Structural and mechanical responses to concentric and eccentric functional overloading of the "Muscle-Tendon Unit" in young and older individuals.

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Thesis submitted to the university of Nottingham for the degree of Doctor of Philosophy

September 2017

Author's Declaration

I, Jonathan Quinlan, declare that the work in this thesis was carried out in accordance with the regulations of the University of Nottingham. The production of MRI images was carried out via a collaboration with Dr. S Francis based at the Sir Peter Mansfield MRI Centre, University of Nottingham. However, the subsequent analysis was carried out by the author.

Date: 29/09/2017

Signed: 

Dedication and acknowledgements

I would first like to thank both Prof Marco Narici and Prof Ken Smith for their continual support and guidance throughout my PhD programme. I appreciate all their efforts and all the knowledge that they have passed onto me.

I think it is only fair that I commend everybody within the group who has put up with me over the years. You will all know how much you have helped me in this PhD process; whether it was reading endless drafts, lending me your limbs for practice scans, or the needed pint at the Mallard.

"*Where's Martino?" – "Not sure, he's always 10 minutes late".*
Martino, I must personally thank you for everything in the last 4 years. You taught me the way of ultrasound. You taught me to think critically. And you also taught me to tell you 10 minutes early that what I meant. I am so grateful for all the support that you have given me.

It is safe to say that I would not be the person I am today if it wasn't for my family; nor would I have made it as far as this submission. Thank you for routing for me. As a family, the last year has been difficult for us all, but at least we were all in it

together. Mam and Dad, thank you for everything you do and everything you have done for me, I am so very proud to call you my parents.

Gemma, you will never know just how much help you have given me in this year. I can honestly say that if it wasn't for you being there for me, I seriously doubt I would have completed this chapter in my life. You stuck by me in my darkest days and kept me on track and I can never thank you enough.

There are many people who have come in and out of my life during these 4 years. While I doubt any of you will read this, I owe you all thanks for sharing in this experience with me. Unfortunately, there are also cherished people who have passed away in this time and to you I thank you for everything that you were, you will be forever remembered.

'Si in te non potes credere, ergo quod credis'

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Abbreviations

AAs – Amino acids

AC – Activation capacity

ACSA – Anatomical cross sectional area

AGEs - Advanced glycation end products

aRTD – Absolute rate of torque development

ADP – Adenosine diphosphate

ATP – Adenosine triphosphate

BF – Bicep Femoris

CON – Concentric

CSA – Cross sectional area

ECC – Eccentric

ECM - extracellular matrix

EMG – Electromyography

F/V – Force / velocity

HP - Hydroxylysyl pyridinoline

L/T – Length – tension

LP - Lysyl pyridinoline

PINP - procollagen type 1 N-terminal propeptide

PICP - procollagen type 1 C-terminal propeptide

MMPs - Matrix metalloproteinases

MPB – Muscle protein breakdown

MPS - Muscle protein synthesis

MTJ - Myotendinous junction

MVC – Maximal voluntary contraction

nRTD – Normalised rate of torque development

OC – Old concentric

OE – Old eccentric

OM – Old males

PT – Patellar tendon

RE – Resistance exercise

RER – Rough endoplasmic reticulum

RMS – Root mean squared

RTD – Rate of torque development

TIMPs - Tissue inhibitors of matrix metalloproteinases

Tm – Tropomyosin

Tn – Troponin

TnC - Troponin C

TnI - Troponin I

TnT - Troponin T

TPT – Time to peak twitch

TTPE – Time to peak EMG

VL – Vastus laterallis

Xtrl – Control

YC – Young concentric

YE – Young eccentric

YM – Young males

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1 Chapter 1 – Introduction

1.1 Skeletal Muscle

Skeletal muscle is the most abundant tissue within the human body, occupying 40-45% of the total content (S. Lindstedt, 2016). The fundamental role of skeletal muscle is the production of force which consequently maintains posture and also facilitates the completion of a multitude of motor tasks varying from simple to complex (Reid and Fielding, 2012). The diversity in these motor demands requires skeletal muscle to produce a variety of contractions; some powerful, some precise and controlled and some which must be sustained for a prolonged period.

In addition to a critical role in locomotion, skeletal muscle also has a significant bearing upon whole body metabolism (Zurlo *et al.*, 1990). Skeletal muscle is the primary store of amino acids (AAs), which are the essential precursors required around the whole body; when needed, these can be released into the blood stream. Skeletal muscle also provides one of the largest deposits for glucose and store for glycogen (Thiebaud *et al.*, 1982). The maintenance of glucose homeostasis is essential for

the prevention of metabolic conditions, such as type II diabetes mellitus (DeFronzo, Bonadonna and Ferrannini, 1992)

Significantly, skeletal muscle is an extremely pliable tissue in terms of both structure and function. In turn, this means skeletal muscle undergoes many positive changes in response to exercise whether it is increased strength or hypertrophy (Franchi *et al.*, 2014; Brook *et al.*, 2015). Nonetheless, this pliable nature also means it is susceptible to negative changes in response to ageing, disease or inactivity (Narici and Maffulli, 2010; Mitchell *et al.*, 2012; Wall *et al.*, 2014), i.e. loss of function and muscle mass.

1.1.1 Structure

The structure of skeletal muscle takes the form of a hierarchical collection of contractile proteins, enveloped by a connective tissue framework. The smallest structure of skeletal muscle is termed the sarcomere, which will be discussed in section 1.1.1.1, however a chain of sarcomeres forms a myofibril. Myofibrils themselves collate in parallel to form an individual muscle fibre; often also known as myofibres, muscle fibres are typically 1-100μm in diameter and can reach lengths of up to 30-40cms. A bundle of 10-100 muscle fibres together

consequently forms a muscle fascicle, which is contained by a connective tissue wrapping entitled the perimysium (also referred to as the extracellular matrix). Multiple fascicles are collated into bundles which are eventually enveloped by epimysium, this partition constitutes the entire muscle; this is additionally covered by a thicker layer of connective tissue called fascia.

