Human aging and expression of proteins interacting with the ryanodine receptor in skeletal muscle

H. Willemse,[1] P.N. Smith,[2] P.G. Board,[1] M.G. Casarotto[1] and A.F. Dulhunty,[1] [1]Department of Molecular Bioscience, The John Curtin School of Medical Research, The Australian National University, P.O. Box 334, Canberra, ACT 2601, Australia and [2]Trauma and Orthopaedic Research Unit, Building 6, Level 1, Canberra Hospital, P.O. Box 11, Woden, ACT 2606, Australia.

Sarcopenia is the progressive decline in muscle function with age, characterized by reduced force generating capacity and loss of muscle mass. Aging skeletal muscle shows a decrease in specific force that is only partially attributed to muscle atrophy. A change in fibre type distribution has also been observed, with fast twitch fibres decreasing and slow twitch fibres increasing. It has been suggested that excitation contraction coupling (ECC) may be impaired in aging by an uncoupling of the two Ca^{2+} channels facilitating ECC, the dihydropyridine receptor (DHPR) and the ryanodine receptor (RyR1). This uncoupling is possibly due to a decrease in expression of the DHPR α_{1s} subunit (Delbono *et al.*, 1995; Renganathan *et al.*, 1997). In skeletal muscle, the C-terminal tail of the DHPR β_{1a} subunit activates the RyR1 and this interaction may contribute to EC coupling (Rebbeck *et al.*, 2011). Apart from the DHPR, there are many other proteins that modulate RyR1 activity. One of these is the FK506 binding protein 12 (FKBP12) which stabilizes the RyR1 in the closed state and its dissociation from the RyR1 causes a leaky channel. A decreased EC coupling Ca^{2+} transient has also been ascribed to the dissociation of FKBP12 from the RyR1 due to increased nitrosilation occurring in aged muscle (Andersson *et al.*, 2011).

Human muscle samples were obtained from 42 donors (age 40-90) undergoing knee and hip replacements. The samples were taken from the *gluteus minimus, gluteus medius* or *vastus medialis* depending on the operation. Details of the samples can be seen in the Table below. The muscle was immediately processed in the operating theatre in phosphate buffered saline containing 1mM EGTA and snap frozen in liquid nitrogen. The fibre type distribution in muscle homogenate was determined for each muscle sample by SDS PAGE using an 8% polyacrylamide (99:1) gel containing 35% glycerol and a runtime of 20h at 140V and 4°C. Densitometry was used to determine the slow and fast twitch percentages. Expression levels of the DHPR α_{1s} and β_{1a} subunits relative to that of actin were determined in muscle homogenates using western blot and densitometry. Muscle homogenates were used as we have found that most of the β_{1a} is lost during microsomal vesicle preparation. The levels of FKBP12 to RyR1 were determined in microsomal vesicles prepared from the same donors using western blot and densitometry.

Muscle	Female	Age range	Male	Age range
Vastus medialis	6	54-74	8	59-77
Gluteus minimus	11	51-85	10	45-72
Gluteus medius	4	51-84	1	72

Thus far we have analysed the fibre distribution of 6 *vastus medialis* (4 female, 2 male), 5 *gluteus minimus* (2 female, 3 male) and 2 *gluteus medius* (1 female, 1 male) samples. There is a higher fraction of fast twitch fibres in *vastus medialis*, whereas *gluteus minimus* has a higher fraction of slow twitch fibres. Clear trends are visible in the female *vastus medialis* and *gluteus minimus* samples where fast twitch fibre percentage decreases with age and the slow twitch percentage increases. More data points are needed for the male muscles. Preliminary western blot results show a decrease of FKBP12 relative to the RyR1 with age. Levels of α_{1s} and β_{1a} are being processed. It appears that the levels of several of the proteins that modulate the RyR1 and are associated with it may change with age and could affect the release of Ca^{2+} during ECC and muscle function.

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