Systemic administration of IGF-I enhances oxidative status and reduces contraction-induced injury in skeletal muscles of \textit{mdx} dystrophic mice

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The absence of dystrophin and resultant disruption of the dystrophin glycoprotein complex renders skeletal muscles of dystrophic patients and dystrophic \textit{mdx} mice susceptible to contraction-induced injury. Strategies to reduce contraction-induced injury are of critical importance, because this mode of damage contributes to the etiology of myofiber breakdown in the dystrophic pathology. Transgenic overexpression of insulin-like growth factor-I (IGF-I) causes myofiber hypertrophy, increases force production, and can improve the dystrophic pathology in \textit{mdx} mice. In contrast, the predominant effect of continuous exogenous administration of IGF-I to \textit{mdx} mice at a low dose (1.0–1.5 mg·kg$^{-1}·$day$^{-1}$) is a shift in muscle phenotype from fast glycolytic toward a more oxidative, fatigue-resistant, slow muscle without alterations in myofiber cross-sectional area, muscle mass, or maximum force-producing capacity. We found that exogenous administration of IGF-I to \textit{mdx} mice increased myofiber succinate dehydrogenase activity, shifted the overall myosin heavy chain isoform composition toward a slower phenotype, and, most importantly, reduced contraction-induced damage in tibialis anterior muscles. The deficit in force-producing capacity after two damaging lengthening contractions was reduced significantly in tibialis anterior muscles of IGF-I-treated (53 ± 4%) compared with untreated \textit{mdx} mice (70 ± 5%, $P < 0.05$). The results provide further evidence that IGF-I administration can enhance the functional properties of dystrophic skeletal muscle and, compared with results in transgenic mice or virus-mediated overexpression, highlight the disparities in different models of endocrine factor delivery.

insulin-like growth factor-I; fiber type; myosin heavy chain; muscular dystrophy

ABSENCE OF THE CYTOSKELETAL PROTEIN DYSTROPHIN IN DUCHENNE MUSCULAR DYSTrophy (DMD) disrupts the dystrophin glycoprotein complex (DGC), a cluster of proteins spanning the sarcolemma that are important in providing cytoskeletal support and regulating intracellular signaling (27, 40). The \textit{mdx} mouse, a widely used animal model of DMD, also harbors a mutation in dystrophin and lacks functional levels of dystrophin protein. Although \textit{mdx} mice exhibit a more benign phenotype compared with DMD patients, the compromised structural integrity of dystrophin-deficient skeletal muscle renders it highly susceptible to contraction-mediated injury (5, 9, 20, 22). In general, contraction-induced damage occurs when activated muscles are subjected to forced lengthening. The susceptibility to contraction-induced damage in dystrophic muscle may be attributed to an aberration in the link between the cytoskeleton and plasma normally provided by a functional DGC, which disrupts the normal transmission of forces during contraction and places extra stress on the muscle fiber membrane or myofibrillar proteins (18).

In \textit{mdx} mice, several studies have shown that fast-twitch muscles such as the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles are highly susceptible to contraction-induced damage (5, 9, 20). In addition, it has been demonstrated that skeletal muscles composed of fast-twitch fibers are more susceptible to deleterious effects of dystrophin deficiency and preferentially affected compared with predominantly slow-twitch skeletal muscles in \textit{mdx} mice (7) and α-sarcoglycan-null mice (8). Similarly, fast-twitch fibers in DMD patients appear to be particularly vulnerable to dystrophin deficiency compared with slow-twitch fibers, as evidenced by the earlier onset of degeneration and regeneration in type IIb muscle fibers compared with other fiber types (39). Assessment at the cellular (single fiber) level demonstrated that the increased susceptibility to contraction-induced damage in skeletal muscles of \textit{mdx} mice does not arise from factors within contractile apparatus but, likely, from abnormalities at the level of the sarcolemma (18).

