Calcium and strontium contractile activation properties of single skinned skeletal muscle fibres from elderly women 66–90 years of age

Susan M. Ronaldson1 · D. George Stephenson2 · Stewart I. Head3,4

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Abstract
The single freshly skinned muscle fibre technique was used to investigate Ca2+- and Sr2+-activation properties of skeletal muscle fibres from elderly women (66–90 years). Muscle biopsies were obtained from the vastus lateralis muscle. Three populations of muscle fibres were identified according to their specific Sr2+-activation properties: slow-twitch (type I), fast-twitch (type II) and hybrid (type I/II) fibres. All three fibre types were sampled from the biopsies of 66 to 72 years old women, but the muscle biopsies of women older than 80 years yielded only slow-twitch (type I) fibres. The proportion of hybrid fibres in the vastus lateralis muscle of women of circa 70 years of age (24%) was several-fold greater than in the same muscle of adults (<10%), suggesting that muscle remodelling occurs around this age. There were no differences between the Ca2+- and Sr2+-activation properties of slow-twitch fibres from the two groups of elderly women, but there were differences compared with muscle fibres from young adults with respect to sensitivity to Ca2+, steepness of the activation curves, and characteristics of the fibre-type dependent phenomenon of spontaneous oscillatory contractions (SPOC) (or force oscillations) occurring at submaximal levels of activation. The maximal Ca2+ activated specific force from all the fibres collected from the seven old women use in the present study was significantly lower by 20% than in the same muscle of adults. Taken together these results show there are qualitative and quantitative changes in the activation properties of the contractile apparatus of muscle fibres from the vastus lateralis muscle of women with advancing age, and that these changes need to be considered when explaining observed changes in women’s mobility with aging.

Keywords Human aging · Calcium · Skeletal muscle contraction · Skeletal muscle fibre types

Introduction
There have been a large number of reports of a loss of skeletal muscle mass and force generation capacity as a result of the aging process (Aniansson et al. 1980, 1983; Grimby et al. 1984; Brooks and Faulkner 1988, 1991; Edstrom and Larsson 1987; Jubrias et al. 1997; Lexell et al. 1983, 1988; Sipilä and Suominen 1991; Lamboley et al. 2015). However, it is not yet clear to what extent this reduction in force is due to an intrinsic loss of force generating capacity of the contractile proteins in a particular type of fibre, to the change in the fibre type composition, or to a reduction of the cross-sectional area of the muscle.

Several studies have shown a decrease or selective atrophy of specific muscle fibre types. In particular, it appears that fast-twitch muscle fibres are more prone to atrophy or damage with age (Aniansson et al. 1980; Larsson et al. 1978; Lexell et al. 1988; Lexell 1995). Brocca et al. (2017) found there was a switch from fast-twitch to slow-twitch fibres in the leg muscles from older men, while Meznaric et al. (2018) found that there was a shift in the myosin heavy chain phenotype to the slower myosin heavy chains in the neck muscles of ageing males. They attributed this shift in the fibre type portion to a greater loss of fast-twitch motor neurons during ageing.

In an earlier study using intact skeletal muscles Brooks and Faulkner (1988) found that the maximum force
generated per cross-sectional area was less in whole fast-twitch muscle from old mice. However, in a later study using single skinned muscle fibres they found that there was no difference in the maximum force per cross-sectional area generated by muscle fibres from young and old mice (Brooks and Faulkner 1994). They did, however, note that the Ca\(^{2+}\) sensitivity of the contractile proteins was less in muscle fibres from old mice compared with adult mouse muscle fibres (Brooks and Faulkner 1994). In a study from our laboratory on the effect of ageing on whole fast-twitch EDL muscles in mice (Chan and Head 2010), we showed that muscles from old mice were stiffer and produced less specific force with a shift towards slow-twitch contractile properties as evidenced by a slowing of relaxation and increased resistance to fatigue. Interestingly, from the point of view of the present study, the effect of these age-related changes was greater in female mice compared to male mice (Chan and Head 2010).

Straight et al. (2018) using single skinned fibres from ageing men and women found that the type I, type IIA and hybrid I/IIA fibres were less sensitive to Ca\(^{2+}\) when compared with young controls. In a study using single chemically skinned fibres from young and old men Ochala et al. (2007) found a reduced specific force in fibres from old men.

