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Increased catalase expression improves muscle function in *mdx* mice

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Abstract

It has been well established that oxidative stress contributes to pathology associated with Duchenne muscular dystrophy (DMD). I hypothesized that overexpression of the antioxidant enzyme catalase would improve muscle function in the *mdx* mouse, the mouse model of DMD. To test this hypothesis, neonatal *mdx* mice were injected with a recombinant adeno-associated virus driving the catalase transgene. Animals were killed 4 or 6 weeks or 6 months following injection. Muscle function was generally improved by catalase overexpression. Four weeks following injection, extensor digitorum longus specific tension was improved twofold, while soleus was similar between groups. Resistance to contraction-induced injury was similar between groups; however, resistance to fatigue was increased 25% in catalase-treated soleus compared with control muscle. Six weeks following injection, extensor digitorum longus specific tension was increased 15%, while soleus specific tension was similar between treated and untreated limbs. Catalase overexpression reduced contraction-induced injury by 30–45% and fatigue by 20% compared with control limbs. Six months following injection, diaphragm specific tension was similar between groups, but resistance to contraction-induced injury was improved by 35% and fatigue by 25%. Taken together, these data indicate that catalase can improve a subset of parameters of muscle function in dystrophin-deficient skeletal muscle.

Duchenne muscular dystrophy (DMD) is generally diagnosed during childhood when children fail to achieve developmental milestones and begin to exhibit signature defects in locomotion (for review, see Nowak & Davies, 2004). Over time, the muscles of these patients are replaced by progressively more fibrous tissue, resulting in wheelchair confinement when the legs can no longer support the weight of the body, usually before the teenage years. Once wheelchair bound, patients experience profound atrophy and usually die in the early twenties due to either respiratory failure or cardiomyopathy. The underlying cause of this disease is the absence of a functional dystrophin protein. This protein is responsible for linking cytoskeletal actin to the cell membrane and ultimately to the extracellular matrix through the dystrophin–glycoprotein complex (DGC). In these patients, not only is dystrophin lacking, but protein expression, localization and organization of DGC components also fails, resulting in a loss of signalling from that structure (Straub & Campbell, 1997; Blake *et al.* 2002).

Duchenne muscular dystrophy is modelled by the *mdx* mouse, which is also dystrophin deficient (Bulfield *et al.* 1984). Limb muscles of these mice experience a period of profound injury with muscle necrosis, immune cell infiltration, inflammation and loss of function, at approximately 4 weeks of age and recovery by approximately 8 weeks of age (Dangain & Vrbova, 1984; Disatnik *et al.* 1998). Pathology beyond this period progresses at a very slow rate relative to the human disease until approximately 15 months of age (Pastoret & Sebillé, 1993, 1995). In contrast, the diaphragm experiences a progressive decline throughout the lifespan of the animal that more closely recapitulates the advancing nature of DMD (Stedman *et al.* 1991).

While the underlying cause of DMD is dystrophin deficiency, a number of secondary pathologies contribute to the phenotype. Increased free radical injury has been detected in the skeletal muscles of DMD patients and in *mdx* mice (Jackson *et al.* 1984; Mechler *et al.* 1984; Murphy & Kehrner, 1986, 1989; Hauser *et al.* 1995; Haycock *et al.* 1996). Importantly, increased oxidant stress prior to necrosis and functional deficit was observed at only 3 weeks of age in *mdx* mice (Disatnik *et al.* 1998). Given that this occurs prior to the initial necrosis suffered by the limb muscles at approximately 4 weeks, it is reasonable to suggest that free radicals contribute to disease pathology. In addition, even at young ages, an increased susceptibility to free radical damage was demonstrated in *mdx* mice (Rando *et al.* 1998b). Taken together, the evidence suggests that a key problem in DMD may be a compromised antioxidant system, allowing the formation of free radicals and subsequent damage, including impairment of muscle function. This early oxidative stress may lead to the initial necrotic bout seen in *mdx* skeletal muscle and may be an early causative event in initiation and progression of human DMD. While the free radical component of the disease has been well documented, numerous clinical trials using antioxidants have failed to attenuate disease symptoms (Mendell & Wiechers, 1979; Stern *et al.* 1982; Tamari *et al.* 1982; Hunter *et al.* 1983; Gamstorp *et al.* 1986; Fenichel *et al.* 1988). This may indicate that oxidant damage has a role early in disease initiation or progression, but not later.