Investigators in our laboratory have demonstrated previously that continuous administration of a relatively low dose (~1 mg·kg$^{-1}·$day$^{-1}$) of recombinant insulin-like growth factor-I (IGF-I) protein to \textit{mdx} mice for 8 wk increased succinate dehydrogenase (SDH) activity and improved fatigue resistance in diaphragm, EDL, and soleus muscles (10, 11) and prolonged the time course of the isometric twitch ($P_c$) in the EDL and soleus muscles (10). Interestingly, this IGF-I regimen did not alter myofiber cross-sectional area, muscle mass, or maximum force-producing capacity ($P_c$), indicating that the predominant effect of low-dose IGF-I administration to \textit{mdx} mice is a shift toward a more oxidative muscle phenotype. The dose of IGF-I is clearly a critical factor in determining its effects in skeletal muscle, because transgenic or postnatal gene transfer methodologies that increase IGF-I to much higher levels have different effects on muscle phenotype (3, 4). The principal effects of high-level IGF-I overexpression in skeletal muscle are myofiber hypertrophy and increased muscle mass via activation of IGF-I receptor-mediated intracellular signaling cascades that ultimately increase protein synthesis and reduce protein degradation (25, 35). As such, the growth-promoting characteristics of IGF-I, when delivered by these methods, have been used to ameliorate the decline in skeletal muscle mass and function associated with aging (4), chronic left ventricular dysfunction (30), excessive angiotensin II levels (34), de-
nervation (32), and various myopathies including muscular dystrophy (3, 33).

Undoubtedly, gene manipulation will eventually have tremendous implications for muscular dystrophy and other muscle diseases. However, several obstacles such as vector design, the possibility of insertional mutagenesis, and achieving systemic delivery to therapeutic levels must be overcome for these methodologies to reach widespread clinical use (31). As such, there is an immediate need for clinically relevant treatment strategies for muscular dystrophies.

Previous studies have demonstrated that slow-twitch muscles from mdx mice are less susceptible to contraction-induced damage than fast-twitch muscles (7). On the basis of these findings, and the fact that continuous low-dose exogenous IGF-I administration increases the oxidative capacity of skeletal muscles (10), we hypothesized that a similar regimen could reduce contraction-induced damage in mdx mice, in part by inducing a shift in overall muscle phenotype toward more slow muscle characteristics.

MATERIALS AND METHODS

Experimental design. All procedures were approved by the Animal Experimentation Ethics Committee of The University of Melbourne and conformed to the Guidelines for the Care and Use of Experimental Animals described by the National Health and Medical Research Council (Australia). Male C57BL/10ScSnmdx/J (dystrophic) mdx mice (20–23 wk of age) were randomly assigned to either untreated or IGF-I–treated groups (with at least 7 mice per group). Treated mice received recombinant human IGF-I (GroPep, Thebarton, South Australia) exogenously at a dose of ~1.5 mg/kg body mass daily for a period of 8 wk via miniosmotic pump, as described previously (10, 11). Investigators in our laboratory have shown previously that systemic administration of IGF-I by miniosmotic pump increases IGF-I levels in the blood (10, 11), rather than increasing muscle IGF-I levels, as in transgenic mice (3). For some experiments, age-matched C57BL/10ScSn mice were used as wild-type controls (n = 3). Some histological assessments of myofiber membrane permeability also required some mice (n = 3 per group) to be injected intraperitoneally with 10 μg body wt Evan’s blue dye (EBD; 1% mass/vol) 20 h before experimentation, as our group has described previously (28).

Skeletal muscle contractile properties measured in situ. Contractile properties of TA muscle were measured in situ as described previously (28). Mice were anesthetized with pentobarbital sodium (Nembutal; Rhone Merieux, Pinkenba, Queensland, Australia; 60 mg/kg ip), and the right TA muscle was exposed by a single incision. The tendon of the TD was cut several millimeters distal to the end of the muscle and tied securely to the lever arm of a dual-mode servomotor platform, and a pin was passed behind the patellar tendon to immobilize the knee. The TA muscle was stimulated by supramaximal (10 V) 0.2-ms square-wave pulses for 300 ms in duration, delivered via two wire electrodes adjacent to the femoral nerve. Optimum muscle length (L0) was determined from Pt, and Po was recorded from the TA muscle and tied securely to the lever arm of a dual-mode servomotor platform, and a pin was passed behind the patellar tendon to immobilize the knee. The TA muscle was stimulated by supramaximal (10 V) 0.2-ms square-wave pulses for 300 ms in duration, delivered via two wire electrodes adjacent to the femoral nerve. Optimum muscle length (L0) was determined from Pt, and Po was recorded from the TA muscle.

