The cellular response of skeletal muscle in the first week after a change in activity: clues to signalling pathways whose responses may inform stimulation regimes and improve functional outcomes.

Jarvis JC[1], Fisher LM[2], Fisher AG[3], Coulson JM[4]

Institutes of Ageing and Chronic Disease [1, 2, 3] and Translational Medicine [4] University of Liverpool, UK

Abstract

The outcome of all FES is determined to some extent by the properties of the skeletal muscles that are the effectors. Muscle cells change their properties in response to changes in activity: both the stimulation used to prepare the muscle for FES and the subsequent functional use of the muscle affect the protein expression within the stimulated muscle cells. Transcriptional analysis of muscle samples taken at various times after the initiation of stimulation and with various pattern of stimulation can reveal the signalling pathways that are invoked by changes in activity, and the progressive change in protein expression that underlie the desired increases in strength and endurance. Understanding the cellular response of the muscle may allow us to create models of the response space and potentially to optimise the pattern of delivery of stimulation to maximise benefit and minimise inconvenience.

***Keywords**: Muscle adaptation, electrical stimulation of muscle*

Introduction

There are rather few studies that have followed the response of skeletal muscle cells to electrical stimulation to measure the time course of changes in transcription elicited by substantial changes in activity. We have used implantable electrical stimulators to investigate the time- and frequency-dependence of changes in the transcription of genes in the rat tibialis anterior muscle. We have followed expression of the contractile proteins myosin type 2B, 2A and 1, proteins involved in growth/atrophy, and proteins involved in mitochondrial biogenesis. We have also measured the behaviour of transcription factors that may have a role in sensing the need for adaptive change, such as a relative shortage of ATP.

Material and Methods

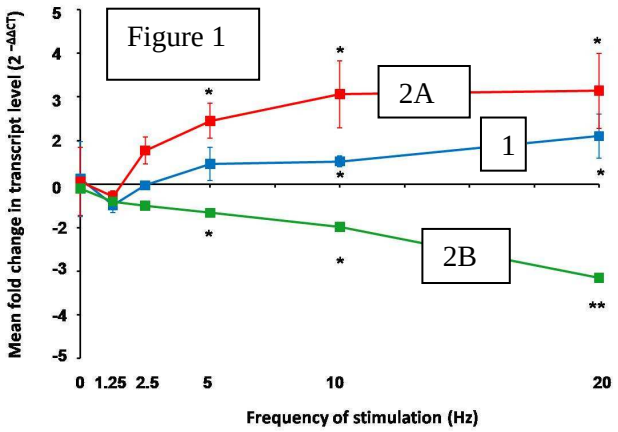
We stimulated rat hind limb muscles with a pattern of stimulation that we have previously shown to cause a substantial shift over five or six weeks in contractile properties (Jarvis et al 1996). We harvested muscles at 3, 6, 12, 24, 72 and 168 hours after the start of stimulation. In this way we studied the early transcriptional changes that in due course lead to the expected changes of muscle mass, protein content, and functional profile such as speed of contraction and endurance.

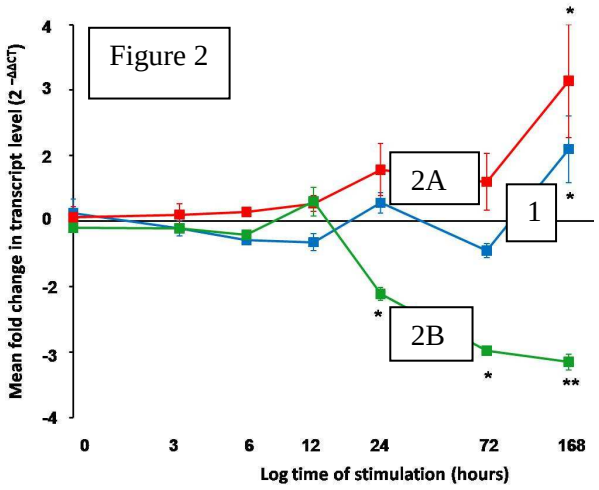
Muscle samples were snap-frozen then processed to extract RNA. This was tested for quality then used to generate complementary DNA. The amount of transcript for each of 11 genes was determined by QRTPCR. The abundance of each transcript was measured relative to the abundance of the transcript for 18S ribosomal RNA, which we found was the most appropriate reference gene for these studies. Several other reference genes are commonly used in transcriptional analysis, but we found that of these 'typical' reference transcripts glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta-2 micro-globulin (B2M), glucose-6-phosphate1-dehydrogenase (G6PD), beta-actin (ACTB), RNA polymerase II (POLR2E) were all unsuitable in this context because their CT value (related to transcript level) changed with different amounts of stimulation. This is not surprising, because continuous activity is a major transcriptional stimulus, but it does mean that the choice of reference gene is very important in studies of transcription in electrically stimulated muscle.

Results

We found that the transcripts for the contractile proteins changed with consistent patterns: In experiments in which groups of animals were stimulated at different frequencies for one week, the transcript level for myosin type 2B changed at

the lowest frequency, followed by myosin type 2A whereas myosin type 1 changed only with greater amounts of continuous stimulation (Figure 1). In a similar way, when a single continuous stimulation frequency of 20Hz was chosen, the transcript level for type 2B changed earlier (approx 12 hours) than the transcript for type 2A (approx 24 hours) which in turn changed earlier than the transcript for type 1 (approx 72 hours) (Figure 2).





On the other hand some of the transcription factors showed behaviour that was not simply graded with time or activity. For example, the transcript for PGC1 alpha showed a transient rise to a peak 12-fold higher than control after 6 hours but then returned to baseline after 12 hours and stayed at a low level thereafter. In contrast the transcription factor PPAR delta showed a gradual rise over one week.

Discussion.

Stimulation with implantable stimulators in an animal model can be used to investigate the relationship between activity and phenotype with some precision. Fixed known frequencies or patterns of activation can be used and the response documented either early by transcriptional analysis or later by functional analysis of parameters such as force or speed or endurance.

We have shown that transcriptional analysis can detect differences in response with good resolution between, for example 6 hours and 12 hours or between 2.5 Hz and 5 Hz

These data will inform models of the response elements in skeletal muscle that we purposefully invoke when we apply Functional Electrical Stimulation. Since some of them display threshold type behaviour, we may be able to use transcript levels as indicators of beneficial response, or indicators of over-stimulation. For example, we might choose a stimulation pattern in which we wish to induce mitochondrial biogenesis, but avoid induction of a slow isoform of myosin. Transcriptional analysis of a very small muscle sample would provide an indication of whether the intended outcome was achieved.

References:

Muscle Nerve. 1996 Nov;19(11):1469-75.
Fast-to-slow transformation in stimulated rat muscle.
Jarvis JC, Mokrusch T, Kwende MM, Sutherland H, Salmons S.

Communicating author

Dr Jonathan C Jarvis

Dept Musculoskeletal Sciences

Institute of Ageing and Chronic Disease

University of Liverpool

Sherrington Bdg Ashton St

Liverpool L69 3GE

UK