© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 276-284 doi:10.1242/jeb.128017

REVIEW

Muscle structural assembly and functional consequences

Marco Narici[1,*], Martino Franchi[1] and Constantinos Maganaris[2]

ABSTRACT

The relationship between muscle structure and function has been a matter of investigation since the Renaissance period. Extensive use of anatomical dissections and the introduction of the scientific method enabled early scholars to lay the foundations of muscle physiology and biomechanics. Progression of knowledge in these disciplines led to the current understanding that muscle architecture, together with muscle fibre contractile properties, has a major influence on muscle mechanical properties. Recently, advances in laser diffraction, optical microendoscopy and ultrasonography have enabled *in vivo* investigations into the behaviour of human muscle fascicles and sarcomeres with varying joint angle and muscle contraction intensity. With these technologies it has become possible to identify the length region over which fascicles and sarcomeres develop maximum isometric force *in vivo* as well as the operating ranges of fascicles and sarcomeres during real-life activities such as walking. Also, greater insights into the remodelling of muscle architecture in response to overloading and unloading, and in ageing, have been obtained by the use of ultrasonography; these have led to the identification of clinical biomarkers of disuse atrophy and sarcopenia. Recent evidence also shows that the pattern of muscle hypertrophy in response to chronic loading is contraction-mode dependent (eccentric versus concentric), as similar gains in muscle mass, but through differing addition of sarcomeres in series and in parallel (as indirectly inferred from changes in fascicle length and pennation angle), have been found. These innovative observations prompted a new set of investigations into the molecular mechanisms regulating this contraction-specific muscle growth.

KEY WORDS: Skeletal muscle, Hypertrophy, Atrophy, Sarcopenia, Muscle contraction

Introduction

The relationship between muscle structure and function has been a matter of interest to anatomists and physiologists since the Renaissance. This period represented a new era for medical science as the fine details of the human body were revealed through the use of anatomical dissections. This enabled great advancement in medical knowledge as, before this period, understanding of human anatomy was based on Galen's dissection work on animals (Barbary macaques), as human dissections were forbidden in ancient Rome. Many of Galen's assumptions on the anatomy of the human body were proved wrong by Andreas Vesalius, one of the greatest contemporary scholars of the time, who shortly after obtaining his doctorate in 1537 was appointed Professor of Surgery and Anatomy at the University of Padua. Just 6 years after his appointment at Padua University, Vesalius published his treatise *De Humani Corporis Fabrica* (1543) in seven books (*Libri Septem*) (Fig. 1A). In his treatise, Vesalius gives a highly detailed description of each muscle of the human body, through a series of artistic illustrations of 'muscle men' (Fig. 1B), attributed to Titian's pupil Jan Stephen van Calcar. Vesalius' drawings and descriptions provided accurate anatomical details of muscle insertions, position and actions but not of the arrangement of muscle fibres because the technique he used of engraving on woodblocks followed by printing probably did not enable him to achieve sufficient accuracy to illustrate muscle fibres.

Such details were instead provided almost a century later (1627) by Casserius (Giulio Cesare Casseri; Fig. 2A), pupil of Hieronymus Fabricius (Girolamo Fabrici d'Acquapendente), through the use of a different drawing technique: engraving on copper plates. Working at the Universita d'Artista, at the time a branch of Padua University, Casserius was able to produce drawings based on his copper engravings that clearly showed the arrangement of muscle fibres (Fig. 2B) *in situ*.

Even greater morphological details of individual muscles were provided by the illustrations of the British architect Sir Christopher Wren, who is best known for having designed St Paul's Cathedral in London. In 1670 in Willis' treatise *De Motu Musculari*, Wren produced highly detailed anatomical drawings clearly illustrating the architectural features of different muscles of the body, differentiating between parallel-fibred, uni-pennate, bi-pennate and multi-pennate muscles (Fig. 3).

However, appreciation of the functional meaning of different muscle fibre arrangements seems to start only with the work of the Danish scientist Nicolaus Steno(nis) who, working under the court of the Duke of Florence, Ferdinand II de' Medici, published in 1667 the treatise *Elementorum Mythologiae Specimen* (Fig. 4). Steno used geometric models to represent muscles as parallelepiped integrations of fibres and was the first to describe changes in muscle architecture with muscle contraction as follows: '*dum contrabitur musculus, anguli eius acuti siunt ampliores*' (with muscle contraction, angles that are acute become greater) (Fig. 5). A contemporary of Steno, who was probably inspired by his work as well as by that of Galileo, was the Neapolitan mathematician, physicist and physiologist Giovanni Alfonso Borelli (1608–1679), often described as the father of biomechanics. In his *De Motu Animalium* (1680), he applied to biology the rigorous analytical methods introduced by Galileo in the field of mechanics. Borelli was the first to estimate the forces of fusiform and pennate muscles required for equilibrium in various joints and demonstrated that the levers of the musculoskeletal system magnify motion rather than force, requiring muscles to produce forces much greater than those opposing motion (Fig. 6).

However, the mathematical relationship between muscle dimensions and mechanical output was actually formalized two centuries later by the German physiologist Ernst H. Weber, in his

[1]University of Nottingham, Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, Faculty of Medicine and Health Sciences, MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, Derby Royal Hospital, Derby DE22 3DT, UK. [2]Research Institute for Sport and Exercise Sciences, Faculty of Science, Liverpool John Moores University, Liverpool L3 3AF, UK.

*Author for correspondence (marco.narici@nottingham.ac.uk)

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 276-284 doi:10.1242/jeb.128017

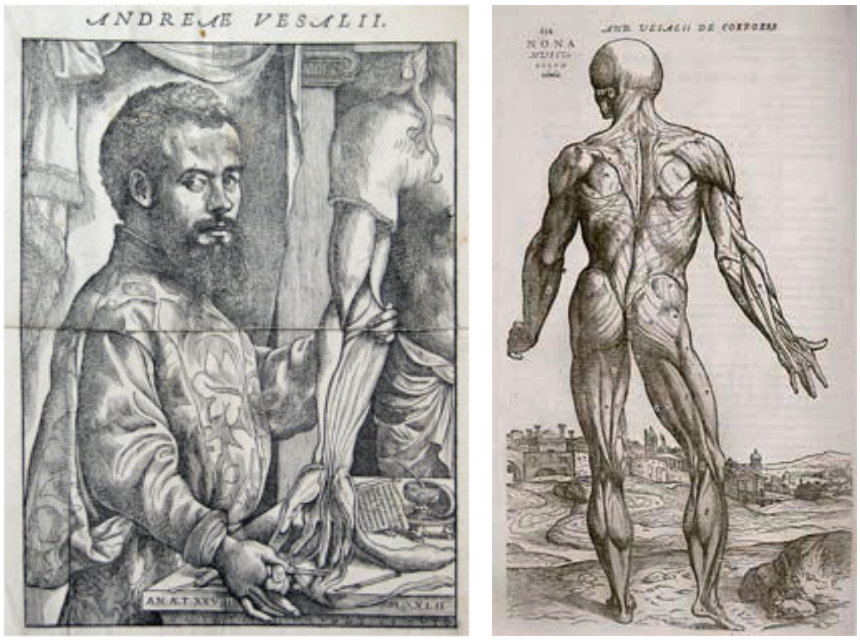

Fig. 1. *De Humani Corporis Fabrica*. (A) Portrait of the 28 year old Andreas Vasalius, taken from *De Humani Corporis Fabrica* (1543). (B) Illustration from this treatise showing details of the muscular system of the human body. Individual muscles are accurately represented though details of muscle fibre arrangement are missing. Reproduced as public domain images from: http://catalogue.museogalileo.it/biography/NielsSteensenNicolasSteno.html.

