The physiological effects of IGF-1 (class 1:Ea transgene) over-expression on exercise-induced damage and adaptation in dystrophic muscles of mdx mice

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Abstract Duchenne muscular dystrophy (DMD) is a genetic disorder in which muscle weakness and fragility contribute to ongoing muscle degeneration. Although exercise-induced muscle damage is associated with adaptation that protects normal muscle from further damage, exploiting this process to protect dystrophic muscle has been avoided for fear of inducing excessive muscle degeneration. However, muscle-specific over-expression of the class 1:Ea isoform of insulin-like growth factor-1 (IGF-1) reduces myofibre necrosis in dystrophic mdx mice (a model for DMD) and, therefore, may enhance the adaptation process in response to eccentric exercise. To test this hypothesis, we evaluated the effect of transgenic class 1:Ea IGF-1 over-expression on the susceptibility to muscle damage and subsequent adaptation in 12-week-old dystrophic mdx and non-dystrophic control mice. Experiments were conducted in vivo using a custom-built isokinetic mouse dynamometer to measure the deficit in joint torque (indicating muscle damage) after 20 maximal lengthening (eccentric) contractions. Adaptation to this damaging exercise was evaluated by repeating the protocol 7 days after the initial exercise. The over-expression of IGF-1 significantly increased the normalised joint torque in non-dystrophic mice and appeared to ameliorate the muscle weakness in dystrophic mice. All mice displayed a marked reduction in the susceptibility to muscle damage on day 7; however, this adaptation was unaffected by IGF-1, showing that IGF-1 does not protect the dystrophic muscles of adult mdx mice against damage resulting from maximal lengthening contractions.

Keywords Insulin-like growth factor · Eccentric muscle damage · Isokinetic dynamometer · Duchenne muscular dystrophy · Mdx mouse · Dystrophy · Muscle damage · Muscle adaptation · Mouse

Introduction

Duchenne muscular dystrophy (DMD) is the most common of the genetic muscular dystrophies (affecting about one in 3,500 newborn males) in which mutations in the gene encoding dystrophin result in defects in the dystrophin-associated glycoprotein complex at the sarcolemma. Dystrophin plays a crucial role in connecting the contractile cytoskeleton within the myofibre to the extracellular matrix and, where this link is defective, forces generated by contraction tear the sarcolemma resulting in myofibre damage leading to necrosis [23, 45]. Although many treatments for DMD have been proposed, few have proven to be effective in extending life and functional capabilities for more than a few years: corticosteroids remain the most widely used intervention for DMD but the effects are variable and there can be adverse side effects [1]. Voluntary exercise as a treatment modality has shown conflicting results with evidence of both beneficial [7, 8, 25, 46] and deleterious outcomes [8, 11]. The precise effects and potential benefits of different forms of exercise on skeletal muscle function in the dystrophic condition remains poorly understood, although regular, low-intensity exercise appears beneficial [18, 42].
Exercise-induced muscle damage (EIMD) is a common event that can occur in normal daily life as well as in sporting activities and high-intensity training [16]. It is more pronounced in activities involving lengthening muscle actions (also referred to as eccentric contractions) in which the contracting muscle is forcibly lengthened as occurs when walking down stairs or squatting. The extent of EIMD can be considerable if an individual is not accustomed to such activities. However, the initial bout of damaging exercise is followed by a period of muscle repair and adaptation resulting in a muscle that is less susceptible to damage when performing subsequent bouts of equivalent exercise. This phenomenon has been termed the ‘repeated bout effect’ [31]. The use of this adaptive or protective effect has been proposed as a possible therapy for neuromuscular disorders such as the muscular dystrophies; however, its effects have yet to be quantified.

The increased susceptibility to EIMD in dystrophic muscles from mdx mice has largely precluded the investigation of the potential benefits of the physiological adaptations that arise from the use of “eccentric” exercise as a treatment modality for DMD patients [2]. However, persons with mild dystrophic conditions (e.g. Becker’s muscular dystrophy) have shown similar responses to high-intensity eccentric exercise as non-dystrophic people [27]. The effects of chronic exercise, such as voluntary wheel running and treadmill running, have shown mixed outcomes for skeletal muscle function in dystrophic mdx mice. Young mdx mice that begin voluntary wheel running at 3–4 weeks of age display improved muscle function including increased fatigue resistance and force production [7, 8, 11]. Whereas forced treadmill running, designed to worsen the dystrophy progression in mdx mice, has been linked to muscle weakness and impaired calcium handling [10, 12] and downregulation of IGF-1 [34] in muscles of mdx mice.

