Myotoxin induced regeneration of fast-twitch EDL skeletal muscles from aged mice results in fibre branching and increased susceptibility to dystrophic type eccentric damage

S.I. Head, A.S.J. Lee, A.J. Kee, P.W. Gunning and E.C. Hardeman, School of Medical Sciences, University of New South Wales, NSW 2052, Australia.

Duchenne muscular dystrophy is an X-linked recessive disorder characterized by progressive wasting of skeletal muscle. It is caused by the absence of dystrophin, a protein that, in normal muscle fibres, is located in close proximity to the internal face of the sarcolemma. Recent work from my laboratory carried out using aged mdx mice, which also lack dystrophin, has lead to the proposal that it is the branched fibres, formed during the repeated bouts of regeneration, which are responsible for the terminal phase of muscle damage(Head, 2010; Chan, Head & Morley, 2007). In this hypothesis, damage results from the structural weakening of the muscle fibres at multiple branch points, and hence is not a primary consequence of the absence of dystrophin. In order to further test this hypothesis myotoxin (notexin) was used to induce injury in EDL muscles from aged (28 months old female C57BL6) WT mice. The resulting regenerated muscles contained "dystrophin positive" branched fibres. Mice were anaesthetised with a mixture of Ketamine (0.1 mg/g) and Xylazine (20 μg/g), a 1 cm incision on the lateral side of both hindlimbs was made to expose EDL. Notexin (0.2 μg) was injected intramuscularly into one EDL muscle, the contralateral EDL served as a control. Animals were allowed to recover for 21 days with daily monitoring and analgesic care. Post recovery the animals were killed with halothane and the EDL removed and attached to a force transducer (approved UNSW ethics). After the contractile analysis the muscle was detached from the force transducer, weighed and processed for histology. Single EDL muscle fibres were enzymatically isolated using 3mg/ml collagenase Type I (Sigma), and suspended in a relaxing solution containing 50mM EGTA in order to view individual fibres. 38% (n=3) of the regenerated fibres had branches while only 2% (n=3) of the control fibres had a small degree of branching. The regenerated muscles were significantly heavier than the contralateral muscle, 11.9mg ± 0.8 *vs* 9.8mg ± 0.2, absolute force was significantly larger in the regenerated muscle, 169mN ± 8 *vs* 145mN ± 8 while there was no significant difference in specific force 230mN/mm^2 ± 15 *vs* 231mN/mm^2 ± 14, (all case n=10 with means ± SEM & t-test). A lengthening/eccentric contraction was performed three times, at intervals of 5 min with a strain of 33% of fibre length. Regenerated muscles has a significant force deficit of 11% ± 3 (n=3), the force from control muscles was not affected (n=3). This study shows that notexin induced regeneration produces muscles that contain a population of branched fibres, the regenerated muscles have normal contractile properties and an increased susceptibility to eccentric damage. I propose that this increase in susceptibility to mild eccentric damage is the result off the presence of regenerated branched fibres within the muscle.

Head SI (2010). Branched fibres in old dystrophic mdx muscle are associated with mechanical weakening of the sarcolemma abnormal Ca^{2+} transients and a breakdown of Ca^{2+} homeostasis during fatigue. *Experimental Physiology* **95**, 641-56.

Chan S, Head SI, Morley JW (2007). Branched fibers in dystrophic mdx muscle are associated with a loss of force following lengthening contractions. *American Journal of Physiology. Cell Physiology* **293**, C985-92.