Susceptibility to sarcomere injury induced by single stretches of maximally activated muscles of \textit{mdx} mice

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Submitted 6 June 2003; accepted in final form 15 October 2003

Consolino, Christina M., and Susan V. Brooks. Susceptibility to sarcomere injury induced by single stretches of maximally activated muscles of \textit{mdx} mice. \textit{J Appl Physiol} 96: 633–638, 2004; 10.1152/japplphysiol.00587.2003.—The purpose was to investigate the contribution of mechanical damage to sarcomeres to the greater susceptibility of dystrophic muscle fibers to contraction-induced injury. Single stretches provide an effective method for studying mechanical factors that contribute to the initiation of contraction-induced injury. We hypothesized that, after single stretches, the deficits in isometric force would be greater for muscles of \textit{mdx} than C57BL/10 mice, whereas membrane damage would be minimal for all muscles. Extensor digitorum longus (EDL) and soleus muscles of mice were removed under anesthesia with Avertin (tribromoethanol). During the plateau of a maximum isometric contraction in vitro, muscles were stretched through single strains of 20–60% fiber length. Isometric force was remeasured 1 min later, and muscles were then incubated in procion orange dye to identify fibers with membrane damage. Force deficits at 1 min were two- to threefold greater for EDL muscles of \textit{mdx} compared with C57BL/10 mice, whereas no significant differences were observed between soleus muscles of \textit{mdx} and C57BL/10 mice. For all muscles, membrane damage was minimal and not significantly increased by single stretches for either strain of mice. These data support a critical role of dystrophin maintaining sarcomere stability in EDL muscles, whereas soleus muscles retain abilities, in the absence of dystrophin, not different from control muscles to resist sarcomere damage.

muscular dystrophy; force deficit; damage

THE LACK OF DYSTROPHIN in muscles of patients with Duchenne muscular dystrophy and in \textit{mdx} mice leads to ongoing muscle fiber degeneration (10, 32). A common cause of degeneration in muscles of control animals is contraction-induced injury. A high susceptibility to contraction-induced injury of muscles in \textit{mdx} mice is supported by the twofold larger decrease in isometric force observed for muscles from \textit{mdx} compared with C57BL/10 mice immediately after a protocol of five lengthening contractions in vitro (25). The structure and location of dystrophin suggest that it functions to provide support to the muscle fiber plasma membrane (13), but the 57% decrease in force observed by Petrof and his coworkers (25) for muscles of \textit{mdx} mice after five lengthening contractions was accompanied by muscle fiber membrane disruptions in only \(\sim\)15% of the fibers. Thus membrane damage did not account entirely for the greater force deficits of dystrophin deficient muscles of \textit{mdx} mice. The “unexplained” portion of the force deficit may reflect greater fatigue or other metabolic factors in muscles of \textit{mdx} mice, damage to force-generating or force-transmitting structures in addition to membrane damage, or some combination of these factors.

Contraction-induced injury is initiated in control muscles by mechanical disruption of the ultrastructure of sarcomeres that are excessively stretched (5, 9, 20, 23). A major contributing factor to the likelihood of sarcomeres being stretched excessively and damaged is the development of heterogeneity in sarcomere lengths (12, 15, 19). Sarcomere length heterogeneity allows sarcomeres to experience large strains even during relatively small stretches of the muscle fibers (5, 20). Whether dystrophin plays a role in maintaining sarcomere stability is not known. Our working hypothesis is that the dystrophin glycoprotein complex (DGC) shunts contractile forces laterally from the myofibrils through the plasma membrane to the extracellular matrix and that a lack of dystrophin results in a mechanically compromised cytoskeleton, which increases sarcomere heterogeneity during contractions, increasing the susceptibility to contraction-induced injury.

