

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 over-expression 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 (rAAV) driving the catalase transgene. Animals were sacrificed 4 or 6 weeks or 6 mo following injection. Muscle function was generally improved by catalase over-expression. Four weeks following injection, extensor digitorum longus (EDL) specific tension was improved 2-fold 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 solei compared to control. Six weeks following injection, EDL specific tension was increased 15% while soleus was similar between treated and untreated limbs. Catalase over-expression reduced contraction induced injury by 30-45% and fatigue by 20% compared to control limbs. Six months following injection, diaphragm specific tension was similar between groups, however 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.

Introduction

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, 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 signaling from that structure (Straub & Campbell, 1997; Blake *et al.*, 2002).

DMD is modeled 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 four weeks of age and recovery by approximately eight 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 & Kehrer, 1986, 1989; Hauser *et al.*, 1995; Haycock *et al.*, 1996). Importantly, increased oxidant stress prior to necrosis and functional deficit was observed at only three 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 over-expression has been shown to yield longer life spans in mice (Schriner *et al.*, 2005) and reduce disuse atrophy in rats (Dodd *et al.*, 2010). In contrast, SOD over-expression caused an increase in oxidative damage and created a muscle pathology similar to a muscular dystrophy likely 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 is to determine the extent to which enhanced H₂O₂ buffering will prevent dystrophic muscles from suffering a decline in muscle function. It is hypothesized that catalase over-expression will result in improved resistance to fatigue and reduced contraction induced injury in dystrophic skeletal muscle. Further, I will also determine the extent to which muscle condition at the time of sacrifice alters the effects of catalase over-expression. It is anticipated that severely damaged muscle will be less improved than muscle that is 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 IACUC at the University of Pennsylvania where the study was conducted.

Animal Treatment. Neonatal mdx mice from the colony were injected with recombinant adeno-associated virus (rAAV) 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 hind limb (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. Mice were sacrificed at 4 (n=6) and 6 (n=5) weeks of age. In addition, another group of neonatal mdx mice were injected in the sub-xyphoid region for diaphragm infection with 1×10^{12} gc catalase virus (n=6) or given a sham injection (n=8) (Moline *et al.*, 2010). These mice were allowed to age for 6 months.

Upon animal sacrifice, 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 anesthesia. 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.

Viral preparation. To prepare virus, human catalase cDNA (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 sequence of the insert and inverted terminal repeats (ITR) 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 AAV genomes containing AAV2 ITR's were packaged by triple transfection of 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 performed according to standard techniques (Barton *et al.*, 2005; Barton, 2006a; Barton *et al.*, 2010) 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 (Ontario, Canada) interfaced with a Dell Dimension 2400 desk top computer and controlled with DMC software (version 3.2). Muscles were placed in oxygenated Ringers solution (95% O₂ and 5% CO₂ at 22°C) such that the proximal and distal tendons were attached to clamps (for diaphragm, 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 (Lo) was determined using standard techniques followed by supramaximal stimulation (Extensor digitorum longus (EDL) – 120 Hz; soleus (Sol) – 100 Hz; Diaphragm – 100 Hz) in order to achieve tetanic contractions. Each muscle performed three 500 msec tetanic contractions at Lo with 5 minutes between each trial. Cross sectional area 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 five minutes between each. Stimulation conditions for lengthening contractions for both muscles were 80 Hz for 500-msec followed by 200-msec where the muscle was lengthened 10% beyond Lo. The soleus and one diaphragm strip were also stimulated each second for 10 minutes in order to determine resistance to fatigue (200 µsec pulse, 100 Hz, 330 msec duration). The high frequency of stimulation used in this investigation may limit comparisons made to in vivo muscle function.