1.1.1.1 Sarcomere organisation

As aforementioned, the sarcomere is the smallest functional structure in skeletal muscle, which is responsible for the production of force during contraction. Sarcomeres contain two types of myofilament, actin and myosin; whereby actin is relatively thin and myosin relatively thick.

The Actin filament is comprised of two interwoven strands of polymerized actin monomers. Additionally, actin filaments have two regulatory proteins attached, troponin (Tn) and tropmyosin (Tm). Tn is located regularly along the actin filament, every seven actin monomers, and is essential for the detection of and interaction with calcium ions (Ca^{2+}). The Tn complex is made up of three components: troponin C (TnC) which binds to Ca^{2+},

troponin T (TnT) which binds Tm and finally troponin I (TnI) which acts as an inhibitory subunit.

The myosin filament is composed of two heavy chains (MHCs) and also two pairs of lighter chains (MLCs); the MHC form will dictate the particular fibre phenotype. The myosin molecule itself consists of two major fragments: 1) the globular head (S1 fragment) which contains ATPase and binds to the actin and 2) the S2 fragment, which is the flexible portion which bind with one another to form the thick filaments.

Within the muscle fibres, the thick myosin filaments are organized in such a way that it permits the thin actin filaments to slide in between them (Figure 1.1). Actin and myosin are also arranged in a hexagonal lattice, meaning each myosin filament is surrounded by six individual actin filaments onto which it can bind; each actin filament can interact with three myosin filaments. The actual definition of a sarcomere is the structure the spans from one Z line to another which usually equates to 2.0 – 2.5μm long at resting length. There are also four other points of reference within a sarcomere, the M-line, the A-band, the I-band and the H-band. The A-band constitutes the region where there is large overlap between actin and myosin, whereas

the I-band is the region in where there is no overlap at all, hence where only actin filaments are found. The H-band is a region of the A-band at which there is no overlap of actin filaments, the middle of this region is terms the M-line, where several structural proteins are found which embed the myosin filaments.

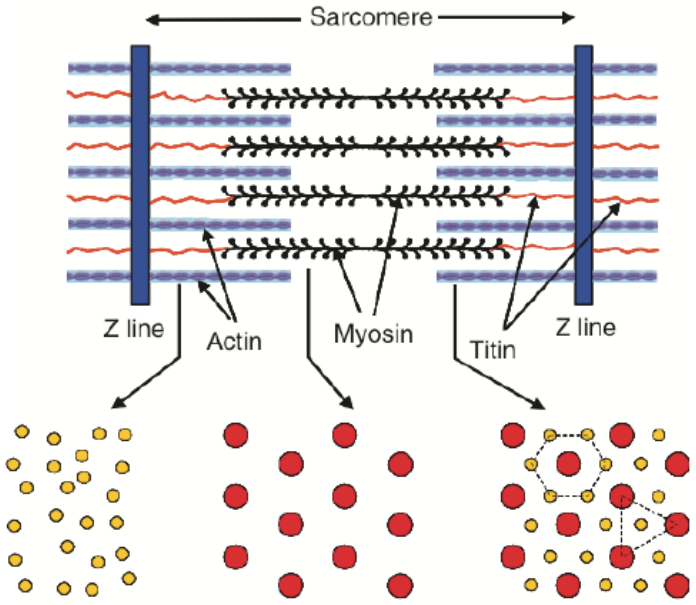


Figure 1.1: The geometric organisation of a sarcomere and the spatial arrangement of actin and myosin. Taken from (DA Jones, 2004)

1.1.1.2 Muscle functional output

The structure of skeletal muscle and the organisation of the sarcomere allows for effective force output. The mechanics of muscle contraction and hence force output is explained by the

sliding filament theory (1.1.2.1). Significantly, force output can be influence by both the length of the sarcomere (1.1.2.2) and by the velocity of contraction (1.1.2.3).

1.1.1.3 Muscle contraction – sliding filament theory

As mentioned in section 1.1.1.1, there are two main filamentous proteins involved in muscle contraction; actin and myosin. Huxley explained how muscle contraction takes place with the now well established sliding filament theory (Huxley and Simmons, 1971). The sliding filament theory, as the name suggests involves the sliding and consequent interdigitation of the two filamentous proteins; this sliding occurs due to cross-bridges between actin and myosin. However, it is important to note that only myosin filaments slide as the actin filaments remain fixed to the cytoskeleton.

The first stage of the cross-bridge cycle is attachment of the myosin head to the actin filament. This is facilitated through the influx of Ca^{2+}, which binds to the TnC located on actin and consequently reveals the myosin-binding site which was blocked by Tm, allowing the globular myosin head to attach. At this point the attached inorganic phosphate is released strengthening the cross-bridge which also initiates the power

stroke of the cycle; resulting in the production of force and shortening of the sarcomere. Finally, Adenosine triphosphate (ATP) binds to the myosin head which forces the detachment of myosin from the actin binding site. Simultaneously, the ATP is converted to adenosine diphosphate (ADP) and inorganic phosphate. The cycle then repeats until sufficient force is produced, or until the sarcomeres are unable to shorten further.

1.1.1.4 Tension – length (T/L) relationship

The degree of overall force output is dependent on many factors; however, the mechanical generation of force is dictated by the extent of myosin-actin overlap. During isometric contraction (i.e. no change in sarcomere length during contraction), it is the amount of overlapping of these two filament proteins which would decree the amount of force output; hence the length of the sarcomere itself. This principle is known as the length – tension (L/T) relationship. This idea was composed through work by Gordon (Gordon, Huxtey and Jitliant, 1966) and also by Huxley (Huxley, 1974) in which they constructed a sarcomere L/T curve (Figure 1.2) to illustrate how the sarcomere length affected the production of force. There are three main regions of the curve, the ascending limb (A), the plateau region (B) and the descending limb (C). Within region

(A) there is an increase in force for an increase in sarcomere length, in region (B) the force is constant for changes in sarcomere length and (C) where force decreases for an increased sarcomere length.