Our purpose was to investigate the underlying causes of the higher susceptibility to contraction-induced damage of muscles of \textit{mdx} compared with control mice. We induced injury with single lengthening contractions in vitro of extensor digitorum longus (EDL) and soleus muscles of \textit{mdx} and C57BL/10 mice. Single lengthening contractions cause force deficits without fatigue and provide a valuable tool for investigating relationships between mechanical events in contracting muscles and the initiation of injury (4, 5, 20). Injury was evaluated through measurements of the decrease in force immediately after the stretch and quantification of the number of fibers that took up dye from the bath, indicative of membrane disruption. We hypothesized that, after single stretches of any given strain, the force deficits would be greater for muscles of \textit{mdx} mice than for muscles of C57BL/10 mice, whereas the extent of membrane damage would be minimal for muscles of both strains of mice.

METHODS

\textbf{Animals}. Specific-pathogen-free male \textit{mdx} mice (C57BL/10ScSn-Dmd\textsuperscript{mdx/1}) and C57BL/10 control mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at \(\sim\)5 mo of age. For several weeks before experimentation, mice were housed in a specific-pathogen-free barrier facility at the University of Michigan in the Unit for Laboratory Animal Medicine. Thirty-three C57BL/10 mice, mean body mass of 30.9 \(\pm\) 0.6 (SE) g and 24 \textit{mdx} mice, 34.1 \(\pm\) 0.5 g, provided a total of 187 muscles. Fifty-six EDL muscles and 56 soleus muscles from C57BL/10 mice, and 40 EDL and 35 soleus muscles from \textit{mdx} mice were examined. All experimental procedures were conducted in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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conducted in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. 85-23 (NIH), Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Operative procedure. For each procedure, animals were anesthetized with intraperitoneal injections of Avertin (tribromoethanol; 400 mg/kg). Supplemental doses were administered as needed to maintain adequate levels of anesthesia throughout the experiment. In most cases, experiments were conducted on the EDL and soleus muscles of both the right and left legs for each mouse. Each muscle was isolated, and a 5-0 silk suture was tied securely to the distal and proximal tendons. The muscle was carefully removed from the animal and placed in a horizontal bath containing buffered mammalian Ringer solution (composition in mM: 137 NaCl, 24 NaHCO₃, 11 glucose, 5 KCl, 2 CaCl₂, 1 MgSO₄, 1 NaH₂PO₄, and 0.025 turbucararine chloride) maintained at 25°C and bubbled with 95% O₂-5% CO₂ to stabilize pH at 7.4. One tendon of the muscle was tied securely to a force transducer (model BG-50, Kulite Semiconductor Products) and the other tendon to the lever arm of a servomotor (model 305B, Aurora Scientific, Richmond Hill, ON, Canada). After removal of the muscles, animals were euthanized with an overdose of anesthetic and administration of a pneumothorax.

Measurement of contractile properties. Muscles were stimulated between two stainless steel plate electrodes. The voltage of single 0.2-s stimulation pulses was increased in a stepwise manner until a single lengthening contraction of each muscle was initiated at the length (Lₒ) that resulted in maximal twitch force (3). Muscle length was measured with calipers. The mean values for Lₒ were 13.5 ± 0.1 and 12.1 ± 0.1 mm for EDL and soleus muscles, respectively, of C57BL/10 mice and 13.9 ± 0.1 and 12.3 ± 0.1 mm for EDL and soleus muscles of mdx mice. With the muscle held at Lₒ, the force developed during trains of stimulation pulses was recorded, and stimulation frequency was increased until the maximum isometric tetanic force (Pₒ) was achieved. For EDL muscles, 300-ms trains of pulses were used, and 900-ms trains were used for soleus muscles. A stimulus frequency of ~130 Hz was typically needed to achieve Pₒ for both EDL and soleus muscles of mdx mice, whereas frequencies of ~165 and ~120 Hz elicited Pₒ for EDL and soleus muscles, respectively, of C57BL/10 animals. For each muscle, optimum fiber length (Lₒ) was calculated by multiplying Lₒ by previously determined Lₒ/Lₒ ratios for EDL and soleus muscles of 0.45 and 0.71, respectively (3).