The use of insulin-like growth factor-1 (IGF-1) has also been investigated as a method for the amelioration of dystrophic symptoms in the mdx mouse model of DMD. It has recently been demonstrated that transgenic over-expression of class 1:Ea IGF-1 (originally referred to as mIGF-1) in mdx mice reduces the amount of myofibre necrosis in young mdx/IGF-1 mice [4, 39] and improved excitation–contraction coupling in adult (20- to 24-week-old) mdx/IGF-1 mice [37]. Furthermore, systemic administration of IGF-1 reduces the deleterious effects of forced treadmill running [10] and increases resistance to fatigue [19] in mdx mice.

Given the improvements in skeletal muscle function brought about by IGF-1 treatment, it was of interest to determine if muscle-specific over-expression of IGF-1 would facilitate the adaptation process in normal and dystrophic mdx mice. To further evaluate the effects of exercise on dystrophic muscle in mdx mice, we have used a custom-built isokinetic mouse dynamometer [24] to determine the extent of EIMD and subsequent adaptation induced by a series of repeated lengthening contractions. The effects of muscle-specific over-expression of IGF-1 (class 1:Ea) on the extent of initial damage (measured by a reduction in joint torque and shift in optimal joint angle) and subsequent adaptation was determined in mdx and control (non-dystrophic) mice.

Materials and methods

Animals

Five inbred mouse strains were used in these studies: all mice were derived from colonies established at The Animal Resources Centre, Murdoch, Western Australia with the transgenic class 1:Ea IGF-1 mice originally provided by Prof. Nadia Rosenthal, EMBL, Italy. All mice were housed individually in standard cages with the ambient temperature maintained at 22°C and the light–dark cycle fixed at 12:12 h. Water and food (rat and mouse chow) was available ad libitum. All animal procedures were in accordance with the guidelines and approval of the University of Western Australia Animal Ethics Committee.

Experiments were performed on 12-week-old male mice to determine the effects of the class 1:Ea isoform of IGF-1 driven by the skeletal muscle-specific myosin light chain promoter (widely referred to as mIGF-1) on the susceptibility to and recovery from EIMD. These effects were examined in both normal non-dystrophic mice bred on an FVB/N background and in dystrophic mice on a C5710ScSn×FVB/N background. For the non-dystrophic group, the transgenic mice (referred to as IGF-1(+)) [33, 39] were compared with non-transgenic littermate FVB/N control mice (hereafter referred to as IGF-1(−)). In the dystrophic group, the transgenic mice (mdx/IGF-1(+)) were generated by crossing mdx mice with IGF-1(+) mice [4, 41] and transgene negative littermate mice (mdx/IGF-1(−)) were used as dystrophic controls. These transgenic and littermate null mdx/IGF-1 mice were thus both on a background parental C57/FVB strain (resulting from crossing C57Bl/10ScSn and FVB mice). Therefore, the C57/FVB mice represent a non-dystrophic control for the mdx/IGF-1(−) and mdx/IGF-1(+).

The absence of dystrophin in mdx mice results in an abrupt onset of muscle pathology at 3 weeks of age at which time necrotic tissue occupies about 20–40% (or more) of the muscle. The damage peaks around 4 weeks and then decreases significantly to stabilise by 12 weeks of age to a relatively low level of active damage where only about 5% of the tissue is affected [22, 30]. We have previously shown that over-expression of mIGF-1 strikingly reduced the extent of myofibre damage by up to 97% in tibialis anterior muscle of young (3-week-old) mdx mice.
Although a similar effect was not evident in adult (12-week-old) mdx mice, this might have been difficult to detect with such low levels of muscle damage (only approximately 1–2% of the tissue was affected) [39]. Any protective effect of mIGF-1 over-expression in adult mdx mice may only become evident when the animal is exposed to a series of damaging eccentric contractions.

The effects of IGF-1 on skeletal muscle function and the susceptibility to EIMD in both normal and dystrophic muscle were examined in an initial experiment that involved a bout of lengthening muscle actions, referred to as day 0. The adaptation to this initial bout of damaging lengthening muscle actions was determined by a second bout at day 7. The extent of EIMD and subsequent adaptation were quantified by the decrease in maximal torque production and change in optimal joint angle towards longer muscle lengths. These markers are robust mechanical measures of muscle damage that recover in a predictable time-course [35]: they are not subject to the high variability between individual mice, complicated by issues of representative tissue sampling, that is seen with biochemical and histological markers of muscle damage [13, 22, 26, 38].