When the sarcomere length is high (>3.5μm), often when the muscle fibres have been stretched, the active muscle force is either very low or non-existent; this occurs as there is either little or no interdigitation of the actin and myosin filaments. However, as the sarcomere length begins to decrease (i.e. moving backwards along region C) the amount of force produced continues to increases until region (B) is reached; at which point the force production is maximal due to the optimal actin-myosin cross-bridging. From this point onwards (A), as the sarcomere shortens further (<2.5μm) the force significantly reduces as double overlapping of the two filaments begins to occur. Thus, the length of the sarcomere during isometric contraction dictates the overall force output.

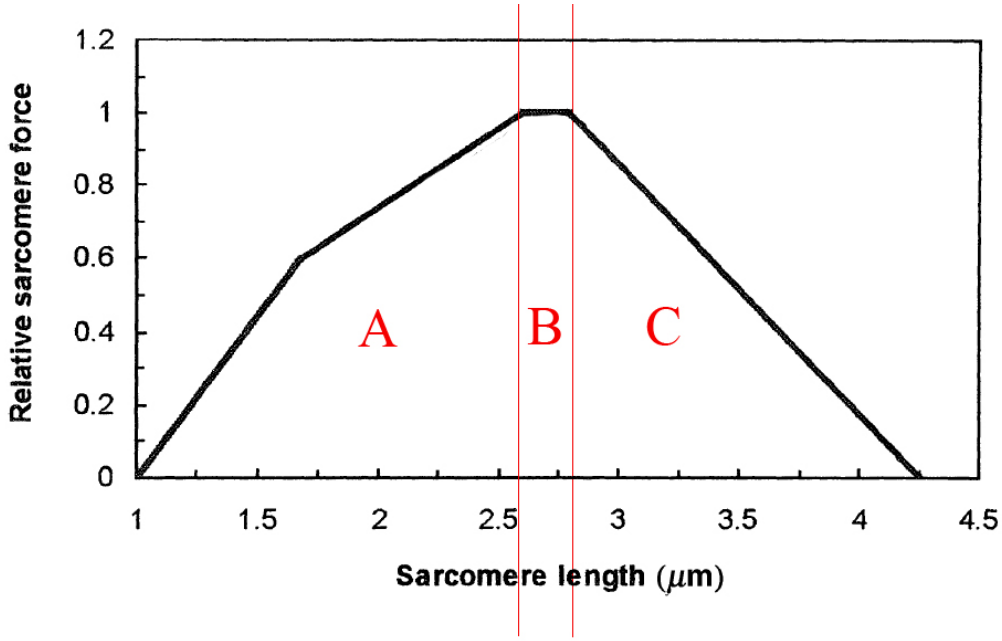


Figure 1.2: Diagram represents the Force / Length relationship. (A) represents the ascending limb, (B) represents the optimal region, and (C) is the descending limb. Adapted from (Maganaris, 2001).

1.1.1.5 Force – Velocity (F/V) relationship

Nonetheless, while it is shown above that length affects force output during isometric contraction; the existence of isometric contraction in everyday scenarios is rare. One other variable that alters force output, is the velocity of the contraction, this relationship is known as the force – velocity (F/V) relationship, which was first constructed with work on isolated fibres by Hill (Hill and Sec, 1938) and Katz (Katz, 1939) and is demonstrated in figure 1.3. One can clearly see there are two regions of this

relationship; shortening and stretching. The shortening region reflects the properties of a concentric (CON) contraction, while the stretching region reflects eccentric (ECC) contractions. The work conducted by Hill investigated the effect of changing shortening velocity on the production of force. As one attempts to move a relatively light weight or load, the contraction will happen at a relatively fast velocity as the force required is low. In fact, if the weight is suitably minimal, i.e. approaching to, or has reached 0, then the velocity of contraction will be at its highest physiologically possible. However, if the weight/load increases, then the force required would increase; therefore, the velocity of contraction will inherently become lower. As the force demands continues to rise, the velocity will continue to decrease until it reaches 0, at which point the contraction is now termed 'isometric'. The definition of an isometric contraction, is that of a contraction that exerts force, without any change in sarcomere dimensions. Significantly, the force produced during an isometric contraction is the maximal possible force that can be produced during a CON contraction.

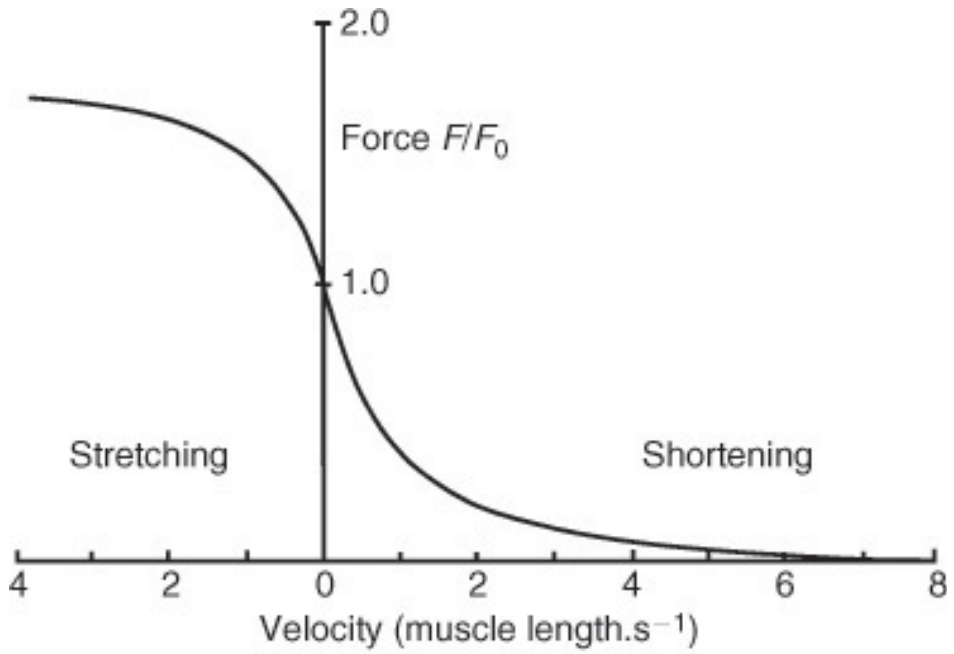


Figure 1.3: Diagram represents Force / Velocity relationship. Taken from (DA Jones, 2004)

While Hill focussed upon shortening, CON contractions, Katz instead focussed upon the muscle's ability to produce tension during stretching. When the external force applied onto the muscle is continually increasing, it will eventually reach a level of which is greater than the muscle's ability to produce force. In this scenario, the muscle will become forcibly lengthened, however it will still be producing force, this is what is known as an eccentric (ECC) contraction. Katz observed that the relative force produced during stretch rose above that of both CON contraction and even that of an isometric contraction. Moreover,

it was also observed that the relative force reached a plateau with increasing velocity, rather than the eventual drop off seen in CON contraction. Significantly, the relative force produced during stretching reached as high as 1.8 times that of isometric contraction (Katz, 1939; Lombardi and Piazzesi, 1990).