Protocol for inducing injury. Immediately after a protocol of repeated contractions, the decrease in force reflects fatigue as well as injury (22, 28). The injury could be mechanical, chemical, or metabolic in nature, with the chemical pathway activated by an increased permeability in the plasma membrane and the metabolic pathway activated by the depletion of energy stores (20). To focus on the mechanical factors that contribute to the initiation of injury, other factors must be minimized or eliminated. Our laboratory’s approach has been to induce injury by using single lengthening contractions (4, 5, 18–20). A single lengthening contraction is unlikely to result in significant fatigue or depletion of energy stores. Furthermore, on the basis of the observation that, after a series of five maximum lengthening contractions (25), muscles from mdx mice showed membrane damage in ~15% of the fibers in a cross section, we anticipated that single lengthening contractions would result in membrane damage to only a small percentage of the fibers.

Each muscle was exposed to a single stretch initiated from the plateau of an isometric contraction at the stimulation frequency that elicited Pₒ. At time 0, stimulation was initiated and muscles were held with no movement for either 100 or 300 ms to allow nearly maximal activation of EDL or soleus muscles, respectively. Stimulation was terminated at the end of the lengthening ramp. Pₒ was measured immediately before a single stretch and again 1 min after a single stretch was completed. The changes between the isometric forces measured before and after the stretch, as expressed as a percentage of the force before the stretch. The size of the stretches was varied to alter the severity of the stimulus for inducing injury. For maximally activated EDL muscles of C57BL/10 mice, a single stretch of 30% strain was necessary to induce a significant force deficit (5). On the basis of our hypothesis that muscles of mdx mice would be more susceptible to injury, our expectation was that muscles of mdx mice might be injured by stretches of lesser strain. In addition, we expected soleus muscles to be more resistant to injury than EDL muscles (20). Consequently, in an attempt to induce injury with single stretches for both soleus and EDL muscles and to test for a lower threshold for injury of muscles of mdx mice, we used stretches of 20, 30, 40, 50, or 60% strain relative to Lₒ. All stretches were performed at a velocity of two fiber lengths per second (L/fs).

Muscle fiber membrane damage. Injury to the plasma membrane has been assessed in numerous (7, 21, 25) studies using light-microscopic analysis of muscle cross sections that reveal the presence of various dyes, known to be excluded from cells with intact membranes, within the cytoplasm of muscle fibers. In the present study, immediately after stretches of 30 and 40%, muscles were incubated while held at Lₒ in a 0.2% procion orange solution at 30°C for 30 min and washed 2 × 5 min in Ringer solution at 30°C (21).

Either immediately after final assessments of maximum isometric force or after incubation in procion orange, muscles were trimmed of tendons, blotted, weighed, and rapidly frozen in isopentane cooled by dry ice. The mean wet masses of 17.3 ± 2.0 and 14.3 ± 0.2 mg for EDL and soleus muscles of C57BL/10 mice, respectively, were significantly lower than the respective values for muscles of C57BL/10 mice of 11.7 ± 2.0 and 10.1 ± 0.1 mg. Total muscle fiber cross-sectional area was determined by dividing the wet mass by the product of Lₒ and the density of mammalian skeletal muscle (1.06 mg/mm³). Maximum isometric specific force (kN/m²) was determined by dividing Pₒ by the total muscle fiber cross-sectional area. Before the stretches, values for specific Pₒ for EDL and soleus muscles of mdx mice were 172.6 ± 2.8 and 182.1 ± 4.2 kN/m², respectively. These values were significantly lower than the values of 234.5 ± 3.4 and 231.1 ± 3.7 kN/m², respectively, for EDL and soleus muscles of C57BL/10 mice.

Frozen cross sections of 10-μm thickness were cut and stained with hematoxylin (nuclear stain) and eosin-phloxine (cytoplasmic stain), and muscle fiber structure was examined by light microscopy. Stained sections were visualized on a microscope (Leitz Laborlux, Leica, Wetzlar, Germany) and captured with a video camera (Diagnostic Instruments, Sterling Heights, MI) using the BioQuant image analysis system (Nashville, TN). A second set of sections was mounted with DPX mountant and viewed with a fluorescent microscope to identify the presence of procion orange dye. Fields were photographed under identical conditions at ×160 on the Bioquant system. Each image was imported into Microsoft Photodraw (Microsoft, Redmond, WA), and the same brightness and contrast were applied to include only those fibers that had taken up procion orange. Saved gray-scale images were then imported into Scion image (Scion, Frederick, MD) to determine the 256 gray-level counts. MatLab 12 (The Mathworks, Natick, MA) was used to calculate the percentage of the cross section positive for procion orange. The number of dye-positive fibers was also counted for each image to establish the relationship between dye-positive fibers and pixel percents. For both mdx and C57BL/10 mice, a minimum of six muscles per stretch protocol was analyzed.

