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Age-related remodelling of the myotendinous junction in the mouse soleus muscle

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ABSTRACT

The age-related loss of muscle mass and function predominantly affect muscles of the lower limbs and have largely been associated with decline in muscle fibre size and number, although the exact mechanisms underlying these losses are poorly understood. In addition, consistent reports that the loss of muscle strength exceeds that which can be explained by declines in muscle mass has widened the search for causes of sarcopenia to include supporting tissues such as the extracellular matrix and tendons. Although the changes to both muscle and tendon with age are well characterised, little work has focused on the interface between these two tissues, the myotendinous junction (MTJ). Given the crucial role for this structure in force transfer between muscle and tendon, we asked whether the myotendinous junction underwent structural changes with age in lower limb muscle. We used whole muscle to assess gross muscle and tendon morphology, and immunohistochemistry to determine fibre and MTJ profile number in young (6 months), middle aged (18 months) and elderly (24 months) C57BL/6 female mice. MTJ length was quantified using serial cross sections of the soleus muscle. We found an apparent 3.5-fold increase in MTJ profiles per cross section with no increase in fibre number in old mice, and found this to be a result of a doubling in length of the MTJ region with age. This coincided with an increase in proximal tendon length (31%), as well as an increase in collagen deposition between 6 and 24-months of age consistent with an expansion of the fibre termination area. These findings uncover a previously undescribed effect of ageing on the MTJ and open up new lines of investigation into the role of this structure in the age-related loss of muscle function.

1. Introduction

Sarcopenia, the age-related loss of muscle mass and function (Baumgartner et al., 1998), results from age-related changes to the muscle itself, such as fibre loss (Rowe, 1969; Lexell et al., 1988; Sheard and Anderson, 2012) and fibre atrophy (Scelsi et al., 1980; Rowan et al., 2012) thought to be caused by age-related denervation (Oda, 1984; Cardasis and LaFontaine, 1987; Chai et al., 2011) and/or disruption to the protein synthesis-degradation balance leading to a net loss of protein (Furuno et al., 1990; Clavel et al., 2006; Samengo et al., 2012). In addition, changes to the surrounding environment and muscle supporting tissues include changes to the extracellular matrix (Kovanen et al., 1987; Mohan and Radha, 1980) as well as compromised force delivery caused by age-related changes to the tendon. The ageing tendon has been shown to undergo conformational changes leading to increased cross-sectional area (Magnusson et al., 2003) and altered mechanical properties (Kubo et al., 2003; Li et al., 2013). While the effects of ageing on muscle and tendon individually have been well characterised, little work has focused on the association between these two ageing tissues.

Myotendinous junctions (MTJs) make up the interface between muscle fibre and tendon and represent the main site of force transfer between these two tissues upon muscle fibre contraction (Tidball, 1991). As the efficiency of force transmission relies on area of contact between muscle and tendon, the muscle membrane is highly folded at the MTJ (Gelber et al., 1960; Korneliussen, 1973), increasing surface area and thereby area of contact between muscle and tendon (Fig. 1). In addition, the high levels of mechanical stress at the MTJ increase the risk of membrane rupture, which is counteracted by an elevated expression of structural proteins such as dystrophin, integrins, and laminin at these sites compared with non-junctional sarcolemma (Tidball and Law, 1991; Paul et al., 2002).

The MTJ is also characterised by presence of Acetylcholine esterase (AChE), the function of which at this site is largely unknown (Young et al., 2000). Because MTJs are exposed to high levels of mechanical stress they are common sites of injury (Nikolaou et al., 1987). For this reason, MTJs are highly plastic and undergo remodelling in response to changes in muscle loading by denervation (Tidball and Quan, 1992a),

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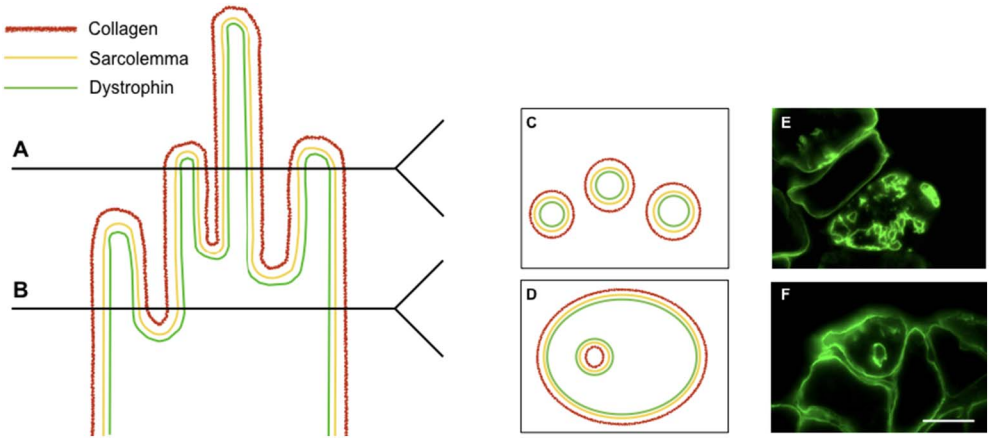

