Insulin-like growth factor-I analogue protects muscles of dystrophic mdx mice from contraction-mediated damage

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Contraction-mediated injury is a major contributing factor to the pathophysiology of muscular dystrophy and therefore therapies that can attenuate this type of injury have clinical relevance. Systemic administration of insulin-like growth factor-I (IGF-I) has been shown to improve muscle function in dystrophic mdx mice, an effect associated with a shift towards a more oxidative muscle phenotype and a reduced susceptibility to contraction-mediated damage. The actions of IGF-I in vivo are modulated by IGF binding proteins (IGFBPs), which generally act to inhibit IGF-I signalling. We tested the hypothesis that an analogue of IGF-I (LR IGF-I), which has significantly reduced binding affinity for IGFBPs, would improve the dystrophic pathology by reducing the susceptibility to muscle injury. Dystrophic mdx and wild-type (C57BL/10) mice were administered LR IGF-I continuously (∼1.5 mg kg⁻¹ day⁻¹) via osmotic mini-pump for 4 weeks. Administration of LR IGF-I reduced the susceptibility of extensor digitorum longus, soleus and diaphragm muscles to contraction damage, as evident from lower force deficits after a protocol of lengthening contractions. In contrast to the mechanism of protection conferred by administration of IGF-I, the protection conferred by LR IGF-I was independent of changes in muscle fatigue and oxidative metabolism. This study further indicates that modulation of IGF-I signalling has therapeutic potential for muscular diseases.

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The muscular dystrophies are a group of genetic disorders characterized by progressive skeletal muscle wasting and weakness. The most severe of these conditions is Duchenne muscular dystrophy (DMD), affecting ∼1 in 3500 live male births (Emery, 2002). Duchenne muscular dystrophy results in death from cardiac and/or respiratory failure, usually before 30 years of age (Emery, 2002). Although there is currently no cure for DMD and existing therapies are inadequate, endocrine-based approaches have definite therapeutic potential (Krag et al. 2004; Schertzer et al. 2006).

Duchenne muscular dystrophy results from the loss of the membrane-stabilizing cytoskeletal protein, dystrophin (Koenig et al. 1988; Krag et al. 2004), which renders muscles fragile and more susceptible to contraction-induced injury (Moens et al. 1993; Consolino & Brooks 2004; Lynch, 2004). Protecting dystrophin-deficient muscles from the self-injury that occurs during everyday activities is of clinical significance, since contraction-mediated damage is a major contributor to the dystrophic pathology (Petrof et al. 1993; Lynch, 2004). In addition, it is important to characterize the underlying metabolic, endocrine and structural mechanisms responsible for muscle fibre susceptibility to contraction-induced injury and thereby identify strategies that may ameliorate the progression of the dystrophic pathology.

We have shown previously that administration of recombinant human insulin-like growth factor I (rhIGF-I, ∼1.5 mg kg⁻¹ day⁻¹) to mdx mice improved fatigue resistance in extensor digitorum longus (EDL), soleus and diaphragm muscles, and reduced the susceptibility...
of tibialis anterior (TA) muscles to contraction-mediated injury (Gregorevic et al. 2002, 2004; Schertzer et al. 2006). While the improved resistance to muscle fatigue was attributed to an IGF-I-induced shift to an overall slower muscle phenotype, the mechanism for the reduced susceptibility to contraction-induced injury has yet to be elucidated. The actions of IGF-I are modulated by six IGF-binding proteins (IGFBPs), which bind the majority of circulating IGF-I (Jones & Clemmons, 1995). Although IGFBPs are thought to generally inhibit the actions of IGF-I, their role in skeletal muscle is not well understood (Firth & Baxter, 2002). While the effects of exogenous administration of IGF-I to dystrophic mice are probably modulated through interactions with various IGFBPs, whether circumventing IGF-I interactions with IGFBPs can attenuate the dystrophic pathology has received only limited attention (Scherzter et al. 2007).

