Twitch kinetics of adult and aged EDL muscle from an α-actinin-3 knockout mouse

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The actin-binding protein α-actinin-3 is specifically expressed in fast glycolytic muscle fibres. Homozygosity for a common polymorphism in the ACTN3 gene results in complete deficiency of α-actinin-3 in about 16% of individuals worldwide. Although α-actinin-3 deficiency does not cause disease in α-actinin-3 knockout mice, recent studies suggest that the absence of α-actinin-3 is detrimental to sprint and power performance in elite athletes (Yang *et al.*, 2003). Recent studies also suggest that in young mice there is an alteration in the metabolic profile of the fast muscle such that fast-twitch, glycolytic fibres have slower-twitch, more oxidative properties without an alteration in the overall expression of myosin 2B. To determine the effect of α-actinin-3 deficiency on the twitch kinetics of whole skeletal muscle, we studied isolated extensor digitorum longus (EDL) muscles from α-actinin-3 knockout mice and wild-type littermate controls. Animals were examined in 2 age groups: "Adult" (8 weeks to 6 months) and "Aged" (20 to 22 months).

Animals were sacrificed with an overdose of halothane (ethics approval UNSW). The EDL muscle was dissected from the hindlimb and tied by its tendons to a force transducer at one end and a linear tissue puller at the other. It was placed in a bath continuously superfused with Krebs solution, with composition (mM): 4.75 KCl, 118 NaCl, 1.18 KH_2PO_4, 1.18 $MgSO_4$, 24.8 $NaHCO_3$, 2.5 $CaCl_2$ and 10 glucose, with 0.1% fetal calf serum and continuously bubbled with 95% O_2-5% CO_2 to maintain pH at 7.4. The muscle was stimulated by delivering a supramaximal current between two parallel platinum electrodes. At the start of the experiment, the muscle was set to the optimum length L_0 that produced maximum twitch force. All experiments were conducted at room temperature (~22°C to 24°C). Muscles were subjected to fatiguing stimulation consisting of one-second, 100-Hz stimuli given every 2 seconds over a period of 30 seconds. Twitches were recorded both before and immediately after this fatigue protocol, and twitch half-relaxation times were measured from these recordings.

Before fatigue, the half-relaxation times of knockout (KO) muscles were not significantly different from wild-types (WT) in either age group. However, following the fatigue protocol, the half-relaxation times of knockouts were significantly higher than those of wild-type muscles, and this difference was more pronounced in the younger ("adult") age group. In the younger ("adult") age group, there was a 6.2 ms difference (20.5 ± 0.8 ms, n=13 for KO *versus* 14.3 ± 0.6 ms, n=12 for WT, $p < 0.001$) while in older ("aged") animals there was a 2.8 ms difference (17.0 ± 0.9 ms, n=10 for KO *versus* 14.2 ± 0.5 ms, n=11 for WT, $p < 0.05$).

These data suggest that in fatigued muscles, α-actinin-3 deficiency is associated with longer half-relaxation times and this lengthening is more pronounced in younger animals. This increase in half-relaxation time may be related to the observation that absence of α-actinin-3 is detrimental to sprinting performance in elite athletes.

Yang, N, MacArthur, DG, Gulbin, JP, Hahn, AG, Beggs, AH, Easteal, S & North, K. (2003). ACTN3 genotype is associated with human elite athletic performance. *American Journal of Human Genetics* **73,** 627-631