Western blotting was done as previously described with minor modifications (Selsby & Dodd, 2005). Gastrocnemius from four and six week old animals or diaphragm from six 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 in reducing buffer, 25 µg protein was loaded into 4-20% gradient gels. Transfer was accomplished by using the I-blot system from Invitrogen (Carlsbad, CA). Primary antibodies were used as described below in 1% milk dissolved in TTBS overnight at 4°C. Primary antibodies were used as follows: catalase (1:1000, Sigma); beta actin (1:2000, Neomarkers). Secondary antibodies were incubated for one hour at room temperature at 2x primary dilution such that an antibody used at 1:1000 for primary would receive a 1:2000 dilution secondary in 1% milk dissolved TTBS. Blots were exposed to SuperSignal (Thermo Scientific, Rockford, IL) for 3-4 minutes and emitted light captured with film. Following development, blots were quantified using Kodak software (Rochester, NY) 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 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 a paired T-test. Muscle function from catalase and control injected diaphragms were compared using a 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 is presented as means \pm SEM unless otherwise noted.

Results

Limb muscle function at 4 weeks of age. rAAV delivery caused an approximate 2-fold increase in catalase protein expression four weeks following injection (Figure 1). Muscle mass was similar between groups for the soleus, EDL, and tibialis anterior (TA), however, the gastrocnemius was approximately 20% smaller in the Cat limbs compared to Con ($p < 0.05$; Table 1). As it is well recognized that muscle injury is associated with edema, I reasoned that muscle mass may be smaller because of less water infiltration. To determine the percent water weight, sections of gastrocnemius were lyophilized for three days. Water weight was reduced approximately 15% ($p < 0.05$; Figure 2) in catalase treated muscle compared to control.

EDL cross sectional area was similar between groups, however, tetanic force was increased nearly 2-fold in Cat limbs compared to Con (Table 2) as was specific tension (Figure 3). There was no enhanced protection from damage caused by lengthening contractions in the Cat group compared to Con at 4 weeks (Figure 3). Solei from Cat injected limbs had a similar cross sectional area, tetanic force (Table 2) and specific tension (Figure 3) when compared to solei taken from control limbs. Solei 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 to control muscles (Figure 3).

Limb muscle function at 6 weeks of age. rAAV delivery caused an approximate 3-fold increase in catalase expression six weeks following injection (Figure 1). Muscle mass was significantly reduced in the soleus, EDL, TA and gastrocnemius by 17-25% ($p < 0.05$) in Cat limbs compared to Con (Table 1). Water weight was similar between groups (Figure 2).

At 6 weeks of age, cross sectional area in the EDL was reduced 25% ($p < 0.05$) in Cat limbs compared to Con, however, tetanic force was maintained (Table 2). Specific tension was increased in Cat EDL compared to Con EDL by 15% ($p < 0.05$; Figure 3). Catalase over-expression improved resistance to contraction-induced injury by 30-45% when compared to control muscles in each of four subsequent muscle stimulations (Figure 3). In similar fashion to 4-week-old soleus muscles, solei from Cat injected limbs had a similar cross sectional area, tetanic force (Table 2) and specific tension (Figure 3) when

compared to solei taken from control limbs. Additionally, catalase over-expression decreased the rate of fatigue and force was 20% greater ($p < 0.05$; Figure 3) during the final contraction in Cat compared to Con.

Diaphragm muscle function at 6 months of age. At six months of age, diaphragmatic catalase expression was similar between virally injected and control injected mdx mice (Figure 1). Two diaphragm strips were removed from the diaphragms of Cat and Con animals for function testing six months following injection. As strip size may vary from strip to strip, I report only specific tension (Force/cross sectional area), as it eliminates strip size as a variable. Specific tension was similar between groups (Figure 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 mo old diaphragm from contraction-induced injury in subsequent contractions by 7%, 20%, 25%, and 35% compared to control animals, respectively, however, only reached significance during the final contraction ($p < 0.05$; Figure 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 limbs compared to control (Figure 4).