book titled Handwörterbuch der Physiologie (Weber, 1846), which reports one of the very first calculations (0.836 kg cm^{-2}) of human muscle maximum isometric force normalized to cross-sectional area (estimated as volume/muscle length in cadavers). In 1944, Haxton refined the estimate of force (**F**) per cross-sectional area (CSA) (**F**/CSA, specific force) of human skeletal muscle by correcting for moment arms, accounting for pennation, and by accurately measuring the physiological cross-sectional area (PCSA, i.e. the area of the cross-section of a muscle perpendicular to all its fibres) of the plantarflexor muscles obtained by inclusion of all fibres along the muscle length (Fig. 7). Although Haxton's measurement of PCSA in the cadaveric specimens of his study is correct, as it accounts for the cross-section of all muscle fibres, the PCSA of his living subjects was estimated by multiplying the anatomical cross-sectional area for the ratio of physiological to anatomical CSA (ACSA, which is orthogonal to the muscle belly but not to all muscle fibres of pennate muscles) measured in cadavers (Haxton, 1944). Despite this limitation, the real novelty of Haxton's study was that of relating the maximum force of a muscle to its PCSA; this ratio is now referred to as 'specific force', a quantity that represents the intrinsic force-generating potential of muscle independent of muscle size. As in the plantarflexors, PCSA is about 1.3-fold greater than ACSA; the average value of **F**/CSA obtained by Haxton (38.2 N cm^{-2}) is similar to that reported by Close (1972) for mammalian muscles (15–30 N cm^{-2}).

Although the pioneering work of these early scholars has been fundamental for identifying differences in muscle structural design and for relating this to the ability to generate force and movement, information on muscle structure in living humans could only be obtained through inference from anatomical dissections and from observations on animal muscle.

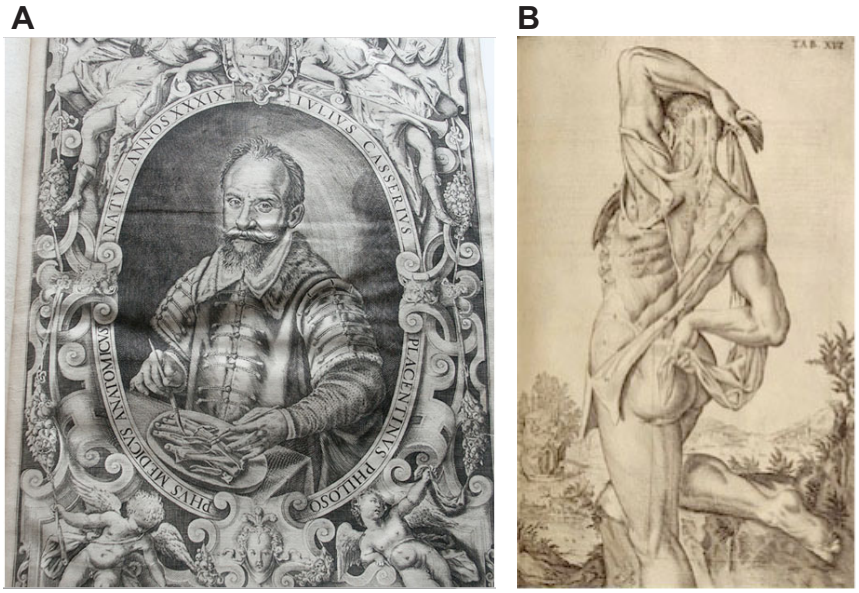

Fig. 2. *Tabulae Anatomicae*. (A) Portrait of Giulio Cesare Casseri. (B) Illustration of a copper engraving from Casseri's *Tabulae Anatomicae* showing the fine details of muscle anatomy. Contrary to Vesalius' drawings, the technique used by Casseri enabled illustration of muscle fibres and their orientation. Reproduced as public domain images from: https://commons.wikimedia.org/wiki/Category:Giulio_Cesare_Casseri#/media/File:Giulio_Casserio._Line_engraving_by_G._van_Veen._Wellcome_V0001024.jpg.

Journal of Experimental Biology (2016) 219, 276-284 doi:10.1242/jeb.128017

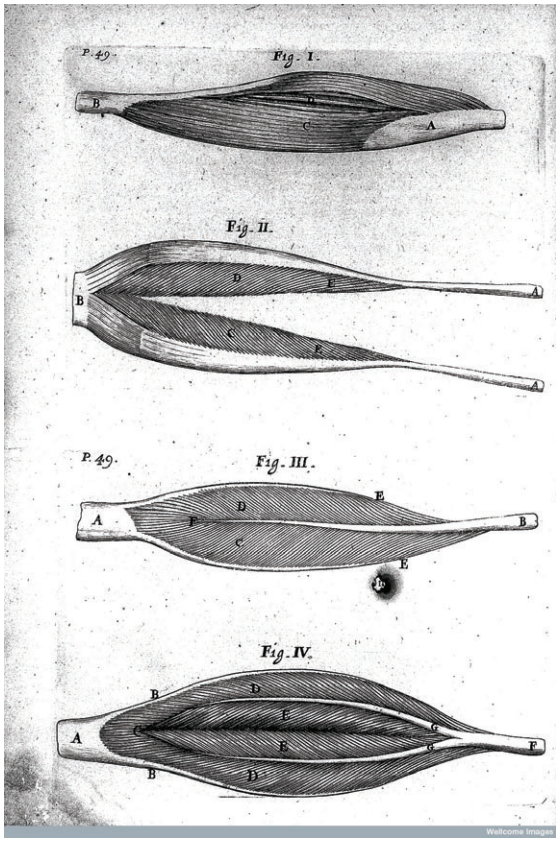

Fig. 3. Structure of muscle from an ox. This drawing is attributed to Christopher Wren in Willis' *De Motu Musculari* (1670; The Wellcome Institute for the History of Medicine, London). It is one of the first to illustrate uni-pennate, bi-pennate and multi-pennate muscle arrangements. Reprinted with permission of the Wellcome Library.

Only with the introduction of modern imaging techniques such as MRI and ultrasound it has become possible to measure in humans key parameters of muscle architecture (muscle volume, fascicle length and pennation angle), enabling us to obtain *in vivo* accurate values of PCSA and muscle-specific force (13–25 N cm^{-2}; Narici et al., 1992; Morse et al., 2005), in line with those reported by Close (1972) for non-human mammalian skeletal muscle. Also, recent advances in MRI diffusor tensor imaging (Fig. 8) have made it possible to obtain three-dimensional reconstructions of human and rodent muscle fascicle length and pennation angle (Sinha et al., 2006; Heemskerk et al., 2005), which are likely to yield more realistic values of PCSA.