Control mdx IGF-I mdx

<table>
<thead>
<tr>
<th>Property</th>
<th>Control mdx</th>
<th>IGF-I mdx</th>
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<tbody>
<tr>
<td>Body mass, g</td>
<td>39.0 ± 1</td>
<td>39.9 ± 1</td>
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<tr>
<td>Heart mass, mg</td>
<td>145.1 ± 5</td>
<td>145.9 ± 4</td>
</tr>
<tr>
<td>Liver mass, g</td>
<td>1.6 ± 1</td>
<td>1.6 ± 1</td>
</tr>
<tr>
<td>EDL mass, mg</td>
<td>14.1 ± 1</td>
<td>14.5 ± 1</td>
</tr>
<tr>
<td>Tibial length, mm</td>
<td>17.9 ± 1</td>
<td>17.7 ± 1</td>
</tr>
<tr>
<td>Soleus mass, mg</td>
<td>12.5 ± 1</td>
<td>13.0 ± 1</td>
</tr>
<tr>
<td>TA mass, mg</td>
<td>108.0 ± 5</td>
<td>108.9 ± 3</td>
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</tbody>
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Values are means ± SE. Insulin-like growth factor-I (IGF-I) was administered at ~1.5 mg kg−1day−1. EDL, extensor digitorum longus; TA, tibialis anterior.

Histology and immunofluorescence. TA muscles were surgically excised, weighed on an analytical balance, mounted in embedding medium, frozen in thawing isopentane, and then stored at ~80°C. A portion of each frozen muscle sample was cryosectioned transversely (8 μm) through the mid-belly region. Muscle sections used for EBD analysis were incubated for 5 min in methanol at ~20°C and air-dried at room temperature, and EBD-positive fibers were identified using fluorescent microscopy with a wide-band interference green (WIG) filter (excitation 520–550 nm; emission 565+ nm), as described previously (28). Muscle sections were stained with hematoxylin and eosin to determine general muscle architecture, the percentage of fibers with centrally located nuclei, and the cross-sectional area (CSA) of individual myofibers. Muscle sections were reacted for SDH activity and analyzed as described previously (11, 12, 26). Median values for CSA and SDH activity were calculated from at least 300 individual muscle fibers per sample. Mice were killed by cardiac excision, and heart, liver, EDL, and soleus masses were recorded.

Tibial length was determined using digital calipers and was measured from the knee to ankle joint along the longitudinal axis of the left tibia.

Muscles (n = 3 per group) were homogenized, and myosin heavy chain (MHC) proteins were separated by SDS-PAGE as described previously (13, 29, 36). Because the TA muscle is composed of predominantly fast-twitch fibers, only MHC Ila/Ix and MHC Iib isoforms could be detected reliably by SDS-PAGE, although transcripts could be detected for the other MHC isoforms.

