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Of mice and men: filling gaps in the TBC1D1 story

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Skeletal muscle is the major tissue for the increased glucose disposal caused by insulin or exercise. Each stimulus elevates GLUT4 glucose transporter translocation to skeletal muscle's cell surface membranes, but distinct signalling pathways lead to this common outcome. Insulin's proximal signalling events include activation of the insulin receptor, phosphatidylinositol 3-kinase, and Akt2. The signalling events necessary for exercise-induced glucose transport may involve increased cytosolic calcium, AMP-activated protein kinase and other mechanisms. The prevalence of obesity, insulin resistance and diabetes provides motivation for understanding the exercise pathway in humans because in many insulin resistant conditions, exercise-stimulated glucose transport is normal, presenting an attractive therapeutic target.

Recently, two related Rab-GTPase activating proteins (GAPs) known as TBC1D1 and TBC1D4 (also called Akt substrate of 160 kDa or AS160) were recognized to potentially link the proximal signalling of insulin and/or exercise with GLUT4. TBC1D4's phosphorylation in response to insulin was discovered by Gustav Lienhard's group using 3T3-L1 adipocytes and was subsequently found in rat skeletal muscle with insulin or contraction (Bruss *et al.* 2005). Reviewing TBC1D4's relationship with GLUT4 is helpful before considering TBC1D1. Insulin's activation of Akt2 causes TBC1D4 phosphorylation on multiple Akt phosphomotifs, thereby inhibiting TBC1D4's activation of Rab-GTPase proteins associated with GLUT4 vesicles and/or causing TBC1D4's release from GLUT4 vesicles. Insulin-induced phosphorylation of TBC1D4 on key insulin-responsive motifs enhances TBC1D4's association with 14-3-3 proteins, which may regulate GLUT4 vesicle traffic. Akt2 is not essential for exercise-stimulated glucose transport, but mutation of four phosphomotifs on TBC1D4 caused a small reduction in contraction-stimulated glucose uptake in mouse muscle. A mutation in TBC1D4's calmodulin binding domain (CBD) also caused a modest decline in glucose uptake with contraction, but not with insulin. Simultaneous mutation of the CBD and phosphomotifs did not reduce contraction-stimulated glucose uptake below the values found with either mutation alone. These results suggest a modest role for TBC1D4 in contraction-stimulated glucose uptake, but TBC1D4-independent mechanisms (potentially involving TBC1D1) are likely to be essential for most of the contraction's effect.

TBC1D1 and TBC1D4 have significant sequence similarity, including a GAP domain and a CBD. The sequence surrounding a key Akt phospho-site of TBC1D4 (T642) is nearly identical to the sequence surrounding TBC1D1's T596, but TBC1D4 includes a greater number of predicted Akt phosphomotifs. Although AMPK can phosphorylate both proteins, TBC1D1 includes an important AMPK phosphomotif (Ser237) that TBC1D4 lacks. In L6 cells, Ser237 phosphorylation of TBC1D1 is increased with AMPK activation, but not by insulin. Preventing the increase in Ser237 phosphorylation by expressing mutated TBC1D1 in HEK-293 cells blocks the AMPK-associated increase in 14-3-3 binding by TBC1D1 (Chen *et al.* 2008). Insulin or electrically simulated contractions enhances TBC1D1 phosphorylation on various sites in rodent muscle, but only contraction elevates phosphorylation on Ser237 (Funai *et al.* 2009). Contraction-stimulated (but not insulin-stimulated) glucose uptake was partially reduced in muscle of mice expressing TBC1D1 mutated on four phosphomotifs (including Ser231, homologous to human Ser237) (An *et al.* 2010). Evidence from cells and rodent muscle links TBC1D1 Ser237 phosphorylation to both 14-3-3 binding and contraction-stimulated (but not insulin-stimulated) glucose transport. But is this relevant to humans?

The results of the study by Frøsig *et al.* (2010) in this issue of *The Journal of Physiology* fill an important gap in the TBC1D1 story by demonstrating that *in vivo* exercise (cycle ergometery) by humans can increase the phosphorylation of skeletal muscle TBC1D1 on the key Ser237 site. The increased Ser237 phosphorylation occurred with each of three protocols which involved a nearly 3-fold range of work-rates (222–658 W) and 40-fold range of duration (0.5–20 min). Furthermore, TBC1D1's capacity for 14-3-3 binding was increased in muscle by each exercise protocol. The exercise effects on Ser237 phosphorylation and 14-3-3 binding were rapid and sustained, as would also be expected for exercise-stimulated glucose transport.

What is the mechanism for increased phosphorylation of Ser237-TBC1D1 after exercise? There is not a straightforward experimental approach for directly answering this question in humans undergoing *in vivo* exercise. Accordingly, Frøsig *et al.* used mice to probe the specific roles of AMPK isoforms in contraction-induced Ser237 phosphorylation. Studying α1- and α2-AMPK knockout mice and wild-type controls, they demonstrated that contraction-stimulated Ser237 phosphorylation was unaltered in mice deficient in α1, but greatly diminished in muscles from mice lacking α2. Earlier research demonstrated that α2-AMPK knockout mice compared to wild-type controls have normal contraction-stimulated glucose transport, but it is uncertain if the residual contraction effect on Ser237 in α2-knockout mice plays a role in contraction-mediated glucose transport. It is also unknown if the residual Ser237 phosphorylation in α2-knockout mice is attributable to a compensatory increase in α1-AMPK activity or to another kinase. A recent publication found that electrically stimulated muscle contractions activated an AMPK-related kinase known as sucrose non-fermenting AMPK-related kinase (SNARK), and that a mutation of SNARK that attenuated contraction-stimulated SNARK activity was accompanied by decreased contraction-stimulated glucose transport (Koh *et al.* 2010). Furthermore, exercise by humans similar to the 2 and 20 min protocols used by Frøsig *et al.* also activated skeletal muscle SNARK. However,

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the effect of SNARK on TBC1D1 Ser237 phosphorylation remains to be assessed.

What will future chapters of the TBC1D1 story reveal? Do α2-AMPK and/or SNARK regulate Ser237 phosphorylation in human skeletal muscle? Is Ser237 phosphorylation necessary for exercise-induced glucose transport in human skeletal muscle? Does TBC1D1's CBD participate in exercise-stimulated glucose transport? Do exercise effects on TBC1D1 have functional roles other than increased glucose transport? The TBC1D1 story is far from finished.

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