Long R3 IGF-I (LR IGF-I) is an analogue of IGF-I that exhibits virtually no binding to IGFBPs owing to its different amino acid sequence and altered structural conformation (Tomas et al. 1996; Yang et al. 1999; Tomas, 2001). The aim of this study was therefore to examine the effects of administration of LR IGF-I on dystrophic skeletal muscles of mdx mice. We tested the hypothesis that LR IGF-I would confer protection to dystrophic muscles by reducing their susceptibility to contraction-induced injury and altering oxidative metabolism.

**Methods**

**Animals**

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 as described by the National Health and Medical Research Council of Australia. Male C57BL/10ScSn (BL/10) and C57BL/10ScSn-mdx/J dystrophic (mdx) mice (8-10 weeks old; n = 32 total) were obtained from the Animal Resource Centre (Canning Vale, Western Australia, Australia), and housed in the Biological Research Facility at The University of Melbourne under a 12 h light-12 h dark cycle, with drinking water and standard chow provided ad libitum.

The BL/10 and mdx mice were randomly assigned to either a control or a LR IGF-I-treated group (n = 8 per group). The LR IGF-I was infused continuously via a subcutaneously implanted osmotic mini-pump (Alzet Corporation, Cupertino, CA, USA). A subgroup of mice (n = 3) from each untreated group received the vehicle (saline + 10 mmol l−1 HCl) via osmotic pump to control for the method of administration. Treated mice were administered LR IGF-I (GroPep, Thebarton, South Australia, Australia) at a daily dose of ~1.5 mg kg−1 body mass, which is similar to the dose of rhIGF-I we have administered previously to mice (Gregorevic et al. 2002, 2004; Schertzer et al. 2006). The LR IGF-I was dissolved in 10 mmol l−1 of HCl and sterile isotonic saline at a concentration of 9.3 mg ml−1 for administration to BL/10 mice and 10.9 mg ml−1 for mdx dystrophic mice (to account for the greater body mass of age-matched mdx mice). The LR IGF-I solution was loaded into osmotic pumps with a pumping rate of ∼0.125 μl h−1 and an operational duration of 28 days (Gregorevic et al. 2002). Pumps were primed in isotonic saline at 4°C for 24 h prior to implantation. Mice were anaesthetized with 100 mg kg−1 ketamine and 10 mg kg−1 xylazine (i.p.) such that they were unresponsive to tactile stimuli. A small incision was made in the skin of the upper back and a small subcutaneous pocket created by blunt dissection. Primed osmotic mini-pumps were inserted in the pocket under the skin with the delivery portal directed distally. The incision was closed with Michel clips (Aesculap, Germany) and the animal allowed to recover from anaesthesia. Following the 4 week treatment period, pumps were aspirated to ensure contents had been administered accordingly.

**Muscle function**

At the completion of treatment, mice were anaesthetized with sodium pentobarbitone (Nembutal, Sigma-Aldrich, NSW, Australia; 60 mg kg−1 i.p.) such that they were unresponsive to tactile stimuli. Supplementary doses were administered as required to maintain the appropriate depth of anaesthesia. Isometric contractile properties of the EDL and soleus muscles and diaphragm muscle strips were assessed in vitro as described in detail previously (Gregorevic et al. 2002, 2004). Since the width of individual diaphragm muscle strips will vary depending on the surgeon performing the excision, measures of absolute peak twitch force (Pf) and tetanic force (Pm) are not valid and must be normalized with respect to overall muscle cross-sectional area (specific force, spm, in kN m−2).

