Calpain-3 deficiency results in the perturbation of specific Ca^{2+}-handling proteins in skeletal muscle of mice

B.P. Frankish and R.M. Murphy, Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia.

Calpain-3 is a muscle-specific protease, expressed almost exclusively within skeletal muscle. Calpain-3 is both dependent on, and sensitive to, very low Ca^{2+} concentrations, vital to its function as a proteolytic enzyme. The specific functions that calpain-3 fulfills within skeletal muscle are unidentified, and consequently there is an overall poor understanding of calpain-3 as a whole. It is known however, that as a result of absent or non-functional calpain-3 (calpain-3 deficiency), humans develop limb-girdle muscular dystrophy type 2A (LGMD2A). We explored the expression of specific Ca^{2+}-handling proteins, in mice that either did or did not express the calpain-3 protein within skeletal muscle.

Genetically modified C57BL6/129Sv calpain-3 mice (3-4 months old) were sacrificed using a lethal overdose of isoflurane (4% vol/vol) in accordance with La Trobe University Animal Ethics Committee, and the *extensor digitorum longus* (EDL) and *soleus* (SOL) muscles were excised. Male wild-type (WT) and calpain-3 deficient (C3D) mice were used for experiments, where the abundances of specific Ca^{2+}-handling proteins such as calsequestrin, SR Ca^{2+}-ATPase (SERCA) and parvalbumin were examined in both the EDL and SOL muscles. Using Western blotting, the expression of the sarcoplasmic reticulum (SR) Ca^{2+}-buffering protein, calsequestrin 2 (CSQ2) protein was increased ~75% (WT: 1.26 ± 0.24 ; C3D: 2.25 ± 0.32, mean ± SEM, $P<0.05$, unpaired t-test) and the myoplasmic Ca^{2+}-buffering protein, parvalbumin, was decreased ~50% (WT: 2.92 ± 0.55 ; C3D: 1.34 ± 0.27, mean ± SEM, $P<0.05$, unpaired t-test) in the predominantly slow-twitch SOL muscle from C3D mice compared to WT mice. However, other Ca^{2+}-handling proteins including SR Ca^{2+}-ATPase (SERCA1 and SERCA2a) and CSQ1 were not found to be altered in the SOL muscle. The abundance of SERCA1, SERCA2a, CSQ1, CSQ2 and parvalbumin proteins were not altered in the fast-twitch predominant EDL muscle from C3D compared to WT mice.

These findings suggest that the Ca^{2+}-regulation within the slow-twitch predominant SOL muscle of C3D mice is altered, indicating that as a result of calpain-3 deficiency Ca^{2+}-buffering in slow-twitch skeletal muscle is likely to be affected.