Published in final edited form as:

Muscle Nerve. 2012 December ; 46(6): 948–950. doi:10.1002/mus.23536.

A marginal level of dystrophin partially ameliorates hind limb muscle passive mechanical properties in dystrophin-null mice

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Abstract

Introduction—The goal of this study was to determine whether a minimal level of dystrophin expression improves the passive mechanical properties of skeletal muscle in the murine Duchenne muscular dystrophy model.

Method—We compared the elastic and viscous properties of the extensor digitorum longus muscle (EDL) in *mdx3cv* and *mdx4cv* mice at 6, 14 and 20 months of age. Both strains are on the C57Bl/6 background, and both lose the full-length dystrophin protein. Interestingly, *mdx3cv* mice express a near full-length dystrophin at ~5% of the normal level.

Result—We found that the stress-strain profile and the stress relaxation rate of the EDL in *mdx3cv* mice were partially preserved in all age groups compared to that of age-matched *mdx4cv* mice.

Discussion—Our results suggest that a low-level of dystrophin expression may treat muscle stiffness in Duchenne muscular dystrophy.

Keywords

Dystrophin; passive properties; EDL muscle; *mdx3cv* mice; *mdx4cv* mice

Introduction

Loss of dystrophin causes Duchenne muscular dystrophy (DMD). A major clinical manifestation of DMD is muscle stiffness.^{1,2} Although the full spectrum of muscle stiffness is not completely understood, the change in muscle passive properties may play a critical role. We recently demonstrated that *mdx* (a DMD model) muscle is much stiffer than normal muscle.^{3,4} Here, we asked the question if low-level dystrophin expression can reduce muscle stiffness. We compared the passive mechanical properties of the extensor digitorum longus (EDL) muscle in BL6, *mdx4cv* and *mdx3cv* mice. Both *mdx4cv* and *mdx3cv* mice are murine DMD models on the BL6 background. These strains were generated by N-ethylnitrosourea mutagenesis. The *mdx4cv* mice have a nonsense mutation in exon 53 that leads to premature termination of translation. The *mdx3cv* mice have a point mutation in intron 65 that results in low level expression (~5%) of a near full-length dystrophin.^{5–8}

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Disclosure

No conflicts of interest, financial or otherwise, are declared by the authors

Materials and Methods

Experimental mice

Experiments were performed on male BL6, *mdx4cv* and *mdx3cv* mice. All animal experiments were approved by the Animal Care and Use Committee of the University of Missouri and were in accordance with NIH guidelines.

Passive property assay

The passive (elastic and viscous) properties of the EDL muscle were evaluated as described previously.³ Briefly, the EDL muscle was subjected to a six-step lengthening protocol in the absence of electrical stimulation. At each step, the EDL muscle was stretched at an increment of 10% of the optimal length (Lo). The viscous property was determined by measuring the stress relaxation rate (SRR) while holding the EDL muscle at 110% of Lo. The EDL muscle cross sectional area (CSA) was calculated according to the published protocol.³

Hydroxyproline assay

Hydroxyproline is the main component of fibrotic tissue. To evaluate fibrosis, we quantified the hydroxyproline content by colorimetry. The lyophilized EDL muscle (without tendons) was hydrolyzed in 1 ml 6N HCL for 3 hours at 115°C and then neutralized with 10N NaOH to a final pH of ~7.5. The muscle lysate was oxidized with chlormatine-T to generate a red-purple color. The hydroxyproline content was determined by measuring the color absorbance at 558 nm.^{3,4}

Statistical analysis

Data are presented as mean $\pm$ standard error of mean. Statistical significance was determined by one-way ANOVA followed by Bonferroni *post hoc* analysis using the SPSS software (IBM Corporation, Armonk, NY). Difference was considered significant when $p < 0.05$.

Results

Morphometric properties of the EDL muscle

Irrespective of the age, the EDL muscle of *mdx3cv* and *mdx4cv* mice consistently showed muscle hypertrophy. The weight and CSA of the EDL muscle from these 2 strains were significantly higher than those of age-matched BL6 mice (Fig. 1A and B).

Passive properties of the EDL muscle

Consistent with our previous reports,^{3,4} the EDL muscle of *mdx4cv* mice was significantly stiffer than that of BL6 mice. Compared to BL6 muscle, *mdx4cv* muscle yielded a significantly much higher resistance force (passive stress) before it reached the peak (Fig. 1C to E). Further, the post-peak stress of *mdx4cv* muscle showed a more significant rapid decline than that of BL6 muscle (Fig. 1C to E). As expected, there was also an age-associated increase of muscle stiffness in *mdx4cv* mice (Fig. 1C to E).