Following determination of the force–frequency relationship, muscles were subjected to a contraction-induced injury protocol (EDL and soleus muscles and a diaphragm muscle strip) or a fatigue protocol (EDL and soleus muscles from the contralateral limb; Gregorevic et al. 2002, 2004, 2006). The protocol and assessment of contraction-induced injury has been described in detail elsewhere (Gregorevic et al. 2006; Schertzer et al. 2006). Briefly, isolated muscles were stimulated to produce maximal isometric force and then subjected to a lengthening contraction [at a velocity of 2 fibre lengths (L0) s−1] at progressively increasing magnitudes of stretch (5, 10, 20, 30 and 40% of L0), with Pf determined prior to each lengthening contraction. Muscles were allowed to recover for 2 min between each contraction in order to prevent fatigue. The contraction-induced injury protocol examined the functional deficits following successive...
lengthening contractions of increasing magnitudes of strain. Thus, the protocol provides a means for examining cumulative injury to normal and dystrophic muscles, beginning with lengthening contractions of small magnitude, with subsequent successive lengthening contractions at progressively larger strains. Such protocols are able to distinguish injury susceptibility between muscles of dystrophic mdx and wild-type control mice. The strains of low magnitude mimic those that are likely to occur during everyday activities, whereas those of larger magnitudes are similar to those that occur to limb muscles during sudden falls or to diaphragm muscles during coughing bouts under restricted breathing conditions.

Muscle fatigability was assessed using a standard fatigue protocol as described in detail previously (Gregorevic et al. 2002). Muscles were stimulated maximally once every 4 s for 4 min, with maximal force reported every minute. During recovery, $P_o$ was determined at 5, 10 and 15 min following completion of the fatigue protocol. Muscles were trimmed of tendons and any adherant non-muscle tissue, blotted on filter paper, weighed on an analytical balance, snap frozen in thawing isopentane, and stored at −80°C for histological and biochemical analyses. The heart and liver were also trimmed carefully, blotted once on filter paper, and weighed on an analytical balance.

**Histological analysis**

Transverse muscle sections (8 μm thick) were cut from the mid-belly region of each muscle using a cryostat. Sections were stained with Haematoxylin and Eosin for examination of fibre cross-sectional area (CSA) and central nucleation (a marker of the regenerating muscle fibres; Harcourt et al. 2005). Collagen content within each muscle cross-section was determined with Van Gieson’s stain, as described previously (Harcourt et al. 2005). Sections were imaged using an upright microscope (BH-2; Olympus, Japan) with camera (Spot model 1.3.0; Diagnostic Instruments). Digitized images were analysed using Leica IM50 image software (version 4.0; Leica Microsystems Imaging Solutions Ltd, Cambridge, UK) controlled by Spot Diagnostic software (version 2.1; Diagnostic Instruments). Section thickness was calculated in a double-blinded manner. The median fibre CSA was determined by measuring the circumference of no less than 200 adjacent fibres from the centre of each cross-section. Mean diaphragm muscle strip thickness was determined at no less than 10 points along the entire muscle section.

**Insulin-like growth factor I levels**

Serum IGF-I levels were quantified using a mouse IGF-I immunoassay kit (MG 100; R&D Systems, Minneapolis, MN, USA). Immediately prior to diaphragm excision, blood was sampled intraventricularly via cardiac puncture, and allowed to coagulate for 30 min at room temperature after extraction. The blood was centrifuged at 1000 g for 15 min to separate out serum, which was removed, frozen in liquid nitrogen and stored at −80°C for analysis of IGF-I according to the manufacturer’s instructions and as described previously (Schertzer & Lynch, 2006).

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**Table 1. Morphological parameters from BL/10 and mdx mice after 4 weeks of continuous LR IGF-I administration**

