Diffusibility and glycogen association of AMPK in rat skeletal muscle with and without in vitro stimulation

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The 5′-AMP-activated protein kinase (AMPK) functions as an intracellular fuel sensor that affects metabolism, and AMPK is activated in skeletal muscle in response to exercise and energy storage utilization (Jorgensen, Jensen & Richter, 2007). Mammalian AMPK is a heterotrimeric complex with a carbohydrate-binding module (CBM) in the β2-subunit (AMPKβ2) that shows high affinity for glycogen mimics (Koay *et al.*, 2010). It has recently been demonstrated that glycogen-binding is blocked by a new AMPKβ phosphorylation site (Thr-148) within the CBM (Oligschlaeger *et al.*, 2015) .

To investigate this finding in rat skeletal muscle, male Sprague-Dawley rats (6-8 mo old) were sacrificed using a lethal overdose of isoflurane in accordance with La Trobe University Ethics Committee. The extensor digitorium longus (EDL) muscle was excised and stimulated *in vitro* in order to activate AMPK as well as utilise glycogen storage. The stimulated and contralateral control muscles were then homogenized in a physiological K+ based solution with pCa >10 (Murphy *et al.*, 2012).

Muscles were stimulated at 30 V with ten 50 Hz tetani for 0.5 s every two seconds, repeated every 2 min until peak force declined to < 20% of original (taking ~1 h), which resulted in ~33% increase in phosphorylated acetyl CoA carboxylase (p-ACC), a downstream product of activated AMPK. An enzymatic glycogen content assay showed ~28% glycogen utilization during stimulation. Individual muscle fibres were isolated from control and stimulated muscles and AMPKβ2 content measured in the diffusible component (Murphy *et al.*, 2012). Diffusibility of AMPKβ2 decreased ~20% in stimulated compared to control muscles, indicating a pool of AMPKβ2 becomes bound in muscle as a consequence of stimulation. Amylase treatment, which is able to identify proteins associated with glycogen, indicated that the bound AMPKβ2 was not associated with glycogen. A phospho-specific AMPKβ-Thr-148 antibody was used in an immunoprecipitation (IP) assay to detect the AMPKβ phosphorylation, and it was found that the entire pool of AMPKβ2 was phosphorylated in both control and stimulated muscles. This finding further confirmed that skeletal muscle AMPKβ2 is not associated with glycogen *in vivo*, and that activation of AMPK by muscle contraction does not dephosphorylate AMPKβ2. These findings confirm that when AMPKβ2 is phosphorylated at Thr-148, AMPK does not associate with glycogen.

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