In the present study we investigated the calcium (Ca\(^{2+}\))—and strontium (Sr\(^{2+}\))—activation properties of freshly dissected single skeletal muscle fibres from elderly women (60–90 years) using the skinned muscle fibre technique. Strontium (Sr\(^{2+}\)) was also used as an activator because it allows unequivocal differentiation between type I, type II and hybrid (type I/type II) fibres (O’Connell et al. 2004; Lamboley et al. 2013). This is because the sensitivity to Sr\(^{2+}\) ions depends on the troponin C (TnC) isoform (slow, fast or both slow and fast) expressed in the respective fibre, which in turn, is tightly correlated with the myosin heavy chain (MHC) expressed (MHCI or MHCII) in the fibre (Bortolotto et al. 2000, O’Connell et al. 2004; Lamboley et al. 2013). Moreover, the Sr\(^{+}\)-activation curves of hybrid fibres permit direct estimation of the fraction of TnC isoforms present in the fibre (O’Connell et al. 2004).

Materials and methods

Ethics approval, subject selection and age grouping

Prior to obtaining a muscle biopsy we conducted an activity profile interview with each woman to ensure our subjects met a minimum activity level in their day to day activities. Human ethics approval was obtained from LaTrobe University and Austin Hospital, Melbourne, Australia. Human Research Ethics Committees and informed written consent was given by each woman prior to surgery.

The age of the women used in this study ranged from 66 to 90 years (n = 7). We split the data obtained from the 28 freshly dissected muscle fibres into two age bins; (1) a circa 70 year old age group which contained five women 66–70 years of age (mean 69.80 ± 1.11 years), in this group the statistics were performed on 16 fibres and (2) a ≥ 80 year age group (mean 85 ± 5 years) containing two women, in this group the statistics were performed on 12 fibres. Each fibre segment was carefully dissected from the muscle biopsy under oil by an experienced operator under a high-powered dissection microscope (magnification ×40–100), fibres were only selected if they were free from any visual imperfections and were supple and flexible, any fibres with signs of mechanical damage from the surgery or were fragile and showed signs of damage when manipulated between the fine jewellers forceps were discarded.

Preparation

Single muscle fibres were dissected from fresh muscle biopsies obtained from elderly women undergoing orthopaedic surgery for total hip replacement or repair of a fractured neck of femur. Each woman’s activity profile was obtained from interview and medical records data prior to the scheduled surgery. Care was taken to select only women with an active lifestyle prior to surgery.

The muscle sample was dissected by an orthopaedic surgeon from the vastus lateralis muscle in elderly women undergoing orthopaedic surgery. The muscle biopsy was obtained within 5–15 min of the commencement of the surgery. The muscle biopsy was blotted thoroughly on Whatman’s filter paper (No. 1) immediately upon its dissection from the vastus lateralis muscle to remove any excess interstitial fluid, then placed in a jar of cold paraffin oil at 2 °C. The muscle biopsy was then placed in a thermos flask containing ice and transferred immediately to the laboratory.

The dissection of the muscle fibres from the muscle biopsy was generally commenced within 60 min of its removal from the vastus lateralis muscle. The muscle biopsy was transferred from the cold paraffin into a Petri dish containing cold paraffin. Lynch et al. (1993) showed that this biopsy procedure had no effect on the activation characteristics of human skeletal muscle biopsy samples. Dissection of muscle fibres took place under oil. The muscle fibre was mechanically skinned (Stephenson and Williams 1981) and tied at one end with silk thread and the other end was mounted between a pair of fixed forceps and attached to a force transducer (801 SensoNor Horten, Norway). Once a single muscle fibre had been dissected the remainder of the biopsy sample was stored under cold paraffin at 2 °C for up to 6 h during which further fibres were dissected out. Our use of fresh fibres from chilled biopsies is in contrast to the majority of human single muscle fibre studies which use...
chemically skinned fibres dissected from biopsies stored at − 20 °C in glycerol-containing skining solutions. Fresh fibres are less brittle and produced greater maximum Ca2+ activated specific forces than fibres obtained from biopsies stored at − 20° for days or weeks.

The length of the fibre was adjusted such that the preparation was just taut, and the diameter of the skinned muscle fibres was measured under oil. The sarcomere length (SL) of the skinned muscle fibres was then measured by laser diffraction (mean SL 2.71 ± 0.04 μm; Stephenson and Williams 1981).

### Solutions

Solutions were prepared according to standard procedures described by Stephenson and Williams (1981). The composition of the solutions is given in Table 1.