<table>
<thead>
<tr>
<th></th>
<th>BL/10</th>
<th>LR IGF-I</th>
<th>mdx</th>
<th>LR IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body mass (g)</td>
<td>27.2 ± 0.7</td>
<td>29.5 ± 0.4</td>
<td>31.8 ± 0.9*</td>
<td>33.2 ± 0.6</td>
</tr>
<tr>
<td>Extensor digitorum longus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (mg)</td>
<td>8.8 ± 0.3</td>
<td>8.5 ± 0.2</td>
<td>14.6 ± 0.4*</td>
<td>15.0 ± 0.6</td>
</tr>
<tr>
<td>EDL/BM (mg g⁻¹)</td>
<td>0.32 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.46 ± 0.01*</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>CSA (μm²); 95% CI of median</td>
<td>1021–1114</td>
<td>1073–1180</td>
<td>1317–1427*</td>
<td>1035–1123†</td>
</tr>
<tr>
<td>Percentage of fibres centrally nucleated</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>62.6 ± 1.8*</td>
<td>65.2 ± 2.8</td>
</tr>
<tr>
<td>Percentage collagen infiltration</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>2.1 ± 0.3*</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (mg)</td>
<td>6.7 ± 0.2</td>
<td>6.2 ± 0.1</td>
<td>10.1 ± 0.4*</td>
<td>9.8 ± 0.4</td>
</tr>
<tr>
<td>Soleus/BM (mg g⁻¹)</td>
<td>0.25 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>CSA (μm²); 95% CI of median</td>
<td>1096–1139</td>
<td>1138–1179</td>
<td>1162–1249*</td>
<td>1058–1129†</td>
</tr>
<tr>
<td>Percentage of fibres centrally nucleated</td>
<td>0.3 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>57.5 ± 2.6*</td>
<td>56.4 ± 2.6</td>
</tr>
<tr>
<td>Percentage collagen infiltration</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>3.6 ± 0.6*</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>Diaphragm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness (μm)</td>
<td>341 ± 7</td>
<td>349 ± 9</td>
<td>493 ± 15*</td>
<td>557 ± 16</td>
</tr>
<tr>
<td>CSA (μm²); 95% CI of median</td>
<td>649–680</td>
<td>645–679</td>
<td>391–426</td>
<td>387–428</td>
</tr>
<tr>
<td>Percentage of fibres centrally nucleated</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>27.8 ± 1.4*</td>
<td>27.1 ± 0.8</td>
</tr>
<tr>
<td>Percentage collagen infiltration</td>
<td>3.7 ± 0.8</td>
<td>4.2 ± 0.8</td>
<td>14.8 ± 2.3*</td>
<td>10.9 ± 1.5</td>
</tr>
</tbody>
</table>

Abbreviations: BM, body mass; CI, confidence interval; CSA, cross-sectional area; and EDL, extensor digitorum longus. *P < 0.05 BL/10 versus mdx; †P < 0.05 control versus LR IGF-I within respective strain of mouse. (n = 8 per group).
Citrate synthase activity

Citrate synthase activity was determined in whole muscle homogenates using an assay kit (CS0720; Sigma-Aldrich, USA). The EDL, soleus and diaphragm muscle samples were thawed on ice and prepared according to methods we have described previously (Schertzer et al. 2005). Total muscle protein was determined in triplicate by the method of Bradford, and the protein concentration of all samples equalized. Citrate synthase activity was determined in triplicate based on the formation of TNB (2-nitro-5-thiobenzoic acid) at a wavelength of 412 nm at 25°C on a spectrophotometer (Multiskan Spectrum, Thermo Labsystems, Boston, MA, USA). In each well, 8 μl of sample was added to a reaction medium containing 178 μl assay buffer, 2 μl of 30 mM acetyl coenzyme A and 10 mM TNB acid. The baseline assay solution absorbance was recorded, reactions were initiated by addition of 10 μl oxaloacetic acid, and the change in absorbance measured every 15 s for 2 min.

Statistical analysis

All values are expressed as means ± s.e.m. unless specified otherwise. Groups were compared using two-way analysis of variance where appropriate. For all comparisons, the level of significance was set at \( P < 0.05 \). Bonferroni’s post hoc multiple comparison procedure was used to determine significant differences between groups. The CSA data were analysed using the Anderson–Darling Normality Test. Muscle fibre CSA was not normally distributed; therefore, the 95% confidence interval of the median was used and differences were considered significant when no overlap existed between the 95% confidence interval of the median.

