Furthering our insights into the ATPases in muscle

R.M. Murphy,[1] B.P. Frankish,[1] D. Christiansen[2] and G.D. Lamb,[3] [1]Department of Biochemistry and Genetics, La Trobe Institute of Molecular Science, La Trobe University, Melbourne, VIC 3086, Australia, [2]Institute of Sport, Exercise and Active Living, College of Sport and Exercise Science, Victoria University, Melbourne, VIC 8001, Australia and [3]School of Life Sciences, La Trobe University, Melbourne, VIC 3086, Australia.

Skeletal muscle is a heterogeneous tissue, being comprised of two major cell or fibre types (Type I or Type II) distinct in metabolic and/or contractile properties. A large proportion of ATP consumption in skeletal muscle is due to the activities of the ATPases, sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) and the sodium potassium ATPase (NKA). The activities and/or abundance of these ATPases are often fibre type specific, and studies at individual muscle fibre level are necessary to elucidate how these proteins might play a functional role in skeletal muscle overall. Adding complexity, the functions of these ATPase proteins are often related to regulatory proteins, specifically phospholamban (PLBN) and sarcolipin for SERCA, and phospholemmen (or FXYD1) for NKA.

We have undertaken numerous studies using segments of single muscle fibres obtained from rat and human skeletal muscle (Lamboley *et al.*, 2013,2014; Murphy *et al.*, 2009). Force measurements were examined, where fibres were mechanically-skinned by removing the surface membrane (sarcolemma) and exposed to various solutions. Ca^{2+}-release from the SR was invoked chemically (with caffeine and low [Mg^{2+}]) and the amount of Ca^{2+} release ascertained from the time integral of the force response after loading the SR for various set periods. Following force measurements, fibres were collected for later analyses by Western blotting. Type I and Type II fibres obtained from predominantly fast-twitch or slow-twitch muscles of the rat show distinctly different profiles for both Ca^{2+}-release and Ca^{2+}-uptake (Murphy *et al.*, 2009). Human muscle fibres also show distinct fibre type profiles, although most fibres from the *vastus lateralis* are oxidative fibres, being either Type I or Type IIa. These showed less disparity in properties such as maximal rate of Ca^{2+} uptake by the SERCA and maximal SR Ca^{2+} content, as well as in the abundance of the relevant proteins responsible for these functions (Lamboley *et al.*, 2013,2014).

In the current work, segments of individual fibres were dissected from the *vastus lateralis* muscle of humans, obtained by needle biopsy following injection of 1% xylocaine in the skin and fascia. We examined the content of PLBN and FXYD1 in Type I and Type II muscle fibres. In muscle from well-trained endurance athletes, PLBN was present in all fibres that expressed MHC I, regardless of whether these fibres co-expressed Type IIa MHC isoforms. PLBN was completely absent from any fibres that expressed MHC IIx isoform. When a fibre expressed only MHC IIa they expressed up to 25% of the amount of PLBN seen in a MHC I fibre (with total protein taken into account) (n=43 fibres, N=4 subjects). In a cohort of young, habitually active individuals, FXYD1 was ~40% more abundant in Type I *vs* Type II fibres (determined in muscle fibre pools from 12 individuals, $P<0.05$).

Our work adds to understanding how muscle fibres are able to differentially regulate Ca^{2+} uptake into the SR. It demonstrates that when muscle is examined at the single fibre level it is possible to obtain the most meaningful mechanistic data about the function of ATPases as well as their regulatory properties.

Lamboley CR, Murphy RM, McKenna MJ, Lamb GD. (2013). Endogenous and maximal sarcoplasmic reticulum calcium content and calsequestrin expression in Type I and Type II human skeletal muscle fibres. *J Physiol* **591**: 6053-68.

Lamboley CR, Murphy RM, McKenna MJ, Lamb GD. (2014). Sarcoplasmic reticulum Ca^{2+} uptake and leak properties and SERCA isoform expression in type I and type II fibres of human skeletal muscle. *J Physiol* **592**: 1381-95.

Murphy RM, Larkins NT, Mollica JP, Beard NA, Lamb GD. (2009). Calsequestrin content and SERCA determine normal and maximal Ca^{2+} storage levels in sarcoplasmic reticulum of fast- and slow-twitch fibres of rat. *J Physiol* **587**: 443-60.