1.1.2 Modes of contraction: ECC vs CON

As mentioned above, the relative forced produced during stretching, i.e. an ECC contraction, is 1.8 times greater than isometric contraction *in vitro*. However, when investigated *in vivo* this difference is somewhat lessened, nonetheless, force output still remains 1.2 times that of isometric contraction (Aagaard, Simonsen, Andersen and Magnusson, 2000). Additionally, the force produced by *in vivo* ECC contraction is also superior to CON (Westing *et al.*, 1988). Not only do ECC contractions provide a significantly higher force output than either CON or isometric contractions but they do so with a lower metabolic demand (Hoppeler, 2016; Lindstedt, 2016). The reasoning for these stark differences comes down to the fundamental mechanics of these contractions.

Unlike isometric contractions, CON and ECC contractions always occur with some degree of velocity, as explained by the F/V

relationship (subsection 1.1.2.3) this influences force production. CON contractions will inherently produce less force than an isometric contraction; this occurs as the greater the velocity of contraction, the less cross-bridges formed (Huxley, 1957) and the greater the rate of detachment (Rome *et al.*, 1999). Moreover, during fast shortening contractions there is inherently less time for the myosin S2 complex to attach, resulting in partial extension of this complex. In turn, if the S2 complex is not fully extended, i.e. partially compressed, then the force output will be lower (Figure 1.4A). Thus, if the velocity of contraction is zero, i.e. isometric, not only will more cross bridges be formed, but the myosin S2 complex will be able to fully stretch (Figure 1.4B); the result of these will be higher force output (Huxley and Simmons, 1971). However, ECC force output even exceeds that during isometric contraction. It has been proposed that ECC contraction can produce higher levels of force due to the greater stretch of the myosin S2 complex (Fig 1.4C), even at low velocities. Significantly, while many of the myosin heads will remain attached as the velocity of ECC contraction increases, the cross bridges will continue to be stretched further and inevitably some will be forced to detach from actin. Nonetheless, it is proposed that these recently detached myosin heads will be able to quickly reattach,

maintaining tension and force but significantly doing so at very low metabolic cost (Lombardi and Piazzesi, 1990; Jones, Round and de Haan, 2004). The concept of lower metabolic demand during ECC compared to CON was even demonstrated in early observations by Bigland-Ritche and Wood. They observed that the energy cost during cycling was six-fold greater in shortening contraction (CON) as opposed to lengthening contraction (ECC) (Bigland-Ritchie and Woods, 1976). While the Huxley model goes some way to explain differences in force output during either a CON or ECC contraction; it is still perplexing that the force output during ECC exceeds isometric contraction. If the sarcomere is forcibly lengthened, the myofibrils will be stretched beyond the actin-myosin overlap zone; thus, this suggests the potential involvement of structural proteins to maintain force production (Herzog *et al.*, 2008; Herzog, 2014).

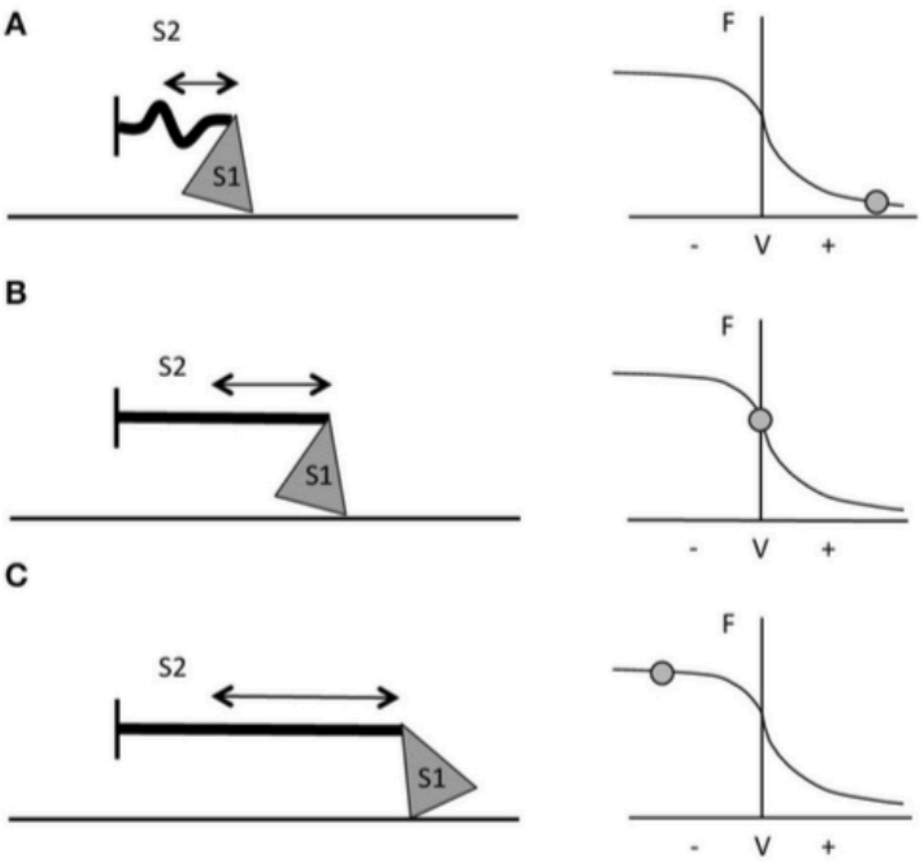


Figure 1.4: Representation of the additional stretch of myosin during eccentric contractions. The figures show the action of myosin during CON contraction (A), isometric contraction (B) and ECC contraction (C). Taken from (Franchi, 2017).