Results

Morphological properties

As we and others have reported previously, mdx mice had a greater body mass than age-matched BL/10 mice \( (P < 0.05) \), greater EDL and soleus masses (66 and 51%, respectively), and a 45% greater mean diaphragm thickness \( (P < 0.05; \text{Table 1}) \). Administration of LR IGF-I did not alter body mass, EDL or soleus muscle mass, or diaphragm thickness in either BL/10 or mdx mice. Heart and liver mass were also unaffected by LR IGF-I administration (data not shown).

The EDL and soleus muscles from mdx mice exhibited a 29 and 8% greater median fibre CSA than those from BL/10 mice, respectively \( (P < 0.05; \text{Fig. 1}) \). Diaphragm median CSA was 63% lower in mdx mice compared with BL/10 mice \( (P < 0.05) \). Administration of LR IGF-I reduced median fibre CSA by 21% in EDL and by 9% in soleus muscles from mdx mice \( (P < 0.05) \), but had no effect on fibre CSA in the diaphragm.

All muscles from mdx mice exhibited a significant proportion of centrally nucleated fibres and increased collagen infiltration compared with muscles from BL/10 mice (Table 1). Administration of LR IGF-I had no effect on central nucleation or on the extent of collagen infiltration in BL/10 or mdx mice.

Figure 1. Individual and median data for EDL (A), soleus (B) and diaphragm (C) myofibre CSA

* Indicates a significant difference in median myofibre CSA between EDL, soleus and diaphragm muscles from mdx compared with BL/10 mice \( (P < 0.05) \). † Indicates a significant decrease in median myofibre CSA in EDL and soleus muscles after LR IGF-I treatment \( (P < 0.05) \).
Muscle function assessed in vitro

Extensor digitorum longus muscles from mdx mice had a 23% greater $P_0$ than those from BL/10 mice ($P < 0.05$; Table 2). However, when $P_0$ was normalized for muscle cross-sectional area, $sP_0$ was 25% lower than in BL/10 mice ($P < 0.05$; Table 2). The rate of force development ($dP/dt$) in the EDL muscle during twitch and tetanic responses was faster in mdx mice than in BL/10 mice ($P < 0.05$, main effect; Table 2). The $P_0$ was 6% higher in the soleus muscles of mdx mice, but $sP_0$ was 20% lower in than in BL/10 mice ($P < 0.05$; Table 2). For the diaphragm, $sP_0$ of muscle strips was 46% lower in mdx than in BL/10 mice ($P < 0.05$; Table 2). Administration of LR IGF-I had no effect on the contractile properties of the EDL, soleus or diaphragm muscles in either mdx or BL/10 mice (Table 2).

Muscle susceptibility to contraction-induced injury

The EDL muscles from mdx mice were more susceptible to injury during lengthening contractions than those from BL/10 mice. A greater deficit in $P_0$ was evident after lengthening contractions that were 20% beyond optimal fibre length (i.e. $L_f + 20\%$, $P < 0.05$; Fig. 2). The force deficit was less in EDL muscles from treated compared with untreated mdx mice, at $L_f + 40\%$ ($P < 0.05$).

The force deficit after lengthening contractions was greater in soleus muscles from mdx mice compared with BL/10 mice, at stretches beyond $L_f + 20\%$ ($P < 0.05$; Fig. 2). Soleus muscles from LR IGF-I treated mdx mice had a reduced force deficit compared with those from untreated mdx mice at lengthening contractions beyond $L_f + 30\%$ ($P < 0.05$).

For the diaphragm muscle strips, mdx mice exhibited a greater force deficit after lengthening contractions than BL/10 mice at stretches beyond $L_f + 10\%$ ($P < 0.05$). Importantly, LR IGF-I treated mdx mice had a reduced force deficit compared with untreated mdx mice, which was significant following stretches of the diaphragm strip beyond $L_f + 30\%$ ($P < 0.05$).

