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Phosphorylation of C18ORF25 regulates skeletal muscle function

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In efforts to identify novel regulators of skeletal muscle function, we have shown that the uncharacterised protein C18ORF25 is phosphorylated at serine-67 (S67) following different exercise modalities in human muscle biopsies, and that this protein is a novel exercise-regulated AMPK substrate. To characterise the function of this protein, we generated a C18ORF25 whole-body mouse knockout (KO) and used isolated soleus (SOL) muscles together with single mechanically-skinned muscle fibres to probe muscle contractile function.

Experiments were approved by The University of Melbourne Animal Ethics Committee and mice anaesthetised with isoflurane (4% in oxygen, 1 L/min). Isolated muscles were bathed in carbogen bubbled Krebs at 30 °C and isometric contractions elicited via supramaximal pulses (26 V, 0.2 ms), with force-frequency responses determined between 10 and 130 Hz using 500 ms stimulation trains. Fatigue resistance was assessed by maximally stimulating muscles once every 4 s for 4 min with recovery measured 5, 10, and 15 min after fatigue. In skinned fibres, sarcoplasmic reticulum (SR) Ca^{2+} loading capacity was estimated from the area of the force response to 30 mM caffeine following different loading intervals at pCa 7 (= $-\log_{10}[Ca^{2+}]$). SR Ca^{2+} leak was assessed by the area of the 30 mM caffeine response obtained after the SR had been loaded with Ca^{2+} for a set time and then exposed to a leak solution (0.5 mM EGTA).

Specific force was significantly reduced in SOL muscles from KO mice as compared to wild-type (WT) across all frequencies tested (~ 1.5-fold decrease, $P<0.05$), with no differences in fatigue resistance or recovery from fatigue. In SOL skinned fibres, maximal SR Ca^{2+} loading was significantly decreased in fast-twitch fibres of KO mice as compared to WT (~ 2-fold decrease, $P<0.05$). Passive SR Ca^{2+} leak was also significantly increased in fast-twitch fibres of KO mice as compared to WT (% leak: 66.8 ± 5.0 *vs* 49.5 ± 5.3). The reduced SOL muscle function of KO mice was not due to differences in the Ca^{2+}-sensitivity of the contractile apparatus at a single fibre level. Thus, loss of C18ORF25 attenuates SOL muscle contractile function and this is likely due to impaired SR Ca^{2+} handling.

C18ORF25 was then re-expressed into left or right extensor digitorum longus of KO mice, with S67 mutated to either an Ala(A) (phospho-dead) or Asp(D) (phospho-mimetic), respectively, in a paired design. As compared to the S67A mutant, muscles expressing the S67D mutant generated significantly greater specific force at all frequencies tested ($P<0.05$) and showed improvements in tetanic contractile kinetics such as the rate of force development and relaxation. Thus, the reduced muscle function of KO mice could be reversed following re-expression of a C18ORF25 S67 phospho-mimetic mutant.

Taken together, these results demonstrate phosphorylation of S67 on C18ORF25 regulates skeletal muscle function and identifies C18ORF25 as a novel regulator of muscle function.