Discussion

Dystrophic muscle suffers profound injury, partially due to oxidative damage. This sort of injury is apparent even before obvious necrosis that occurs at approximately four 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 over-expression would protect muscle function. Using a novel approach, I injected neonatal mdx mice in a single hind limb with a virus causing a 2-3 fold increase in catalase while the contralateral limb was given a sham injection. Animals were killed at four and six weeks, corresponding to the early (4 wk – severe injury) and late phase (6 wk – 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 four weeks, while at six weeks muscle function improvement was more robust. Additional neonatal mdx mice were injected in the sub-xyphoid 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 sacrifice (6 mo – moderate-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 lab group (Selsby *et al.*, unpublished observations), however, at four weeks of age soleus and EDL mass were smaller than previously reported (Buetler *et al.*, 2002). Muscle masses in untreated limbs are consistent with earlier reports using 6-7 week old mdx mice (Murphy *et al.*, 2010; Selsby *et al.*, 2010). Of note is the remarkable muscle growth occurring between four and six weeks of age. This observation is consistent with previous investigations (Selsby *et al.* 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 (Moline *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). Further, 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 four week time point in a study of similar design (Buetler *et al.*, 2002). Differences in the response of the soleus and EDL compared to 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 edema, and hence, a lower percentage of water in the muscle as I found in gastrocnemii 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 as well as 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 as to 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 over-expression in this study. For example, 4-week-old limb muscle is severely damaged (Dangain & Vrbova,

1984; Anderson *et al.*, 1987) and in this study catalase over-expression 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 partially 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 over-expression resulted in improved fatigue resistance.

The effectiveness of catalase over-expression in maintaining muscle function points to the importance of H₂O₂ in disease related muscle damage. More specifically, as H₂O₂ 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 H₂O₂ is largely dependent upon superoxide dismutase activity, which catalyzes the reaction of superoxide to H₂O₂. Indeed, superoxide dismutase activity is higher in dystrophic muscle compared to control (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 H₂O₂ can lead to superoxide production by the mitochondria (Viola & Hool, In press), which in dystrophic skeletal muscle may contribute to a feed forward mechanism.

In partial support of my hypothesis, catalase over-expression successfully improved muscle function in a muscle-condition dependant 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 possibility of 1) free radicals being a contributing cause of disease onset rather than an effect and 2) disease phases that are pathologically distinct where an early phase is free radical dependent and a later phase that is not. In total, these data support the hypothesis that catalase over-expression prior to necrosis will improve muscle function. Moreover, given that H₂O₂ 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 over-expression can rescue dystrophin-deficient muscle.

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Table 1. Muscle masses from 4 (n=6) and 6 (n=5) wk old mdx mice

	TA (mg)	EDL (mg)	Soleus (mg)	Gastroc (mg)
4 wk Control	9.2 ± 1.2	2.9 ± 0.4	1.9 ± 0.3	29.9 ± 2.9
4 wk Catalase	9.9 ± 1.2	3.1 ± 0.2	2.1 ± 0.1	24.4 ± 2.7*
6 wk Control	45.4 ± 2.6	8.6 ± 0.6	6.7 ± 0.4	114 ± 6.9
6 wk Catalase	35.8 ± 1.6*	6.4 ± 0.5*	5.6 ± 0.5*	86 ± 5.0*

Data are expressed as mean ± SEM. * indicates significantly different when compared to control (p<0.05). TA – tibialis anterior; EDL – extensor digitorum longus; Gastroc – gastrocnemius; wk – week.

Table 2. Cross sectional area and tetanic force from 4 (n=5) and 6 (n=5) wk old mice				
	Soleus CSA (mm ²)	Soleus Tetanic Force (mN)	EDL CSA (mm ²)	EDL Tetanic Force (mN)
4 wk Control	0.38 ± 0.03	45.75 ± 8.5	0.88 ± 0.14	46.1 ± 10
4 wk Catalase	0.39 ± 0.02	37.4 ± 5.1	0.90 ± 0.07	79.9 ± 8*
6 wk Control	0.95 ± 0.03	121 ± 7	1.61 ± 0.11	247 ± 13
6 wk Catalase	0.84 ± 0.05	102 ± 11	1.23 ± 0.05*	225 ± 44

Data are expressed as mean ± SEM. * indicates significantly different when compared to control (p<0.05). CSA – cross sectional area; EDL – extensor digitorum longus; wk – week

