

## Fast-twitch muscle from old dystrophic mice contain complex branched fibres which are susceptible to contractile damage while slow-twitch are protected

L. Kiriaev,<sup>1</sup> S. Chan,<sup>1</sup> S. Kueh,<sup>2</sup> J. Morley<sup>2</sup> and S.I. Head,<sup>1</sup> <sup>1</sup>School of Medical Sciences, University of New South Wales, NSW 2052, Australia and <sup>2</sup>School of Medicine, Western Sydney University, Penrith, NSW 2751, Australia.

Duchenne muscular dystrophy (DMD) is characterized by progressive wasting of skeletal muscle. Previous work from our laboratory, using the extensor digitorum longus (EDL) muscle from adult (24 weeks) mdx mice, suggests that the branched fibres formed during repeated bouts of regeneration could be responsible for the terminal phase of muscle wasting (Chan & Head, 2011; Chan, Head & Morley, 2007; Head, Williams & Stephenson, 1992). To further test this hypothesis, mice were killed by an overdose of isoflurane and the following muscles dissected and attached to a force transducer:- fast-twitch EDL and slow-twitch *soleus* (SOL) muscles from mdx mice and littermate controls: 2-3 weeks (pre-disease), termed “young”; 6-9 weeks (around the first phase of muscle destruction) termed “adolescent” and 68-112 weeks, termed “old”. After contractile analysis single muscle fibres were enzymatically isolated and suspended in a relaxing solution in order to view individual fibres. In old controls, branching was found in <7% of the 426 fibres counted. In young mdx EDL muscles, <1% of 330 fibres counted were branched, adolescent mdx EDL muscles, 59% of 669 fibres counted were branched, while in old mdx EDL muscles, 98% of 672 fibres counted were branched. In adolescent mdx EDL muscles, 63% of the branched fibres had only one branch, while in old mdx EDL muscles, 66% of the branched fibres contained 4 or more branches. In adolescent mdx SOL muscles, 40% of 172 fibres counted were branched, while in old mdx SOL muscles, 79% of 462 fibres counted were branched. No branches were found in young mdx SOL muscles. In adolescent mdx SOL muscles, 73% of the branched fibres had only one branch, and in old mdx SOL muscles, only 17% of the branched fibres contained 4 or more branches. Muscles were subjected to a series of 10 isometric contractions (1-2 s with 1 min rest). For the EDL, force loss was <1% (n = 9) for young control and  $17.6 \pm 4.0\%$  (n = 6) for young mdx ( $P < 0.001$ ) over the 10 contractions. Muscles from the adolescent control and mdx mice lost <12% force. In old mice, the force loss was  $10.3 \pm 1.0\%$  (n = 15) for control and  $33.8 \pm 4.1\%$  (n = 11) for mdx ( $P < 0.0001$ ). For the SOL, force loss was negligible in control and mdx muscles of all age groups. Muscles were subjected to a series of eccentric contractions. In old mdx EDL muscles, the first contraction caused so much force loss that subsequent contractions resembled passive stretches. The first contraction at 10% strain produced a force loss of <1% in young controls and  $14.4 \pm 4.3\%$  (n = 6) in young mdx ( $P < 0.05$ ). For adolescent mice, the force loss was  $1.1 \pm 2.6\%$  (n = 9) in controls and  $13.2 \pm 2.2\%$  (n = 14) in mdx ( $P < 0.05$ ). In old mice, the force loss was  $1.7 \pm 1.7\%$  (n = 11) in controls and  $63.1 \pm 2.1\%$  (n = 5) in mdx ( $P < 0.0001$ ). For the age-genotype interaction,  $P < 0.0001$ . In SOL muscles, eccentric contractions produced negligible force losses in mdx and controls. We have shown that the marked aberrations in cytoarchitecture present in virtually all EDL fibres of very old mdx mice have a distinct association with the increased vulnerability to isometric and eccentric damage of their muscles. The association between degree of branching and degree of damage is further confirmed by the internal control of the SOL muscle, which shows much less complex branching than the EDL and virtually no damage from isometric and eccentric contractions.

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