A small portion (~25 μg) of the TA muscle (n = 3 per group) was mechanically homogenized with a Polytron homogenizer and used for RNA extraction and analysis as described previously (13). Total RNA from TA muscle was isolated using a commercially available kit (no. 74704; Qiagen, Valencia, CA). RNA concentration was determined as UV absorption at 260 nm, and the samples were stored at ~80°C. Semiquantitative RT-PCR was performed on 100 ng of total RNA. RT and PCR were performed using a commercially available kit (no. 74704, Qiagen). A standard RT-PCR protocol was used and consisted of 30 min at 50°C, 15 min at 95°C, and repeated cycles of denaturation (94°C, 45 s), annealing (various temperatures, 45 s), and extension (72°C, 45 s). The common forward primer for all MHCs (except for embryonic MHC) was 5′-GAAGGCCAAGAAGGCGACATC-3′. The reverse primers used for specific MHC isoforms were MHC Ila, 5′-TCTACAGCATCAGAGCTGCC-3′; MHC IIX, 5′-GTGACTTTTCTCTTTGGAGA-3′; MHC Iib, 5′-GTGTTGATTTCTCTTGTCAACC-3′. The forward and reverse primers for embryonic MHC were 5′-GAAGGCCAAGAAGGCGACTCA-3′ and 5′-CCCTACAGGAGAGGACATGC-3′, respectively. The RT-PCR script could be detected for the other MHC isoforms.

Table 1. Morphological properties of dystrophic mdx mice after 8 wk of continuous IGF-I administration via miniosmotic pump

<table>
<thead>
<tr>
<th>Property</th>
<th>Control mdx</th>
<th>IGF-I mdx</th>
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<tr>
<td>Body mass, g</td>
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<td>Liver mass, g</td>
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<td>12.5 ± 1</td>
<td>13.0 ± 1</td>
</tr>
<tr>
<td>TA mass, mg</td>
<td>108.0 ± 5</td>
<td>108.9 ± 3</td>
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Table 2. Contractile properties of TA muscles from dystrophic mdx mice after 8 wk of continuous IGF-I administration via miniosmotic pump

<table>
<thead>
<tr>
<th></th>
<th>Control mdx</th>
<th>IGF-I mdx</th>
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<tbody>
<tr>
<td>Pₚ, mN</td>
<td>683.3 ± 31</td>
<td>649.9 ± 53</td>
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<tr>
<td>dP/dt, mN/ms</td>
<td>96.0 ± 4</td>
<td>90.7 ± 4</td>
</tr>
<tr>
<td>TPT</td>
<td>15.9 ± 1</td>
<td>16.6 ± 1</td>
</tr>
<tr>
<td>1/2RT</td>
<td>13.3 ± 1</td>
<td>14.7 ± 1*</td>
</tr>
<tr>
<td>Pₚ, mN</td>
<td>2,204.4 ± 87</td>
<td>2,065.1 ± 91</td>
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<tr>
<td>sPₒ, kN/m²</td>
<td>198.3 ± 5</td>
<td>186.7 ± 7</td>
</tr>
<tr>
<td>1st lengthening contraction, mN</td>
<td>4,117.1 ± 296</td>
<td>4,536.6 ± 212</td>
</tr>
<tr>
<td>2nd lengthening contraction, mN</td>
<td>3,397.1 ± 203</td>
<td>3,927.2 ± 221</td>
</tr>
<tr>
<td>Maximum force after damage, mN</td>
<td>631.9 ± 61</td>
<td>957.9 ± 34*</td>
</tr>
<tr>
<td>Force deficit, %</td>
<td>70.7 ± 5</td>
<td>53.0 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *IGF-I was administered at ~ 1.5 mg·kg⁻¹·day⁻¹. Pₒ, maximal twitch force; dP/dt, maximal rate of force development during twitch response; sPₒ, specific force (maximum tetanic force/muscle cross-sectional area); TPT, time to peak twitch tension; 1/2RT, twitch half-relaxation time; Pₚ, maximum tetanic force production. Force deficit was determined by calculating the difference between the Pₒ before and after lengthening contractions and is expressed as a percentage of Pₒ determined before the lengthening contractions.

IGF-I reduces contraction injury in dystrophic muscle

Skeletal muscle morphometric and contractile properties. Continuous administration of IGF-I protein for 8 wk did not alter morphometric properties such as body mass, heart mass, liver mass, EDL mass, soleus mass, TA mass, and tibial length (Table 1). Similarly, IGF-I administration did not alter the majority of contractile properties of the TA measured in situ. IGF-I administration did not alter Pₒ, the rate of force development of the twitch, the time required to reach peak twitch force, maximum tetanic force (Pₒ), or maximum tetanic force normalized for overall muscle CSA (specific force; sPₒ; Table 2). However, IGF-I administration prolonged one-half relaxation time of the isometric twitch by 11% (P < 0.05; Table 2).