Recently, catalase overexpression has been shown to yield longer lifespans in mice (Schriner *et al.* 2005) and reduce disuse atrophy in rats (Dodd *et al.* 2010). In contrast, superoxide dismutase overexpression caused an increase in oxidative damage and created a muscle pathology similar to a muscular dystrophy, probably due to an accumulation of H₂O₂ and hydroxyl radical (Rando *et al.* 1998a).

Given the apparent importance of blunting the initial oxidative stress in disease initiation and propagation, the purpose of this investigation was to determine the extent to which enhanced H₂O₂ buffering would prevent dystrophic muscles from suffering a decline in muscle function. It is hypothesized that catalase overexpression would result in improved resistance to fatigue and reduced contraction-induced injury in dystrophic skeletal muscle. Further, in this study I also determined the extent to which muscle condition at the time of death altered the effects of catalase overexpression. It was anticipated that severely damaged muscle would be less improved than muscle that was moderately damaged by DMD.

Methods

Ethical approval

All animal procedures were done in accordance with the guiding principles of animal use established by the American Physiological Society and were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania where the study was conducted.

Animal treatment

Neonatal *mdx* mice from the University of Pennsylvania colony were injected with recombinant adeno-associated virus pseudotype 2/8 expressing human catalase. To inject pups, a technique called cryosedation was used. Briefly, pups were placed on ice with a thin plastic barrier until they could be handled without excessive movement, allowing accurate and precise injection (Balice-Gordon & Lichtman, 1993). Mice were injected with 1×10^{12} gc of virus delivered in 50 μ l phosphate-buffered saline to the lower right hindlimb (Cat), and contralateral limbs were given a sham injection with an equal volume of saline (Con). The injection site for each limb was on the anterior surface with a depth of approximately 1 mm, as previously described (Barton, 2006b, 2010). This technique is effective for achieving transgene expression in the soleus, gastrocnemius, tibialis anterior and extensor digitorum longus (EDL). Mice were killed at 4 ($n = 6$) and 6 weeks of age ($n = 5$). In addition, another group of neonatal *mdx* mice were injected in the subxyphoid region for diaphragm infection with 1×10^{12} gc catalase virus ($n = 6$) or given a sham injection ($n = 8$; Morine *et al.* 2010). These mice were allowed to age for 6 months.

Muscle mechanics testing was performed on the muscles at the Physiological Assessment Core at the University of Pennsylvania. All animals were given an injection of a ketamine/xylazine cocktail to induce a surgical level of anaesthesia and killed by cervical dislocation. Limb muscles were removed and prepared for muscle function analysis (described below) or frozen in liquid nitrogen. Alternatively, the diaphragm was cut into strips so that two independent function tests could be performed. Removal of the diaphragm resulted in animal death.

Viral preparation

To prepare virus, human catalase complementary DNA (NM 001752.3) was amplified from a vector previously used for successful catalase expression (Zhu *et al.* 2000) and inserted into an expression vector (pZac) containing a truncated desmin promoter to drive muscle specific gene expression (Li *et al.* 1993; Barton, 2010). After confirming the sequence of the insert and inverted terminal repeats in the plasmid, it was amplified for viral preparation. Vectors were produced according to the previously described pseudotyping protocol by the Vector Core of the University of Pennsylvania (Gao *et al.* 2002). Briefly, recombinant adeno-associated virus genomes containing AAV2 inverted terminal repeats were packaged by triple transfection of HEK 293 cells with a *cis*-plasmid containing the catalase transgene, an adenovirus helper plasmid and a chimeric *trans*-plasmid containing the AAV2 *rep* gene fused to the capsid gene of the AAV8 serotype.