Muscle fatigue and recovery

The muscle fatigability and the ability to recover force-producing capacity after fatigue were not different in EDL muscles between BL/10 and mdx mice (Fig. 3A). However, soleus muscles from mdx mice were more resistant to...
fatigue than those from BL/10 mice ($P < 0.05$; Fig. 3B). Treatment with LR IGF-I had no effect on the fatiguability or recovery of force in either the EDL or the soleus muscles from BL/10 or mdx mice.

### Serum IGF-I levels and citrate synthase activity

Serum IGF-I concentration was lower in BL/10 mice than in mdx mice ($P < 0.05$, main effect; Fig. 4). Treatment with LR IGF-I reduced serum IGF-I concentration as a main effect across strains ($P < 0.05$, main effect).

Citrate synthase activity was higher in EDL and soleus muscles from BL/10 mice compared with mdx mice ($P < 0.05$; Fig. 5). There was no difference in citrate synthase activity in diaphragm muscles between BL/10 and mdx mice. Treatment with LR IGF-I had no effect on citrate synthase activity in the EDL, soleus or diaphragm muscles from BL/10 or mdx mice.

### Discussion

The most important finding of this study was that systemic administration of LR IGF-I reduced the susceptibility of the EDL, soleus and diaphragm muscles of mdx mice to contraction-mediated injury, independent of alterations to markers of muscle oxidative metabolism. This has...
important clinical significance, given that contraction-induced damage is a major contributor to the fibre degeneration and progressive muscle wasting in muscular dystrophy (Lynch, 2004). Furthermore, therapies that improve diaphragm muscle function in mdx mice are especially relevant, since the diaphragm most closely resembles the pathology in DMD (Stedman et al. 1991), and because respiratory function is a key predictor of mortality in DMD (Emery, 2002).

Despite its mechanism of action being to circumvent the inhibitory actions of IGFBPs, administration of LR IGF-I did not alter muscle mass, maximal force-producing capacity or specific force in either BL/10 or mdx mice. It did, however, reduce serum levels of IGF-I in BL/10 and mdx mice. In contrast, high-level muscle-specific expression of IGF-I causes significant muscle hypertrophy (Barton et al. 2002). The findings of this study are consistent with previous studies in which a similar dose of rhIGF-I also did not alter muscle mass or force-producing capacity (Gregorevic et al. 2002, 2004; Schertzer et al. 2006). Administration of LR IGF-I reduced the median myofibre CSA in both the EDL and the soleus muscles of mdx mice. Such changes in median myofibre CSA have not been reported previously with rhIGF-I administration (Gregorevic et al. 2002, 2004; Schertzer et al. 2006), suggesting that IGFBPs may play an important role in regulating myofibre size.

In our previous studies, we have shown that rhIGF-I administration increased resistance to fatigue in EDL and soleus muscles of mdx mice (Gregorevic et al. 2002, 2004). However, in the present study, we found that LR IGF-I administration did not alter fatigue resistance in either fast- or slow-twitch muscles. The different effects of LR IGF-I and rhIGF-I administration indicate that IGFBPs may play a role in regulating muscle oxidative capacity. Muscle citrate synthase activity was not affected by LR IGF-I treatment, indicating that muscle oxidative capacity was unaffected; findings that are consistent with the data for muscle fatigue. This was an unexpected finding, given we had hypothesized that protection from contraction-induced injury would be attributed to a shift in fibre proportions towards an overall slower, more oxidative muscle phenotype.

![Figure 4. Serum IGF-I levels](image)

**Figure 4. Serum IGF-I levels**

* Indicates that BL/10 mice had lower serum IGF-I levels than mdx mice ($P < 0.05$, main effect). † Indicates that following 28 days of continuous LR IGF-I infusion, levels of IGF-I were reduced when compared with control values in the respective strain of mouse ($P < 0.05$, main effect).