The strongly buffered Ca2+ solutions of different free Ca2+ concentrations were prepared by mixing specific proportions of EGTA-containing solutions (solution A) and Ca-EGTA-containing solution (solution B). The strongly buffered Sr2+ solutions of different free [Sr2+] were prepared by mixing specific proportions of EGTA-containing solutions (solution A) and Sr-EGTA-containing solution (solution C). It was important that solution B did not have excess total Ca compared with EGTA to ensure that the pH did not change when solution A was mixed with solution B. An excess Ca compared with EGTA in solution B inevitably causes a decrease in the pH of a mixture of solution A and solution B because the excess Ca coming from solution B will bind to EGTA coming from solution A, releasing two protons for each Ca that becomes bound to EGTA in the mixed solution. A change in pH must be avoided when mixing two EGTA containing solutions because the ionised [Ca2+] is very sensitive to pH. Note that solution C contained 40 mM total Sr and 50 mM EGTA to ensure that the concentration of ionised Sr2+ was <0.3 mM and would not significantly affect the pH when solution C was mixed with solution A. The apparent binding constants for Ca2+ (KappCa) and Sr2+ (KappSr) to EGTA at pH 7.10 and in the presence of 1 mM Mg2+ used to determine the pCa (− log [Ca2+]) and pSr (− log [Sr2+]) values in our solutions were KappCa = 4.78 × 106 M−1 (Stephenson and Williams 1981) and KappSr = 1.53 × 107 M−1 (West and Stephenson 1993).

Following mounting, the single skinned muscle fibre into a relaxing solution (solution A Table 1) containing EGTA (50 mM) and allowed to equilibrate for 5 min. Before activation the fibre was immersed in a pre-activating solution containing HDTA (solution D Table 1) to facilitate a rapid [Ca2+] rise (Moisescu and Thieleczek 1978) when the fibre was placed in the Ca2+ activating solutions (Solution B Table 1) after which it was returned to the relaxing solution (Solution A Table 1). This procedure was repeated for activation of muscle fibres in Sr2+ solutions (Solution C Table 1; Fig. 1). All experiments were performed at room temperature (22 ± 1 °C).

### Activation properties

Assessment of the effects of Ca2+ and Sr2+ activation was achieved by construction of force-pCa and force-pSr curves for the contractile responses of each individual fibre. The steady state tension developed in each solution was expressed as a percentage of the maximum tension developed in the sequence (Fig. 1). The curves were thus generated using a modified form of the Hill equation using GraphPad Prism Software (GraphPad Software Inc., 5755 Oberlin Dr # 110 San Diego, Ca 92121). The modified Hill equation is: Relative tension (%) = 100/(1 + ([Ca50]/[Ca2+])n) where n is the Hill co-efficient for Ca2+ and [Ca50] is the calcium concentration required for half-maximal tension activation. An equivalent equation was used for the Sr2+ activation curves. The following activation properties were measured from the force activation curves generated for each muscle fibre from the two age groups, when all data points fell within 5% of the fitted curves: Ca2+ and Sr2+ threshold for contraction (pCa10 and pSr10, corresponding to 10% maximum force), sensitivity to Ca2+ and Sr2+ (pCa50 and pSr50, corresponding to 50% maximum force) and related differential sensitivity (pCa90-pSr90) and steepness of the activation curves (Hill co-efficient: nCa and nSr).

As described by Bortolotto et al. (2000), the Sr2+-data points for some fibres could not be well fitted by simple Hill-curves (i.e. not all data points fell within 5% of the best fitted Hill-curve). In such instances, the Sr2+-data points were well fitted by a biphasic curve generated by the

### Table 1

Chemical composition of stock solutions of relaxing solution (Solution A), calcium activating solution (Solution B), strontium activating solution (Solution C) and a pre-activating solution (Solution D)

| Chemicals | Solution A | Solution B | Solution C | Solution D |
|-----------|------------|------------|------------|------------|
| HEPES     | 60         | 60         | 60         | 60         |
| EGTA      | 50         | 50         | 50         | 0.1        |
| HDTA      | –          | –          | –          | 49.1       |
| Mg total  | 10.3       | 8.12       | 8.5        | 8.5        |
| Ca total  | –          | 49.5       | –          | –          |
| Sr total  | –          | –          | 40         | –          |
| NaN3      | 1          | 1          | 1          | 1          |
| ATP       | 8          | 8          | 8          | 8          |
| CP        | 10         | 10         | 10         | 10         |
| Mg2+      | 1          | 1          | 1          | 1          |

