Editorial

What is the mechanism for in vivo loss of skeletal muscle function in elderly women?

With rapid ageing of the global human population, a deeper understanding of the molecular changes within old myofibres and the benefits of regular exercise is of increasing importance for the design of interventions to help maintain healthy muscle function well into old age. In this issue of Acta Physiologica, the paper by Venturelli et al. (2015) provides comprehensive old human data that are very valuable to the field of gerontology, specifically to the age-related loss of skeletal muscle mass and function, known as sarcopenia. This paper addresses the impact of advanced age, as compared with disuse, on sarcopenia, by measuring the function of muscles in vivo and isolated myofibres in vitro for both upper and lower limbs in groups of very old women aged approx. 87 years, who were either mobile or non-ambulatory (in wheelchairs for about 2 years with relative disuse of leg muscles), compared with healthy young females aged approx. 25 years (n = 8 women per group). Studies on such very old human muscles, especially using frail non-ambulatory subjects, are rare because few willing subjects are available, the muscle biopsy is invasive and there are logistical and ethical complications; many other studies use humans aged around 70 years.

The in vivo data indicate that muscle mass is relatively unaffected by age for the upper limbs, compared with the lower limbs, and that disuse accelerates the loss of muscle mass in the legs (Venturelli et al. 2015). There is discordance between their measurements of muscle function in vitro (no changes) and in vivo (striking loss of absolute strength with age, more pronounced in lower limbs and exacerbated by disuse). The authors conclude that while intrinsic contractile properties of myofibres in vitro may be relatively unaffected by age, the in vivo contractile function is compromised by adverse changes in the myofibre environment and tissue architecture, including progressive denervation of myofibres (probably exacerbated by disuse) and likely other changes including extracellular matrix (ECM) composition.

However, other studies of isolated old human myofibres in vitro using additional measurements indicate that while absolute force (measured at maximal Ca²⁺ concentrations) may be similar between young and old myofibres, the kinetics of contraction and force generation at lower Ca²⁺ concentrations may differ: this may be especially relevant under physiological conditions and compromise contraction capacity of old muscles in vivo. Comment is made below on this aspect, along with other factors that influence such measurements of muscle function, the impact of disuse and exercise, the molecular mechanisms that cause sarcopenia and feasible interventions.

In vitro vs. in vivo measurements

A muscle biopsy provides the opportunity to analyse the function of individual myofibres (along with cell and molecular characterization), and this is especially useful where the fibre type of each myofibre studied is identified (e.g. fast type II or slow type I contractile and molecular properties). Such information is of increasing interest for ageing studies of humans. The paper by the Italian group (Venturelli et al. 2015) analysed maximum specific tension (Po) as a measure of function for segments of isolated human myofibres in vitro, but found no differences in the intrinsic contractile properties of muscles in all groups. This is intriguing. Studies of immobilized old human muscles using both in vitro and in vivo measurements of muscle function have been also conducted by the group in Copenhagen (Hvid et al. 2011, discussed by Head 2011), but there are many important differences. The studies of Hvid et al. (2011) used short-term immobilization (up to 2 weeks using lower limb cast immobilization) for healthy mobile males aged about 67 years of age, sometimes classified as ‘young ageing’. These males were about 20 years younger than the approx. 87-year-old females (Venturelli et al. 2015), and many secondary consequences can occur over two decades of ageing. Regardless, it is interesting that the Copenhagen group found no effect of age on maximum Ca²⁺-activated force in single myofibres (Head 2011, Hvid et al. 2011) in agreement with observations for the much older muscles in the present paper (Venturelli et al. 2015, see * in Fig. 1).

The Copenhagen study (using 67-year-old male muscles) also measured Ca²⁺ sensitivity (this was not
carried out by Venturelli et al. (2015) and showed that immobilization induced changes in this parameter that were dependent on age and affected by myofibre type (Hvid et al. 2011). Similar effects of age on contractile response to a range of Ca$^{2+}$ concentrations were reported by a recent Australian paper (Lamboley et al. 2015). Using isolated myofibres from leg muscles of old (approx. 70 years) and young (approx. 22 years) men and women, this study examined the stored intracellular sarcoplasmic reticulum (SR) Ca$^{2+}$ content as well as contractile properties and found reduced Ca$^{2+}$ sensitivity in type II myofibres as well as less releasable SR Ca$^{2+}$ in both myofibre types (Lamboley et al. 2015): see different force–pCa curves in Figure 1. Their observation of reduced maximum force in old type II myofibres appears to contrast with both of the other studies mentioned (Hvid et al. 2011, Venturelli et al. 2015). A number of important distinctions can be made between these studies including the temperatures at which skinned myofibre measurements were made (approx. 22–23 °C for Hvid et al. 2011 and Lamboley et al. 2015, but much lower at 12 °C for Venturelli et al. 2015); myofibre-type classification (Lamboley and Hvid report data for type I and Ila fibres, whereas Venturelli et al. (2015) report data for type I, Ila and Ila-x fibres); and low numbers of Ila myofibres, $n = 4–6$ (Venturelli et al. 2015). These combined factors could contribute to the different conclusions. Thus, it appears that depending on the parameter measured and experimental conditions used, some age-related changes are evident in old isolated myofibre in vitro.

