, suggesting that the primary effect of dystrophin deficiency may be the malfunctioning of ion channels rather than a fragile sarcolemma.

Hence, in these studies, it does not appear that dystrophin’s primary role is to mechanically strengthen the sarcolemma and that the initiating event in the dystrophic process may not necessarily be mechanical damage during contraction. Popular alternative candidates for the initial step are a pathological calcium homeostasis due to one or more of the following: aberrant ion channel functioning; sarcolemmal ion channel dysfunction, especially with reference to mechanosensitive channels; and reactive oxygen species activity (1, 7, 23).

Interestingly, the aged control muscles were also somewhat damaged by the lengthening contractions. This finding is consistent with those of Brooks and Faulkner (3), who found that aged mice ~24 mo old were more susceptible to contraction-induced damage than younger mice. Our analysis of muscle stiffness provides a possible explanation of this. We found that older muscles had increased stiffness compared with younger muscles. This may mean that older muscles are less compliant and less able to absorb the strain as the muscle is stretched, rendering it more susceptible to damage.

In summary, we have observed that the force deficits in mdx muscle following mild lengthening contractions are associated

Fig. 9. Muscle stiffness, as measured by the percent change in force for every 1% change in length during the ramp phase of the lengthening contractions. There were no differences between mdx and control in either age group, but older muscles showed significantly more stiffness than younger muscles, in both mdx and controls. **0.001 < P < 0.01; *0.01 < P < 0.05. n = 5 Muscles for younger control; n = 6 for younger mdx; n = 3 for older control; n = 8 for older mdx.
with the degree of fiber branching. Given this association, it is important to isolate the possible effects of fiber branching from the direct effects of dystrophin deficiency when interpreting the results of similar studies in mdx mice. The effect of fiber branching can be removed by using mice that are as young as possible or by using individual fibers that have no branches. In this way, the direct effect of dystrophin deficiency can be more easily seen and provide a clearer understanding of what is the primary initiating event in the pathogenesis of muscular dystrophy.

GRANTS

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