Contractile and fatigue properties of α-actinin-3 knockout fast-twitch EDL muscle

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The actin-binding protein α-actinin-3 is one of the two isoforms of α-actinin that are found in the Z-discs of skeletal muscle, and is specifically expressed in fast glycolytic (Type 2B) muscle fibres. Homozygosity for a common polymorphism in the ACTN3 gene results in complete deficiency of α-actinin-3 in about 1 billion people worldwide. Although α-actinin-3 deficiency does not cause disease, recent studies suggest that the absence of α-actinin-3 is detrimental to sprint and power performance in elite athletes (Yang *et al.*, 2003). To determine the effect of α-actinin-3 deficiency on the physiological properties of skeletal muscle, we studied isolated extensor digitorum longus muscles (EDL) from a specially developed α-actinin-3 knockout mouse. Animals aged 8 to 10 weeks 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).

α-actinin-3-deficient muscles showed similar levels of damage to wild-type muscles following eccentric contractions of 20% strain, 1.6 ± 2.0% in wild-types and 2.6 ± 1.5% in knockouts, suggesting that the presence or absence of α-actinin-3 does not influence mechanical stability of the sarcomere. α-actinin-3 deficiency does not result in a loss of fast glycolytic fibres (expressing myosin 2B). However, α-actinin-3-deficient muscles were 9% lighter than α-actinin-3-positive muscles, with a corresponding 9% reduction in cross-sectional area. Knockouts displayed longer twitch half-relaxation times; the half-relaxation time of 15.7 ± 0.6 ms in knockouts was 2.6 ms longer than the half-relaxation time of 13.2 ± 0.6 ms in wild-types ($p = 0.008$). α-actinin-3-deficient muscles showed significantly better recovery from fatigue; 30 minutes following the fatigue protocol knockouts recovered to 86.1 ± 1.1% of their original force, but wild-types recovered to only 78.4 ± 1.9% of original ($p = 0.013$). In combination, these data suggest that α-actinin-3 deficiency results in fast-twitch, glycolytic fibres developing slower-twitch, more oxidative properties while not affecting the mechanical strength of the fibre. This alteration in the metabolic profile of the fast muscle would be detrimental to optimal sprint and power performance but beneficial for endurance activities.

Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S & North K. (2003). *American Journal of Human Genetics*, **73:** 627-31.