All solutions contained (mM): K+ 117, Na+ 36. The pH was 7.10 ± 0.01 at 22 °C in all solutions.
following equation: Relative tension (%) = α/(1 + ([Sr501]/[Sr2+])n1 + β/(1 + ([Sr502]/[Sr2+])n2), where α and β represent the percentage of the two phases (α + β = 100%) and Sr501, Sr502 are the strontium concentrations corresponding to the half-maximal activation of the two phases. For the hybrid fibres we do not provide Hill parameters as there aren’t enough data points (distinct pSr’s) to determine pSr501/pSr502 and two Hill coefficients.

**Fibre classification**

The Sr2+-dependent activation properties of individual muscle fibres identifies three groups which are commonly accepted to relate directly to the type of myosin isoform expressed (Schiaffino and Reggiani 2011): type I (slow-twitch expressing TnC slow (cardiac) isoform and MHC I), type II (fast-twitch, expressing TnC fast isoform and MHC II isoforms) and hybrid (type I/ type II, expressing TnC fast/TnC slow and MHC I/MHC II isoforms) (O’Connell et al. 2004; Lamboley et al. 2013). This classification is based on the much higher force sensitivity to Sr2+ of fibres expressing the TnC slow isoform (and MHC I), than the TnC fast isoform (and MHC II isoforms) (Bortolotto et al. 2000; O’Connell et al. 2004; Lamboley et al. 2013). The hybrid fibres are characterised by biphasic force-pSr curves and express both the slow and fast TnC isoforms and a combination of MHC I and II isoforms (Bortolotto et al. 2000; O’Connell et al. 2004). Moreover, the Sr+-activation curves of hybrid fibres permit direct estimation of the fraction of TnC isoforms (MHC I/MHC II isoforms) present in the fibre (O’Connell et al. 2004) from the percentage ratio (α/β) of the two phases of the Sr2+-activation curve.

**Force oscillations of myofibrillar origin**

All slow-twitch (type I) fibres and some fast-twitch (type II) and hybrid fibres displayed spontaneous oscillatory contractions or ‘SPOC’ of myofibrillar origin at submaximal levels of force activation. The highly Ca2+- and Sr2+-buffered activation solutions used in this study (containing 50 mM EGTA) eliminates the possibility that the force oscillations were in any way caused by oscillations in Ca2+ or Sr2+ concentration. Indeed, in previous studies, we have shown that these oscillations are maintained even after treatment of the fibres with detergent to disrupt and extract all membrane compartments (Stephenson and Williams 1981). Moreover, we have shown that such force oscillations at submaximal levels of activation are caused by myosin interactions with the actin filaments (Smith and Stephenson 2009).

**Maximum Ca2+ activated specific force**

Skinned muscle fibres swell when exposed to relaxing solutions and the amount of swelling between an intact fibre at rest in normal physiological solution and after the fibre is skinned and exposed to a relaxing solution varies with the sarcomere length, being larger at longer than at shorter sarcomeres. This is because an intact fibre behaves iso-volumetrically when stretched, with the fibre diameter (transverse spacing of filament lattice) changing inverse-proportionally with the square root of sarcomere length. In contrast, the diameter (transverse spacing of filament lattice) changes little when a skinned fibre is stretched (Matsubara and Elliott 1972). Consequently, the difference between the fibre diameter (transverse spacing of filament lattice) in relaxing solution at a particular sarcomere length after the fibre was...
skinned and the diameter of the fibre when the fibre was intact at same sarcomere length, is greater at longer than at shorter sarcomere lengths because the fibre diameter of an intact muscle fibre is essentially more compressed at longer than at shorter sarcomere lengths compared with that of a skinned fibre. Therefore, when measuring the maximum Ca$^{2+}$-activated specific force it makes sense to express the maximum Ca$^{2+}$-activated force developed by the fibre per unit cross-sectional area before the fibre swells. The maximum Ca$^{2+}$ activated specific force was, therefore, calculated only for fibres where the fibre diameter was measured in oil, as described by Fink et al. (1990) after mechanical skinning and before the skinned fibre was exposed to solutions. The fibre cross-sectional area was calculated assuming it to be circular.

