Sarcoplasmic reticulum Ca^{2+} leak is increased in type I muscle fibres of old human subjects

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We investigated the role of ryanodine receptor (RyR) oxidation and resultant sarcoplasmic reticulum (SR) Ca^{2+} leak as an underlying mechanism for our recent observation that SR Ca^{2+} content is reduced in both type I and type II muscle fibres in aged compared to young adult human subjects.

Fibre segments from *vastus lateralis* muscle, obtained by needle biopsy from 9 healthy young (25 ± 4.8 yr) and 9 old (71 ± 4.3 yr) adults, were mechanically skinned and the SR Ca^{2+} uptake properties characterized. The relative SR Ca^{2+} leak through RyRs in young and old muscle fibres was gauged from the net SR Ca^{2+} uptake achieved in a load solution at pCa 6.7 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 for 5 min with 10 mM DTT, a potent reducing agent, before loading the SR maximally in the presence of 1 mM Mg^{2+}. In both sets of experiments, net SR Ca^{2+} uptake was ascertained from the time-integral of the force response upon releasing all SR Ca^{2+} with a caffeine-low [Mg^{2+}] solution. Each fibre segment was subsequently classified as type I or II by western blotting. In type I fibres, the maximal SR Ca^{2+} content reached after loading in the presence of 10 mM Mg^{2+}, relative to that after loading in 1 mM Mg^{2+}, was significantly higher (p<0.05) in old compared to young subjects (mean ± SEM: 96 ± 3 %, n=28, and 87 ± 1 %, n=15, respectively), which indicates greater Mg^{2+}-blockable Ca^{2+} leakage through the RyRs in the type I fibres of the old subjects. No such difference was observed in type II fibres (81 ± 3 %, n=11 and 82 ± 3 %, n=15, respectively). Furthermore, treatment with DTT before repeating the SR load-release cycle significantly increased the maximal SR Ca^{2+} uptake in type I fibres of old subjects but not young subjects (maximal SR Ca^{2+} content following DTT treatment: 105 ± 2 % (n=12) (p<0.05) and 98 ± 1 % (n=7) of that before DTT treatment in type I fibres of old and young subjects, respectively, and 99 ± 2 % (n=5) and 95 ± 2 % (n=7) in type II fibres, respectively), indicating that the Ca^{2+} leakage in the type I fibres of the old subjects was decreased by the reducing treatment.

Western blotting of total homogenates showed a higher proportion of type I fibres in the old subjects (ratio of myosin heavy chain I (MHCI) in old (n=11) compared to young subjects (n=10) was 1.24 ± 0.14, and for MHCII it was 0.62 ± 0.11). After taking the shift in fibre types into account, it appeared that the amounts of CSQ1 and CSQ2 (normalised to total fibre protein) were both approximately 20% higher in old compared to young subjects. Interestingly, there was no significant difference in the density of either the RyRs or dihydropyridine receptors in muscle of old compared to young subjects.

The greater Ca^{2+} leakage occurring through the RyRs in type I fibres of old subjects, which seemingly results from increased RyR oxidation and/or nitrosylation, could have deleterious effects on Ca^{2+} movements and muscle function.