Functional significance of muscle architecture

Knowledge of muscle architectural characteristics is fundamental for the understanding of muscle mechanical properties as PCSA and fibre length, together with myosin heavy chain content and fibre-type distribution, are the main determinants of the length–force and force–velocity relationships. This is because the maximum force developed by a muscle is proportional to the number of sarcomeres in parallel, while the maximum shortening velocity is proportional to the number of sarcomeres in series, and hence to fibre length (Gans and Gaunt, 1991; Woittiez et al., 1983; Spector et al., 1980; Lieber and Fridén, 2000). PCSA is normally calculated from the ratio of muscle volume (v), which can be measured by MRI, to fascicle length (L_f), multiplied by the cosine of pennation angle (θ), which can be measured by B-mode ultrasound; that is, PCSA=(v/L_f)×cosθ. This PCSA equation actually represents the projection of PCSA along the tendon, described by Haxton (1944) as the 'reduced PCSA', which is useful for calculating specific force – that is, the force acting along the tendon divided by the (reduced) PCSA. Conversely, PCSA may be obtained by normalizing muscle volume to fibre length (Close, 1972; Woledge et al., 1985); this represents the 'non-reduced' PCSA (Haxton, 1944). Thus, to obtain specific force using the non-reduced PCSA, the force acting along the fibres must be calculated by multiplying the tendon force component by the cosine of pennation angle, and then this product is divided by the non-reduced PCSA (v/L_f). What is important to note is that, as long as pennation angle is taken into account, the two approaches (for calculation of 'reduced PCSA' or for calculation of

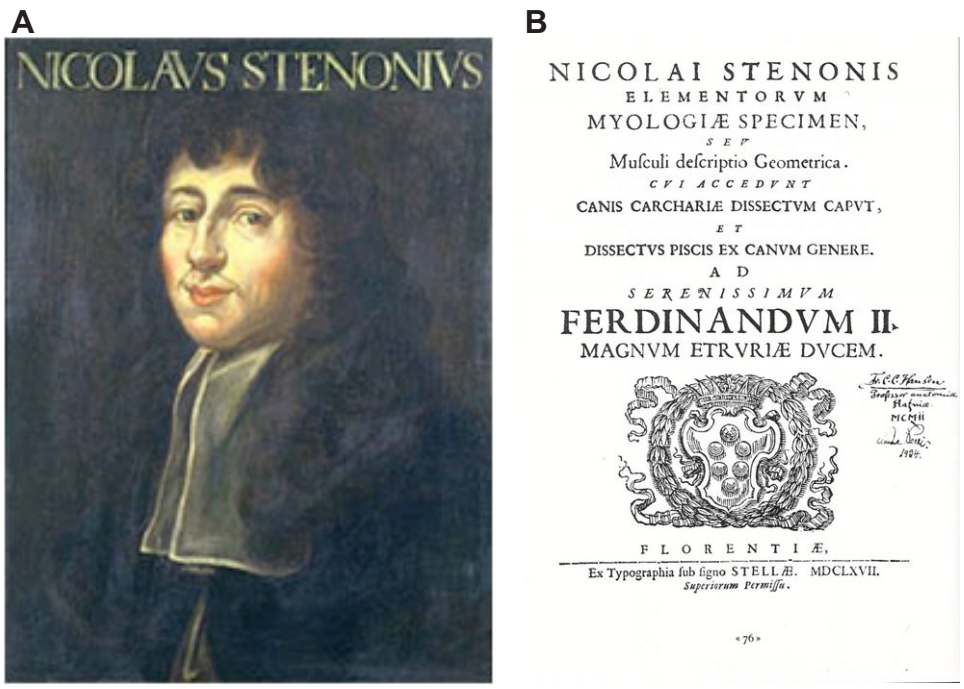

Fig. 4. *Elementorum Mythologiae Specimen*. (A) Portrait of Nicolai Stenonis attributed to Ferdinando II de' Medici's court painter Justus Sustermans (Uffizi Gallery, Florence, Italy). (B) Title page of Steno's treatise *Elementorum Mythologiae Specimen*. Reproduced as public domain picture from: https://en.wikipedia.org/wiki/Nicolas_Steno#/media/File:Portrait_of_Nicolas_Stenonus.jpg.

Journal of Experimental Biology (2016) 219, 276-284 doi:10.1242/jeb.128017

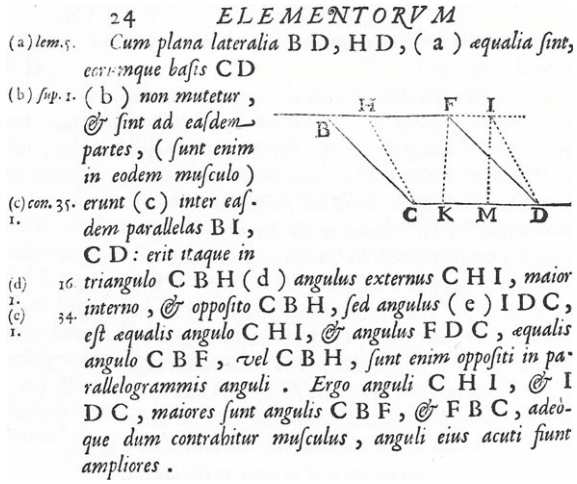

Fig. 5. Stenos' schematic drawing of a pennate muscle. The drawing illustrates a clear appreciation of the increase in pennation angle with muscle contraction, which he describes as '*dum contrahitur musculus, anguli eius acuti siunt ampliores*'. Reproduced as public domain picture from: https://ia800907.us.archive.org/24/items/nicolaistenonise00sten/nicolaistenonise00sten.pdf.

the fibre force component) yield the same mathematical result (Narici, 1999).

Measurements of serial sarcomere number in human muscles were originally obtained from cadaveric specimens (Walker and Schrodt, 1974; Wickiewicz et al., 1983); more recently, however, the use of laser diffraction and optical microendoscopy has enabled measurement of sarcomeres *in vivo* in humans and mice (Llewellyn et al., 2008; Lieber et al., 1994; Mai and Lieber, 1990) and has provided values of sarcomere length at rest and during contraction at different muscle lengths. Through these *in vivo* measurements of sarcomeres it has been possible to show that various muscles operate in different regions of their sarcomere length–force relationship under physiological conditions. Contrary to observations on fish muscles, which showed that sarcomeres operate in the plateau region of their length–tension relationship in order to attain maximum efficiency and power output (Rome et al., 1988), *in situ* optical diffraction studies on frog semitendinosus have shown that sarcomeres operate in the descending limb of the length–tension relationship. It seems, from *in vivo* determinations of muscle length–force properties, that muscles undergoing stretching–shortening cycles during normal movements operate in the ascending limb of the length–force relationship, while muscles that undergo shortening–stretching cycles operate in the descending limb (Rassier et al., 1999). Optical microendoscopy has also been used to measure maximum contraction speed (V_{max}) of the gastrocnemius muscle in mice in response to electrical stimulation (Llewellyn et al., 2008). V_{max} values obtained *in vivo* (8.0 µm s^{-1}) showed good agreement with *in vitro* measurements performed on isolated, chemically activated skinned fibres (range 7.12–13.17 µm s^{-1}). It would thus be of great interest to perform these microendoscopic measurements during contractions of human muscle to obtain *in vivo* values of V_{max}. Furthermore, measurements of optimal sarcomere length *in vivo* would prove useful for estimating sarcomere number, which could easily be obtained by dividing fascicle length measured by ultrasound by the optimal sarcomere length measured by microendoscopy.

Therefore, from a physiological point of view, the most interesting applications of these techniques are the study of muscle contraction *in vivo* and structural remodelling with chronic overloading, unloading and ageing.