Statistical analyses and data presentation. All data are presented as means ± SE. A two-way ANOVA was used to determine the effects of dystrophin deficiency (muscles of mdx compared with C57BL/10 mice) and length change (strain) on the force deficit and dye uptake with the level of significance set a priori at P < 0.05. When significance was detected, Tukey’s post hoc comparison was used to assess the individual difference.

RESULTS

Consistent with previous findings from control muscles (5), single stretches of 30% strain were necessary in the present study to induce force deficits in EDL muscles of C57BL/10 mice.
mice (Fig. 1). In contrast, for EDL muscles of mdx mice, single stretches of only 20% strain resulted in force deficits significantly different from zero. In addition, after stretches of 30, 40, and 50% strain, force deficits were two- to threefold greater for EDL muscles of mdx mice than for those of C57BL/10 mice (Fig. 1). EDL muscles of mdx mice were not tested at 60% strain. In contrast to the greater force deficits observed for EDL muscles of mdx compared with C57BL/10 mice, force deficits were comparable for soleus muscles of C57BL/10 and mdx mice (Fig. 1). The only exception was a slightly greater force deficit for soleus muscles of mdx compared with C57BL/10 mice after single large stretches of 60% strain.

EDL and soleus muscles stretched through strains of 30 and 40% were incubated in the vital dye procion orange. Muscles that did not undergo stretch were also incubated in the dye and served as controls. Representative cross sections are shown in Fig. 2 for unstretched (0%) muscles and for muscles after single 40% stretches. Regression analysis showed excellent agreement ($r^2 = 0.92$) between counts of the total number of dye positive fibers in a cross section and the pixel percent determined by our quantitative image-analysis technique. Muscles of mdx mice showed a trend toward higher levels of dye uptake than muscles of C57BL/10 mice (Table 1), likely explained by the presence of dye within degenerating muscle fibers that are always present to some extent in dystrophic muscle. This explanation is supported by the presence of fibers in our histological sections of muscles of mdx mice displaying faint or complete lack of cosin stain in addition to normally appearing nonnecrotic fibers (not shown). More critical to the present study is that, despite force deficits ranging from ~10 to ~40% induced by stretches of 30 and 40% strain and two- to threefold greater force deficits for EDL muscles from mdx compared with C57BL/10 mice, no differences were observed in the dye uptake for muscles exposed to stretches compared with unstretched controls (Table 1). These findings support the contention that our single-stretch protocol for inducing injury did not result in significant membrane damage. We are, however, unable to discount the possibility that focal membrane disruptions in other regions along the length of the fiber were not detected with this technique because only a single cross section from each muscle was analyzed and the extent to which

![Image](https://www.jap.org/)

**Fig. 1.** Relationship between the size of the stretch and the force deficit 1 min after the stretch. Data are shown for the mean force deficits (expressed as a percentage of prestretch force, %) single stretches of a given strain (expressed as a percentage of optimum fiber length, %L0) for maximally activated extensor digitorum longus (EDL; A) and soleus (B) muscles of C57BL/10 and mdx mice. Values are means ± SE. Error bars are shown where the error bar is larger than the size of the symbol. For both EDL and soleus muscles of both C57BL/10 and mdx mice, force deficit increased with strain ($P < 0.05$). For EDL muscles, significant interactions between strain and the type of mouse indicated that force deficit increased to a larger extent with increases in strain for mdx compared with C57BL/10 mice. *Significant difference between muscles of C57BL/10 and mdx mice, $P < 0.05$.

**Table 1.** Procion orange dye uptake by EDL and soleus muscles that were not stretched (0%) or were exposed to single stretches of 30 or 40% strain.