Assessment of muscle

Contractile function was measured by standard techniques (Barton *et al.* 2005, 2010; Barton, 2006a) at the Physiological Assessment Core of the Wellstone Muscular Dystrophy Cooperative Center at the University of Pennsylvania. Briefly, function was determined with an Aurora dual-mode lever system (Aurora Scientific, Ontario, Canada) interfaced with a Dell Dimension 2400 desktop computer and controlled with DMC software (version 3.2; Aurora Scientific). Muscles were placed in oxygenated Ringer solution (95% O₂ and 5% CO₂ at 22°C) such that the proximal and distal tendons were attached to clamps (for diaphragm, the central tendon and a rib were used as mounting points). One end was connected to a force transducer and the other to an anchor. Bilateral electrodes were placed longitudinally adjacent to the muscle to create a field upon stimulation. Optimum length (L_0) was determined using standard techniques followed by supramaximal stimulation (EDL, 120 Hz; soleus and diaphragm, 100 Hz) in order to achieve tetanic contractions. Each muscle performed three 500 ms tetanic contractions at L_0 with 5 min between each trial. Cross-sectional area (CSA) and specific tension were estimated using standard equations and constants (Brooks & Faulkner, 1988). The EDL and one diaphragm strip were also given a series of five lengthening contractions with 5 min between each. Stimulation conditions for lengthening contractions for both muscles were 80 Hz for 500 ms followed by 200 ms when the muscle was lengthened 10% beyond L_0 . The soleus and one diaphragm strip were also stimulated each second for 10 min in order to determine resistance to fatigue (200 μ s pulse, 100 Hz, 330 ms duration). The high frequency of stimulation used in this investigation may limit comparisons made with *in vivo* muscle function.

Western blotting was done as previously described with minor modifications (Selsby & Dodd, 2005). Gastrocnemius from 4- and 6-week-old animals or diaphragm from 6-month-old animals was powdered on dry ice and lysed using lysis buffer at a 1:10 dilution. Protein concentration was determined by the method of Biuret. Following dilution to 2.5 mg ml⁻¹ in reducing buffer, 25 μ g protein was loaded into 4–20% gradient gels. Transfer was accomplished by using the I-blot system from Invitrogen (Carlsbad, CA, USA). Primary antibodies were used as described below in 1% milk dissolved in Tris buffered saline (TTBS) containing 0.1% Tween 20 overnight at 4°C. Primary antibodies were used as follows: catalase (1:1000, Sigma); β -actin (1:2000, Neomarkers, Fremont, CA, USA). Secondary antibodies were incubated for 1 h at room temperature at twice the primary dilution such that an antibody used at 1:1000 for primary dilution would receive a 1:2000 secondary dilution in 1% milk dissolved in TTBS containing 0.1% Tween 20. Blots were exposed to SuperSignal (Thermo Scientific, Rockford, IL, USA) for 3–4 min, and emitted light was captured with film (Kodak, Rochester, NY, USA). Following development, blots were quantified using Kodak software with the automated band find function, where possible, in order to remove any researcher bias. Following collection of catalase data, membranes were stripped and exposed to the β -actin antibody. To quantify the contribution of water to total muscle weight, a section of gastrocnemius was weighed and subjected to lyophilization. Lyophilization continued until two identical consecutive sample weight readings were obtained. This occurred following 3 days of lyophilization.

Statistics

Catalase and control limbs were compared using Student's paired t test. Muscle function from catalase and control injected diaphragms were compared using Student's unpaired t test. To determine differences in fatigue, the force generated during the final contraction was compared. Significance was determined *a priori* at $P < 0.05$. Data are presented as means $\pm$ S.E.M. unless otherwise noted.