Contraction-induced changes in muscle architecture

Papers on isolated non-human mammalian muscle show that muscle fibre shortening during a maximum isometric contraction can be substantial, especially at shorter lengths at which the aponeurosis and tendon are slack, and it is also a function of the muscle's index of architecture (ia; the ratio of fibre length at optimal muscle length to optimal muscle length) (Huijing et al., 1989). In the rat, specifically, fibre shortening during a maximum isometric contraction is minimal at optimum muscle length (L_0) but at 80% L_0 it can be as large as 40% for the gastrocnemius medialis (ia 0.37) and ∼30% for the semimembranosus (ia 0.76) muscles (Huijing et al., 1989).

Notably, these measurements performed on isolated muscles are in line with those obtained *in vivo* on the gastrocnemius medialis muscle of cats, showing muscle fibre shortening of 28% during

A

B

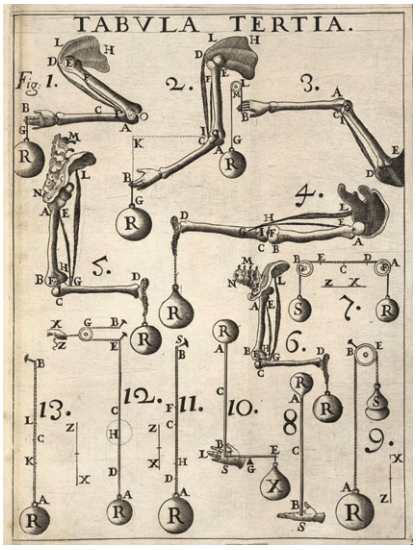

Fig. 6. *De Motu Animalium*. (A) Title page of Giovanni Alfonso Borelli's treatise *De Motu Animalium*. (B) Figure 1 of the treatise, illustrating the use of mathematical models to describe muscles and their mechanical action. In Borelli's drawings, a clear appreciation of different types of muscle fibre arrangement can be recognized. Reproduced as public domain picture from: http://www.design-is-fine.org/post/101352180159/giovanni-alfonso-borelli-plates-from-de-motu.

Journal of Experimental Biology (2016) 219, 276-284 doi:10.1242/jeb.128017

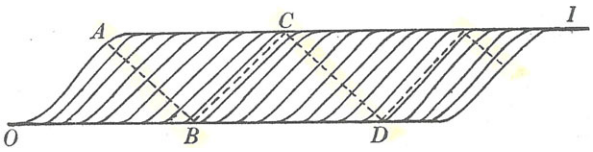

Fig. 7. Haxton's illustration of a pennate muscle. Haxton used this drawing to explain that the physiological cross-sectional area of a muscle is the sum of a number of cross-sectional areas sectioning all muscle fibres at right angles (*A*–*B*+*C*–*D*+…). Reproduced with permission of the *Journal of Physiology*, John Wiley and Son.

maximum contraction at optimal length (Griffiths, 1991). In agreement with these observations on non-human mammalian muscle, *in vivo* ultrasound measurements performed on the human gastrocnemius have shown a shortening of muscle fascicles of ~35–47% during maximum voluntary contractions at optimum ankle joint angle (Kawakami et al., 1998; Maganaris et al., 1998; Narici et al., 1996). This substantial shortening of muscle fascicles *in vivo* brings about a large increase in pennation angle and a consequent reduction in the force generated that is transmitted along the tendon (Alexander, 1998). The shortening of muscle fascicles occurs at the expense of the in-series tendon, which is stretched during the contraction. *In vivo* measurements of gastrocnemius medialis tendon elongation have indeed shown a significant strain of the gastrocnemius medialis tendon during an isometric contraction of the ankle plantarflexors (Maganaris and Paul, 2002). During maximum isometric contractions *in vivo*, joint angle, moment arm, muscle excursion (and thus fascicle length) and tendon stiffness will dictate the degree of shortening of muscle fascicles and influence the region over which the *L*–**F** relationship of the muscle is expressed. By combining measurements of maximum isometric joint moment at different joint angles, Herzog and ter Keurs (1988a) developed a procedure for identification of *L*–**F** relationships in selective multi-articular human muscles and showed that the *L*–**F** relationship of the whole gastrocnemius medialis muscle–tendon unit is expressed *in vivo* over the ascending limb only (Herzog et al., 1991a). Consistent with this finding at the whole-muscle–tendon unit level, muscle fascicle lengths measured at rest and during maximum isometric contraction at different joint angles have shown that the *L*–**F** relationship of the average gastrocnemius medialis muscle fascicle and sarcomere is expressed below the plateau region, in the ascending limb only (Maganaris, 2003). However, the procedure of Herzog and ter Keurs (1988a) showed that the *L*–**F** relationship for the rectus femoris muscle–tendon unit covers a part of both the ascending and descending limbs (Herzog and ter Keurs, 1988b), whereas measurements of muscle architecture *in vivo* during maximum isometric contraction showed that that the *L*–**F** relationship of the soleus and tibialis anterior muscle fascicles and sarcomeres are extended beyond the ascending limb, into the plateau region (Maganaris, 2001). Interestingly, the *L*–**F** relationship *in vivo* may vary not only between different muscles but also for the same muscle between different subjects (Herzog et al., 1991b; Winter and Challis, 2010a,b). The exact reasons why certain muscles operate in the ascending or descending limb of the *L*–**F** relationship during maximum isometric contraction are not known but they seem to be related to its habitual functional demands (Rassier et al., 1999).

The interaction between muscle fascicle shortening and the stretching of the tendon upon contraction not only affects the *in vivo* *L*–**F** relationship during maximum isometric contractions but also becomes particularly relevant for dynamic submaximal contractions habitually performed during locomotion. *In vivo* measurements of the gastrocnemius medialis tendon and fascicles performed during the gait cycle in humans show that when the muscle is active during the single support phase, the gastrocnemius medialis fascicles operate quasi-isometrically, enabling the tendon to be stretched and store elastic energy, which is then released during the push-off phase (Fukunaga et al., 2001). A similar effect has recently been observed in the soleus muscle (Lai et al., 2015). Quasi-isometricity in leg muscle behaviour has also previously been reported during terrestrial locomotion in non-human species (Biewener et al., 1998; Roberts et al., 1997). The role of leg tendon elastic strain energy released during the stance phase in reducing the metabolic cost of locomotion has long been acknowledged (Alexander and Bennett-Clark, 1977; Alexander et al., 1982; Cavagna et al., 1977).

