Single muscle fibre analysis reveals differential fibre type, localization and stimulation responses of enzymes important for glycogen metabolism: insights into muscle glycogen structure and function

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Glycogen is a highly branched polymer of glucose comprising α-1,4-glycosidic bonds with α-1,6-glycosidic bonds at branch points. It is an important energy source within contracting skeletal muscle. To understand function and regulation of proteins it is important to have knowledge about their properties in the physiological environment. We examined how glycogen affects skeletal muscle physiology by examining enzymes essential for muscle glycogen synthesis and degradation using single fibres from quiescent and stimulated rat skeletal muscle. Presenting a shift in paradigm, we have shown that these proteins are differentially associated with glycogen granules.

Male Long-Evans hooded rats (6-8 months old) were sacrificed using a lethal overdose of isoflurane (4% vol/vol) in accordance with the La Trobe University Animal Ethics Committee and the *extensor digitalis longus* (EDL) and soleus (SOL) muscles were excised. Protein diffusibility and/or abundance of glycogenin, glycogen branching enzyme (GBE), glycogen debranching enzyme (GDE), glycogen phosphorylase (GP) and glycogen synthase (GS) were examined in fibres isolated from both EDL and SOL muscles. Compared with SOL muscle, EDL muscle had the highest expression of GDE and GP proteins, similar levels of GS and glycogenin proteins and the lowest expression of GBE protein. Mechanically-skinned fibres exposed to physiological buffer for 10 min showed ~70% total pools of GBE and GP were diffusible (non-bound), whereas GDE and GS were considerably less diffusible. *In vitro* stimulation that resulted in a ~50% decrease in intracellular glycogen, increased diffusibility of GDE, GP and GS by up to 60% yet decreased the diffusibility of GBE by ~20%. Following either degradation of the glycogen granules by breaking α-1,4 linkages of glycogen following amylase treatment or solubilization of the muscle membranes using 1% Triton X-100, it was revealed that many of these glycogen associated proteins were differentially associated with glycogen and indeed subcellular structures. Given differences in enzymes required for glycogen metabolism, the current findings suggest glycogen particles have fibre-type dependent structures. The greater catabolic potential of glycogen breakdown in fast-twitch fibres may account for different contraction induced rates of glycogen utilization.