Deficiency of a fast twitch muscle fibre protein alters muscle adaptation in response to denervation and immobilization

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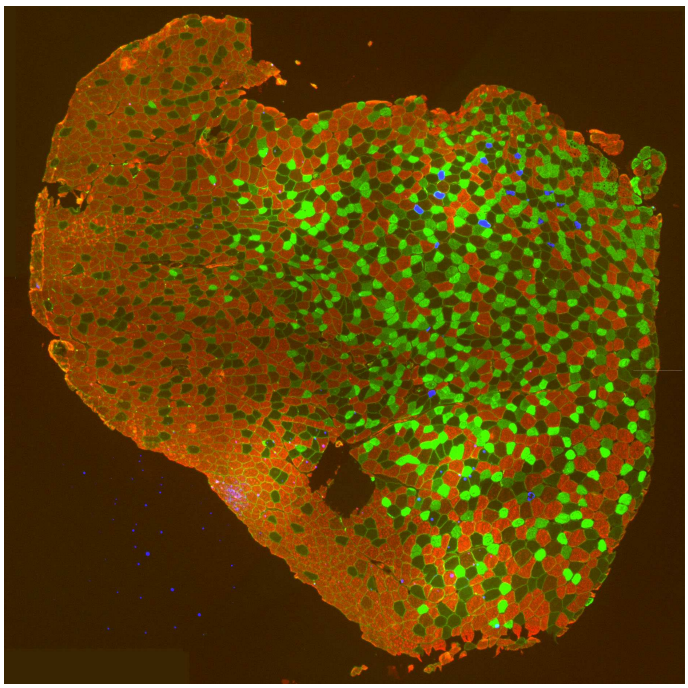
A common null polymorphism (R577X) in the ACTN3 gene results in the absence of fast fibre-specific α-actinin-3 in ~18% of humans worldwide (North *et al.*, 1999). α-actinin-3 deficiency is detrimental to elite sprint athletes and may benefit endurance performance (Yang *et al.*, 2003). We have developed an Actn3 knockout (KO) mouse which mimics the human phenotype. KO mice show reduced grip strength, resistance to fatigue and a shift towards slow/oxidative metabolism in fast fibres, without a shift in fibre type (MacArthur *et al.*, 2007, MacArthur & Seto *et al.*, 2008). Altered levels of regulators of calcineurin (RCAN) in the KO suggest involvement of the calcineurin-dependent signalling pathway.

We aimed to investigate adaptation to muscle disuse hypothesizing that Actn3 genotype would have a local influence on muscle adaptation, irrespective of neural innervation. Separate cohorts of KO and wild-type (WT) mice underwent 2 weeks immobilization and 2 and 8 weeks of denervation and were compared to age matched controls. For these experiments mice were anaesthetized using 3.5% isoflurane in oxygen from a precision vaporizer. Buprenorphine was administered as an analgesic (0.01mg/kg) and mice were monitored regularly for signs of distress during the test period. Tissues were excised after mice were killed *via* cervical dislocation.



Atrophy stress significantly decreased muscle mass and fibre size in both WT ($P<0.01$) and Actn3 KO mice ($P<0.05$). In both immobilization and denervation Actn3 KO myosin heavy chain 2B fibres tended reduce their size less than WT. This was significant with 2 weeks denervation, with Actn3 KO 2B fibres were 75% of KO controls size while WT denervated were 48% of WT controls size ($P=0.008$). Similar trend was seen with immobilization ($P=0.08$). To examine muscle adaptation, the *tibialis anterior* cross section was stained with 2B, 2A and type 1 MyHC antibodies (see figure). After 8 weeks of denervation Actn3 KO mice increased their 2A surface area had lower 2B fibre numbers and surface area compared to WT ($P<0.05$). KO muscle fibres demonstrated a lower threshold to undergo fibre type switch towards a slower phenotype. Additionally they resisted the switch to faster MyHC profile during immobilization, maintaining their percentage of 2A surface area which was reduced in WT ($P<0.01$).

Our results reveal a local effect of α-actinin-3 altering muscle fibre type in response to adaptation which we propose is mediated through the calcineurin signalling pathway. These findings have important implications for understanding local muscle biology signalling and individual responses to muscle disuse/disease and training in the human population.

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