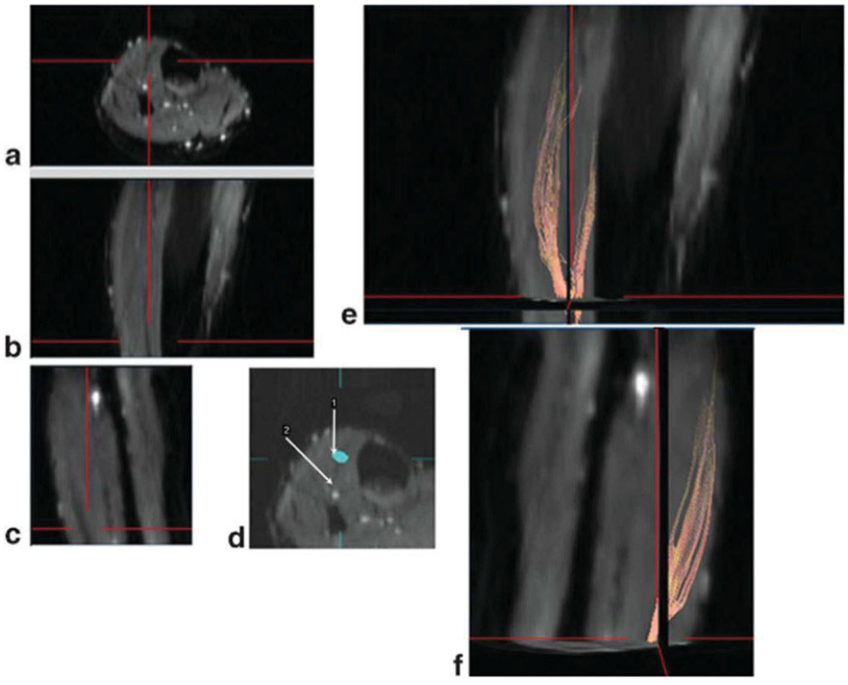

Fig. 8. Three-dimensional reconstruction of the human calf muscle. (A) Acquired axial, (B) reformatted coronal and (C) reformatted sagittal images. (D) Magnified view of the axial image in A with the manually delineated region shown in blue. (E) Three-dimensional coronal view with overlaid fibres. (F) Three-dimensional sagittal view with overlaid fibres. The region of interest is placed across the aponeurosis in the tibialis anterior muscle, and the bipennate structure of the fibres is seen in the three-dimensional coronal view in E. The colour of the fibres corresponds to the fractional anisotropy (red shades indicate higher fractional anisotropy). Numbered regions: (1) tibialis anterior and (2) aponeurosis. Adapted from Sinha et al. (2006) and reproduced with the authors' permission.

Journal of Experimental Biology (2016) 219, 276-284 doi:10.1242/jeb.128017

In terrestrial animals, elastic energy savings of as much as 40–50% of the total mechanical work can be achieved via the tendon stretch–recoil behaviour during fast locomotion (Biewener, 1998). In walking humans, the contribution of elastic strain energy by the Achilles tendon has been estimated to be much lower, around 6% of the total external work (Maganaris and Paul, 2002); however, larger elastic energy contributions are expected in more vigorous activities, such as running.

Remodelling of skeletal muscle with chronic overloading and unloading

It is well established from studies performed on animals in the 1970s and 1980s by Goldspink (1985), Williams and Goldspink (1971, 1973) and Tabary et al. (1972) that skeletal muscle displays great plasticity in response to regimes of overloading and unloading. These pioneering studies on animals showed that sarcomeres in series and in parallel can be either added or removed according to the conditions of chronic loading or unloading. In humans, a common disuse model in which a rapid loss of sarcomeres occurs is the immobilization induced by the use of a plaster cast. Using this model, Narici and Cerretelli (1998) made the unprecedented observation that disuse atrophy in humans is accompanied by a decrease in gastrocnemius medialis muscle fascicle length and pennation angle, respectively indicative of a loss of sarcomeres in series and in parallel. A noteworthy finding of considerable clinical interest is that muscle remodelling with inactivity is an extremely fast process as significant changes in fascicle length may be detected within the first 2 weeks of unloading. Indeed, after just 8 days of a 35 day bed-rest period (the Valdoltra 2007 Bed Rest Study), vastus lateralis fascicle length measured by ultrasound (repeated measures performed in the same location and at full knee extension with a day-to-day intra-class correlation coefficient of 0.91) was found to decrease by 8.6% and pennation angle by 7.0% (Narici et al., 2008; Fig. 9). Assuming a human vastus lateralis sarcomere length of 2.7 µm (Walker and Schrodt, 1974), this equates to a loss of ∼2440 sarcomeres in series after just 8 days of unloading!

Such a rapid remodelling of muscle architecture in response to unloading has been shown to be related to the ability of muscle to sense mechanical signals (change in tension) and convert these stimuli into biochemical events (mechanotransduction) that regulate myofibrillar protein synthesis, and possibly assembly of sarcomeres, thus controlling muscle mass. The regions of skeletal and cardiac muscle that are specialized in mechanotransduction are found in the costameres, represented by regular contact points (focal adhesions) where the extracellular matrix (ECM) comes into contact with the muscle cytoskeleton. Within focal adhesions are cell-surface receptors known as integrins. These are trans-sarcolemmar proteins which connect the ECM to the sarcomere via a chain of cytoskeletal proteins (Hornberger and Esser, 2004). One integrin-associated factor that has been shown to be highly sensitive to changes in mechanical loading (overloading and unloading) is focal adhesion kinase (FAK). Overloading of avian anterior latissimus dorsi muscle was found to cause a marked increase in the content and activity of FAK within 1.5 days of stretch (Flück et al., 1999), while unloading of the human knee extensors causes a decrease in FAK content and activity (of 20% and 30%, respectively) after just 10 days of unilateral limb suspension (De Boer et al., 2007b). Subsequently, Klossner et al. (2009) identified FAK as an upstream modulator of the mechano-sensory pathway of p70S6K. This pathway acts in parallel with the Akt–mTor pathway in regulating protein synthesis. In line with evidence on the role of FAK in the regulation of protein synthesis, a decrease in FAK content (−20%) and activity (−30%), associated with a 50% fall in muscle protein synthesis and a 5% decrease in quadriceps muscle CSA, was found after just 14 days of unilateral lower limb suspension in healthy humans (De Boer et al., 2007a,b). Similarly, a fall in FAK and FRNK (FAK-related regulatory protein) content, correlated with quadriceps muscle atrophy, has been reported after only 8 days of bed rest (Li et al., 2013). In animals, this has been shown to relate to changes in the expression of the costamere component meta-vinculin, which has been found to affect the fibre phenotype (Klossner et al., 2013) and which is a marker of disuse in men (Chopard et al., 2005).

Remodelling of skeletal muscle with ageing

The structural changes of skeletal muscle that occur with ageing are similar to those observed with inactivity, which undoubtedly plays a major role in the loss of muscle mass in old age (sarcopenia). However, the key difference between disuse atrophy and sarcopenia is that while the former only involves a decrease in fibre size, the latter entails both a decrease in size and a decrease in the number of muscle fibres (Narici and Maffulli, 2010). In sarcopenia, as in disuse atrophy, the decrease in muscle mass is accompanied by a decrease in fascicle length and pennation angle (Narici et al., 2003), and these changes in muscle architecture account for about half of the loss in maximum force and shortening velocity (Narici et al., 2005). Nonetheless, even in old age, resistance training has been shown to significantly mitigate/reverse these changes (Morse et al., 2005; Reeves et al., 2004). One particularly noteworthy observation on the muscular adaptations to resistive training both in old and in young individuals recently made by our group (Franchi et al., 2014) is that the pattern, not the amount, of muscle growth depends on loading mode. In two separate studies, one in young and one in older men, we reported that resistive training using either concentric (shortening) or eccentric (lengthening) contractions, matched for neural drive, produced a similar increase in muscle volume but through different architectural adaptations. Whereas concentric training was found to lead to an increase in pennation angle with

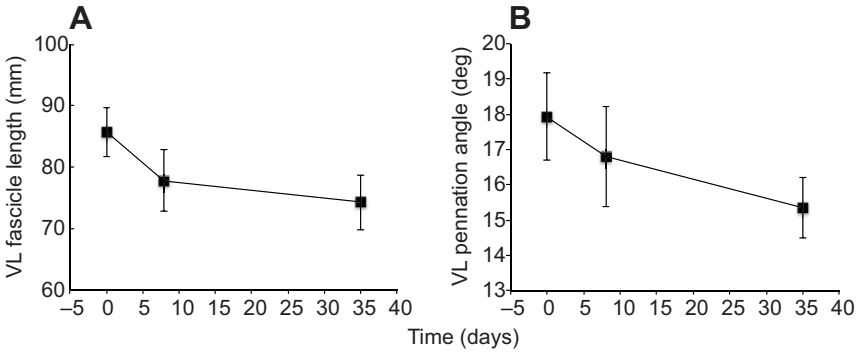

Fig. 9. Disuse atrophy. The effect of 35 day bed rest on (A) fascicle length and (B) pennation angle in healthy men. As can be observed, chronic inactivity leads to a pronounced remodelling of muscle architecture, with fascicles becoming shorter and less pennate. VL, vastus lateralis. Data from Narici et al. (2008).

