Sarcoplasmic reticulum Ca^{2+} leak in human skeletal muscle fibres is not altered with age

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A progressive decrease in skeletal muscle mass and strength is part of the normal ageing process and may contribute to physical disability and loss of independence. In addition to decreased muscle mass, decreases in muscle specific force have also been reported (Larsson, Li & Frontera, 1997).

We recently showed that the sarcoplasmic reticulum (SR) total Ca^{2+} content ($[Ca_T]_{SR}$) of the human aged muscle fibres was significantly decreased compared to those of the young adult fibres in both type I and type II fibres (Lamboley *et al.*, 2012). This difference could be an important factor contributing to muscle weakness in the elderly. However, the mechanisms underlying impaired SR Ca^{2+} storage in ageing muscle remains to be elucidated. In this study we investigated the role of SR Ca^{2+} leak as an underlying mechanism for defective Ca^{2+} handling in age-dependent muscle weakness.

The study was approved by the Human Research Ethics Committees at Victoria University and La Trobe University. Fibre segments, obtained by needle biopsy, from *vastus lateralis* muscle of twelve healthy young (25 ± 4.8 yr) and ten old (71 ± 4.3 yr) adults were mechanically skinned and their specific force, contractile apparatus properties and SR Ca^{2+} leakage properties characterised. Direct activation of the contractile apparatus was performed by activating the skinned fibre segment in strongly Ca^{2+} buffered solutions with pCa (= $-\log_{10}[Ca^{2+}]$) between 6.7 and 4.7. Passive leakage of Ca^{2+} out of the SR was measured from the temporal changes in $[Ca_T]_{SR}$. Briefly, the SR was subjected to repeated cycles in which it was loaded to close to its endogenous content and then placed for a set time (5 to 180 s) in a solution containing 2 mM EGTA (at pCa 8.5) so that any Ca^{2+} leaking from the SR could not be recovered. The amount of Ca^{2+} then remaining in the SR was ascertained from the time-integral of the force response upon releasing all SR Ca^{2+} with a caffeine-low $[Mg^{2+}]$ solution. Further experiments were performed to determine whether the SR Ca^{2+} release channels (RyRs) played any role in the observed SR Ca^{2+} leak, by examining the effect of raising the free $[Mg^{2+}]$ in the load solution to 10 mM (at pCa 6.7) so as to block any SR Ca^{2+} leak through the RyRs. Finally, using western blotting, each muscle fibre was subsequently classified as type I or II according to the myosin heavy chain isoform present.

Specific force was significantly decreased in type II fibres of aged subjects (mean ± S.E.M., with n indicating number of fibres examined: 164 ± 9 mN/mm^2 (n = 16) and 202 ± 10 mN/mm^2 (n = 31) in aged and young subjects, respectively) but not in type I fibres (156 ± 12 mN/mm^2 (n = 34) and 159 ± 7 mN/mm^2 (n = 31), respectively). The pCa producing half maximal force (pCa_{50}) was also significantly decreased in aged type II fibres (5.76 ± 0.03 (n = 13) and 5.83 ± 0.01 (n = 10) pCa units in aged and young subjects, respectively) but not in type I fibres (5.91 ± 0.02 (n = 19) and 5.94 ± 0.01 (n = 14) pCa units, respectively). The relative amount of Ca^{2+} left in the SR after 3 min in the leak solution was not significantly different in muscle fibres of aged compared to young subjects, both for type I fibres (71 ± 2 % (n = 5) and 68 ± 4 % (n = 10) of endogenous $[Ca_T]_{SR}$, respectively) and type II fibres (78 ± 3% (n = 6) and 82 ± 2 % (n = 11), respectively). Finally, the presence of 10 mM Mg^{2+} in the load solution had similar effect on maximal SR Ca^{2+} uptake in fibres of aged and young subjects (SR Ca^{2+} content after loading with 10 mM Mg^{2+} was 92 ± 2 % (n = 10) and 99 ± 5 % (n = 16) of that achieved with 1 mM Mg^{2+} in type I fibres of young and aged subjects, respectively, and 82 ± 3 % (n = 12) and 78 ± 5 % (n = 6) in type II fibres, respectively), indicating that there was no difference between young and aged subjects in the extent of passive leak of Ca^{2+} through the RyRs in either fibre type.

In conclusion, the decreases in specific force and Ca^{2+} sensitivity of the contractile apparatus observed here in the type II fibres from aged subjects are likely to be significant factors in muscle weakness in the aged population. Furthermore, we conclude that the decreased $[Ca_T]_{SR}$ seen in both fibre types in age (Lamboley *et al.*, 2012) is not due to greater leak of Ca^{2+} from the SR in the aged fibres.

Larsson L & Li X & Frontera WR. (1997) *American Journal of Physiology – Cell Physiology*, **272**, C638-C649.
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