Fig. 1. Schematic diagram of the myotendinous junction. A side view of the MTJ region of a muscle fibre (left) with extensive invagination of the sarcolemma (yellow). Dystrophin (green) and collagen (red) are both closely associated with the sarcolemma. When a cross section (line A) is taken close to the MTJ termination, the fibre appears as several separate small diameter profiles, each positive for dystrophin inside and collagen outside (C). If a cross section is taken further from the fibre termination (line B), the fibre appears with a normal outer profile, although one invagination is visible in cross section. Because the profile is an invagination, dystrophin now sits on the outer perimeter of the profile and collagen lines the "inside" of the sectioned profile (D). E) Transverse section from mouse muscle immuno-stained for dystrophin showing multiple small profiles when close to the fibre termination, and with a relatively normal circular profile featuring a single invagination when sectioned further from the fibre termination (F). Scale bar 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

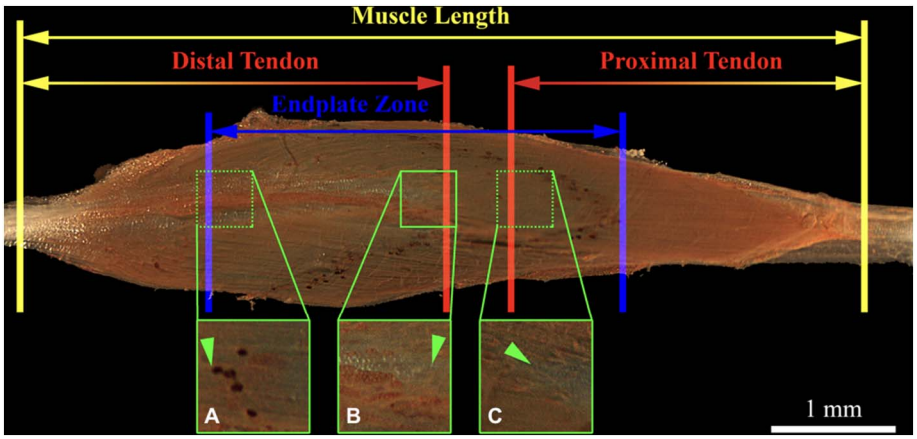

Fig. 2. Whole 24-month AChE stained soleus muscle from anterior lateral perspective. Yellow lines indicate point of muscle distal and proximal termination. Red lines indicate distal and proximal tendon terminations. Inset A indicates AChE positive neuromuscular junctions in the innervation zone, which extends far beyond the muscle belly, the region commonly cited as containing all neuromuscular junctions. Blue lines indicate innervation zone. Insets B and C indicate point of distal and proximal tendon terminations respectively. Dotted lines around insets indicate that these are from the opposite face of the muscle and thus not visible from this view. Scale bar 1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

immobilization (Kim et al., 2007), spaceflight (Roffino et al., 2006) and exercise (Curzi et al., 2013). However, despite the excellent descriptions of MTJ ultrastructure in response to reduced or increased physical activity, no study to date has investigated whether the MTJ undergoes any age-related changes.

In the 2015 paper from our group (Lal and Sheard, 2015), we described a subset of fibres with distinctive morphology. These fibres presented with what appeared to be dystrophin encircled vacuoles in the intracellular space, and were for this reason termed 'DEVILs'. Although DEVILs were then thought to represent degenerating fibres, it was also considered that these profiles might represent MTJs. This was consistent with their location along the tendinous region of the soleus muscle as well as the dystrophin positive structures always appearing near the fibre terminations. Taken together, these characteristics made it possible that the dystrophin structures represented sarcolemmal invaginations, which are a well described feature of MTJs (Fig. 1). However, if representing MTJs, the increased frequency with which these profiles were reportedly observed in old animals remained unexplained, since the total number of fibre terminations should not increase with age unless total fibre number also increased accordingly.

In this study, we therefore sought first to determine whether the fibre morphology previously thought to represent dystrophin-encircled intracellular vacuoles actually represents sarcolemmal invaginations representative of MTJs. We then sought to understand the apparent age-related increase in these features by determining if any structural change to the MTJ could account for the increase in their frequency of observation. Finally, since the MTJ is the muscle-tendon interface, we wanted to determine if a relationship existed between MTJ remodelling and changes to tendon gross morphology with age.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the University of Otago animal ethics committee. Young (6-months), middle aged (18-months) and elderly (24-months) C57BL/6 female mice were used. Food and water were provided *ad libitum* and animals were kept on a 12:12 light/dark cycle. Animals were deeply anesthetized with an intraperitoneal injection of pentobarbital (30 mg/kg), after which they were euthanized by transcardiac perfusion with heparinized phosphate buffered saline followed by fixation-perfusion with 1% (immunohistochemistry) or 2% (tendon measurements) paraformaldehyde in 0.1 M phosphate buffer. Ankles were held at 90° to ensure the muscles were fixed at normal optimal resting length during the perfusion.

2.2. Tissue processing

After euthanasia and fixation soleus muscles were carefully removed from the hind limbs. Tissues to be used for cryosectioning and immunohistochemical staining were submerged and oriented in small aluminium foil boats filled with OCT frozen tissue embedding compound and snap-frozen in liquid nitrogen-cooled isopentane and subsequently stored at −80 °C. Tissues to be used for tendon measurements were stored in Phosphate Buffered Saline (PBS) at 4 °C until needed for imaging after which they were snap-frozen as described above and stored for further analysis.