As mentioned, the Italian study (Venturelli et al. 2015) did not find any age-related changes in the property of single fibre maximum specific tension (Po) for the very old myofibres, in contrast to the striking loss of force generation by the same old muscles in situ, emphasizing that the nature of these measurements can influence the conclusions gained. The loss of muscle function in vivo, despite the preservation of force producing capacity in individual myofibres, reflects the inability to maximally activate the muscle. Using the twitch interpolation technique, Venturelli et al. (2015) showed that old, immobile women could only achieve about 75% of maximum force production voluntarily, as opposed to 95% in the young. While this inability to maximally activate the muscle likely reflects an impaired neural drive, it is important to consider the implications of this on force production at the level of individual myofibres (Fig. 1). While it may not be possible to directly relate the level of voluntary activation of the whole muscle group to an individual myofibre, at submaximal activation levels, force production by myofilaments is highly sensitive to intracellular Ca$^{2+}$, demonstrating that even subtle changes in Ca$^{2+}$ availability can result in large changes in force production.

Therefore, although individual myofibres from very old, immobile women do have the capacity to produce the same force output as young myofibres when fully saturated with calcium in vitro, age-related decreases in motor unit firing rates, SR Ca$^{2+}$ release and/or reduced myofilament calcium sensitivity may all impact on the in vivo force production and consequently muscle function. That these changes to myofilament function appear to be fibre-type specific emphasizes the importance of identifying individual myofibre types and measuring calcium sensitivity when analysing individual isolated myofibres.

The in vitro examination of myofibres isolated from their natural environment has limitations. For a start, one assumption is that these myofibres are representative of all myofibres in the whole intact muscle, yet this is clearly not the case. As discussed by Venturelli et al. (2015), the selection of isolated myofibres for analysis ‘tends to result in the very thin fibres being discarded’. One also needs to consider the fact that branched myofibres (that affect muscle function) are increasingly common in old muscles (Pichavant & Pavlath 2014), and these branched myofibres may also be under-represented (due to technical issues) in the population of isolated myofibres extracted and plated out for in vitro analyses. As emphasized by Venturelli et al. (2015), both innervation and the ECM play critical roles in muscle contraction in vivo, and both are well documented to alter with age, yet neither is fully represented in the in vitro situation.

As mentioned, the Italian study (Venturelli et al. 2015) did not find any age-related changes in the property of single fibre maximum specific tension (Po) for the very old myofibres, in contrast to the striking loss of force generation by the same old muscles in situ, emphasizing that the nature of these measurements can influence the conclusions gained. The loss of muscle function in vivo, despite the preservation of force producing capacity in individual myofibres, reflects the inability to maximally activate the muscle.
The impact of altered oxidative stress must also be considered, because cells in vitro may be exposed to hyperoxic conditions without adequate antioxidant defences, compared with cells in vivo (Lui et al. 2010). Increased oxidative stress can affect protein function by reversible oxidative modification of cysteine residues, or irreversible oxidative damage to macromolecules, and this may obscure differences between young and old myofibres in vitro. The influence of variable levels of reversible oxidative stress on skinned myofibres in vitro was investigated by Lamboley et al. (2015) using the antioxidant dithiothreitol: this had no effect on the specific force of young or old myofibres, but slightly altered the Ca^{2+} sensitivity in type II myofibres from both age groups.

The fidelity of myofibre types throughout ageing is also questioned, because what the myosin isoform identification may reveal about the history of an individual myofibre may not be straightforward, as elegantly discussed by Purves-Smith et al. (2014). While it is widely stated that fast myofibres are preferentially lost with ageing, this is disputed; indeed, Venturelli et al. (2015) reported an increased proportion of MHC-IIx fibres in both upper and lower limbs of very old females. This situation is complicated by the criteria used to distinguish fast- and slow-contracting myofibres; for example, histochemical measurements do not always relate closely to the myosin isoforms and, importantly, there is switching between myosin isoform expression as muscles age, so that the original fibre type and history of an individual myofibre is obscured (Purves-Smith et al. 2014). These and other concerns challenge the broad generalization that fast myofibres are preferentially lost in vivo as muscles age.