Experimental set-up for the mouse dynamometer

Mice were anaesthetised by inhalation of a gaseous mixture of halothane (halothane, 0.3 L/min N₂O, 0.4 L/min O₂). Maintenance of the anaesthetic throughout the experiment was achieved via a flow-through facemask placed over the mouse’s head. The hair on the lower right hind limb was shaved with electric shears and the right foot of the mouse was positioned in an aluminium “shoe” that was attached to a small axial torque transducer fixed to the shaft of the servomotor. The “shoe” was aligned so that the centre of rotation of the ankle joint was coincident with the longitudinal axis of the servomotor shaft (see Fig. 1). Correct alignment was verified by observing the joint torque while passively moving the ankle through a full range of motion (15° dorsiflexion to 55° plantar flexion) under computer control at 10°s⁻¹. The right knee was positioned in a clamp system so that the femur was parallel to the foot and perpendicular to the tibia. The position of the foot perpendicular to the tibia was defined as the neutral position (0°).

Electrical stimulation and muscle contraction

Parallel hook electrodes (Frederick Haer, Bowdoinham, ME, USA) were attached to the common peroneal nerve of the right hind limb through a small skin incision (3–4 mm long) distal to the head of the fibula (Fig. 1, inset). Activation of the anterior crural muscles (tibialis anterior [TA], extensor digitorum longus [EDL], extensor hallucis longus [EHL]) was achieved by electrical stimulation of the common peroneal nerve using a Grass (S88) stimulator and stimulus isolation unit (SIU5, Grass Instruments, Quincy, MA, USA). The nerve was stimulated using a train of 0.1 ms bi-phasic square wave pulses using a frequency and voltage that was optimised for each mouse.

Experimental protocol for exercise-induced muscle damage

The experimental procedure required the establishment of optimal stimulation parameters based on the torque–volt and torque–frequency relationships, which were then used to determine the torque–angle relationship. Following an EIMD protocol of 20 lengthening muscle actions, a recovery period of 10 min was enforced and the previous procedures were repeated to quantify the extent of muscle damage.

Torque–volt relationship

To identify the optimal stimulation voltage to be used in subsequent protocols, the torque–volt relationship was determined for a range of stimulation intensities between 1 and 15 V. Isometric contractions were elicited at a 0° ankle angle from 200 ms trains of pulses delivered at 150 Hz. A rest period of 30 s was enforced between trials to minimise fatigue. The voltage that produced the highest joint torque was used for the subsequent procedures.

Fig. 1 Schematic representation of the hind limb orientation for isokinetic dynamometer testing. The knee joint was fixed at 90° by a mechanical clamp (shaded bar). The ankle joint was aligned with the axis of the torque transducer and the foot fixed in a “shoe” apparatus (front-on view shown in small inset). The muscles of the anterior compartment were activated by stimulation of the common peroneal nerve accessed through a small incision in the skin (large inset)
Torque–frequency relationship

The torque–frequency relationship was established using the optimal stimulation intensity determined from the torque–volt relationship. The isometric joint torque was recorded at 0° ankle angle from 200 ms trains of pulses delivered at 30 s intervals to the common peroneal nerve at stimulation frequencies ranging from 40 to 350 Hz. The frequency at which the highest joint torque was recorded was used for the subsequent procedures.

Torque–angle relationship

The optimal stimulation parameters determined from the torque–volt and torque–frequency protocols were used to establish the torque–angle relationship for the anterior crural muscles. The foot was passively moved by the servomotor to 15° of dorsiflexion and held in this position and a 200-ms train of pulses were delivered to the common peroneal nerve to elicit an isometric contraction. After cessation of the stimulation and relaxation of the muscle, the ankle angle was moved by 5° towards plantar flexion and rested at this new angular position for 30 s prior to the recording of isometric torque. This procedure was repeated at 5° increments until the isometric torque had been recorded at angles up to 55° of plantar flexion.

Each isometric contraction was analysed for the mean peak torque during the final 80 ms of activation. The mean peak torque was plotted against joint angle and a Gaussian fit was applied using Curve Expert v1.36 (Hyams (c) 1995–1998, Starkville, MS, USA). The Gaussian model fitted was of the form:

\[ y = a \exp \left( -\frac{(x-b)^2}{2c^2} \right) \]

The coefficients of this fit are: \( a \) is the amplitude of the Gaussian fitted curve, \( b \) is the centroid (location of peak) and \( c \) is the width at half the peak (a normalised variance). Thus, the anatomical and physiological description of this fitted curve can be interpreted as: \( a \) = the peak torque of the torque–angle relationship, \( b \) = the angle at which peak torque was generated and \( c \) = the width (determined by the steepness or flatness) of the torque–angle relationship. These coefficients were used for subsequent analysis.

