

## Role of glycogen depletion in muscle fatigue

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Fatigue is characterized by a decline in muscle performance that is reversible after a period of rest. It has long been known that a depletion of muscle glycogen occurs during prolonged activity and this is correlated with the inability to maintain workload (exhaustion). However, the mechanism by which decreased glycogen impairs force production is unknown. One possibility is that glycogen depletion reduces calcium release by the sarcoplasmic reticulum, and there are studies on isolated fibres (Chin & Allen, 1996), isolated whole muscles (Helander *et al.*, 2002) and skinned single fibres (Stephenson *et al.*, 1999) that support this idea. The aim of the present study was to use an *in vivo* approach to study intracellular calcium in blood-perfused *tibialis anterior* (TA) muscle of anaesthetized mice when muscle glycogen was depleted. Glycogen depletion was achieved by subjecting the TA to repeated fatigue regimes which involved electrically stimulating the muscle with maximal, repeated tetani until force declined to a steady state.

A genetically encoded calcium indicator (cameleon) was used to measure myoplasmic calcium using two-photon microscopy. The cameleon is based around calmodulin and includes a cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) moiety at opposite ends, such that calcium binding to calmodulin allows fluorescence resonance energy transfer (FRET) between the flanking GFPs. Approximately 8 to 14 days prior to experimentation the cameleon was injected into the superficial layer of the exposed TA and electroporated to aid plasmid entry into the fibres. On the day of experiment, the distal tendon was detached and connected to a force transducer, while the muscle body was positioned horizontally for microscopy. The TA was stimulated with electrodes applied directly to the surface and fatigue was induced by short (0.4 s), repeated (every 4s) maximal tetani (12 min fatigue protocol). Force data were collected continuously, while CFP and YFP ratio images were recorded at various times throughout the fatigue; pre-fatigue, during the fatigue bout, and after recovery during both periods of rest and tetanus. A FRET ratio image was then calculated by dividing the background subtracted YFP signal by the CFP signal, which directly correlates to the level of calcium present in the myoplasm. Two fatigue bouts (15 min apart) were performed on the same muscle in order to see any changes in calcium associated with glycogen depletion. Muscles were also analysed for glycogen content at the start and end of each fatigue using enzymatic assay.

Muscle glycogen significantly fell from a control level of  $152 \pm 16$  mmol·kg<sup>-1</sup>·dw (n=9) to  $40 \pm 5$  mmol·kg<sup>-1</sup>·dw (n=6) at the start of the second fatigue. Initial data show that the tetanic calcium transient fell to  $47 \pm 15\%$  (n=5) of control during the first fatigue bout and to  $50 \pm 18\%$  (n=4) reduction during the second fatigue bout. However, it must be noted that the data obtained from the second fatigue were rather variable given that two values were 81% and 82% and the other two 21% and 17%. Because there was no significant difference in myoplasmic calcium during successive fatigue runs, the hypothesis that a reduction in calcium release occurs concurrently with the decline in force associated with glycogen depletion warrants further investigation.

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