Determining the impact of oxidation on the motility of single muscle-fibres expressing different myosin isoforms

Under oxidative stress, myosin has been shown to be one of the muscle proteins that are extensively modified, leading to carbonylation and cross-linking. However, how oxidation affects the actomyosin interaction in muscle fibres with different metabolic profiles and expressing different myosin heavy chain (MyHC) isoforms has not been previously investigated. Oxidation of myosin isolated from muscle fibres originating from various porcine muscles with a different metabolic profile was studied using a single muscle fibre in-vitro motility assay, allowing measurements of catalytic properties (motility speed) and force-generation capacity of specific MyHC isoforms. In the experimental procedure, single muscle fibres were split in different segments and each segment was exposed to a different concentration of hydrogen peroxide. Speed and force measurements were recorded and compared, to assess the effect of myosin oxidation on motility and force. The MyHC isoform expression in the single muscle fibre was subsequently determined on silver-stained gel SDS-PAGE. Preliminary results indicate a decrease of directionality and speed of the in-vitro motility as a result of an oxidative environment, and the successful use of the assay in determining fibre-specific responses to oxidation. Subsequent analyses will focus on the location of protein modifications on the myosin molecule and on how these modifications induce changes in speed and force.