Journal of Experimental Biology (2016) 219, 276-284 doi:10.1242/jeb.128017

little change in fascicle length, eccentric training produced diametrically different results: a large increase in fascicle length and a smaller increase in pennation angle (Franchi et al., 2014, 2015; Reeves et al., 2009). These findings suggest that the addition of sarcomeres in a muscle in response to chronic overloading follows a direction dictated by the contraction mode. Hence, eccentric training seems to promote muscle growth mainly through the addition of sarcomeres in series (as inferred from the increase in fascicle length), while concentric training results in muscle growth predominantly through the addition of sarcomeres in parallel (as inferred from the increase in pennation angle, a geometric consequence of the addition of new contractile tissue along the tendon aponeurosis) (Kawakami et al., 1993). Fundamentally, the new concept to the current understanding of muscle hypertrophy is as follows: the increase in muscle mass in response to chronic overloading occurs through the addition of sarcomeres both in parallel and in series; however, the direction of muscle growth – that is, the differential addition of sarcomeres in parallel and in series – depends on the mode of muscle contraction (concentric versus eccentric) (Fig. 10; Franchi et al., 2014).

These observations on the structural remodelling of human muscle in response to concentric and eccentric loading are supported by previous animal studies which showed increased longitudinal muscle growth (i.e. greater addition of sarcomeres in series) after downhill (eccentric) compared with uphill running (concentric) exercise in rats (Lynn and Morgan, 1994; Butterfield et al., 2005). Furthermore, other investigations on animal muscle attempted to clarify the molecular, metabolic and myogenic mechanisms that may govern such distinct patterns of muscle remodelling in response to concentric and eccentric loading. In one of these studies, Wretman and colleagues showed marked differences in the phosphorylation of mitogen-activated protein kinases (MAPKs; i.e. ERK1/2, p38) between muscle shortening and lengthening actions (Wretman et al., 2001), highlighting greater MAPK activation in response to stretch stimuli (Martineau and Gardiner, 2001). Moreover, it has been shown that concentric and eccentric growth in mice cardiac myocytes is regulated by different activation of ERK1/2 MAPKs (Kehat et al., 2011). This contraction-specific MAPK activation has also been investigated in human muscle, where it appears to be markedly up-regulated in response to a single eccentric-only bout of resistance training (Franchi et al., 2014).

Current investigations in our laboratory are focused at establishing which specific molecular signalling pathways, activated by these two modes of training, regulate the direction of muscle growth as it is also known that, acutely, different genes are expressed by concentric as opposed to eccentric exercise in humans (Goldspink et al., 2002; Hyldahl et al., 2015; Kostek et al., 2007; Vissing et al., 2013).

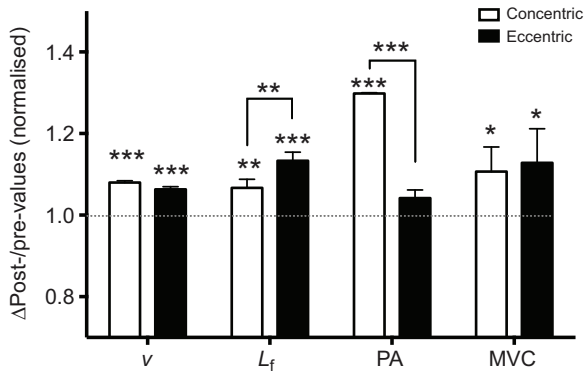

Fig. 10. Contraction-dependent muscle growth in response to eccentric and concentric resistive training in young males. Eccentric and concentric training (matched for relative load and neural drive) result in similar muscle hypertrophy in vastus lateralis but through distinctly different architectural changes. Eccentric training leads to an increase in fascicle length with little change in pennation angle whereas concentric training results in a large increase in pennation angle with a modest increase in fascicle length. *v*, volume; L_f, fascicle length; PA, pennation angle; MVC, maximal voluntary contraction. **P*<0.05, ***P*<0.001, ****P*<0.0001. Adapted from Franchi et al. (2014).

Conclusions

The structural assembly of skeletal muscle, together with muscle fibre properties, has a major influence on muscle mechanical behaviour. Early scholars of the 16th and 17th centuries recognized differences in muscle fibre arrangements and were the first to describe changes in muscle structure upon contraction. These early notions of muscle anatomy and architecture led two centuries later to investigations into the relationship between muscle architecture, assessed in cadavers, and its functional properties. However, it was only in the last century that muscle fibre arrangement and sarcomere structure could be measured *in vivo*, both at rest and during muscle contraction, by laser diffraction, optical microendoscopy, MRI diffusor tensor imaging and ultrasonography. Assessment of tendon mechanical properties *in vivo* also became possible through ultrasound tracking of tendon displacement during muscle contractions. Integration of this information with data on muscle fibre morphological and contractile properties has enabled current investigators to provide unprecedented knowledge on the modus operandi of various muscles *in vivo* with respect to their sarcomere length–tension relationship and the relevance thereof for locomotor performance.

New insights into the structural assembly of muscle fibres with growth, exercise, inactivity and ageing have been obtained through the use of ultrasonography. These observations provide evidence of the great plasticity of skeletal muscle in response to use, disuse and ageing, and biochemical analyses on muscle biopsies obtained in these conditions are revealing the molecular mechanisms regulating skeletal muscle remodelling, and how these differ between contraction mode. This information is of considerable clinical interest as understanding of the mechanisms of skeletal muscle remodelling, and of its functional consequences, is fundamental for the development of innovative clinical approaches to common neuromuscular, orthopaedic and age-related conditions.

Acknowledgements

The authors are grateful to Professor Martin Flück for advice and comments on issues in this manuscript pertaining to mechanotransduction, and to Professor Shantanu Sinha for providing the 3-D image of human calf muscle.

Competing interests

The authors declare no competing or financial interests.

Funding

Partial support by BBSRC project BB/K019104/1 is acknowledged.

References

Alexander, R. McN. (1998). Muscle geometry. *J. Physiol.* **512**, 315.
Alexander, R. McN. and Bennet-Clark, H. C. (1977). Storage of elastic strain energy in muscle and other tissues. *Nature* **265**, 114-117.
Alexander, R. McN., Maloiy, G. M. O., Ker, R. F., Jayes, A. S. and Warui, C. N. (1982). The role of tendon elasticity in the locomotion of the camel (*Camelus dromedarius*). *J. Zool.* **198**, 293-313.
Biewener, A. A. (1998). Muscle-tendon stresses and elastic energy storage during locomotion in the horse. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **120**, 73-87.

Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V. (1998). In vivo muscle force-length behavior during steady-speed hopping in tammar wallabies. *J. Exp. Biol.* **201**, 1681-1694.
Butterfield, T. A., Leonard, T. R. and Herzog, W. (2005). Differential serial sarcomere number adaptations in knee extensor muscles of rats is contraction type dependent. *J. Appl. Physiol.* **99**, 1352-1358.
Cavagna, G. A., Heglund, N. C. and Taylor, C. R. (1977). Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditures. *Am. J. Physiol.* **233**, R243-R261.
Chopard, A., Arrighi, N., Carnino, A. and Marini, J. F. (2005). Changes in dysferlin, proteins from dystrophin glycoprotein complex, costameres, and cytoskeleton in human soleus and vastus lateralis muscles after a long-term bedrest with or without exercise. *FASEB J.* **19**, 1722-1724.
Close, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiol. Rev.* **52**, 129-197.
De Boer, M. D., Maganaris, C. N., Seynnes, O. R., Rennie, M. J. and Narici, M. V. (2007a). Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. *J. Physiol.* **583**, 1079-1091.
De Boer, M. D., Selby, A., Atherton, P., Smith, K., Seynnes, O. R., Maganaris, C. N., Maffulli, N., Movin, T., Narici, M. V. and Rennie, M. J. (2007b). The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse. *J. Physiol.* **585**, 241-251.
Flück, M., Carson, J. A., Gordon, S. E., Ziemiecki, A. and Booth, F. W. (1999). Focal adhesion proteins FAK and paxillin increase in hypertrophied skeletal muscle. *Am. J. Physiol.* **277**, C152-C162.
Franchi, M. V., Atherton, P. J., Reeves, N. D., Flück, M., Williams, J., Mitchell, W. K., Selby, A., Beltran-Valls, R. M. and Narici, M. V. (2014). Architectural, functional, and molecular responses to concentric and eccentric loading in human skeletal muscle. *Acta. Physiol.* **210**, 642-654.
Franchi, M. V., Wilkinson, D. J., Quinlan, J. I., Mitchell, W. K., Reeves, N. D., Smith, K., Atherton, P. J. and Narici, M. V. (2015). Early hypertrophic, architectural and metabolic adaptations of human skeletal muscle to eccentric and concentric loading. *Physiol. Rep.* **3**, e12593.
Fukunaga, T., Kubo, K., Kawakami, Y., Fukashiro, S., Kanehisa, H. and Maganaris, C. N. (2001). In vivo behaviour of human muscle tendon during walking. *Proc. Biol. Sci.* **268**, 229-233.
Gans, C. and Gaunt, A. S. (1991). Muscle architecture in relation to function. *J. Biomech.* **24**, 53-65.
Goldspink, G. (1985). Malleability of the motor system: a comparative approach. *J. Exp. Biol.* **115**, 375-391.
Goldspink, G., Williams, P. and Simpson, H. (2002). Gene expression in response to muscle stretch. *Clin. Orthop. Relat. Res.* **403** Suppl., S146-S152.
Griffiths, R. I. (1991). Shortening of muscle fibres during stretch of the active cat medial gastrocnemius muscle: the role of tendon compliance. *J. Physiol.* **436**, 219-236.
Haxton, H. A. (1944). Absolute muscle force in the ankle flexors of man. *J. Physiol.* **103**, 267-273.
Heemskerk, A. M., Strijkers, G. J., Vilanova, A., Drost, M. R. and Nicolay, K. (2005). Determination of mouse skeletal muscle architecture using three-dimensional diffusion tensor imaging. *Magn. Reson. Med.* **53**, 1333-1340.
Herzog, W. and ter Keurs, H. E. D. J. (1988a). A method for the determination of the force-length relation of selected *in-vivo* human skeletal muscles. *Pflugers Arch.* **411**, 637-641.
Herzog, W. and ter Keurs, H. E. D. J. (1988b). Force-length relation of *in-vivo* human rectus femoris muscles. *Pflugers Arch.* **411**, 642-647.
Herzog, W., Read, L. J. and ter Keurs, H. E. D. J. (1991a). Experimental determination of force-length relations of intact human gastrocnemius muscles. *Clin. Biomech.* **6**, 230-238.
Herzog, W., Guimaraes, A. C., Anton, M. G. and Carter-Erdman, K. A. (1991b). Moment-length relations of rectus femoris muscles of speed skaters/cyclists and runners. *Med. Sci. Sports Exerc.* **23**, 1289-1296.
Hornberger, T. A. and Esser, K. A. (2004). Mechanotransduction and the regulation of protein synthesis in skeletal muscle. *Proc. Nutr. Soc.* **63**, 331-335.
Huijing, P. A., van Lookeren Campagne, A. A. and Koper, J. F. (1989). Muscle architecture and fibre characteristics of rat gastrocnemius and semimembranosus muscles during isometric contractions. *Acta Anat.* **1135**, 46-52.
Hyldahl, R. D., Nelson, B., Xin, L., Welling, T., Groscost, L., Hubal, M. J., Chipkin, S., Clarkson, P. M. and Parcell, A. C. (2015). Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. *FASEB J.* **29**, 2894-2904.
Kawakami, Y., Abe, T. and Fukunaga, T. (1993). Muscle-fiber pennation angles are greater in hypertrophied than in normal muscles. *J. Appl. Physiol.* **74**, 2740-2744.
Kawakami, Y., Ichinose, Y. and Fukunaga, T. (1998). Architectural and functional features of human triceps surae muscles during contraction. *J. Appl. Physiol* **85**, 398-404.
Kehat, I., Davis, J., Tiburcy, M., Accornero, F., Saba-El-Leil, M. K., Maillet, M., York, A. J., Lorenz, J. N., Zimmermann, W. H., Meloche, S. et al. (2011). Extracellular signal-regulated kinases 1 and 2 regulate the balance between eccentric and concentric cardiac growth. *Circ. Res.* **108**, 176-183.
Klossner, S., Durieux, A.-C., Freyssenet, D. and Flueck, M. (2009). Mechano-transduction to muscle protein synthesis is modulated by FAK. *Eur. J. Appl Physiol.* **106**, 389-398.
Klossner, S., Li, R., Ruoss, S., Durieux, A.-C. and Flück, M. (2013). Quantitative changes in focal adhesion kinase and its inhibitor, FRNK, drive load-dependent expression of costamere components. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **305**, R647-R657.
Kostek, M. C., Chen, Y.-W., Cuthbertson, D. J., Shi, R., Fedele, M. J., Esser, K. and Rennie, M. J. (2007). Gene expression responses over 24 h to lengthening and shortening contractions in human muscle: major changes in CSRP3, MUSTN1, SIX1, and FBXO32. *Physiol. Genomics* **31**, 42-52.
Lai, A., Lichtwark, G. A., Schache, A. G., Lin, Y.-C., Brown, N. A. T. and Pandy, M. G. (2015). *In vivo* behavior of the human soleus muscle with increasing walking and running speeds. *J. Appl. Physiol.* **118**, 1266-1275.
Li, R., Narici, M. V., Erskine, R. M., Seynnes, O. R., Rittweger, J., Pišot, R., Šimunič, B. and Flück, M. (2013). Costamere remodeling with muscle loading and unloading in healthy young men. *J. Anat.* **223**, 525-536.
Lieber, R. L. and Fridén, J. (2000). Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve* **23**, 1647-1666.
Lieber, R. L., Loren, G. J. and Fridén, J. (1994). In vivo measurement of human wrist extensor muscle sarcomere length changes. *J. Neurophysiol.* **71**, 874-881.
Llewellyn, M. E., Barretto, R. P. J., Delp, S. L. and Schnitzer, M. J. (2008). Minimally invasive high-speed imaging of sarcomere contractile dynamics in mice and humans. *Nature* **454**, 784-788.
Lynn, R. and Morgan, D. L. (1994). Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *J. Appl. Physiol.* **77**, 1439-1444.
Maganaris, C. N. (2001). Force-length characteristics of in vivo human skeletal muscle. *Acta. Physiol. Scand.* **172**, 279-285.
Maganaris, C. N. (2003). Force-length characteristics of the in vivo human gastrocnemius muscle. *Clin. Anat.* **16**, 215-223.
Maganaris, C. N. and Paul, J. P. (2002). Tensile properties of the in vivo human gastrocnemius tendon. *J. Biomech.* **35**, 1639-1646.
Maganaris, C. N., Baltzopoulos, V. and Sargeant, A. J. (1998). In vivo measurements of the triceps surae complex architecture in man: implications for muscle function. *J. Physiol.* **512**, 603-614.
Mai, M. T. and Lieber, R. L. (1990). A model of semitendinosus muscle sarcomere length, knee and hip joint interaction in the frog hindlimb. *J. Biomech.* **23**, 271-279.
Martineau, L. C. and Gardiner, P. F. (2001). Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension. *J. Appl. Physiol.* **91**, 693-702.
Morse, C. I., Thom, J. M., Birch, K. M. and Narici, M. V. (2005). Changes in triceps surae muscle architecture with sarcopenia. *Acta. Physiol. Scand.* **183**, 291-298.
Narici, M. (1999). Human skeletal muscle architecture studied in vivo by non-invasive imaging techniques: functional significance and applications. *J. Electromyogr. Kinesiol.* **9**, 97-103.
Narici, M. and Cerretelli, P. (1998). Changes in human muscle architecture in disuse-atrophy evaluated by ultrasound imaging. *J. Gravit. Physiol.* **5**, P73-P74.
Narici, M. V. and Maffulli, N. (2010). Sarcopenia: characteristics, mechanisms and functional significance. *Br. Med. Bull.* **95**, 139-159.
Narici, M. V., Landoni, L. and Minetti, A. E. (1992). Assessment of human knee extensor muscles stress from in vivo physiological cross-sectional area and strength measurements. *Eur. J. Appl. Physiol.* **65**, 438-444.
Narici, M. V., Binzoni, T., Hiltbrand, E., Fasel, J., Terrier, F. and Cerretelli, P. (1996). *In vivo* human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. *J. Physiol.* **496**, 287-297.
Narici, M. V., Maganaris, C. N., Reeves, N. D. and Capodaglio, P. (2003). Effect of aging on human muscle architecture. *J. Appl. Physiol.* **95**, 2229-2234.
Narici, M. V., Maganaris, C. and Reeves, N. (2005). Myotendinous alterations and effects of resistive loading in old age. *Scand. J. Med. Sci. Sports* **15**, 392-401.
Narici, M., Flueck, M., Seynnes, O., De Boer, M., Maganaris, C. and Rennie, M. (2008). Structural remodeling of human skeletal muscle with chronic unloading. *Proc. 13th Annual ECSS Congress Estoril/Portugal, July 9–12, 2008*, P257-P258.
Rassier, D. E., MacIntosh, B. R. and Herzog, W. (1999). Length dependence of active force production in skeletal muscle. *J. Appl. Physiol.* **86**, 1445-1457.
Reeves, N. D., Narici, M. V. and Maganaris, C. N. (2004). In vivo human muscle structure and function: adaptations to resistance training in old age. *Exp. Physiol.* **89**, 675-689.
Reeves, N. D., Maganaris, C. N., Longo, S. and Narici, M. V. (2009). Differential adaptations to eccentric versus conventional resistance training in older humans. *Exp. Physiol.* **94**, 825-833.
Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113-1115.
Rome, L. C., Funke, R. P., Alexander, R. McN., Lutz, G., Aldridge, H., Scott, F. and Freadman, M. (1988). Why animals have different muscle fibre types. *Nature* **335**, 824-827.
Sinha, S., Sinha, U. and Edgerton, V. R. (2006). In vivo diffusion tensor imaging of the human calf muscle. *J. Magn. Reson. Imaging* **24**, 182-190.