Nishikawa and colleagues proposed the involvement of the giant protein titin within the mechanics of muscle contraction (Nishikawa *et al.*, 2012). This idea was named the "winding filament theory", and it explained titin's active role in muscle contraction. They suggested that titin acts as an internal spring,

which winds upon the actin filament during contraction, consequently storing and releasing elastic potential energy.

1.2 Tendon function

While skeletal muscle has the role of force production, the primary function of tendon tissue is the transmission of force onto bony levers; thus resulting in joint movement, while also providing joint stability (Kvist, 1994). The tendon concentrates a large amount of force, generated from the muscle, onto a small area, which allows the muscle to operate at a distance. Thus, this allows the muscle belly to exist at an optimal distance from the joint, negating the need for an extended length of muscle from origin to insertion (Cardinale, Newton and Nosaka, 2010). The tendon connects to the muscle in an area known as the myotendinous junction (MTJ); it is here where the collagen fibres extend into the muscle body.

Tendon tissue has several other roles, including the retention of energy during locomotion and significantly providing protection to the muscle fibres against sudden external forces, preventing injury. Tendon also has an important relationship with sarcomere length, influencing the muscles operating range (Reeves, 2006), this will be discussed in detail in section

1.3.1.2. The specific roles each tendon play will vary depending on its anatomical position, for example, the Achilles tendon is essential for energy retention, whereas the patellar tendon is responsible for the transmission of high force during movement.

The patellar tendon extends from the inferior pole of the patella to the tibial tuberosity (tubercle), and is responsible for transmitting force generated within the quadriceps muscle onto the tibia. During knee extension, quadriceps force is transmitted onto the quadriceps tendon at the MTJ, which results in the movement of the patella. Consequently, the patella is shifted upwards pulling the patellar tendon resulting in movement of the tibia, facilitating locomotion.

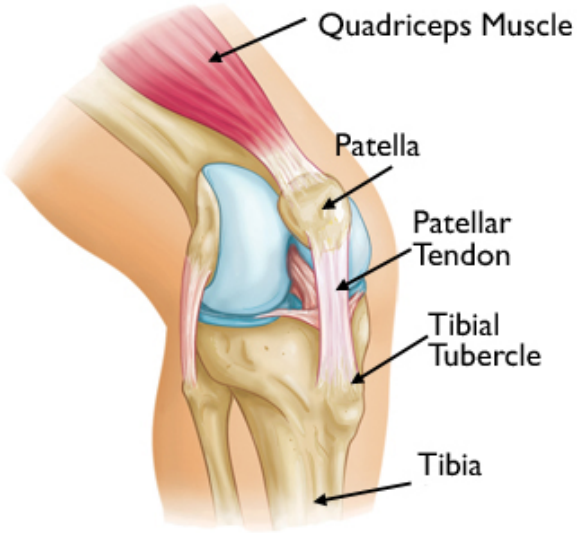


Figure 1.5: Anatomy of the knee and the patellar tendon. Taken from (http://orthoinfo.aaos.org/).

To effectively and efficiently transmit force, the tendon tissue must be capable of withstanding high levels of tensile force, while maintaining a limited degree of elongation. Therefore the tendon does not simply act as an inextensible rigid body, but instead exhibits viscoelastic properties during tension (Johnson *et al.*, 1994). Viscoelasticity is defined as a material (or tissue) which exhibits both viscous and elastic properties while undergoing deformation (elongation) (Butler, Grood and Noyes F R, 1978). It is this specific property of tendon that makes it perfectly suited for its primary role. The viscoelastic properties of tendon are further discussed in 1.2.2.

1.2.1 Tendon structure

Tendons consist of approximately 68% water (Cardinale, Newton and Nosaka, 2010), however, 60-85% of the dry mass is comprised of collagen (specifically tropocollagen), of which type 1 collagen is the most abundant (Kjær, 2004). In addition to collagen, the dry mass also contains elastin, glycosaminoglycans, and tenocytes (Longo, Ronga and Maffulli, 2009a, 2009b). The tendon receives both blood and nerve supply via inlets through the paratenon and consequently the epitenon; the nerve supply is nearly all afferent.

The tendon, like skeletal muscle, is a multi-unit hierarchical structure (fig 1.6). It is composed of small collagen molecules progressing into fibrils, fibre bundles, fascicles and tendon units, which run parallel to the axon of the tendon. The smallest visible structure is the tropocollagen molecule, which is approximately 1.5nm in diameter and 280nm in length. It is here where the characteristic crimping or wavy formation of fibrillar collagen can be seen. In the resting state (i.e. unstrained), tropocollagen molecules are not straight but instead present in a regular sinusoidal pattern. The functional purpose of this crimping is to provide a buffer against small longitudinal elongation.

A collection of tropocollagen molecules constitutes a collagen fibre, which are enclosed by a sheath of connective tissue known as endotenon, binding them together (Kastelic, Galeski and Baer, 1978). Moreover, endotenon allows these fibres to glide

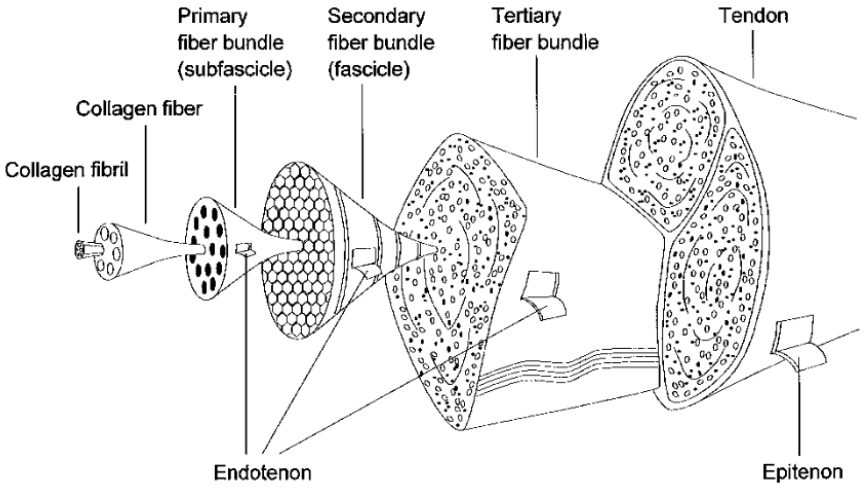


Figure 1.6: The hierarchical structure of tendon tissue, taken from (Kannus, 2000).

over one another during elongation, an essential requirement for tendon tissue during external strain; if not, this would give rise to shear damage. Collagen fibres are surrounded by extracellular matrix (ECM) containing the connective tissue cell, the tenocyte (Wang, 2006), as well as many glycosaminoglycans.