In vivo estimates of maximum specific force are complicated, for example by indirect measures of muscle force based on joint torque measurements that are influenced by joint mechanics and the complex architecture and innervation of multiple muscle groups. By combining in vivo measures of maximum voluntary contraction and resting twitch force, and relating these to physiological cross-sectional area measured by magnetic resonance imaging, Venturelli et al. (2015) revealed an age-related decline in voluntary maximum specific force, but not for specific twitch force (in response to ‘involuntary’ electrical stimulation), in either upper or lower limb muscles. Thus, impaired voluntary muscle activation and reduced muscle mass contributed to the loss in absolute force and in vivo muscle function with ageing and disuse.

With respect to the in vivo situation, the diverse muscles in the human body differ dramatically in their architecture, size, the relative composition of fast and slow myofibres, and the frequency and nature of mechanical loading and contraction. In rodents, there is a wide variation in the extent of sarcopenia between muscles (Ibebunjo et al. 2013, Softe et al. 2015) and this can be further influenced by gender. It is important to note that quadrupedal rodents have similar loading on muscles of both hind and forelimbs, compared with bipedal humans where the forelimbs are relatively unloaded (although provide additional essential functions). It is unclear whether the greater loss of muscle mass of some muscles, for example as is well documented for the human hind limbs, is because these are distal (relative to proximal muscles in forelimb) or is due to greater mechanical loading.

**Denervation, metabolism and the benefits of exercise**

Due to the limitations of invasive sampling of ageing human muscles, the use of animal models (with relative ease of sampling) enables investigations into the time course of molecular changes related to the onset and progression of sarcopenia, combined with many experimental variables. Time course studies of age-related molecular changes in muscles of rodents using microarray and proteomic analyses emphasize major alterations in muscle innervation and metabolism, as well as alterations in the ECM (Ibebunjo et al. 2013, Barns et al. 2014). Metabolism regulates the rates of protein synthesis and degradation, and thus, the net amount of contractile proteins that affect muscle mass and function. In older humans, the complex interactions between these age-related intrinsic molecular changes and the impact of nutrition and exercise are of much interest and remain to be fully elucidated (Denison et al. 2015). Some key questions are as follows: What type of exercise and frequency (perhaps combined with nutritional supplementation) are sufficient to prevent sarcopenia and, especially, to what extent can late-onset exercise reverse sarcopenia and frailty? It is of particular interest to understand the molecular basis for the benefits of exercise, with respect to all components of muscle tissue (including myofibres, nerves, blood vessels and ECM) combined with systemic effects, because such insight provides the basis for potential drug and other interventions to reduce, and ideally reverse, sarcopenia.

There is a wide agreement on the benefits of exercise for all aspects of human health, and this includes the maintenance of old muscle function and the prevention of sarcopenia. The important comparison of the consequences of disuse of muscles in the lower limbs of very old, 87-year-old, immobile and active mobile women by Venturelli et al. (2015) emphasizes the need for loading and exercise to maintain the muscle mass and function. For comparison, the muscles of the upper...
limbs with ‘similar usage’, in the immobile and mobile old women, were relatively well maintained in both groups. The central issue in this paper is that the \textit{in vitro} intrinsic production of contractile force by all old and young myofibres was similar. However, there is a need for the impact of Ca$^{2+}$ dysregulation and levels of oxidative stress on myofibre function to also be considered for such \textit{in vitro} studies. The \textit{in vivo} loss of absolute muscle contraction (strength) in the old women, that was pronounced by disuse, is probably due largely to age-related alterations in other components of the muscle tissue, such as the innervation of the muscles and composition of the ECM that are essential for generation of myofibre contraction and force.

\textbf{Conflict of interest}

There is no conflict of interest.

\textit{M. D. Grounds and G. J. Pinniger}

School of Anatomy, Physiology and Human Biology, The University of Western Australia, Perth, WA, Australia

E-mail: miranda.grounds@uwa.edu.au

\textbf{References}


Head, S.I. 2011. Old men still have the skeletal muscle contractile function to get up and go even after they have had their leg in a cast. \textit{J Physiol} 589, 4639.