Results

Limb muscle function at 4 weeks of age

Recombinant adeno-associated virus delivery caused an approximately twofold increase in catalase protein expression 4 weeks following injection (Fig. 1). Muscle mass was similar between groups for the soleus, EDL and tibialis anterior; however, the gastrocnemius was approximately 20% smaller in the Cat limbs compared with Con limbs ($P < 0.05$; Table 1). As it is well recognized that muscle injury is associated with oedema, I reasoned that muscle mass may be smaller because of less water infiltration. To determine the percentage water weight, sections of gastrocnemius were lyophilized for 3 days. Water weight was reduced by approximately 15% ($P < 0.05$; Fig. 2) in catalase-treated muscle compared with control muscle.

Extensor digitorum longus CSA was similar between groups; however, tetanic force was increased nearly twofold in Cat limbs compared with Con limbs (Table 2), as was specific tension (Fig. 3). There was no enhanced protection from damage caused by lengthening contractions in the Cat group compared with the Con group at 4 weeks (Fig. 3). Soleus muscles from catalase-injected limbs had a similar CSA, tetanic force (Table 2) and specific tension (Fig. 3) when compared with soleus muscles taken from control limbs. Soleus muscles from Cat limbs, however, did have decreased rates of fatigue and had 25% ($P < 0.05$) higher force production in the final contraction when compared with control muscles (Fig. 3).

Limb muscle function at 6 weeks of age

Recombinant adeno-associated virus delivery caused an approximate threefold increase in catalase expression 6 weeks following injection (Fig. 1). Muscle mass was significantly reduced in the soleus, EDL, tibialis anterior and gastrocnemius by 17–25% ($P < 0.05$) in Cat limbs compared with Con limbs (Table 1). Water weight was similar between groups (Fig. 2).

At 6 weeks of age, CSA in the EDL was reduced by 25% ($P < 0.05$) in Cat limbs compared with Con limbs, but tetanic force was maintained (Table 2). Specific tension was increased in Cat EDL compared with Con EDL by 15% ($P < 0.05$; Fig. 3). Catalase overexpression improved resistance to contraction-induced injury by 30–45% when compared with control muscles in each of four subsequent muscle stimulations (Fig. 3). In similar fashion to 4-week-old soleus muscles, soleus muscles from Cat-injected limbs had a similar CSA, tetanic force (Table 2) and specific tension (Fig. 3) when compared with soleus muscles taken from control limbs. Additionally, catalase overexpression decreased the rate of fatigue, and force

was 20% greater ($P < 0.05$; Fig. 3) during the final contraction in Cat compared with Con soleus.

Diaphragm muscle function at 6 months of age

At 6 months of age, diaphragmatic catalase expression was similar between virally injected and control injected *mdx* mice (Fig. 1). Two diaphragm strips were removed from the diaphragms of Cat and Con animals for function testing 6 months following injection. As strip size may vary, I report only specific tension (force/CSA), because this eliminates strip size as a variable. Specific tension was similar between groups (Fig. 4). One strip was used for determination of resistance to contraction-induced injury and the other was used for determination of resistance to fatigue. Treatment tended to protect 6-month-old diaphragm from contraction-induced injury in subsequent contractions by 7, 20, 25 and 35% compared with control animals, respectively; however, this only reached significance during the final contraction ($P < 0.05$; Fig. 4). Treatment also resulted in significant resistance to fatigue, as force generated during the final contraction was 25% ($P < 0.05$) greater in catalase-treated diaphragms compared with control diaphragms (Fig. 4).

