Sarcoplasmic reticulum function in human skeletal muscle during ageing and inactivity

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The aim of our research was to define the physiological alterations occurring in human skeletal muscle fibres during ageing or following inactivity. In both of these conditions, the observed decrease of specific force can result from failure in any of the steps in the excitation contraction-coupling sequence. Force production in a muscle fibre is closely related to the amount of Ca^{2+} released from the SR. For this reason, it is important to investigate the SR function in human skeletal muscle fibres under physiological resting conditions.

Using the mechanically-skinned fibre technique, our project examined individual fibres collected from muscle biopsies of the *vastus lateralis*, to investigate whether SR Ca^{2+} content and associated SR proteins are different between young (~23 yo) and old (~70 yo) adults (Lamboley *et al.*, 2015, Lamboley *et al.*, 2016a) or following a 23 days period of muscle disuse induced by unilateral lower limb suspension (ULLS) (Lamboley *et al.*, 2016b). The endogenous amount of SR Ca^{2+} contained in each fibre could be quantified by pre-equilibrating the fibre in a solution with a known concentration of the very fast calcium-buffer BAPTA and then transferring the fibre to an emulsion of Triton X-100 and paraffin oil in order to lyse all membranous compartments and release any Ca^{2+} from within the fibre (Fryer and Stephenson, 1996). Using a second set of skinned fibres, the relative SR Ca^{2+} leak through the ryanodine receptors (RyRs) was gauged from the net SR Ca^{2+} uptake achieved in a load solution when the cytosolic free [Mg^{2+}] was set at 1 mM or 10 mM, the latter expected to substantially reduce any leak through the RyRs. To examine whether RyR oxidation was responsible for any increased SR leak, in further experiments skinned fibres were treated with dithiothreitol (DTT), a potent reducing agent, before loading the SR maximally.

Our findings indicated that both the endogenous and maximal SR Ca^{2+} content are ~10 to 15% lower in virtually every type I and type II fibre in the old subjects, and also that a caffeine/low Mg^{2+} solution cannot empty the SR of Ca^{2+} to the same extent in the aged fibres as in young adult fibres. Interestingly, the amount of calsequestrin (CSQ) was 20% higher in muscle from aged subjects. Also, a greater Ca^{2+} leakage through the RyRs was found in type I fibres of the old subjects, but not in type II fibres. Furthermore, treatment with DTT significantly increased the maximal SR Ca^{2+} uptake only in type I fibres of old subjects, indicating that the Ca^{2+} leakage in the type I fibres of the old subjects was decreased by the reducing treatment. The density of RyRs and dihydropyridine receptors was not significantly changed in muscle of old compared to young subjects.

Following muscle disuse in young adults, the endogenous SR Ca^{2+} content was ~8% lower in type I fibres and maximal SR Ca^{2+} capacity was lower in both type I and type II fibres (-11 and -5%, respectively). Western blot analyses did not show significant change in the amounts of MHCI and MHCIIa, whereas the amounts of SERCA1 and CSQ increased by ~120% and ~20%, respectively. This research identified important functional changes in the human skeletal muscle fibres with ageing or following 23 days of muscle disuse. In both of these conditions, SR Ca^{2+} content was decreased and it could not be attributed to lower CSQ levels. Instead we suggest that increased leakage of Ca^{2+} out of the SR through the RyRs, due to oxidation/nitrosylation of the RyRs, is possibly the primary cause of the decreased SR Ca^{2+} content seen in such fibres. The effects of the SR Ca^{2+} depletion on Ca^{2+} release could be expected to have significant deleterious effects on the muscle strength and performance and may be significant contributory factors to muscle weakness and atrophy with ageing or following a period of inactivity.

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