α-actinin-3 deficiency does not affect the unloaded shortening biomechanics of single isolated fast-twitch FDB fibres from Actn3 KO mice

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Introduction. We have identified a common null polymorphism in ACTN3 (R577X) that has undergone strong positive selection during recent human evolution. Homozygosity for this allele (577XX) results in absence of α-actinin-3 in the fast-twitch (IIX human) muscle fibres of ~18% of the world's population. The polymorphism does not have any known disease phenotype. However, it is associated with a shift in the metabolic profile of the α-actinin null fast fibers from a predominantly anaerobic production of ATP to an aerobic production of ATP (MacArthur *et al.*, 2007). There is a common misconception that this metabolic shift means that in *Actn3* KO fast-twitch fibres have been switched to a slow-twitch contractile phenotype. This is not correct as has been demonstrated in a series of experiments carried out on muscles form the *Actn3* KO mice, these include skinned fibre experiments and histochemical fiber typing along with isometric contractile measurements made from isolated *extensor digitorum longus* (EDL) fast-twitch muscles (Chan *et al.*, 2011). These studies showed there is no change in the expression of fast myosin isoforms, Force-pCa curve or rise times of the twitch.

Aim. In the current study we measure the unloaded maximum speed of shortening in single intact enzymatically isolated fast twitch muscle fibers to find out whether it is altered by the absence of α-actinin-3.

Methods and results. Littermate control and *Actn3* KO mice were killed by an overdose of the inhalation anaesthetic, halothane or isoflurane. The *flexor digitorum brevis* muscle (FDB) was dissected out and single fibres were obtained by collagenase dissociation. Single intact fibres were plated out on glass coverslips and viewed via a Nikon inverted microscope. Fibres were stimulated to contract by field stimulation from two insulated platinum wires. The stimulation protocol was; single pulses, 10 Hz, 20 Hz, 30 Hz and 100 Hz in order to record single twitch, unfused and fused tetanic contractile responses. Fibre shortening was recorded using a CMOS high-speed camera (hs1200, PCO, Kehlheim, Germany) connected to the side port of the inverted microscope. Adjusting the field of image acquisition on the chip allowed frame rates up to 4,500 frames per second. For analysis, a custom-made program was written consisting of the following steps: after loading the first image of an experiment XYT series, the fibre borders could be manually enclosed with a freehand ROI in order to exclude eventual debris or artifacts within the fibre area. The ROI mask was applied to all images within the series and converted to a binary mask on which border detection was performed. The longest distance between detected borders were taken as fibre length and followed up within subsequent images. A criterion was set to allow for maximum length change in successive images of 2.5 to 5% of initial fibre length. This resulted in a significant reduction in false-positive border detections from areas outside the fibre or floating particles in the solution. The length values were stored in a vector matrix upon which time derivatives were performed for speed of shortening assessment. Each experiment was plotted in an automated results file showing the l(t) and v(t) curves, both in relative (normalized to L_0) as well as absolute values (converting to µm after a grid calibration). Minimum shortening length and maximum speed of shortening did not significantly differ between wild-type (n=18) and *Actn*3 KO fibres (n=22), neither for the variable of genotype nor for stimulation frequency. For instance, both littermate controls and *Actn3* KO recorded unloading shortening speeds of around 5.5 mm/s for single twitches and around 6.5 mm/s at 100Hz stimulations.

Conclusion. This is the first report of unchanged unloaded speed of shortening kinetics in fast-twitch fibres from *Actn*3 KO mice upon either twitch- or tetanic field stimulation. These findings support previous reports that the absence of α-actinin-3 does not change the heavy myosin chain compositions or isometric twitch rise times. We conclude that the *Actn3* polymorphism results in fast-twitch fibres with normal contraction kinetics which have a metabolic shift towards slow-twitch fibre aerobic ATP production pathways.

Chan S, Seto JT, Houweling PJ, Yang N, North KN, and Head SI. (2011) *Muscle and Nerve* **43**: 37-48.

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