Exercise-induced muscle damage

Mice were subjected to an eccentric exercise protocol consisting of 20 lengthening contractions between 15° and 55° plantar flexion with a 30-s rest period between trials. The anterior crural muscles were stimulated at an initial angle of 15° plantar flexion and, after 100 ms when the isometric tension had reached a steady state, the ankle was rotated to 55° plantar flexion at an angular velocity of 1,000°s\(^{-1}\). The total stimulus duration in this protocol was 140 ms. At the completion of the contraction, the foot was immediately and passively returned to the starting position at 10°s\(^{-1}\) under servomotor control.

The servomotor position and velocity, as well as the resultant dorsiflexion torque from the transducer were recorded at 5 kHz and saved to disk for subsequent analysis. After a 10-min rest period, the torque–volt, torque–frequency and torque–angle relationships were re-established. The assessment of eccentric muscle performance was also repeated, but in this instance consisted of only five eccentric contractions. When repeated measures were required at day 7, the incision over the nerve site was sutured and the mice were allowed to recover. After final measurements were completed, the mice were killed via cervical dislocation while under terminal anaesthesia.

Additional evidence of muscle damage after dynamometer exercise

In additional experiments (\( n = 4 \)), alternative markers of muscle damage resulting from the eccentric exercise protocol were obtained including membrane permeability to Evans blue dye (EBD) and histological evidence of myofibre necrosis. Mice were injected intraperitoneally with 1% EBD (Sigma, St. Louis, MO, USA) in phosphate-buffered saline sterilised by passage through a Millex®-GP 0.22 μm filter (Millipore, Bedford, MA, USA). An injection of 1% body weight EBD was administered 24 h before killing the mice (and 24 h after the exercise regime) [24]. At 48 h (2 days) after completion of the dynamometer exercise regime, the tibialis anterior muscles from the exercised (right) and non-exercised (left) limbs were removed, mounted onto tragacanth gum and frozen in liquid nitrogen quenched in isopentane. Frozen sections (10 μm) were cut on a Leica (CM3050) cryostat and alternate sections were mounted for fluorescence imaging of EBD or for haematoxylin and eosin (H&E) staining [38]. Additional H&E sections were obtained from the exercised and non-exercised limbs of several mice immediately following the day 7 exercise regime. After terminal anaesthesia of these mice, both hind limbs were placed in buffered formol saline, processed and embedded in paraffin wax. Five micrometer transverse sections were cut and stained for H&E using standard procedures.

Statistics

The group means±SE were calculated for all dependent variables. Statistical analysis of between group differences for peak torque, optimal joint angle and width of the torque–angle relationship was performed using a repeated-measures analysis of variance (ANOVA) design and post
hoc analyses were performed using the Tukey HSD test. Paired Student’s t tests were used to identify any significant differences in these parameters resulting from EIMD. Significance was accepted at $P \leq 0.05$ with Bonferroni corrections for multiple comparisons.

## Results

There was a significant main effect of mouse strain on body mass (of the 12-week-old male mice) with post hoc analyses (Table 1), indicating that the over-expression of class 1:Ea IGF-1 in non-dystrophic (IGF-1(+)) and dystrophic (mdx/IGF-1(+)) mice significantly increased body mass when compared to their control strains (IGF-1(−) and C57/FVB, respectively; $P<0.05$). Therefore, unless otherwise specified, the joint torque data were normalised to body mass for each animal.

The joint torque–angle relationships recorded at day 0 and day 7 are presented in Figs. 3 and 4 for the non-dystrophic (IGF-1(−) and IGF-1(+)) and dystrophic (C57/FVB, mdx/IGF-1(−) and mdx/IGF-1(+)) mice, respectively. The torque–angle relationships were recorded before and after the 20 damaging lengthening contractions and the data are normalised to the peak joint torque recorded before the lengthening contractions. It should be noted that the peak joint torques and optimal joint angles that are presented in the following sections were derived from curve-fits applied to individual torque–angle relationships.

