Contractile and fatigue properties of aged fast-twitch EDL muscle from an α-actinin-3 knockout mouse

S. Chan,[1] S.I. Head,[1] J.T. Seto[2] and K.N. North,[2] [1]Department of Physiology, University of New South Wales, NSW 2052, Australia and [2]Faculty of Medicine, University of Sydney, Neurogenetics Research Unit , The Children's Hospital at Westmead, Westmead, NSW 2145, Australia.

The actin-binding protein α-actinin-3 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 16% of individuals worldwide. Although α-actinin-3 deficiency does not cause disease in α-actinin-3 knockout mice, recent studies suggest that in young mice (8-10 wks) 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 physiological properties of skeletal muscle from "aged" animals we studied isolated *extensor digitorum longus* muscles (EDL) from "aged" animals 26 to 31 weeks of age. 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).

"Aged" α-actinin-3-deficient muscles showed similar levels of eccentric damage to wild-type muscles following eccentric contractions of 20% strain, 2.6 ± 1.5% in young *c.f.* 1.0 ± 1.7% in "aged" knockouts, suggesting that the absence of α-actinin-3 does not influence the mechanical stability of the sarcomere as the muscle matures. In contrast to younger animals where knockouts showed a slowing of the twitch half-relaxation time, in "aged" knockouts the half-relaxation times were similar (15.4 ± 0.9 ms) to the half-relaxation times (15.8 ± 0.5 ms) recorded in "aged" wild-types. The main effect of aging on the α-actinin-3-deficient muscles was that the significantly better recovery from fatigue reported in young knockouts 30 minutes following a fatigue protocol was even more pronounced, with "aged" knockouts recovering to 85.8 ± 3.0% of their original force, while "aged" wild-types recovered to only 73.4 ± 1.5% of original ($p = 0.004$). These data suggest that the shift in metabolic profile to a slow-twitch profile in α-actinin-3 deficiency becomes even more marked as the animal ages. This may have some beneficial effects on α-actinin-3 deficient "aged" fast-twitch skeletal muscle allowing them to perform repetitive fatiguing tasks at shorter interval than wild-types. Additionally the comparison with "aged" wild-type muscle shows that actinin-3 deficient muscles from young animals appear to be prematurely aged in some of their contractile properties.