Discussion

Dystrophic muscle suffers profound injury, partly due to oxidative damage. This sort of injury is apparent even before obvious necrosis that occurs at approximately 4 weeks of age in muscle taken from *mdx* mice, indicating that free radical generation and injury may play a role in the manifestations of disease initiation and early progression. In this investigation, I sought to determine the extent to which neonatal catalase overexpression would protect muscle function. Using a novel approach, I injected neonatal *mdx* mice in a single hindlimb with a virus causing a two- to threefold increase in catalase, while the contralateral limb was given a sham injection. Animals were killed at 4 and 6 weeks, corresponding to the early (4 weeks, severe injury) and late phase (6 weeks, moderate injury) of the initial necrotic bout observed in dystrophic limb muscles (Dangain & Vrbova, 1984; Anderson *et al.* 1987). Some aspects of muscle function were enhanced at 4 weeks, while at 6 weeks muscle function improvement was more robust. Additional neonatal *mdx* mice were injected in the subxyphoid region with virus driving catalase expression in order to infect the diaphragm or given a sham injection and allowed to live for 6 months before killing (6 months, moderate to severe injury). Treating animals in this fashion resulted in improved resistance to contraction-induced injury and fatigue in the diaphragm.

Muscle masses and force production are similar to previous observations by the laboratory group (J.T. Selsby, K.J. Morine, K. Pendrak, E.R. Barton & H.L. Sweeney, unpublished observations); however, at 4 weeks of age the soleus and EDL mass were smaller than previously reported (Buetler *et al.* 2002). Muscle masses in untreated limbs are consistent with earlier reports using 6- to 7-week-old *mdx* mice (Murphy *et al.* 2010; Selsby *et al.* 2010). Of note is the remarkable muscle growth occurring between 4 and 6 weeks of age. This observation is consistent with previous investigations (J.T. Selsby, K.J. Morine, K. Pendrak, E.R. Barton & H.L. Sweeney, unpublished observations). Muscle function measures were consistent with previous data collected by the Physiological Assessment

Core of the Wellstone Muscular Dystrophy Cooperative Center at the University of Pennsylvania (Morine *et al.* 2010; Selsby *et al.* 2010) as well as other research groups (Murphy *et al.* 2010).

These findings are in good agreement with previous animal work attempting to blunt the free radical component of DMD using broad antioxidants. For example, green tea extract reduced muscle necrosis in *mdx* mice, and iron deprivation reduced heat shock protein expression and improved muscle histology (Bornman *et al.* 1998; Buetler *et al.* 2002). More recently, and consistent with our findings, muscles were more resistant to contraction-induced injury and had improved histological measures following exposure to the antioxidant *N*-acetylcysteine (Whitehead *et al.* 2008). Furthermore, following supplementation with a vitamin E analogue, *mdx* mice were more fatigue resistant (Messina *et al.* 2006).

Time- and muscle-specific changes in muscle mass following increased catalase expression were unanticipated findings. Consistent with our results, it was previously reported that soleus and EDL muscle weights were unchanged at a 4 week time point in a study of similar design (Buetler *et al.* 2002). Differences in the response of the soleus and EDL compared with the gastrocnemius may indicate that the gastrocnemius is injured to a different extent than other limb muscles at this time point. It stands to reason that reduced muscle injury would result in less oedema and therefore a lower percentage of water in the muscle, as I found in gastrocnemius muscles taken from 4-week-old animals. What is less clear is why this was also not found in muscles taken from 6-week-old animals and, to the best of my knowledge, the literature does not provide a basis for comparison. Clearly, the underlying mechanism is more complex than simple water weight changes and should be the subject of future investigations.

While antioxidant use has been largely successful in rodent models, it has consistently failed in clinical trials (Mendell & Wiechers, 1979; Stern *et al.* 1982; Tamari *et al.* 1982; Hunter *et al.* 1983; Gamstorp *et al.* 1986; Fenichel *et al.* 1988). These failures may be due to insufficient dosing and/or poor matching of antioxidant and oxidant, both in terms of chemical interaction and intracellular localization. Additionally, muscles may have already been damaged to such an extent that antioxidants were no longer sufficient for rescue (Thomson, 1985). In light of data generated in this investigation, it raises the question of whether or not early DMD may be pathologically distinct from more advanced DMD.