The effects of IGF-1 over-expression on skeletal muscle function

The peak dorsiflexion joint torque and the angle at which the peak torque was produced are presented in Fig. 2. There was a significant main effect of mouse strain on peak joint torque ($P<0.05$). The non-dystrophic mice over-expressing IGF-1 (IGF-1(+)) produced significantly greater peak joint torques compared to normal FVB mice (IGF-1(−)). Not surprisingly, the peak torque produced by dystrophic mdx mice (mdx/IGF-1(−)) was significantly lower than the normal C57/FVB (non-dystrophic) mice. However, there was no significant difference in peak joint torque between mdx/mIGF-1(+) mice and non-dystrophic C57/FVB mice.

The optimal joint angle for maximum dorsiflexion torque production is displayed for all groups in Fig. 2b. Negative values refer to plantar flexion from the neutral ankle position (0°) and are indicative of a longer optimal muscle length. There was no significant difference in the optimal joint angle for peak torque production for the three normal, non-dystrophic mice groups (IGF-1(−), IGF-1(+) and C57/FVB). The optimal joint angle for the dystrophic mdx mice (mdx/IGF-1(−)), however, was significantly less than that for the normal C57/FVB mice ($P<0.05$), indicating that the peak torque production in these dystrophic mice occurred at longer muscle lengths. Furthermore, the width of the torque–angle relationship was significantly less for mdx/IGF-1(−) (56.6±4.1°) compared to control C57/FVB mice (75.4±8.6°). This may be

### Table 1  Descriptive characteristics for the different experimental groups

<table>
<thead>
<tr>
<th></th>
<th>IGF-1(−) (FVB controls)</th>
<th>IGF-1(+)</th>
<th>C57/FVB (controls for mdx)</th>
<th>Mdx/IGF-1(−)</th>
<th>Mdx/IGF-1(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>27.4±0.7 ($n=15$)</td>
<td>32.9±0.7* ($n=13$)</td>
<td>27.±0.5 ($n=16$)</td>
<td>30.0±0.6 ($n=23$)</td>
<td>32.0±0.8** ($n=10$)</td>
</tr>
<tr>
<td>Number of animals in each group</td>
<td></td>
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<tr>
<td>Day 0 combined</td>
<td>15</td>
<td>13</td>
<td>16</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Days 0 and 7</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>3</td>
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</table>

Data are presented as the mean±SE.

* $P<0.05$; significantly different from IGF-1(−), i.e. FVB controls

** $P<0.05$; significantly different from C57/FVB i.e. mdx controls
attributed to the small joint torques produced when the ankle was at 15° dorsiflexion, resulting in a steep ascending limb of the torque–angle relationship. As was the case for the peak joint torque, there was no significant difference in the optimal joint angle or width of the torque–angle relationship between mdx/IGF-1(−) mice and non-dystrophic C57/FVB mice.

Susceptibility to exercise-induced muscle damage

From Figs. 3 and 4, it can be seen that the 20 damaging lengthening contractions resulted in marked decreases in peak joint torque, although the extent of this force deficit varied among the groups. The extent of muscle damage was quantified as the percentage decrease in peak joint torque, that is, the difference between the peak torques recorded before and 10 min after the eccentric exercise protocol as a percentage of the initial joint torque. As shown in Fig. 5a, the extent of muscle damage in mice that over-express IGF-1 (i.e. the non-dystrophic IGF-1(+) and dystrophic mdx/IGF-1(−)) appeared to be lower than for their littermate controls. This difference, however, was not significant (IGF-1(−) vs. IGF-1(+), P=0.059; mdx/IGF-1(−) vs. mdx/IGF-1(+), P=0.199). Although the dystrophic mdx mice (mdx/IGF-1(−)) experienced significantly greater muscle damage than the non-dystrophic control C57/FVB mice, there was no significant difference between the C57/FVB and mdx/IGF-1(+) mice.

As expected, the damaging eccentric exercise protocol produced a shift in the optimal angle for peak torque production in all groups; the optimal joint angle occurred at a more plantar flexed ankle angle, indicative of longer muscle lengths, following the 20 maximal lengthening muscle actions. Although the shift in optimal joint angle appeared to be smaller for IGF-1(+) mice compared to the littermate control mice (IGF-1(−)), this difference was not significant (Fig. 5b). The change in optimal joint angle was accompanied by a decrease in the width of the torque–angle relationship which was significant in all groups except for IGF-1(+).

