

Investigating the Role of FOXO Transcription Factors in Skeletal Muscle Remodelling

Andrew Fisher, Jonathan Jarvis, Judy Coulson, Lauren Fisher. The University of Liverpool, Liverpool, Merseyside, United Kingdom.

Skeletal muscle is highly plastic and its contractile properties, volume and endurance capacity adapt to changes in load and activity. The FOXO family of forkhead transcription factors are suggested to play a central role in controlling genes expressed during skeletal muscle atrophy, but their role in muscle adaptation to changed activity is unclear. The FOXO family comprises at least three members in mammals: FOXO1, FOXO3 and FOXO4, which regulate expression of numerous transcripts and have been associated with biological processes including metabolism, energy homeostasis, cell cycle and survival. Central to their own regulation is a shuttling system that sequesters FOXO factors in either the nucleus or cytosol.

We are using miniature implantable electrical stimulators that artificially increase muscle activity in rats to monitor molecular changes within muscle fibres. Quantitative real-time PCR (qRT-PCR) is used to measure transcript levels of genes involved in the muscle remodelling response. We have also developed techniques to fractionate nuclear and cytoplasmic proteins to investigate the sub-cellular localisation of FOXO transcription factors within muscle following stimulation. An initial qRT-PCR study of control tissue samples was used to assess biological variation and determine appropriate n numbers for subsequent experiments. We then stimulated rat Tibialis anterior muscle using various patterns and durations of stimulation to identify appropriate parameters. We concluded that transcriptional changes were difficult to quantify at early time points, e.g. 3 hr stimulation, but that significant changes were evident over a stimulation period of 1 week at a frequency of 20Hz. We analysed transcript levels for the FOXO family and show that their expression alters in response to stimulation, suggesting an additional regulatory mechanism. We have designed and validated qRT-PCR primers for a panel of 17 candidate transcripts, regulated by FOXO1 and representing different biological processes, which we are currently screening in our experimental model.