Endogenous and maximal sarcoplasmic reticulum calcium content in human *vastus lateralis* muscle fibres is decreased with ageing

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A progressive decline in skeletal muscle function is part of the normal ageing process and often results in significant losses of functional independence in the elderly. The observed loss of specific force in aged muscle suggests that a Ca^{2+} dependent process may be impaired in ageing. Muscle contraction is initiated when T-tubule membrane depolarization triggers Ca^{2+} release from the adjacent sarcoplasmic reticulum (SR). The subsequent force production is closely related to the amount of Ca^{2+} released from the SR. For this reason, it would be important to have a reliable measurement of SR Ca^{2+} content ($[Ca_T]_{SR}$) in aged human skeletal muscle fibres under physiological resting conditions. The present study examined for the first time, in individual fibres from human skeletal muscle biopsies, whether endogenous SR Ca^{2+} content and maximal SR Ca^{2+} capacity are different between young and aged healthy adults.

The study was approved by the Human Ethics Committees at Victoria University and La Trobe University. A muscle biopsy was taken from the *vastus lateralis* muscle from eleven and nine healthy young (23±0.8 yo) and old (70±0.7 yo) adults, respectively. After injection of a local anesthetic into the skin and fascia (1% lidocaine (Xylocaine)), a small incision was made and a muscle sample taken (~150 mg) using a Bergstrom biopsy needle. Individual fibre segments, obtained from the biopsy, were mechanically skinned under paraffin oil so that they still contained their endogenous Ca^{2+} content. The total amount of endogenous 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 for 20 s and then transferring the fibre to an emulsion of 1% Triton X-100 and paraffin oil (TX-oil) in order to lyse all membranous compartments and release any Ca^{2+} from within the fibre (Fryer & Stephenson, 1996). If the preequilibrating [BAPTA] was chosen such that the fibre produced a finite, non-maximal force response upon lysis, then the total amount of Ca^{2+} present in the fibre can be calculated from the BAPTA concentration in the equilibration solution and the magnitude of the force response. Furthermore, other fibre segments, prior to the TX-oil lysing, were (1) totally depleted from their endogenous SR Ca^{2+} content by a 1 minute exposure to a solution containing 30 mM caffeine and 0.05 mM Mg^{2+} or (2) loaded to their maximal SR Ca^{2+} capacity by a 4 minute exposure to a solution containing 0.2 μM free Ca^{2+} (buffered with 1 mM CaEGTA – EGTA). Finally, using Western blotting, each muscle fibre was classified as type I or II according to the myosin heavy chain isoform present.

When fibres with an endogenous Ca^{2+} content were assayed, the endogenous $[Ca_T]_{SR}$ obtained (expressed relative to intact fibre volume) was significantly decreased in aged subjects compared to young individuals (0.60±0.01 (n=15) and 0.71±0.02 (n=8) $mmol \cdot l^{-1}$, respectively, in type I fibres, and 0.67±0.03 (n=10) and 0.80±0.03 (n=12) $mmol \cdot l^{-1}$, respectively, in type II fibres). By loading the SR of the fibres maximally, the study also revealed that the maximal SR Ca^{2+} capacity was significantly decreased in fibres of aged subjects compared to young adults (1.21±0.04 (n=12) and 1.35±0.05 (n=12) $mmol \cdot l^{-1}$, respectively, in type I fibres, and 1.44±0.03 (n=11) and 1.70±0.03 (n=17) $mmol \cdot l^{-1}$, respectively, in type II fibres).

In conclusion, the present results show that the $[Ca_T]_{SR}$ of the human aged muscle fibres was significantly different from those of the young adult fibres. These results suggest that the appreciable decrease with ageing of the endogenous and maximal $[Ca_T]_{SR}$ in both type I and II fibres could be an important factor in muscle weakness in the elderly. Future studies, using the same mechanically skinned fibre preparation can be used to identify which mechanism(s) or key proteins alter the $[Ca_T]_{SR}$ with ageing.

Fryer M.W. & Stephenson D.G. (1996). Total and sarcoplasmic reticulum calcium contents of skinned fibres from rat skeletal muscle. *Journal of Physiology* **493**, 357-370.