**Statistics**

The data analysed were from muscle biopsies obtained from 7 elderly women. In total, 28 muscle fibres were examined (16 fibres from five women of mean age 69.80 ± 1.11 years and 12 fibres from two women (80.5 and 90 years old). Results were analysed with a one-way analysis of variance (ANOVA, Hewlett Packard statistical program) and/or Student t-test where appropriate, according to levels of significance. The Mann Whitney rank order test was applied when the sample size was small (n < 6) and the variance quite marked (SEM > 4% mean; Witte 1993). Results were significant when P ≤ 0.05.

**Results**

**Muscle fibre populations**

On the basis of their Ca$^{2+}$- and Sr$^{2+}$-activation characteristics three populations of skeletal muscle fibres were identified from the muscle biopsies obtained from the elderly women: type 1 (slow-twitch), type II (fast-twitch) and hybrid (type I/II). The slow-twitch fibres were 10 times more sensitive to Sr$^{2+}$ than the fast-twitch fibres (pSr$_{50}$ 5.71 vs 4.71; see Tables 2 and 3) and their identification was clear-cut. The percentages of the total muscle fibre population were: type 1 (slow-twitch) = 46%, type 2 (fast twitch) = 36% and hybrid (type I/II) = 18%. We should note here that while we do not directly look at the MyHC expressed in our fibres, force-pCa/pSr curves are a recognised method for fibre type classification which is accepted to be in full agreement with MyHC isoforms given the tight coupling between TnC isoforms and MyHC isoforms predicting the type of myosin isoform expressed in the single fibre (Bortolotto et al. 2000; Stephenson and Williams 1981; Schiaffino and Reggiani 2011), for this reason while refer to type 1 (slow-twitch), type 2 (fast twitch) fibres and hybrid (type I/II) fibres for clarity, it needs to be born in mind that we have inferred these fibre type categories from the characteristics of the Force-pCa/pSr curves.

When the data were divided into two age groups: fibres from elderly women circa 70 years (66–72 years) and fibres from women older than 80 years, all fibres investigated from women older than 80 years were of type I (100%), while all three fibre types were present in women of circa 70 years (28.5% type I, 47.5% type II and 24% hybrid (type I/II) fibres.

**Ca$^{2+}$- and Sr$^{2+}$-activation characteristics**

Representative force-pCa and force-pSr activation curves for slow-twitch (type I) fibres from the two age groups (circa 70 years and > 80 years) of women together with the activation curves of a representative fast-twitch (type II) fibre from a 70 years old woman are shown in Fig. 2. As described in the Methods, the following parameters were measured from the activation curves: the Ca$^{2+}$ and Sr$^{2+}$ thresholds for contraction (pCa$_{10}$ and pSr$_{10}$, corresponding to 10% maximum force), sensitivity to Ca$^{2+}$ and...
Sr\textsuperscript{2+} (pCa\textsubscript{50} and pSr\textsubscript{50}, corresponding to 50\% maximum force), related differential sensitivity (pCa\textsubscript{50}–pSr\textsubscript{50}) and steepness of the activation curves (Hill co-efficient: nCa and nSr). As shown in Fig. 2, the Sr\textsuperscript{2+}-activation curves lie much closer to the respective Ca\textsuperscript{2+}-activation curves in slow-twitch fibres compared with the fast-twitch fibres.

**Slow-twitch (type I) fibres**

The Ca\textsuperscript{2+} and Sr\textsuperscript{2+}-activation characteristics of slow-twitch (type I) fibres from the two age groups (circa 70 years and \(>80\) years) are summarized in Table 2. There were no significant differences between the measured Ca\textsuperscript{2+} and Sr\textsuperscript{2+} activation parameters of type I fibres from the two groups. However, as we point out in the “Discussion”, the slow-twitch (type I) fibres from older women (our groups circa 70 years and 80 years) appear to be significantly more sensitive to Ca\textsuperscript{2+} compared with slow-twitch fibres from the vastus lateralis muscles of young adults, mean age 24 years (Lamboley et al. 2013) and more sensitive to both Ca\textsuperscript{2+} and Sr\textsuperscript{2+} compared to slow twitch fibres obtained from young adult males (Fink et al. 1990).