Several collagen fibres consequently form a subfascicle and many subfascicles compile a fascicle (Kannus, 2000). Multiple fascicles collated together, form a tertiary fibre bundle; which is consequently surrounded by endotenon. Finally, a collection a tertiary fibre bundles is termed the 'tendon unit' which is approximately 500μm in diameter. The tendon unit is

surrounded by epitenon, a loose sheath of connective tissue which enables vascular, lympathic and neuronal supply to reach the tendon tissue (Kastelic, Galeski and Baer, 1978). The epitenon is further surrounded by another layer of connective tissue named ‘paratenon’, collectively termed the ‘paratendon’, which acts to reduce the friction against other tissues.

The hierarchical structure of tendon is important for providing mechanical strength and rigidity in the resistance of high levels of tension. In the instance that one microfibril becomes ruptured, the larger fibril unit will maintain the strain and compensate for the individual damaged microfibril. Similarly, if the rupture occurred higher in the hierarchical ladder, the larger tendon subunit would take the additional strain, sharing it amongst its individual smaller subunits. In the case of partial or even full rupture of the tendon, it is often due to a repetitive over straining of the tissue (Lippi, Longo and Maffulli, 2010). During an overstraining scenario, damage will occur to some structures, if insufficient time lapses between exposure to large strain there will be insufficient ‘healthy’ structures to maintain tensions: hence the tendon will rupture.

In addition to the hierarchical structure, orientation of collagen fibres has an impact on both transmission of force and tensile strength. Collagen fibrils are not only aligned longitudinally, but also transversely and horizontally. In addition, longitudinal fibrils cross over one which forms spirals and plaits as discovered via electron microscopy (Chansky and Iannotti, 1991; Jozsa *et al.*, 1991). This multi-orientation structure within tendon tissue is essential to its function, enabling it to withstand external forces of longitudinal, transversal and rotational origin (Kannus, 2000).

1.2.2 Viscoelastic and mechanical function of tendon

As aforementioned, the tendon has viscoelastic properties which allow the tissue to be well suited for its role. The viscosity of a tendon is the ability to resist changing shape in relation to induced elongation, while the elasticity is the tendon's ability to return to its original form once its shape has been manipulated; both of which reflect some of the tendon's biomechanical properties. In order to assess the biomechanical properties of tendon tissue *in vivo*, the combination of ultrasonography and dynamometry is implemented (Maganaris and Paul, 2000; Fukunaga *et al.*, 2001; Magnusson *et al.*, 2008; N. Reeves *et al.*, 2009). The elasticity of the tendon is often considered to be

represented by measures of tendon stiffness, a common biomechanical measure. Stiffness reflects the amount of deformation (elongation) induced by a given load; the value generated represents the amount of force (N) needed to elongate the tissue by 1mm. Stiffness can be calculated as the slope of load/deformation (force/elongation) curve, which is obtained during a ramped isometric contraction (Fig 1.8) whereby stiffness will be higher during lower amounts of elongation for a set amount of force. Another way of considering stiffness is how compliant the tendon is, low stiffness reflects high compliance, and high stiffness reflects low compliance. The stiffness of any particular tendon is dictated by the amount of strain or load, that is experienced. If the tendon is exposed to large loads, it will become stiffer, absorbing less energy making them more efficient and safe at moving heavy loads (Fyfe and Stanish, 1992). In contrast, if the tendon is exposed to low levels of strain, it will become more compliant and absorb more energy (Fyfe and Stanish, 1992).

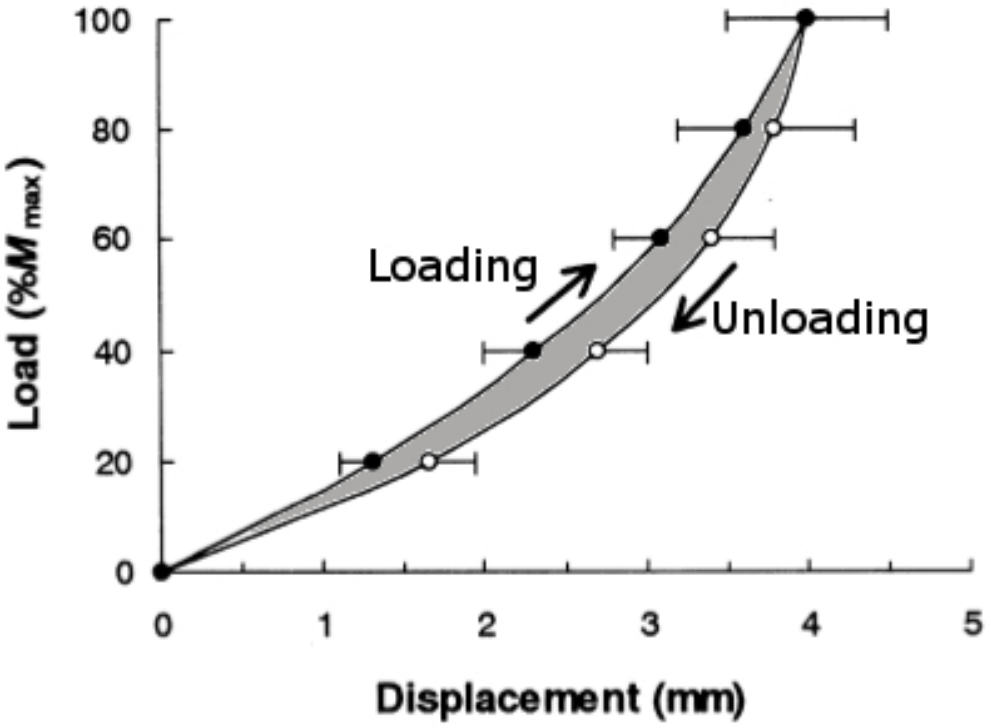


Figure 1.7: The grey area between the loading and unloading curve represents the amount of hysteresis that occurs within the tibialis anterior tendon in vivo. *(Adapted from* Maganaris and Paul, 2000).