Histological evidence of muscle damage after dynamometer exercise

Histological analysis of mdx muscles sampled at 2 days after the initial exercise regime showed a striking increase in areas of inflammatory/necrotic myofibres compared with control non-exercised mdx muscle [Fig. 6a(1) and b(1)]. Even more striking was the increase in EBD-positive myofibres in the exercised muscle compared to the contralateral non-exercised muscle [Fig. 6a(2) and b(2)]. Similar damage was seen in all of the exercised mdx muscles confirming that the dynamometer exercise caused myofibre necrosis [Fig. 6b(1)] and extensive leakiness [Fig. 6b(2)] to dystrophic myofibres. Histological evidence of recent muscle necrosis and subsequent regeneration was also recorded at 7 days after the dynamometer exercise (Fig. 7) with scattered foci of small regenerated fibres consistent with the damage observed at 2 days after exercise.

Fig. 3 Joint torque–angle relationships for IGF-1(−) and IGF-1(+) mice. Data are presented as the mean (±SE) normalised dorsiflexion torque recorded before (open symbols) and after (closed symbols) undergoing 20 maximal lengthening contractions on day 0 and day 7. Data are normalised to the peak joint torque recorded before the damaging lengthening contractions. Negative values refer to plantar flexion from the neutral ankle position (0°)
No striking effect of IGF-1 over-expression on the extent of myonecrosis or EBD staining in the mdx muscles was observed at either 2 or 7 days after exercise; however, quantitative histological analysis was not attempted due to the small number of samples taken combined with well-documented high biological variation between muscles of mdx mice [22].

The effects of IGF-1 over-expression on adaptation to EIMD in dystrophic and non-dystrophic skeletal muscle

Eccentric exercise is associated with a subsequent adaptation process such that the severity of muscle damage is reduced upon repeated exposure to exercise of the same intensity. We examined the effects of IGF-1 on this
adaptation process in dystrophic (mdx) and non-dystrophic mice 7 days after the initial exercise protocol. The procedure for experiments on day 7 were identical to the initial experiments on day 0, and data were analysed to determine the recovery of peak joint torque following the initial damaging exercise, as well as the extent of muscle damage induced by repeating the same eccentric exercise protocol.

The amount of muscle damage, as determined by the percentage decrease in peak torque, was reduced in all experimental groups when the exercise protocol was repeated on day 7 (Figs. 3, 4 and 8a). However, statistical analysis of these force deficits revealed that the decrease in the extent of muscle damage on day 7 was significantly different from day 0 for the IGF-1(−) and mdx/IGF-1(−) mice. This decrease in the extent of muscle damage was taken as evidence of a muscle adaptation process induced by the initial exposure to eccentric exercise and was observed in each group of mice.

The extent of EIMD is influenced by the intensity of exercise such that eccentric contractions performed at lower initial torque levels are likely to result in less muscle damage than those performed at higher intensities [4]. Therefore, to determine if the reduction in EIMD observed at day 7 may have been influenced by exercise intensity, the peak joint torque recorded before the exercise protocol at day 7 was compared to the initial peak joint torque recorded on day 0. As shown in Fig. 8b, the peak torque at day 7 had fully recovered to the initial peak torque level in nearly all groups. Therefore, the eccentric exercise protocols in these mice were performed at equivalent exercise intensities on day 0 and day 7. The torque recovery was slightly lower for the C57/FVB and mdx/IGF-1(−) mice with the mean peak torque at day 7 reaching only 75–80% of the peak torque recorded on day 0.

**Discussion**

The success of any therapeutic intervention can ultimately be determined by the functional assessment of physiological parameters in situ. Such assessments can be made under controlled conditions using a custom-built dynamometer apparatus [3, 28]. Furthermore, the minimally invasive procedures involved in these experiments enable the repeated evaluation of muscle function over time, thus
providing a measure of adaptation [9, 22]. Our results show that the over-expression of class 1:Ea IGF-1 improves skeletal muscle function in normal non-dystrophic mice as determined by an increase in the peak torque production. Although the peak torque and the susceptibility to EIMD in dystrophic (mdx) mice that over-express IGF-1 were not significantly different from their littermate controls, there appeared to be some evidence of restoration of function towards the non-dystrophic control mice. Independent of IGF-1 or dystrophy, there was a marked decrease in the amount of muscle damage that was observed when the eccentric exercise was repeated on subsequent days: this finding was observed in all groups and indicates an adaptation process in response to a single bout of EIMD.