Susceptibility to contraction-induced damage. One of the major findings of this study was that IGF-I administration reduced the force deficit of TA muscles from mdx mice after the contraction-induced injury protocol. Specifically, 5 min after two lengthening contractions, Pₒ was 957.9 ± 34 mN in IGF-I-treated mdx mice and 631.9 ± 61 mN in untreated mdx mice. This corresponded to a significantly lower force deficit of 53 ± 4% compared with 70.7 ± 5% in treated and untreated groups, respectively (P < 0.05; Table 2; Fig. 1). Importantly, IGF-I administration did not affect the force production during lengthening contractions (Table 2; Fig. 1). Functional measurements of TA muscles of age-matched male C57BL/10 wild-type mice (n = 3) demonstrated significantly lower values for Pₒ (1,760.2 ± 82 mN; P < 0.05), higher values for sPₒ (291.0 ± 11 kN/m²; P < 0.05), and lower force deficits 5 min after two lengthening contractions (17.9 ± 1%; P < 0.05) compared with mdx mice (data not shown).

Histology and immunofluorescence. Median and individual values for TA muscle fiber CSA are presented in Fig. 2. The median CSA was not different in TA muscles of untreated mdx mice (2,844 – 3,001 μm²; 95% CI of median; n = 1,951 fibers) compared with mdx mice administered IGF-I for 8 wk (2,738 – 2,899 μm²; 95% CI of median; n = 1,950 fibers). Similarly, the percentage of centrally nucleated fibers identified in transverse sections of TA muscles was not different in mdx mice (87 ± 2%) compared with IGF-I-treated mdx mice (84 ± 1%; Fig. 2).

The percentage of EBD-positive fibers in transverse sections of TA muscles was not different in untreated mdx mice (10 ± 4%) compared with IGF-I-treated mdx mice (8 ± 4%; Fig. 3). The percentage of EBD-positive fibers was slightly higher after contraction-induced damage in TA muscles of mdx mice (main
Effect; $P < 0.05$), but IGF-I administration had no effect on EBD infiltration, because the percentage of EBD-positive fibers was not different in mdx mice (16 ± 5%) compared with IGF-I-treated mdx mice (17 ± 5%; Fig. 3). EBD-positive fibers could not be detected in TA muscles of C57BL/10 wild-type mice ($n = 3$) in control or contraction-induced damage groups (Fig. 3).

Median and individual values for SDH activity in TA muscle fibers are presented in Fig. 4. The median SDH activity was significantly higher in TA muscles of mdx mice after continuous IGF-I administration (6.00–6.14 mM/min; 95% CI of median; $n = 2,205$ fibers) compared with TA muscles of untreated mdx mice (5.64–5.76 mM/min; 95% CI of median; $n = 2,204$ fibers).

**MHC isoform analysis by SDS-PAGE and RT-PCR.** MHC IIa/x and MHC IIb protein isoforms could be detected in TA muscles of mdx mice by SDS-PAGE (Fig. 5A). TA muscles from untreated mdx mice contained 22.9 ± 3% MHC IIa/x and 77.1 ± 3% MHC IIb. IGF-I treatment significantly altered the MHC protein isoform profile such that TA muscles contained a higher percentage of MHC IIa/x (35 ± 4%) and a lower percentage of MHC IIb (65 ± 4%; $P < 0.05$; Fig. 5A).