**Fast-twitch (type II) fibres**

In Table 3 are shown the Ca\textsuperscript{2+} and Sr\textsuperscript{2+}-activation characteristics of fast-twitch (type II) fibres from the circa 70 years old group. There were no type II (fast-twitch) fibres sampled from the muscle biopsies of women older than 80 yrs. The fast-twitch fibres from the circa 70 years old women have significantly higher Sr\textsuperscript{2+} and Ca\textsuperscript{2+} thresholds for contraction (lower pSr\textsubscript{10}, pCa\textsubscript{10} values), lower sensitivity to Sr\textsuperscript{2+} (lower pSr\textsubscript{50} values), larger differential sensitivity (pCa\textsubscript{50}−pSr\textsubscript{50}) and higher Hill coefficients (nCa, nSr) than the slow-twitch fibres from the circa 70 years old women. The sensitivity to Ca\textsuperscript{2+} and Sr\textsuperscript{2+} of fast-twitch (type II) fibres sampled from the circa 70 years old women are higher than in fast-twitch (type II) fibres sampled from young adult males (Fink et al. 1990).
Hybrid fibres (type I/II)

The hybrid fibres were distinguished by their biphasic Sr²⁺-activation curve as mentioned in the Methods section. Representative Sr²⁺- and Ca²⁺-activation curves of hybrid fibres are shown in Fig. 3. All fibres identified as hybrid in this study were found in the younger cohort of elderly women (circa 70 years). No such fibres were sampled from the biopsies of women older than 80 years. As shown by O’Connell et al. (2004), the ratio between the amplitudes α and β of the two phases reflects the percentage ratio between slow and fast TnC isoforms (≈ ratio of MHC I and MHC II isoforms ≈ ratio type I/type II ≈ ratio slow-twitch/fast-twitch) expressed in the respective fibre. The percentage ratio of type I/type II myofibrillar components in the hybrid fibres identified in this study varied between 10%/90% and 50%/50%.

Maximum Ca²⁺ activated specific force

Slow-twitch (type I) fibres

The average value for the maximum Ca²⁺ activated specific force was lower in type I fibres from older women (> 80 years) (18.3 ± 8.4 N/cm², n = 4) than from the women under 70 years of age (26.7 ± 8.0 N/cm², n = 4), but statistically, the results are not significantly different.

Fast-twitch (type II) fibres

There was no statistically significant difference between maximum Ca²⁺ activated specific force in fast-twitch (type 2) skinned muscle fibres from the circa 70 year old women (23.1 ± 5.7 N/cm², n = 4) and the slow-twitch (type I) fibres from either group of women.
Hybrid fibres (type I/II)

The maximum Ca²⁺ activated specific force in hybrid muscle fibres, found only in the circa 70 years old group of women, varied between 16.3 to 40.0 N/cm² and was not statistically different from the values of either the slow- or the fast-twitch fibres in the two groups of women.

Pooled force data from all fibres

As detailed in the “Discussion”, the maximum Ca²⁺ activated specific force in the pooled data from this study was significantly lower by about 20% than the pooled data for young adults from a study from the same laboratory using similar solutions.

Fig. 4 Representative tracings of force oscillations of myofibrillar origin in: A Slow-twitch skinned muscle fibre from a 72-year-old woman (regular force oscillations 0.11 Hz). B Slow-twitch skinned muscle fibre from a 90-year-old woman (regular force oscillations 0.22 Hz). C Slow-twitch skinned muscle fibre from a 90 a woman (irregular force oscillation 0.11 Hz). D Fast-twitch skinned muscle fibre from a 72-year-old woman (regular force oscillations 0.33 Hz). E Fast-twitch skinned muscle fibre from a 72-year-old woman (irregular force oscillations 0.10 Hz). F Hybrid (type I/II) skinned muscle fibre from a 66-year-old woman (irregular force oscillations, 0.22 Hz). Horizontal scale bar = 20 s in all panels

Table 4 Frequency (Hz) of force oscillations of myofibrillar origin (SPOC) in slow-twitch (type I), fast-twitch (type II) and hybrid (type I/II) skinned muscle fibres

| Age               | Slow-twitch     | Fast-twitch     | Hybrid          |
|-------------------|-----------------|-----------------|-----------------|
| c. 70 year women  | 0.15±0.01 (11)  | 0.21±0.07* (3)  | 0.17±0.03 (3)   |
| >80 year women    | 0.19±0.06 (4)   | No fast-twitch fibres | No hybrid fibres |
| Adults            | 0.15±0.01 (7)   | 0.5±0.07* (7)   | 0.18 and 0.23   |

