Evidence of impaired store-operated Ca^{2+} entry in aged mammalian skeletal muscle

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Age-related effects on skeletal muscle function are increasingly recognized as contributing factors to a reduced lifestyle quality in the elderly population. Such effects include a decline in an individual's mobility and independence, possibly due to reduced specific force production and sarcopenia. As a major determinant of muscle force, it has been suggested that Ca^{2+} homeostasis is compromised in aged skeletal muscle (Delbono, 2002). Store-operated Ca^{2+} entry (SOCE) is a mechanism that involves extracellular Ca^{2+} entry in response to Ca^{2+} release from (and hence a reduction in) the intracellular Ca^{2+} stores. SOCE appears to be tailored to the specific needs of different cell types including highly specialized skeletal muscle cells (fibres); where force is produced in response to an increased myoplasmic [Ca^{2+}] due to Ca^{2+} release from the intracellular Ca^{2+} stores (sarcoplasmic reticulum, SR). Reduced SOCE and consequent cell function have been described in aged neuronal cells and aged fibroblasts. Thus, there is an importance to investigate SOCE in aged skeletal muscle because a change in Ca^{2+} handling through SOCE may contribute to the decline in force production.

Young (8-20 weeks) and aged (23 months) C57BL/10 mice were killed by asphyxiation, in accordance to the guidelines set by the Animal Ethics Committee of the University of Queensland. Tibialis anterior muscles were collected for protein analysis. Extensor digitorum longus muscles were rapidly excised, pinned out and fully immersed in paraffin oil. Small bundles of intact fibres were isolated and exposed to a Na^{+}-based physiological solution containing the fluorescent dye, fluo-5N salt. Single fibres were then isolated and mechanically skinned (resulting in the trapping of the dye in the t-system) and transferred to a chamber containing a K^{+}-based internal solution with 1 mM EGTA (100 nM free Ca^{2+}), 1 mM free Mg^{2+} and 50 μM rhod-2. Release of SR Ca^{2+} was evoked by substitution of the bathing solution with a 'low Mg^{2+}' solution, containing 0.01 mM Mg^{2+} and being nominally free of Ca^{2+}. Cytoplasmic rhod-2 and t-system fluo-5N were continuously imaged on an Olympus FV1000 confocal microscope in xyt mode during Ca^{2+} release at 1.0 NA. The net change in t-system fluo-5N signal was used as an indicator of SOCE activity (Launikonis and Ríos, 2007). The protein levels of Orai1 (the integral membrane Ca^{2+} channel thought to be responsible for SOCE) were measured in whole muscle homogenates by Western Blotting.

Substitution of the standard K^{+} -based intracellular solution with a low Mg^{2+} solution induced global SR Ca^{2+} release. This was accompanied by an initial Ca^{2+} uptake in the sealed t-tubules, followed by depletion due to SOCE. SOCE deactivation followed Ca^{2+} reuptake into the SR and reduction in myoplasmic Ca^{2+}. In some fibres, subsequent Ca^{2+} waves were observed, with defined fronts and defined onset of SOCE. This data, together with a high temporal resolution line acquisition, allowed the SOCE activation coupling delay to be measured (start of Ca^{2+} release wave until the beginning of SOCE). SOCE kinetics was analyzed by line-wise signal averaging with a 500Hz resolution. SOCE activation was significantly delayed in aged muscle (38 ± 3.1 ms, n = 4) compared to young mice (27 ± 3.6 ms, n = 6, p = 0.044). This data suggests that SOCE may be delayed in aged skeletal muscle and therefore compromise adequate fine tuning of store-refilling. This may be due to an approximately 50% reduction in Orai1 protein levels observed in aged skeletal muscle relative to skeletal muscle from young mice.

Delbono O. (2007) *Biogerontology*, **3:** 265-270.
Launikonis BS & Ríos E. (2007) *Journal of Physiology*, **583:** 81-97.