As in early clinical trials, it appears as though muscle condition at the time of evaluation was a critical factor in determining the efficacy of catalase overexpression in this study. For example, 4-week-old limb muscle is severely damaged (Dangain & Vrbova, 1984; Anderson *et al.* 1987), and in the present study catalase overexpression was not effective in reducing contraction-induced injury. By contrast, 6-month-old diaphragm, a moderately to severely damaged muscle (Stedman *et al.* 1991), was partly protected by treatment with a virus driving catalase expression despite similar catalase expression at the time of evaluation. Six-week-old limb muscle, a mild to moderately damaged muscle, was more resistant to contraction-induced injury at each contraction. It is interesting that regardless of injury status, catalase overexpression resulted in improved fatigue resistance.

The effectiveness of catalase overexpression in maintaining muscle function points to the importance of H_2O_2 in disease-related muscle damage. More specifically, as H_2O_2 is well known to degrade into the hydroxyl radical, it is likely that the hydroxyl radical plays a key role in free radical injury associated with DMD. The origin of H_2O_2 is largely dependent upon superoxide dismutase activity, which catalyses the reaction of superoxide to H_2O_2 . Indeed, superoxide dismutase activity is higher in dystrophic muscle compared with control muscle (Kaczor *et al.* 2007). Increased superoxide dismutase activity is also strongly suggestive of an involvement of superoxide in the necrosis of dystrophic skeletal muscle. Interestingly, recent evidence indicates that H_2O_2 can lead to superoxide production by the mitochondria (Viola & Hool, 2010), which in dystrophic skeletal muscle may contribute to a feedforward mechanism.

In partial support of my hypothesis, catalase overexpression successfully improved muscle function in a muscle-condition-dependent fashion. Given the struggles of early clinical trials, it is likely that muscle condition is a limiting factor of antioxidant interventions, and future clinical trials should account for this possibility. Notably, these data point to the importance of blocking peroxide-mediated oxidation in early disease and raise the possibilities firstly, of free radicals being a contributing cause of disease onset rather than an effect and secondly, of disease phases that are pathologically distinct, with an early phase is free radical dependent and a later phase that is not. In total, these data support the hypothesis that catalase overexpression prior to necrosis will improve muscle function. Moreover, given that H_2O_2 is the primary substrate of catalase, it may provide some insight regarding the source of free radical generation within dystrophin-deficient muscle. While it is noteworthy that transitioning from animal models to human patients has been largely unsuccessful, given these and other data, this avenue should not be abandoned. Future work will focus on the contrast of early and later disease to determine the extent to which catalase overexpression can rescue dystrophin-deficient muscle.

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Figure 1. Representative Western blot showing virally mediated catalase overexpression along with corresponding β -actin controls

A, a recombinant adeno-associated virus causing overexpression of catalase was injected into a single hindlimb of *mdx* mice, while the contralateral limb was given a sham injection. Animals were killed at 4 ($n = 6$) or 6 weeks of age ($n = 5$). The blot depicts two sets of paired comparisons (gastrocnemius muscles from the same animal) from each time point. Optical density was determined and is shown for 4- (filled and open bars) and (hatched and shaded bars) 6-week-old animals. **B**, recombinant adeno-associated virus driving catalase expression was also injected in the subxyphoid region of neonatal mice, while control mice were given a sham injection. Mice were killed 6 months later, and catalase expression was determined. * Significantly different from corresponding control value ($P < 0.05$).

Figure 2. Percentage water weight in 4- and 6-week-old gastrocnemius muscles
Gastrocnemius muscles were removed and freeze-dried in order to determine the percentage water weight following 4 ($n = 6$) and 6 weeks ($n = 4$) of catalase overexpression in *mdx* mice. * Significantly different from corresponding control value ($P < 0.05$).