Physiological effects of class 1:Ea IGF-1 over-expression

IGF-1 is a potent anabolic agent produced by the liver, skeletal muscle and other tissues, and IGF-1 administration has been widely proposed as a therapeutic treatment for muscle wasting and neurodegenerative disorders. In accordance with observations of Barton et al. [4], we noted a significant increase in body mass for 12-week-old transgenic males for both non-dystrophic and dystrophic mice. For non-dystrophic mice of this same colony, the difference in body mass between transgenic (31.0 g; n=6) and non-transgenic (27.8 g; n=8) males at 12 weeks was not statistically significant [40]; whereas this difference was significant when examining a larger sample in the present

Fig. 7 Histological evidence of muscle damage at 7 days after initial bout of dynamometer exercise. Representative images of transverse (a, l and 2; b, l and 2) and longitudinal (a, 3; b, 3) sections (stained with H&E) of the non-exercised (a) and exercised limb (b) of an mdx/IGF-1(−) mouse shown at low and high magnification. The number of (small darkly stained) foci of recent necrosis/regeneration (e.g. arrow) are far greater after exercise (b). The many mature myofibres with central nuclei (in both a and b) are myofibres that have regenerated previously (and subsequently matured) in these 12-week-old mdx mice.

Fig. 8 a The extent of muscle damage, measured as post-exercise torque relative to pre-exercise torque, induced by 20 eccentric contractions on day 0 (open bars) and day 7 (solid bars). b Recovery of peak torque, measured as the pre-exercise peak torque on day 7 relative to the pre-exercise torque on day 0. *p<0.05, day 7 significantly different from day 0.
study (IGF-1(+)=32.9 g; n=13; and IGF-1(−)=27.4 g; n=
15). In contrast, the systemic administration of recombinant
IGF-1 (1 mg/kg) had no effect on body mass in 12-week-
old male mdx or control mice [19]. However, comparisons
between effects of muscle-specific over-expression of class
1 Ea IGF-1, other transgenic models of IGF-1 over-
expression and systemic administration of IGF-1 protein
can be complicated by a range of factors related to the
complexity of IGF-1 signalling [41].

In the present study, we showed an approximately 20%
increase in normalised joint torque in response to muscle-
specific over-expression of IGF-1 in non-dystrophic mice.
The increase in normalised joint torque was not significant
in dystrophic mice, although this is also in contrast to the
systemic administration of IGF-1 where specific force
measurements in non-dystrophic mice decreased for the
EDL muscle and increased slightly in the soleus muscle
[19]. The reason for these differences is unclear, although it
may reflect a different form of adaptation to IGF-1 treat-
ment depending on the mode of administration (spe-
cific transgene produced within the myofibres compared
with systemic protein delivery) or it may be a consequence
of the different normalisation procedures used in the two
studies. In the present study, the joint torque normalised to
body mass in both the mdx/IGF-1(+) and mdx/IGF-1(−)
mice was lower than the non-dystrophic control mice,
which is consistent with a low specific force production by
mdx muscle reported previously [19].

Susceptibility to exercise-induced muscle damage

The increased susceptibility to EIMD in the dystrophic
IGF-1(−) mice compared to the non-dystrophic C57/FVB
mice is consistent with other in situ studies in mdx mice [5]
and in the canine model of DMD (golden retriever muscular
dystrophy) [9]. Comparing the extent of EIMD in both
normal and dystrophic IGF-1(−) mice (40% mean torque
deficit) to IGF-1(+) mice (30% mean torque deficit) suggests that the over-expression of class 1:Ea IGF-1 has
the potential to reduce the susceptibility to EIMD.
Although this difference was not significant, the findings
may be confounded by the differences in maximal force
production. That is, as the IGF-1(+) mice produced
significantly greater normalised joint torques than control
IGF-1(−) mice, it can be assumed that the muscles of these
transgenic mice experienced larger, and potentially more
damaging, forces. A similar observation was made by
Barton et al. [4] in isolated whole dystrophic EDL muscles
who reported comparable force deficits in transgenic mdx/
IGF-1(+) and mdx/IGF-1(−) mice. However, when the
stimulation parameters were adjusted such that the eccentric
exercise was performed at the same initial force level, the
extent of EIMD was significantly reduced in mdx muscles
that over-expressed IGF-1 [4]. These findings may have
significant functional implications as they imply that over-
expression of IGF-1 in dystrophic muscles results in less
muscle damage when eccentric exercise is performed at the
same intensity (force) compared with control dystrophic
muscles (that lack IGF-1).