However, while measures of tendon stiffness are common place in research, and of course hold importance; it is also essential to consider the tendon stress and strain. Strain is the amount of elongation that occurs in relation to the original length, whereas stress is defined as the amount of force per mm^2. Both strain and stress take into consideration dimensions of the tendon, length or CSA respectively; whereas stiffness doesn't

consider either. Significantly, it is known that the dimensions of the tendon can influence the obtained valued of stiffness. If a tendon is larger in size, i.e. larger CSA, the force is better dispersed through the tendon meaning a larger force can be put through the tendon; therefore this would produce an increase in tendon stiffness. Similarly if the tendon is longer in length, the elongation would be larger, therefore a decreased value of stiffness (Butler, Grood and Noyes F R, 1978). In short, stiffness is directly proportional to the cross-sectional area of the tendon and inversely proportional to its length (Equation 1). Therefore, it is preferable to use a measure of tendon compliance which also considers the tendon dimensions. Thus, in knowing tendon length and CSA, in addition to quadriceps force, it is possible to calculate Young's modulus which fundamentally represents stiffness normalised to tendon dimensions (equation 1). Young's modulus is therefore independent of individual tendon dimensions and gives a true reflection of the biomechanical properties. In plotting a stress-strain curve it is possible to observe the viscoelastic properties of tendon tissue (Fig 1.9).

$$Young's\ modulus = \frac{Quadriceps\ force\ /\ Cross\ sectional\ area}{Elongation\ /\ Original\ length} = \frac{Stress}{Strain}$$

Equation 1.1: The calculation of Young's modulus

While a load-displacement curve will provide values for stiffness from the gradient, it will also allow values of elastic hysteresis to be calculated, provided both the loading and unloading phases of the ramped isometric contraction are obtained. Hysteresis is the amount of energy that is dissipated within the tendon due to internal friction. The degree of hysteresis can be obtained by calculating the area between the loading and unloading curves as shown by Fig 1.8, the greater the area the greater the amount of energy dissipated. Tendon tissue is said to be responsible for 50-60% of the total work done during locomotion (Voigt *et al.*, 1995) due to the storage and release of elastic energy (Alexander, 2002). However this may be more appropriate for spring-like tendon structures such as the Achilles tendon (Magnusson *et al.*, 2008).

Region I is named the toe region of the curve (Butler, Grood and Noyes, 1978), whereby at this stage the curve is concave due to the waviness of the collagen fibre bundles, which become straightened at approximately 1.5% of strain. In region II the tendon begins to show a linear response to stress. Region II may also be known as the elastic region, as during this phase, if the stress were removed the tendon would return to its

original length; this remains true up until 4% of strain. During this linear phase, the gradient of the curve will calculate the Young's modulus. However when the tendon becomes strained above 4%, microfailure of the collagen structures begins to occur (Wang, 2006). During region III, the tendon displays viscous properties, as it will now no longer return to its original length. When levels of strain surpass 8%, macroscopic failure occurs and if the strain continues beyond this point, the tendon is likely to rupture.

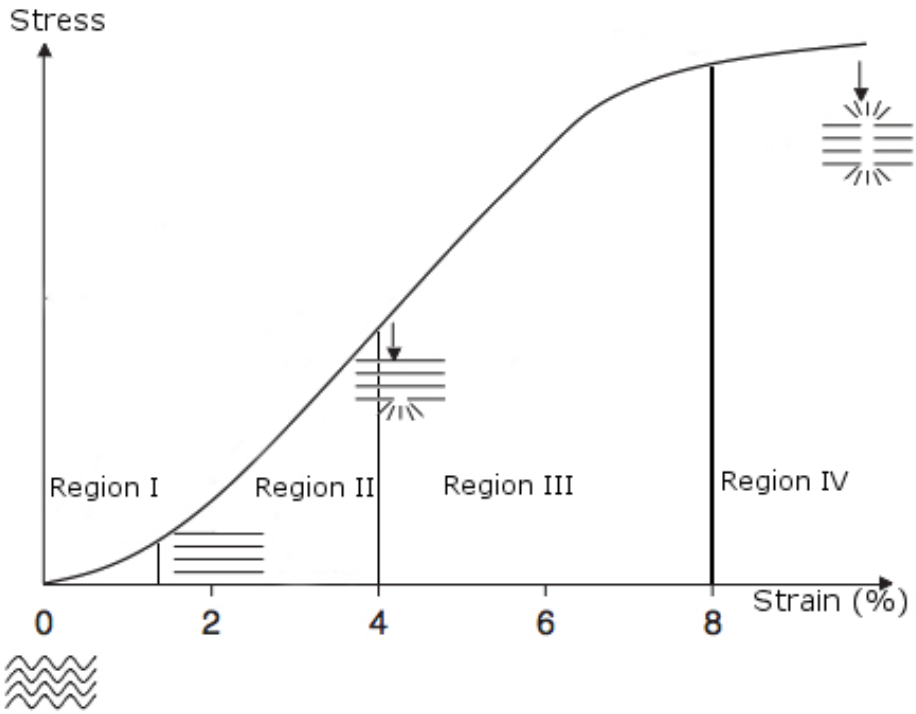


Figure 1.8: The relationship between stress and strain, obtained in vitro, from isolated human tendon collagen fibers. (Adapted from Wang, 2006).