Figure Legends

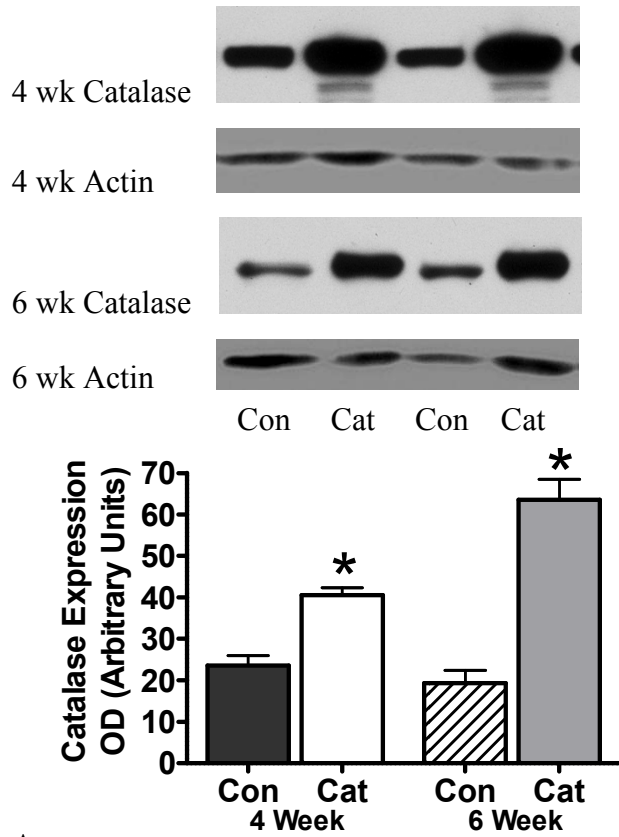
Figure 1) Representative Western blot showing virally mediated catalase over-expression along with corresponding actin controls. (A) An rAAV causing over-expression of catalase was injected into a single hind limb of mdx mice while the contralateral limb was given a sham injection. Animals were sacrificed at four (n=6) or six (n=5) weeks of age. The blot depicts two sets of paired comparisons (gastrocnemius from the same animal) from each time point. Optical density was determined and is shown for four (black and white bars) and six week (striped and gray bars) old animals. (B) rAAV driving catalase expression was also injected in the sub-xyphoid region of neonatal mice while control mice were given a sham injection. Mice were sacrificed six months later and catalase expression determined. * indicates significantly different from corresponding control (p<0.05).

Figure 2) Percent water weight in four and six week old gastrocnemius muscles. Gastrocnemii were removed and freeze-dried in order to determine the percent water weight following four (n=6) and six (n=4) weeks of catalase over-expression in mdx mice. * indicates significantly different from corresponding control (p<0.05).

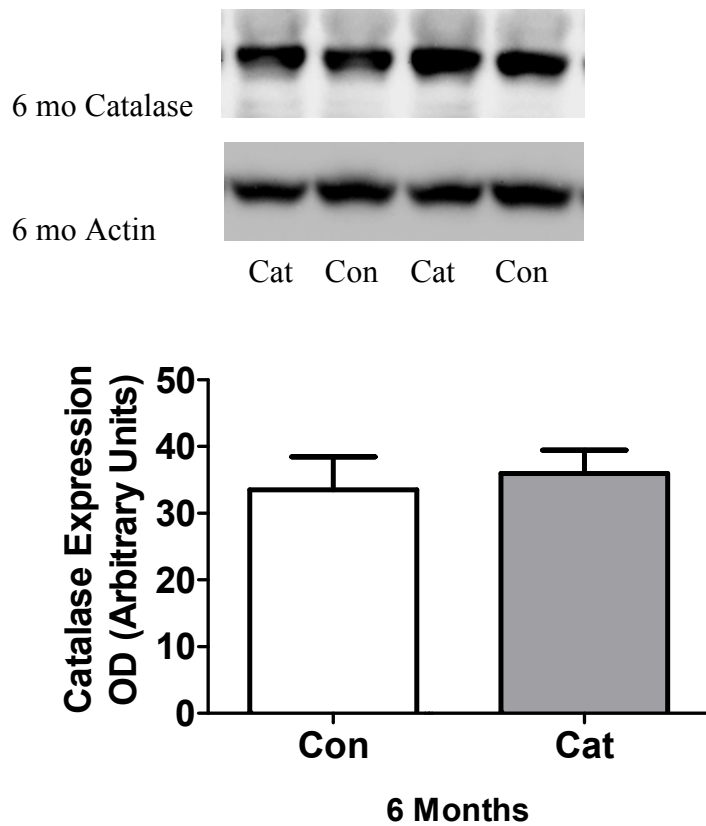
Figure 3) Catalase over-expression in neonatal dystrophin-deficient muscle protects muscle function. (A) Specific force was measured in four (black and white; n=5) and six (striped and gray; n=5) week old soleus and EDL. (B) Peak force produced during five lengthening contractions was recorded. Catalase over-expression did not protect EDL from contraction-induced injury in muscle from four-week-old animals (n=6). Data is expressed as relative to peak force produced during the first lengthening contraction. In six-week-old animals (C; n=5), catalase over-expression protected EDL from contraction induced injury in each subsequent contraction. Data is expressed as relative to peak force produced during the initial lengthening contraction. (D) In catalase over-expressing solei relative force produced during the final contraction of a fatigue protocol was greater than control muscles at both four (n=5) and six (n=5) weeks of age. Data is expressed relative to peak force during the initial contraction (4 wk Con – 10 ± 1 N/cm²; 4 wk Cat – 8 ± 2 N/cm²; 6 wk Con