![Figure 5. Citrate synthase activity in the EDL (A), soleus (B) and diaphragm muscles (C)](image)

**Figure 5. Citrate synthase activity in the EDL (A), soleus (B) and diaphragm muscles (C)**

* Indicates an increased citrate synthase activity in the EDL and soleus muscles from BL/10 mice compared with mdx mice ($P < 0.05$).
(Schertzer et al. 2006). This is the first study to demonstrate an IGF-I-mediated protection from contraction-induced injury, independent of a shift in muscle oxidative capacity.

The mechanism by which LR IGF-I and rhIGF-I confer protection from contraction-mediated damage in dystrophic skeletal muscle is not clear. Studies in transgenic mice with muscle-specific overexpression of IGF-I have reported an increase in the number of dihydropyridine receptors (DHPRs; Renganathan et al. 1997). Dihydropyridine receptors are located on the t-tubule membrane and form a direct physical link with ryanodine receptors (RyR; sarcoplasmic Ca$^{2+}$-release channels). The interaction between these two receptors is an important component of excitation–contraction (E–C) coupling (Anderson et al. 1994). It has been hypothesized that alterations in E–C coupling following IGF-I treatment may influence susceptibility to injury by increasing the number of DHPRs, thus improving E–C coupling immediately after lengthening contractions (Schertzer & Lynch, 2006). One study has shown that up to 75% of the deficit in force-producing capacity after lengthening contractions could be explained by disruptions at the t-tubule–sarcoplasmic reticulum interface (Ingalls et al. 1998). Furthermore, reductions in the susceptibility to contraction-induced injury are also noted in transgenic mdx:IGF$^{++}$ mice when muscles are subjected to equivalent contractile forces (Barton et al. 2002).

Dystrophin-deficient myofibres are fragile and more susceptible to contraction-mediated damage, which can result in membrane tears, calcium influx and pathological myofibre degeneration and regeneration. Damage from everyday contractions can lead to repeated bouts of muscle fibre degeneration and subsequent regeneration, which can potentially compromise the overall regenerative capacity of the muscle (Zacharias & Anderson 1991; Reimann et al. 2000), leading to infiltration of adipose and fibrotic tissue and significant functional deficits. Reducing the susceptibility of dystrophic skeletal muscles to contraction-mediated injury could potentially reduce the magnitude of the muscle fibre degeneration and thus help maintain a more stable muscle phenotype. For these reasons, we believe that any treatment that can reduce muscle injury has significant therapeutic potential for DMD.

Consistent with our previous findings, soleus muscles from mdx mice fatigued less during repeated intermittent stimulation than soleus muscles from BL/10 mice (Gregorevic et al. 2004). Interestingly, citrate synthase activity was lower in the mdx soleus compared with the BL/10 soleus, which was surprising given that citrate synthase is one of the rate-limiting enzymes in oxidative metabolism and controls one of the initial steps of the citric acid cycle (Wiegand & Remington, 1986). This suggests that other factors are also important in determining muscle fatigue resistance.

We found a higher concentration of IGF-I in the serum of mdx mice than of BL/10 mice, a finding that has been demonstrated previously in mice of a similar age (De Luca et al. 1999) and which is thought to contribute to the greater regenerative potential observed in muscles of mdx mice (Brooks, 1998). Continuous LR IGF-I administration caused a reduction in serum IGF-I concentration, which may be attributed to reduced endogenous production of IGF-I. It is not possible to distinguish between endogenous IGF-I and exogenous LR IGF-I with current techniques.

In summary, our findings demonstrate for the first time that modulating IGF signalling by circumventing the actions of IGF-binding proteins conferred protection from contraction-induced injury that was independent of changes to muscle oxidative capacity. Protection from contraction-mediated damage, especially in the diaphragm, is of clinical significance for DMD, since respiratory function is a key predictor of mortality in these patients.

### References


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