Figure 3. Catalase overexpression in neonatal dystrophin-deficient muscle protects muscle function

A, specific force was measured in 4- (filled and open bars; $n = 5$) and 6-week-old (hatched and shaded bars; $n = 5$) soleus and extensor digitorum longus (EDL). B, peak force produced during five lengthening contractions was recorded. Catalase overexpression did not protect EDL from contraction-induced injury in muscle from 4-week-old animals ($n = 6$). Data are expressed relative to peak force produced during the first lengthening contraction. In 6-week-old animals (C; $n = 5$), catalase overexpression protected EDL from contraction-induced injury in each subsequent contraction. Data are expressed relative to peak force produced during the initial lengthening contraction. D, in catalase overexpressing soleus muscles, relative force produced during the final contraction of a fatigue protocol was greater than for control muscles at both 4 ($n = 5$) and 6 weeks of age ($n = 5$). Data are expressed relative to peak force during the initial contraction (Con at 4 weeks, 10 ± 1 N

cm^{-2} ; Cat at 4 weeks, $8 \pm 2 \text{ N cm}^{-2}$; Con at 6 weeks, $11 \pm 1 \text{ N cm}^{-2}$; and Cat at 6 weeks, $10 \pm 1 \text{ N cm}^{-2}$). * Significantly different from corresponding control value ($P < 0.05$).

Figure 4. Treatment with a virus driving catalase expression partly maintains muscle function in dystrophin-deficient diaphragms

Neonatal *mdx* mice were injected in the subxyphoid region with a virus causing overexpression of catalase or given a sham injection. Muscle function was measured 6 months later. *A*, specific force was not improved by catalase overexpression (Con, $n = 8$; Cat, $n = 6$). *B*, catalase overexpression tended to reduce contraction-induced injury; however, this only reached significance for the final contraction. Forces are expressed relative to peak force produced during the first contraction (Con, $n = 4$, $6.3 \pm 0.7 \text{ N cm}^{-2}$; Cat, $n = 5$, $6.1 \pm 0.9 \text{ N cm}^{-2}$). *C*, in catalase overexpressing diaphragms the relative force produced during the final contraction of a fatigue protocol was significantly greater than for control diaphragms (Con, $n = 7$, $7.7 \pm 1.3 \text{ N cm}^{-2}$; Cat, $n = 6$, $7.4 \pm 1.1 \text{ N cm}^{-2}$).

*Significantly different from control value ($P < 0.05$).

Table 1Muscle masses from 4- ($n = 6$) and 6-week-old *mdx* mice ($n = 5$)

	Tibialis anterior (mg)	Extensor digitorum longus (mg)	Soleus (mg)	Gastrocnemius (mg)
Control, 4 weeks old	9.2 ± 1.2	2.9 ± 0.4	1.9 ± 0.3	29.9 ± 2.9
Catalase, 4 weeks old	9.9 ± 1.2	3.1 ± 0.2	2.1 ± 0.1	24.4 ± 2.7*
Control, 6 weeks old	45.4 ± 2.6	8.6 ± 0.6	6.7 ± 0.4	114 ± 6.9
Catalase, 6 weeks old	35.8 ± 1.6*	6.4 ± 0.5*	5.6 ± 0.5*	86 ± 5.0*

Data are expressed as means ± S.E.M.

* Significantly different when compared with control muscle ($P < 0.05$).

Table 2

Cross-sectional area (CSA) and tetanic force from 4- ($n = 5$) and 6-week-old *mdx* mice ($n = 5$)

	Soleus CSA (mm ²)	Soleus tetanic force (mN)	Extensor digitorum longus CSA (mm ²)	Extensor digitorum longus tetanic force (mN)
Control, 4 weeks old	0.38 ± 0.03	45.75 ± 8.5	0.88 ± 0.14	46.1 ± 10
Catalase, 4 weeks old	0.39 ± 0.02	37.4 ± 5.1	0.90 ± 0.07	79.9 ± 8*
Control, 4 weeks old	0.95 ± 0.03	121 ± 7	1.61 ± 0.11	247 ± 13
Catalase, 4 weeks old	0.84 ± 0.05	102 ± 11	1.23 ± 0.05*	225 ± 44

Data are expressed as means ± S.E.M.

* Significantly different when compared with control muscle ($P < 0.05$).