$- 11 \pm 1 \text{ N/cm}^2$; 6 wk Cat $- 10 \pm 1 \text{ N/cm}^2$). * indicates significantly different from corresponding control ($p < 0.05$).

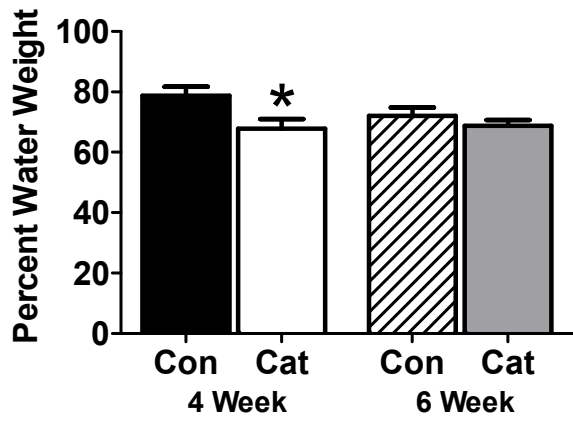
Figure 4) Treatment with a virus driving catalase expression partially maintains muscle function in dystrophin deficient diaphragms. Neonatal mdx mice were injected in the subxyphoid region with either a virus causing over-expression of catalase or given a sham injection. Muscle function was measured six months later. (A) Specific force was not improved by catalase over-expression (Con $n=8$; Cat $n=6$). (B) Catalase over-expression tended to reduce contraction induced injury, however, only reached significance in the final contraction. Forces are expressed relative to peak force produced during the first contraction (Con $n=4$; Con $- 6.3 \pm 0.7 \text{ N/cm}^2$; Cat $n=5$; Cat $6.1 \pm 0.9 \text{ N/cm}^2$). (C) In catalase over-expressing diaphragms relative force produced during the final contraction of a fatigue protocol was significantly greater than control diaphragms (Con $n=7$; Con $- 7.7 \pm 1.3 \text{ N/cm}^2$; Cat $n=6$; Cat $- 7.4 \pm 1.1 \text{ N/cm}^2$). * indicates significantly different from control ($p < 0.05$).