<table>
<thead>
<tr>
<th></th>
<th>0%</th>
<th>30%</th>
<th>40%</th>
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<tbody>
<tr>
<td><strong>EDL muscles</strong></td>
<td></td>
<td></td>
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<tr>
<td>C57BL/10 mice</td>
<td>0.44 ± 0.16</td>
<td>1.32 ± 0.34</td>
<td>0.92 ± 0.36</td>
</tr>
<tr>
<td>Mdx mice</td>
<td>1.48 ± 0.49</td>
<td>3.07 ± 1.21</td>
<td>4.23 ± 1.47</td>
</tr>
<tr>
<td><strong>Soleus muscles</strong></td>
<td></td>
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<tr>
<td>C57BL/10 mice</td>
<td>2.39 ± 0.83</td>
<td>0.73 ± 0.28</td>
<td>0.74 ± 0.23</td>
</tr>
<tr>
<td>Mdx mice</td>
<td>2.09 ± 0.47</td>
<td>2.93 ± 0.63</td>
<td>2.80 ± 0.69</td>
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Values are means ± SE given in pixel percent. EDL, extensor digitorum longus. Despite a trend toward greater dye uptake in muscles of mdx compared with C57BL/10 mice ($P = 0.08$ for EDL muscles and $P = 0.06$ for soleus muscles), there were no differences in the percentage of dye-positive area for stretched compared with unstretched EDL ($P = 0.52$) or soleus ($P = 0.86$) muscles.
the procion orange dye can diffuse along the length of a fiber is not well characterized.

Although the magnitude of the stretch (strain) is an excellent predictor of force deficit after single stretches of maximally activated muscles (5), for a stretch of a given strain, the force deficit induced is lower during submaximal contractions (20), supporting the importance of the combination of force and strain as predictors of the severity of injury. To determine whether differences between muscles of *mdx* and C57BL/10 mice in the force produced during lengthening contractions explained the results shown in Fig. 1, the work inputs during single stretches were compared. Work input was calculated by integrating the force-time curves, dividing the value by the duration of the ramp stretch to give the average force developed, and multiplying average force by the size of the stretch. The work done to stretch the muscles through a given strain is shown normalized to muscle wet mass in Fig. 3. Figure 3 shows that the greater force deficits for any given strain demonstrated by EDL muscles of *mdx* compared with C57BL/10 mice (Fig. 1) were observed despite lower work inputs during the stretch. Furthermore, despite lower specific $P_o$ for soleus muscles of *mdx* compared with C57BL/10 mice, there were no differences between soleus muscles of *mdx* and C57BL/10 mice for the work input during single stretches (Fig. 3), indicating that the comparable force deficits for muscles of *mdx* compared with C57BL/10 mice were not due to lower work input for muscles of *mdx* mice.

**DISCUSSION**

The DGC has been hypothesized to aid in the maintenance of the integrity of the muscle fiber membrane in control muscle fibers, especially when muscles are stretched (18, 25). Support for the hypothesis that dystrophin and the DGC impart structural support to the membrane is provided by the observation that muscles lacking dystrophin show greater increases in permeability of the membrane with contractile activity than control muscles, both in vivo (7) and in vitro (25). The experiments in the present study did not attempt to eliminate membrane damage for muscles of *mdx* mice; rather membrane damage was monitored and demonstrated to be minimal and relatively unaffected by the single stretches we used to induce injury. The observation in the present study that EDL muscles of *mdx* mice showed greater force deficits after single lengthening contractions compared with control muscles without an increase in dye-positive fibers supports the usefulness of the single-stretch protocol for comparing the initial mechanical injury to force-generating or force-transmitting structures. On the basis of these findings of greater force deficits, in the absence of fatigue, depletion of energy stores, or significant membrane damage, we conclude that differences in force deficits resulting from single stretches were due primarily to differences in the extent of disruption during contractions of the ultrastructure of force-generating or force-transmitting structures within or between sarcomeres. These studies suggest that, in addition to a compromised membrane, the lack of dystrophin in EDL muscles of *mdx* mice results in a mechanically compromised cytoskeleton.