The marginal level of dystrophin expression significantly improved the stress-strain profile in *mdx3cv* mice. The pre-peak stresses profile was identical to that of BL6 (Fig. 1C to E). The post-peak stresses also showed a trend of normalization, although it was still significantly different from BL6 (Fig. 1C to E).

The viscous property of the EDL muscle was compromised in *mdx4cv* mice (Fig. 1F to H). Compared to that of age-matched BL6, *mdx4cv* muscle yielded a significantly higher SRR

(Fig. 1F to H). In *mdx3cv* mice, the viscous property of the EDL muscle was partially improved. The SSR of *mdx3cv* muscle was corrected from the peak to 0.1 second. However, in the subsequent time frames (from 0.1 to 1.5 seconds), the SSR of *mdx3cv* muscle was significantly higher than that of BL6 muscle (Fig. 1F to H).

The content of fibrotic tissue in the EDL muscle

At 6 and 20 months, the hydroxyproline level of the *mdx4cv* EDL muscle was significantly higher than that of the age-matched BL6 EDL muscle (Fig. 1I). Compared to *mdx4cv* mice, the hydroxyproline content appeared to be reduced in *mdx3cv* mice. However, this difference was not statistically significant (Fig. 1I).

Discussion

The major finding of this study is that an approximately 5% level of normal dystrophin partially preserved the passive properties of the EDL muscle. The relationship between the level of dystrophin expression and disease severity has been studied extensively. In human patients, it was found that a dystrophin level higher than 20% is associated with mild symptoms.^{9,10} In *mdx* mice, it has also been demonstrated that a 20–30% level of normal dystrophin expression is sufficient to reduce histopathology and improve muscle strength.^{11,12} Recently, we found that ~5% of normal level of dystrophin significantly improved physiological and clinical outcomes, although it did not stop muscle degeneration and necrosis.⁸ Here, we studied whether such a marginal level of dystrophin expression improves muscle passive properties.

We examined the elastic and viscous properties of the EDL muscle in BL6, *mdx4cv* and *mdx3cv* mice. To study disease progression and also to compare with our previous report on the BL10 background *mdx* mice, we studied 6, 14 and 20-month-old male mice.^{3,4} The difference between *mdx4cv* and BL6 is quite similar to the difference between *mdx* and BL10. However, the magnitude is lower for the *mdx4cv*/BL6 comparison.^{3,4} This may reflect the influence of the genetic background. Our data suggest that dystrophin deficiency indeed increases muscle stiffness.

We have previously shown that 5% dystrophin expression increased specific muscle force in *mdx* and dystrophin/utrophin double knockout (u-dko) mice and it further prolonged the life span of u-dko mice.^{8,13} Based on these findings, we hypothesized that 5% dystrophin expression may also improve the passive mechanical properties. Indeed, the stress-strain profile and the relaxation rate were both significantly shifted in the normal direction (Fig 1). Furthermore, a correlative change in muscle fibrosis was observed (Fig 1I). Our data suggest that a minimal level of dystrophin expression may still hold significant therapeutic benefit.

Acknowledgments

This work was supported by grants from the National Institutes of Health (AR-49419, DD), Muscular Dystrophy Association (DD) and NIH training grant T90DK70105 (CH). We thank Mrs. Marianne Abdo, Ms. Juveria Nayeem and Ms. Alexandra Kellogg for technical assistance.

Abbreviations

BMD	Becker muscular dystrophy
CSA	Cross sectional area
DMD	Duchenne muscular dystrophy

EDL	Extensor digitorum longus
Lo	Optimal length
SSR	Stress relaxation rate

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Figure 1. Passive properties of the EDL muscle are partially protected in *mdx3cv* mice
 Comparison of the EDL muscle weight (A) and EDL CSA (B) in BL6, *mdx4cv* and *mdx3cv* mice. Age-matched comparison of the EDL muscle stress-strain profile in BL6, *mdx4cv* and *mdx3cv* mice at 6 (C), 14 (D) and 20 (E) months of age. Age-matched comparison of the SSR for the EDL muscle in BL6, *mdx4cv* and *mdx3cv* mice at 6 (F), 14 (G) and 20 (H) months of age. n=9–13 per group in panels A to H. (I) Hydroxyproline content in the EDL muscle. n = 6–8 per group. *, *mdx4cv* mice are significantly different from all other age-matched mouse strains. #, *mdx3cv* mice are significantly different from age-matched BL6 mice. †, BL6 mice are significantly different from all other age-matched mouse strains. ‡, *mdx4cv* mice are significantly different from all other age-matched BL6 mice. Data are presented as mean ± standard error of mean. $p < 0.05$.