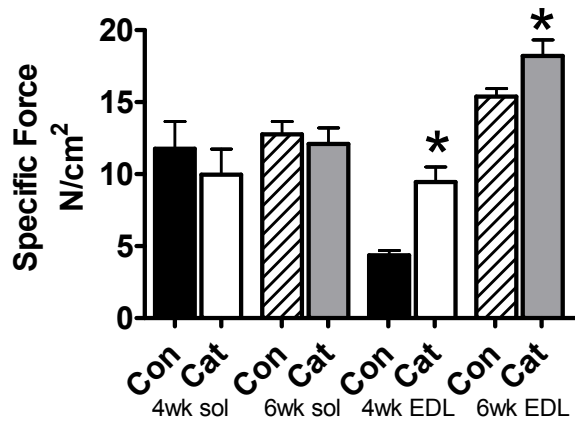


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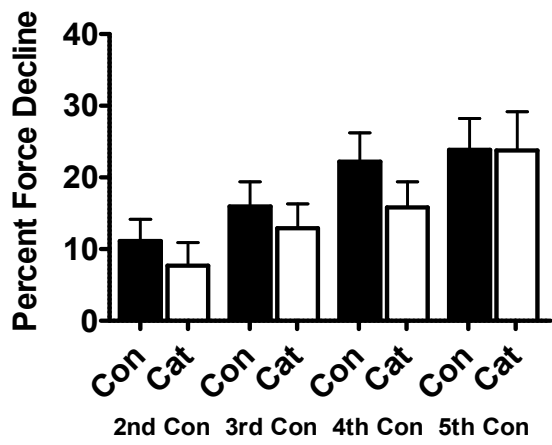


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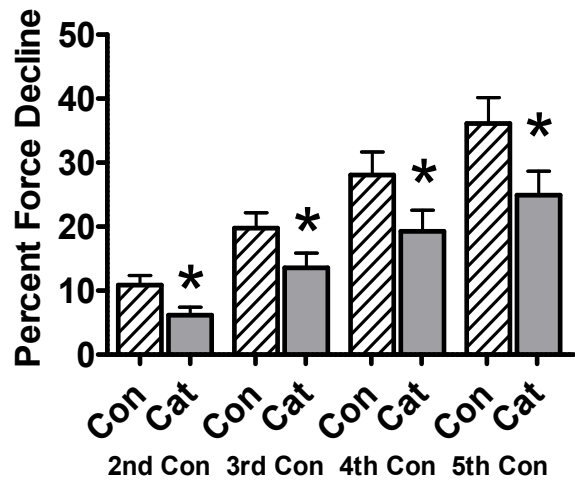