Our conclusion is weakened, somewhat, by the indirect nature of our measurement of sarcomere damage. Direct demonstration of damage to sarcomeres immediately after the occurrence of the injury is possible through numerous morphological techniques (1, 5, 9). Despite the ability to clearly demonstrate sarcomere damage at both the light- and electron-microscopic levels, considering that one mouse EDL muscle, with 6-mm fiber lengths, 50-µm fiber diameters, and ~1,500 fibers, contains over 7 billion sarcomeres, the sheer enormity of the sample involved in assaying sarcomeres makes quantitative analysis of such direct assessments for purposes of comparisons essentially a practical impossibility. An additional difficulty in the present study with direct assessments of damage in muscles of *mdx* mice is the persistent background histopathological lesions of greater force deficits, or membrane damage with subsequent chemical changes, or membrane damage with subsequent chemical changes were not likely significant factors in inducing damage, in the absence of direct demonstration of greater sarcomere...
damage to muscles of *mdx* compared with control mice, we cannot rule out these other mechanisms underlying the greater force deficits we observed.

In control muscle fibers, contraction-induced injury appears to be initiated when individual sarcomeres are stretched excessively (5, 9, 19, 23), resulting in irreversible physical damage to structural proteins (11, 27). A major factor contributing to the likelihood of sarcomeres being excessively stretched is the existence of heterogeneity in sarcomere lengths along the lengths of fibers (12, 15, 19). The degree of heterogeneity in sarcomere length increases with increasing fiber length and is increased further by activation (6, 12). The conclusion is that, when a muscle is activated or stretched, interactions between force and strain influence the distribution of a stretch among different sarcomeres in series. This importance of both force and stretch for the development of sarcomere length heterogeneity explains the significant linear relationship of force deficit with work after single lengthening contractions of whole muscles (4, 5) and single permeabilized muscle fibers (18, 20).

Multiple regression analyses performed on the present data indicated that, as previously demonstrated for control muscles, strain and work input were essentially equivalent predictors of the magnitude of injury for muscles of *mdx* mice (data not shown). In one-variable regression models of force deficit, either strain or work gave coefficients of determination of \( r^2 \approx 0.50 \) for EDL muscles of both *mdx* and C57BL/10 mice and over 0.50 for soleus muscles from either group of mice. These observations that the variations in force deficit were predicted well by work for muscles of *mdx* mice further support the hypothesis that the damage induced during single lengthening contractions was primarily mechanical damage to sarcomeres as is the case for control muscles (5, 18, 20).

Strong linear relationships between work and force deficit hold for whole muscles (4, 5) and single permeabilized muscle fibers (16) from mice and for both fast and slow single permeabilized muscle fibers of rats (20). Consequently, parameters of the work input-force deficit relationship can be used to describe the threshold work required to induce sarcomere injury and the sensitivity to sarcomere injury of a muscle for a given work input (4, 20). For EDL muscles of *mdx* compared with C57BL/10 mice, the lower work input during single lengthening contractions of any given strain coupled with a higher force deficit resulted in a steeper relationship between work input and force deficit, providing further support that EDL muscles of *mdx* mice are more easily injured. The steeper work input-force deficit relationship for EDL muscles of *mdx* compared with C57BL/10 mice is consistent with our working hypothesis that dystrophin plays a role in maintaining sarcomere stability. The high susceptibility to injury of EDL muscles in *mdx* mice observed in the present study is consistent with other reports on the response of fast-twitch muscles of *mdx* mice after protocols of lengthening contractions (25). In contrast to studies of repeated contractions (25), however, the greater force deficits observed in the present study for EDL muscles of *mdx* compared with C57BL/10 mice after single lengthening contractions were not associated with significant uptake of procion orange by muscle fibers of *mdx* mice, suggesting that in the present study membrane disruption was not a factor in the higher force deficits.