Mean± standard error of the mean (n = force traces showing SPOC)
*Significant difference (P≤0.02) between frequency of SPOC in fast-twitch (type II) skinned muscle fibres from circa 70 years old women and those from young adults (Fink et al. 1990)
Frequency of force oscillations of myofibrillar origin (SPOC)

Slow-twitch (type I) fibres

Figure 4 shows representative force responses in submaximally activated fibres where the SPOC phenomenon was observed and the results are summarized in Table 4. In slow-twitch (type I) skinned muscle fibres the frequency of SPOC was not significantly different between the two age groups of elderly women. The SPOC phenomenon was very regular in the slow-twitch (type I) fibres from the circa 70 year old women, but showed irregularities in some slow-twitch fibres from women > 80 years (Fig. 4B, C).

Fast-twitch (type II) fibres

The SPOC phenomenon was observed in a small number of fast-twitch (type II) skinned muscle fibres from elderly women, circa 70 years old. As shown in Table 4 and Fig. 4D, the force oscillations in these fibres were not always regular and had frequencies that were not significantly different from those in slow-twitch (type I fibres).

Discussion

The results obtained in this study show that with advancing age, in women, there are notable changes in the vastus lateralis muscle viewed from the perspective of fibre type ratio and single fibre contractile properties. Based on their functional properties with respect to activation by Sr\(^{2+}\) the fibres could be grouped into three distinct categories: slow-twitch (type I), fast-twitch (type II) and hybrid (type I/II) fibres. All three fibre types were present in the biopsies of women of circa 70 years of age, but no fast-twitch or hybrid fibre was sampled from biopsies of women older than 80 years (> 80 years). This may be a consequence of taking one small sample from a specific region in the women of advanced age, where there are slow motor units containing large numbers of slow-twitch muscle fibres all present in one area rather than being distributed throughout the muscle as is the case with younger subjects (Piasecki et al. 2016). This is because slow motor units in people of advanced age reinervate adjacent denervated fast-twitch muscle fibres and transform them into slow-twitch fibres (Piasecki et al. 2016).

Hybrid fibres may represent fibres in different states of transformation/degeneration/regeneration (Begam ans Roche 2018; Matsuura et al. 2007). The decline in the population of fast-twitch fibres in older animals and humans is possibly due to their increased susceptibility to damage (Aniansson et al. 1980; Larsson et al. 1978; Lexell et al. 1988; Lexell 1995, Chan and Head 2010) and also due to preferential loss of fast motor units as a consequence of the loss of fast motor neurons during advanced ageing (Piasecki et al. 2016).

Moreover, it appears that there are distinct functional differences between fibres of the same type in women of advancing age and young adults. For example, a study on the vastus lateralis muscle from young adults (16 males, seven females, age 24 ± 5 years) (Lamboley et al. 2013) using the same activating solutions and equipment as in the present study, and a similar system for fibre type classification, showed that the pCa\(_{50}\) values for slow-twitch (type I) and fast-twitch (type II) fibres from young adults were 5.93 ± 0.01 (n = 40) and 5.83 ± 0.01 (n = 30) respectively. In comparison, the slow- and fast-twitch fibres from the vastus lateralis muscle of elderly women (both our groups circa 70 years of age and > 80yrs) had significantly greater pCa\(_{50}\) values (6.09 ± 0.03, n = 13 for slow-twitch fibres and 6.05 ± 0.02, n = 10 for fast-twitch fibres). On average, the results show that muscle fibres from elderly women (circa 70 years of age and > 80yrs) produce 50% of their maximum Ca\(^{2+}\)-activated force at Ca\(^{2+}\) concentrations that are lower by a factor of 1.4 for slow-twitch fibres and 1.7 for fast-twitch fibres compared with fast- and slow-twitch fibres biopsied from the vastus lateralis muscle of young adults (Lamboley et al. 2013). The Ca\(^{2+}\)-activation curves of both slow- and fast-twitch fibres also appear to be significantly less steep in fibres from elderly women (our group combined circa 70 years of age and > 80yrs) than in fibres from young adults of both sexes (mean 24 years) (Lamboley et al. 2013) as indicated by the smaller Hill-coefficients in the elderly women (n\(_{Ca} = 2.11\) (old female) vs 4.4 (young adults both sexes pooled) for slow-twitch and 3.38 (old female) vs 5.3 (young adult both sexes pooled) (for fast-twitch fibres). Lamboley et al. (2013) pooled their young adult data from the different sexes so we cannot comment on sex differences in their data.