Spector, S. A., Gardiner, P. F., Zernicke, R. F., Roy, R. R. and Edgerton, V. R. (1980). Muscle architecture and force–velocity characteristics of cat soleus and medial gastrocnemius; implications for motor control. *J. Neurophysiol.* **44**, 951-960.

Tabary, J. C., Tabary, C., Tardieu, C., Tardieu, G. and Goldspink, G. (1972). Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J. Physiol.* **224**, 231-244.

Vissing, K., Rahbek, S. K., Lamon, S., Farup, J., Stefanetti, R. J., Wallace, M. A., Vendelbo, M. H. and Russell, A. (2013). Effect of resistance exercise contraction mode and protein supplementation on members of the STARS signalling pathway. *J. Physiol.* **591**, 3749-3763.

Walker, S. M. and Schrodt, G. R. (1974). I segment lengths and thin filament periods in skeletal muscle fibers of the Rhesus monkey and the human. *Anat. Rec.* **178**, 63-81.

Weber, E. F. (1851). *Ueber die Langenverhaltnisse der Fleischfasern der Muskeln im allgemeinen. Berichte uber die Verhandlungen der Koniglich Sachsischen Akademie der Wissenschaften zu Leipsig, Mathematisch-physische Classe*. Leipzig: Weidmannsche Buchhandlung.

Wickiewicz, T. L., Roy, R. R., Powell, P. L. and Edgerton, V. R. (1983). Muscle architecture of the human lower limb. *Clin. Orthop. Relat. Res.* **179**, 275-283.

Williams, P. E. and Goldspink, G. (1971). Longitudinal growth of striated muscle fibres. *J. Cell Sci.* **9**, 751-767.

Williams, P. E. and Goldspink, G. (1973). The effect of immobilization on the longitudinal growth of striated muscle fibres. *J. Anat.* **116**, 45-55.

Winter, S. L. and Challis, J. H. (2010a). The expression of the skeletal muscle force-length relationship in vivo: a simulation study. *J. Theor. Biol.* **262**, 634-643.

Winter, S. L. and Challis, J. H. (2010b). The force-length curves of the human rectus femoris and gastrocnemius muscles in vivo. *J. Appl. Biomech.* **26**, 45-51.

Woittiez, R. D., Huijing, P. A. and Rozendal, R. H. (1983). Influence of muscle architecture on the length-force diagram of mammalian muscle. *Pflugers Arch.* **399**, 275-279.

Woledge, R. C., Curtin, N. A. and Homsher, E. (1985). Energetic aspects of muscle contraction. *Monogr. Physiol. Soc.* 14, 1-357.

Wretman, C., Lionikas, A., Widegren, U., Lännergren, J., Westerblad, H. and Henriksson, J. (2001). Effects of concentric and eccentric contractions on phosphorylation of MAPK(erk1/2) and MAPK(p38) in isolated rat skeletal muscle. *J. Physiol.* **535**, 155-164.