It is noteworthy that the optimal joint angle for peak
torque production in mdx/IGF-1(−) mice occurred in a
more plantar flexed position compared to the control (C57/
FVB) mice or mdx/IGF-1(+) mice, thus indicating a longer
optimal muscle length in the control dystrophic muscles
(that lack IGF-1). This observation may have a significant
bearing on the interpretation of our results for two reasons.
Firstly, the reason for the longer optimal muscle length
is unknown, but may result from: (1) an increase in series
compliance within the muscle, which may be due to the
presence of local regions of damaged sarcomeres [20],
and/or (2) a greater number of sarcomeres in series, which
has been proposed as a consequence of the adaptation
process brought about by exercise-induced muscle damage
[29]. Irrespective of the mechanisms involved, the longer
optimal muscle length in mdx/IGF-1(−) mice indicates an
increased exposure to muscle damage prior to the eccentric
exercise protocol. Therefore, a shorter optimal muscle
length in mdx/IGF-1(+) mice suggests that IGF-1 may
reduce the susceptibility to muscle damage during the
performance of routine daily activity (i.e. at submaximal
levels of activation).

The second implication of this observation relates to the
eccentric exercise protocol used to induce muscle damage.
It has been established that the extent of force deficit
following a series of eccentric contractions is greater when
the stretches are applied at longer initial muscle lengths
[17] which has been attributed to a larger portion of the
stretch occurring on the descending limb of the length–
tension relationship where sarcomeres are inherently unstal-
bile [32]. The eccentric protocol in the present study was
performed at a fixed range of ankle rotation (15° to 55° of
plantar flexion). Therefore, the applied stretch would cover
less of the descending limb of the length–tension relation-
ship in the animals that had a longer optimal muscle length
(on the proviso that the torque–angle relationship is directly
related to the length–tension relationship). Consequently,
we would expect to see a greater extent of muscle damage
in mdx/IGF-1(−) mice if the stretches were applied over a
comparable range of muscle lengths.

The fact that the mdx mice that over-express class 1:Ea
IGF-1, despite producing more force and being stretched to
relatively longer muscle lengths, did not experience
significantly greater muscle damage than background mdx
mice, supports the proposal that IGF-1 has the potential
to reduce EIMD in dystrophic muscles. Further evidence in
support of this notion is the ability of IGF-1 analogues to
reduce the susceptibility of dystrophic muscle to EIMD [15]. The mechanisms underlying this protective effect is as yet unclear although, as IGF-1 analogues have significantly reduced affinity for IGF-1 binding proteins, the normal inhibitory effect of these binding proteins on IGF-1 signaling is likely to be reduced.

Adaptation to EIMD

The decrease in the extent of muscle damage when the eccentric exercise protocol was repeated 7 days after the initial exercise bout is an indication of the repeated bout effect that has been shown to occur after a single episode of eccentric exercise [31]. This adaptation process is likely to result from the stimulation of various signalling pathways leading to increased gene expression and the net production of skeletal muscle proteins [43]. It is unclear whether the stimulus for adaptation arises from direct mechanical strain of the cytoskeleton during the lengthening muscle actions or a consequence of the inflammation/repair process that follows the initial structural damage to the muscle. In the present study, the level of adaptation in response to EIMD was greatest in the two conditions that displayed the largest amount of initial damage (see IGF-1(−) and mdx/IGF-1(−) in Fig. 8a), which further supports the proposal that the extent of muscle damage is a major factor in initiating the adaptation process.

Although all groups of mice showed evidence of adaptation to EIMD, the over-expression of IGF-1 did not facilitate this adaptation process in either the dystrophic (mdx) or non-dystrophic mice. This may not be altogether surprising, as the initial EIMD is likely to have induced an inflammatory response involving elevated levels of the pro-inflammatory cytokine tumour necrosis factor (TNF). Although the situation is very complex [21], there is strong evidence that TNF can interfere with IGF-1 signalling [6, 14], thus the beneficial effects on muscle repair and development that might be expected by the over-expression of IGF-1 may have been impaired by the inflammatory response to the initial EIMD. Clearly, inflammatory mediators such as TNF and IL-6 play important and complex roles in the repair and regeneration of skeletal muscle following injury [44] and in dystrophic muscle [36]. However, manifestation of the central role of mechanically mediated signal transduction in skeletal muscle adaptation through an IGF-1 pathway [43] in situations where inflammation is present in vivo remains to be clarified.

The efficacy of exercise as a treatment modality for DMD patients is unclear due to a lack of well-controlled studies evaluating the physiological effects of different exercise regimes on muscle function. This study has helped to redress this problem and has shown that the recovery and adaptation of dystrophic mdx muscle in response to EIMD is as good as that of normal healthy muscle. Furthermore, the over-expression of IGF-1 has the potential to reduce the susceptibility of dystrophic mdx mice to EIMD, although the extent of this benefit should be examined further in experiments that take into account the role of exercise intensity and relative muscle lengthening.

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