The percentage of hybrid fibres (type I/II) appears to be several-fold higher in the circa 70 years of age group of elderly women (24%) than in muscle fibres sampled from the vastus lateralis muscle of young adults (<5%, Lamboley et al. 2013; <10% Fink et al. 1990). Care must be taken not to give this observation undue weight because this difference could be due to our small sample size and the possibility of sampling biases in the selection of muscle tissue to be biopsied. Nevertheless it is interesting to note that it does provide some further support for the proposal that muscle remodelling is occurring somewhere around the seventh decade of life in women. A proportion of this remodelling is likely a consequence of the preferential loss of fast twitch-motor neurons with advancing age (Lexell 1995; Lexell and Taylor 1991). It should also be noted that the seven women in our study were all undergoing surgery for hip problems which may also be responsible for a degree of muscle remodelling.

The average maximum Ca\(^{2+}\)-activated specific force expressed per cross sectional area of the skinned fibre before
swelling, measured in this study, was about 20% lower in the pooled (our group combined circa 70 years of age and > 80 years) fibres from all elderly women (24.3 N/cm²) compared with the average value for adult fibres from the vastus lateralis (30.0 N/cm²) obtained in a previous study from the same laboratory using similar procedures and solutions, but where the solutions contained, in addition, 10 mM caffeine (Fink et al. 1990). In a review on measuring specific force in human single chemically skinned muscle fibres Kalakoutis et al. (2021) observed a five-fold difference in mean specific force data reported from different laboratories. Importantly, for the comparison we are making here with earlier studies from our laboratory using the same solutions and equipment (Fink et al. 1990; Lamboley et al. 2013) Kalakoutis et al. (2021) observed a consistent specific force reported for research groups from the same laboratory using similar technique and solutions.

Note that when the force measurements were made at 15 °C (Trappe et al. 2003) no difference was observed between the maximum Ca²⁺ activated specific force in chemically skinned fibres of the same type from young and elderly women. However, Trappe et al. (2003) found that under their conditions, the maximum specific force was about 40% greater in fast-twitch than in slow-twitch fibres. Considering the decline in the number of fast-twitch fibres in the vastus lateralis muscle of elderly women, such a difference would translate to lower overall forces per cross-sectional area of the vastus lateralis in elderly women.

The spontaneous oscillatory contractions, or force oscillations that originate from the interaction of myofibrillar proteins (SPOC, see Fig. 4 and Table 4) when skinned fibres are submaximally activated are characterised by the frequency of the oscillations, which is fibre type specific, and their regularity (Smith and Stephenson 1994). The presence of these oscillations is an indication of the health of the single fibres obtained from the human biopsy demonstrating that the contractile proteins have not deteriorated either in the sampling process or for the six hours when they were used in the laboratory. The SPOC are a function of the contractile protein as all the membrane systems have either been mechanically removed or chemically removed by the high EGTA relaxing solution. The frequency of SPOC in fast-twitch (type II) skinned muscle fibres from circa 70 year old women was significantly different, approximately half of that in the equivalent fibre type from young adults (Fink et al. 1990). In general, SPOC in the fast-twitch (type II) fibres of the elderly women was more similar to SPOC observed in the hybrid (type I/II) fibres from young adults than in the fast-twitch (type II) fibres of young adults (Fink et al. 1990), further suggesting that the contractile proteins of the fast-twitch (type II) muscle fibres may have been altered in some way by the aging process. Miller et al. (2013) also showed that aging slows myosin actin cross-bridge kinetics in women, leading to decrements in whole body dynamic contractile performance.

Taken together the results described in this paper show that with advancing age, in women, there are changes in the activation properties of the contractile apparatus of muscle fibres from the vastus lateralis muscle. There is also an increase in the number of slow-twitch fibres and hybrid fibres and a decrease in fast-twitch fibres. These observed changes occur on the background of a robust capacity of old and senescent muscle to regenerate functional architecture (Lee et al. 2013) and need to be taken into consideration when explaining observed changes in women’s mobility with aging.

Author contributions DGS, SIH and SMR conceived the study. SMR collected biopsies and performed the experiments. SIH, SMR and DGS wrote the main manuscript text and SH and SR prepared all figures and tables. All authors reviewed the manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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