Semiquantitative RT-PCR of total RNA with gene-specific primers demonstrated that IGF-I administration increased MHC IIA transcript by 244 ± 44% and MHC IIX by 248 ± 45% compared with untreated TA muscles of mdx mice ($P < 0.05$; Fig. 5). IGF-I administration did not alter mRNA levels...
of MHC IIb but reduced mRNA levels of embryonic MHC isoform to 52 ± 20% of untreated TA muscles of mdx mice (P < 0.05; Fig. 5B). These results indicate that the shift in MHC isoform profile toward a slower phenotype induced by continuous low-dose IGF-I administration is regulated at the level of transcription. It also is clear that the TA muscles of mdx mice contain predominantly MHC IIb and MHC IIx isoforms, because they were the only protein isoforms reliably detected by SDS PAGE and analysis of mRNA isoforms required only 18 cycles of PCR amplification compared with 30 and 35 cycles of amplification for MHC IIa and embryonic MHC, respectively (Fig. 5B).

DISCUSSION

The most important finding of this study was that continuous IGF-I administration at a relatively low dose (1.5 mg·kg⁻¹·day⁻¹) for 8 wk significantly reduced contraction-induced damage in TA muscles of mdx mice, providing further support for the notion that IGF-I administration can enhance the functional properties of dystrophic skeletal muscle. This is pertinent because skeletal muscles lacking dystrophin are highly susceptible to contraction-mediated damage, and this mode of injury contributes to the etiology of the dystrophic pathology. As such, studies on contraction-based damage assays and modalities to prevent or reduce this mode of injury in dystrophic muscle are needed (16).

The findings provide further evidence that the predominant effect of exogenous low-dose IGF-I administration in mice is a shift toward a slow muscle phenotype (10, 11). We found an increased median SDH activity and altered MHC composition in the TA muscles of mdx mice after IGF-I administration. In agreement with our previous studies, we found that IGF-I treatment shifted the MHC isoform profile from MHC IIb to MHC IIa/x in TA muscles of mdx mice (11). This effect was regulated at the transcription level, because IGF-I administration caused an increase in the mRNA levels of MHC IIa and MHC IIx, whereas MHC IIb was unchanged. The net effect of these changes was consistent with a small but significant shift from a fast to an overall slower phenotype. Interestingly, IGF-I administration decreased transcript levels of the embryonic MHC isoform. The link between this effect and muscle regeneration, muscle function, or the susceptibility to contraction-induced damage has yet to be identified.

We found that the reduction in susceptibility to contraction-induced damage after IGF-I administration was not linked to changes in EBD uptake. Previous studies have demonstrated that a single lengthening contraction (of a similar magnitude to that used in this study) did not result in a significant increase in procion orange uptake in myofibers of EDL or soleus muscles of mdx mice (7), whereas a series of five lengthening contractions significantly increased procion orange uptake in the diaphragm of mdx mice (22). Thus it appears that the extent of membrane damage is dependent on the number of lengthening contractions and/or the muscle tested. We found that as few as two lengthening contractions resulted in small but significant membrane damage in TA muscles of mdx mice, an effect not
seen in muscles from C57BL/10 mice. However, based on the force deficit in TA muscles of mdx mice after this injury protocol, membrane damage seems to play only a minor role (if any) in the lengthening contraction-mediated decrease in maximum force production, suggesting that IGF-I did not confer its protective effect via an increase in membrane stability. It also is possible that IGF-I administration could have improved force production in a subset of muscle fibers after contraction-induced damage.

Models of high-level IGF-I overexpression (e.g., transgenic mice) have indicated clearly that IGF-I can cause skeletal muscle hypertrophy and increase force generation in mdx mice (3). A recent study demonstrated that virus-mediated IGF-I overexpression in 9-mo-old mdx mice did not increase maximum force production of TA muscles and had less obvious effects on muscle morphology compared with transgenic approaches (1). On the basis of the ability of IGF-I to ameliorate the initial wave of myonecrosis in mdx mice (33), it has been suggested that transgenic overexpression before the onset of the initial phase of degeneration in mdx mice is critical for its beneficial effects (1). Virus-mediated IGF-I overexpression was found not to protect TA muscles from contraction-induced damage induced by a single lengthening contraction (1). As such, differences in the dose of IGF-I with the use of virus-mediated overexpression compared with the relatively low dose of IGF-I administered exogenously in the present study may be critical for its differential effects on dystrophic skeletal muscles.

