Eccentric damage is accentuated in aged dystrophin-deficient EDL muscles from dystrophic mice (MDX)

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Duchenne Muscular Dystrophy (DMD) is second most common fatal inherited condition of humans affecting 1 in 3500 live male births. Due to the large size and high mutation rate of the dystrophin gene, 1/3 of all cases of DMD are the result of a new spontaneous mutation. DMD is characterized by a severe and progressive loss of skeletal muscle and marked CNS and cognitive defects. Death usually occurs in the late teens or early twenties due to the failure of respiratory muscles or cardiac complications. The most commonly used animal model of DMD is the *mdx* mouse in which the long M-isoform of dystrophin is absent from the skeletal musculature. In the *mdx* mouse the proximal limb muscles undergo a process of degeneration which affects 100% of the skeletal muscle fibres in some muscles (Tanabe *et al.*, 1986), the fibres then undergo a process of regeneration and repair. During this period there is a striking change in the morphology of the regenerated *mdx* EDL fibres; up until 15 weeks they are normal cylindrical shaped with no splits or deformities, by 17 weeks 30% of fibres have simple splits and relatively mild deformities, while by 40 weeks in excess of 90% of the EDL fibres have multiple splits and quite striking gross abnormalities (Head *et al.*, 1992). We are hypothesizing that these split, morphologically abnormal fibres are weaker and more susceptible to damage by lengthening contractions of a moderate strain than age matched morphologically normal fibres. We used male *mdx* and male littermate controls from our new line of *mdx* mice (N1F1 *mdx* mice). This provides us with dystrophin-positive controls on an identical genetic background to the dystrophin-deficient animals. This is important because the majority of studies on *mdx* mice use a separate wild type colony as a control and it has been shown that there is a remarkable degree of genetic variation between recently divergent mouse strains (Adams *et al.*, 2005). Animals were killed by an overdose of Halothane (ethics approval granted by UNSW). The EDL muscles were attached at one end to a force transducer and to a servo controlled linear tissue puller at the other end. The muscles were then placed in an organ bath, superfused with oxygenated Krebs and externally stimulated *via* two platinum plates attached to a current amplifier driven by an AM-systems stimulator. The muscles were set to optimal length L_o. The muscles were tetanically stimulated at 100hz, and once the force reached its maximal plateau the lengthening contraction (12, 15 or 20% plus L_o) was given as a ramp, hold and release. The ramp speed was 1mm/sec and the total duration of stimulation was 5 seconds. This protocol was repeated twice at 5 minute intervals to allow recovery from fatigue. The isometric force was recorded after a 20 minute recovery. The muscles were then removed, weighed and single fibres enzymatically (collagenase 1) isolated and viewed on a confocal microscope in order to count the number of split fibres. In aged (45-52 weeks) *mdx* animals, 100% of fibres were split and there was an irreversible 35% ± 9.8 (n=6) drop in isometric force as a result of our lengthening contraction protocol. In contrast, there were no split or deformed fibres in age matched controls, and isometric force was largely unaffected (force drop 3.8%± 4.8% n=5) by this relatively mild eccentric contraction protocol. Numerous previous studies (e.g. Yeung *et al.*, 2003) have demonstrated that dystrophin-deficient fast-twitch muscles are damaged by eccentric contractions; we hypothesise that the extent of the eccentric damage is significantly increased by the presence of deformed fibres.

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