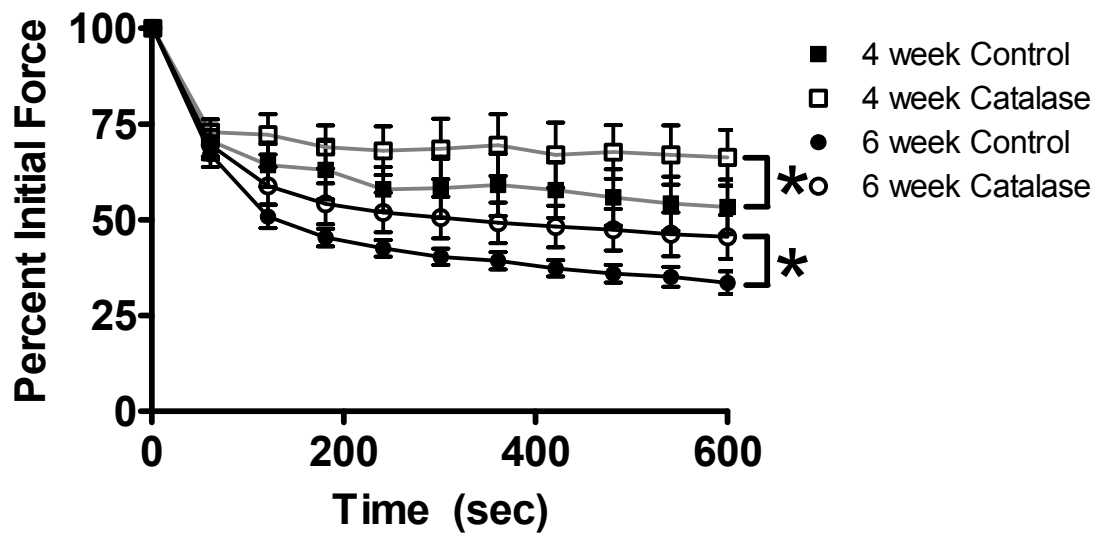
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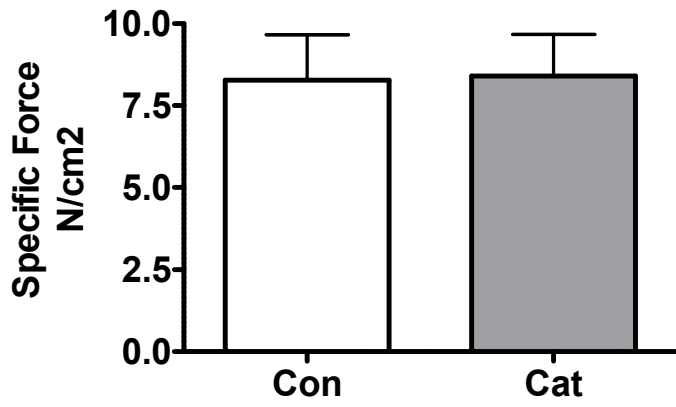
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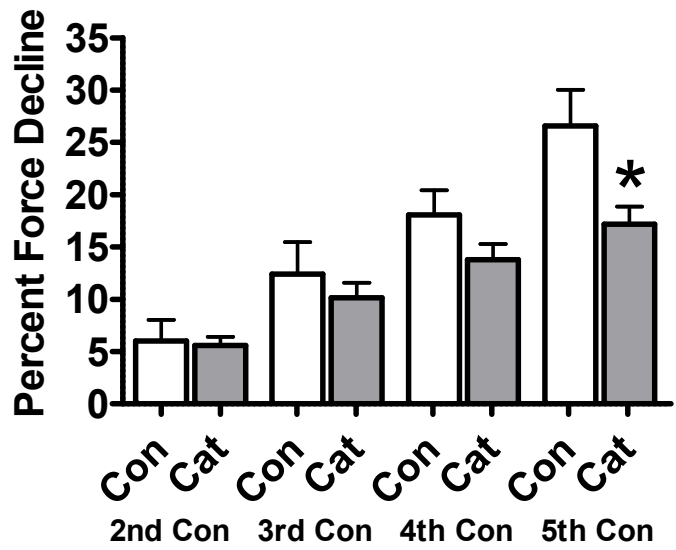
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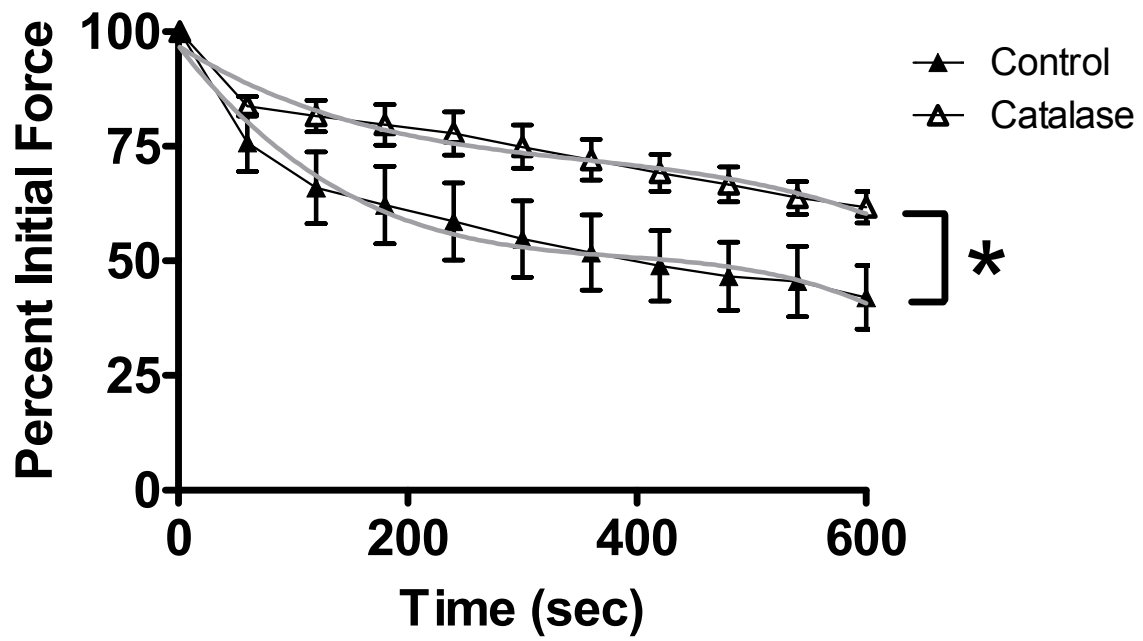
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