Whereas EDL muscles of *mdx* mice were highly susceptible to contraction-induced injury, the observation of no differences in force deficits between dystrophic and control soleus muscles was contrary to our hypothesis. The usefulness of any contraction protocol for discriminating between control and dystrophic muscles requires that the effect of the protocol be within a certain range. For protocols that are too benign, muscles in neither *mdx* nor control mice may be significantly affected, whereas protocols that are too rigorous may overwhelm any difference between dystrophic and control muscles. The wide range of single stretches incorporated in the present study, resulting in a large range of force deficits, from 0 to 70%, provided a sufficient test for differences in susceptibility of muscles to damage. The observation of no differences in the force deficits for soleus muscles of *mdx* and C57BL/10 mice over this wide range provides strong support for the conclusion that disruption of the DGC was not detrimental to the ability of soleus muscles to maintain structural stability. Previous reports that Duchenne muscular dystrophy preferentially affects fast fibers (30) is consistent with the observation in the present study of a greater susceptibility to injury for fast EDL muscles of *mdx* compared with C57BL/10 mice but not for soleus muscles, which contain a significant population of slow fibers. In contrast, the majority of previous studies of *mdx* mice have failed to demonstrate significant differences in the phenotype associated with dystrophin deficiency between EDL and soleus muscles (8, 17). A notable exception is the greater damage to soleus muscles than EDL muscles of *mdx* mice with aging reported by Pastoret and Sebille (24). Although increased concentrations of essentially all cytoskeletal components found in slow compared with fast muscles (26, 30, 31) may help explain the ability of dystrophin-deficient soleus muscles to resist damage, how susceptibility to damage is linked to the degenerative phenotype associated with muscular dystrophies remains unclear.

A somewhat surprising outcome of the present study was that after single stretches of physiological strains the force deficits for EDL and soleus muscles of C57BL/10 mice were not different. This observation is in contrast to other reports that fast-twitch muscle fibers are more susceptible to contraction-induced injury than slow-twitch muscle fibers (14, 20). The velocity of 2 \( \text{L/s} \) used in the present study represents only 20\% of the maximum shortening velocity for the EDL muscles but 50\% for soleus muscles (3). McCully and Faulkner (22) and Warren et al. (28) performed repeated stretches at different velocities, with each group providing some evidence that the higher stretch velocities produced greater force deficits. In contrast, Lynch and Faulkner (16) reported that the velocity of stretch did not influence the force deficit for single permeabilized muscle fibers, whereas Brooks and Faulkner (4) observed that velocity was significant only when high velocity (\( >8 \text{L/s} \)) was combined with large strains (50\% relative to \( L_0 \)). To investigate whether the higher relative velocity of stretch for soleus compared with EDL muscles contributed to the results of the present study, single stretches were also performed at 0.8 \( \text{L/s} \), which is 20\% of the maximum velocity for soleus muscles, and at 5 \( \text{L/s} \), which is 50\% of maximum velocity for EDL muscles. Consistent with the single-stretch experiments of Lynch and Faulkner (16) and Brooks and Faulkner (4), we found comparable force deficits for EDL and soleus muscles at physiological strains regardless of the velocity of stretch (data not shown). Differences in the magnitude of injury between
EDL and soleus muscles may become apparent only after repeated lengthening contractions of activated muscles (29).

If dystrophin functions as a mechanical link between the cytoskeleton and the plasma membrane, forces generated by the contractile apparatus would be transmitted in part through the DGC to the membrane and to the extracellular matrix. In the absence of the DGC, therefore, forces that would normally be shunted by these proteins during a contraction may be borne in larger proportion by myofibrillar proteins as well as place greater stress on the muscle fiber membrane. As discussed previously, higher forces borne by the myofibrillar apparatus would exacerbate heterogeneity in sarcomere lengths and thereby heighten the likelihood of damage to individual sarcomeres. The results of the present study support a role for the DGC in the maintenance of the structural stability of sarcomeres. Thus activities involving either single or repeated contractions that are innocuous for muscles in control animals may be injurious to dystrophic muscles. Furthermore, the comparable abilities of soleus muscles of mdx and control mice to resist sarcomere damage suggest that the role of the DGC for maintaining structural stability is different in EDL and soleus muscles.

ACKNOWLEDGMENTS

The authors thank Cheryl Hassett for assistance in data collection.

GRANTS

Financial support was provided by National Institute on Aging Grants AG-15434 and AG-00114.

REFERENCES