A relatively low dose of IGF-I administration did not cause myofiber hypertrophy in mdx mice in this or previous studies (10, 11), but a similar regimen of exogenous IGF-I administration caused myofiber hypertrophy and increased force production in laminin-deficient dystrophic 129 ReJ/byidb mice (17). Hence, discrepancies regarding the effects of IGF-I on muscle hypertrophy, oxidative potential, and phenotype may be due to the dose and timing of IGF-I employed and the experimental animal model used to test its effects. Further research is required to discern the intracellular signaling cascades that may be responsible for these differences.

Assessment at the cellular (single fiber) level has shown that rat slow-twitch muscle fibers are less susceptible to contraction-induced injury than fast-twitch fibers, at a given percentage of strain beyond optimum length (19). In addition, slow-twitch muscle fibers required a greater strain beyond optimum length to achieve the same force deficit as fast-twitch fibers (19). One study has reported that fast and slow muscles of laminin-deficient dystrophic mice were not more susceptible to lengthening contractions compared with muscles from control mice (14). However, that study showed that fast (but not slow) muscles of dystrophic and control mice were susceptible to contraction-induced damage. Similar studies have not been performed to compare fast- and slow-twitch fibers from muscles of dystrophin-deficient mdx mice. An inviting hypothesis is that dystrophic muscle fibers composed of MHC IIB are more susceptible to contraction-induced injury than fibers composed predominantly of MHC I or IIa/x. It has been suggested that the difference in fiber susceptibility to contraction-mediated injury is dependent on chronic activity patterns, rather than fiber type composition (37). It is highly unlikely that exogenous IGF-I administration altered the chronic activation patterns of TA muscles in the current study. It is noteworthy that the assessment of contraction-induced damage in dystrophic muscle used in the present study is dramatically different from that of other studies which concluded that oxidative capacity does not protect muscle fibers from eccentric contraction-induced damage, such as, for example, one study that employed up to 900 cyclic lengthening contractions over a 30-min period (21), a protocol that would void any physiological differences between muscles of dystrophic and wild-type mice.

In addition to alterations in MHC isofrom composition, our working hypotheses to explain the reduction in contraction-induced damage after IGF-I treatment in dystrophic muscle include alterations in components of excitation-contraction coupling (24) in the absence of myofiber hypertrophy and/or changes in titin isoforms (15), which may be associated (but not caused) by changes in MHC isoforms. Support for this hypothesis comes from the findings of Renganathan et al. (24), who showed that transgenic mice that overexpressed IGF-I had a significant (52%) increase in the number of dihydropyridine receptors (DHPR) in EDL muscle. Thus, given that excitation-contraction uncoupling has been proposed as a contributing mechanism to the force deficit following contraction-mediated damage (23, 38), an increase in the number of DHPR may attenuate disrupted excitation-contraction coupling. Although this is speculative, similar mechanisms may account for the effects of IGF-I on the force deficit immediately following contraction-induced damage. It has been proposed that different titin isoforms in fast and slow muscles may contribute to the differential susceptibility to contraction-induced injury (15, 19).

In summary, exogenous administration of IGF-I at a relatively low dose was associated with a reduced susceptibility of dystrophic muscles to contraction-induced injury. The predominant effect of this treatment protocol to mdx mice was to induce a shift toward a more oxidative, fatigue-resistant, slow-twitch muscle phenotype in the absence of changes to myofiber CSA, muscle mass, or maximum force-producing capacity (10). Our results, when compared with other models of IGF-I overexpression, highlight the disparities between different modes of endocrine factor delivery. IGF-I has many effects on skeletal muscle, and these may differ depending on the age of the host, the IGF-I isoform used (2), and the dose and/or route of IGF-I administration. Discerning these effects and the underlying signaling events responsible for these differences is important for the development of successful treatments for skeletal muscle wasting disorders, especially myopathies.

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GRANTS

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