As mentioned in section 1.2.1, if the tissue is consistently taken to this level of strain (overstraining), the individual begins to risk experiencing a repetitive overstrain rupture.

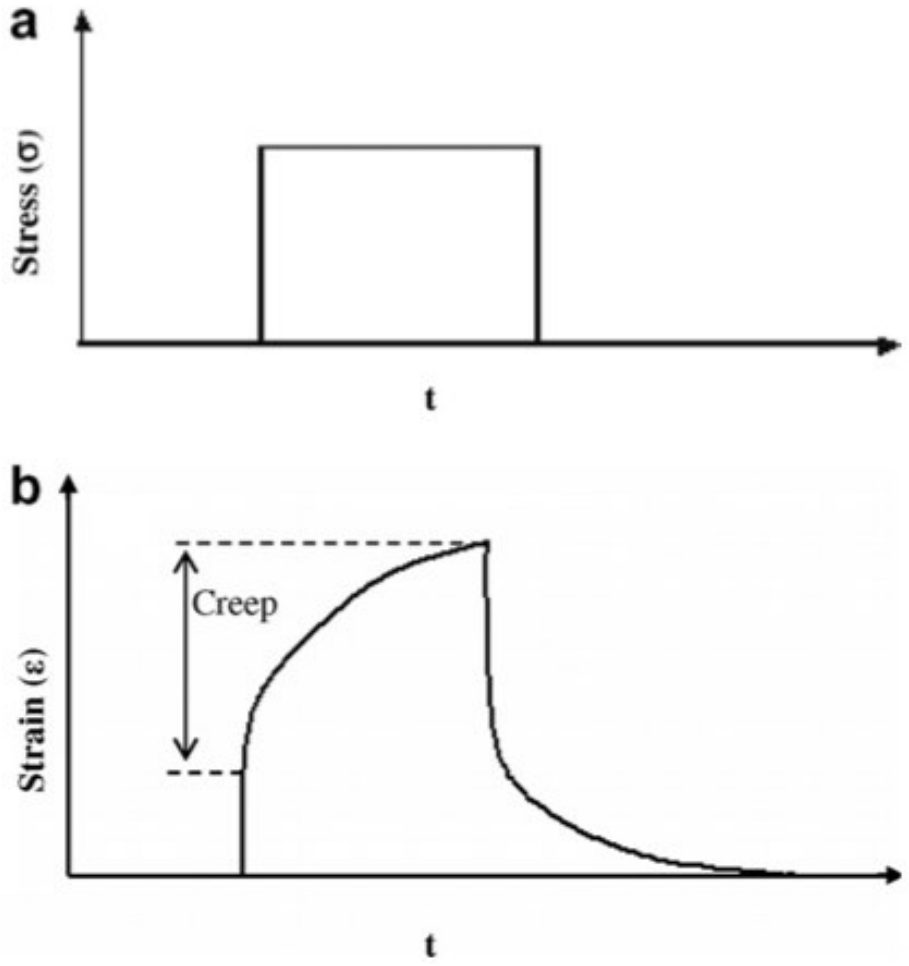


Figure 1.9: The theoretical model of creep. Both (A) and (B) occur simultaneously. (A) reflects the application of a constant stress over time whereas (B) shows that as the duration of contraction increases, non-linear creep begins to occur (Pearson, Burgess and Onambele, 2007).

One final important biomechanical property of tendon tissue is the response observed when a constant load is applied (fig 1.10). Significantly, the tendon deformation develops with time dependent characteristics (Cohen, Hooley and McCrum, 1976;

Pearson, Burgess and Onambele, 2007; Cardinale, Newton and Nosaka, 2010). Moreover levels of strain have been shown to increase in response to consecutive equal force contractions (Maganaris, Baltzopoulos and Sargeant, 2002). This biomechanical phenomenon is known as 'creep' and it presents an important point of consideration for research studies that wish to measure tendon properties.

1.2.3 Tendon turnover

As aforementioned, a large majority of total tendon mass is comprised of water, however approximately 60-85% of the dry mass is collagen; in particular type 1 collagen is the most abundant (Kjær, 2004). The Production of collagen molecules is one of the main aspects of protein turnover within the tendon tissue. The process begins with the translation and synthesis of small pro-α-chains within the rough endoplasmic reticulum (RER) located in the tenocyte. These pro-α-chains consist of approximately 300 repeated Gly-X-Y AA triplets (Ottani *et al.*, 2002), which are essential for the formation of the collagen triple helix in later stages.

Following production of the pro-α-chain, the chain will undergo a host of intracellular, posttranslational modification with the

RER lumen. The first modification is the hydroxylation of both lysine and proline, forming hydroxylysine and hydroxyproline respectively by appropriate enzymatic reactions (Canty and Kadler, 2002). In addition to the above reaction, oxidation of lysine and hydroxylysine results in the production of aldehyde groups by the actions of lysyl oxidase (Eyre, Paz and Gallop, 1984). Additionally enzymatic glycosylation of hydroxylysine residues occurs, resulting in galactosyl- or glucosyl- galactosyl-hydroxylysine (Ottani *et al.*, 2002). Moreover, non-enzymatic glycosylation may occur at this stage causing the occurrence of advanced glycation end products (AGEs). The result of these AGEs is the stiffening of tendon tissue (Bailey, 2001; Couppé *et al.*, 2015). Significantly, AGEs pose a point of interest within diabetes mellitus; whereby it is believed those with type 2 diabetes experience a greater degree of AGEs and hence have stiffer tendon tissue (Couppé *et al.*, 2015).

Following the intracellular modifications, the triple helix structure of a procollagen molecule is assembled. As previously discussed, the repetition of glycine residues allows each chain to coil around one another, forming the triple helix. Procollagen molecules are consequently packed into Golgi vacuoles, which are then transported to the cell surface for exocytosis (Gelse,

2003). Following excretion, cleavage of the terminal carboxypeptides, procollagen type 1 N-terminal propeptide (PINP) and procollagen type 1 C-terminal propeptide (PICP) occurs via the enzyme procollagen peptidase. The cleavage of the terminal peptides allows for the formation of a collagen molecule, followed by assembly into the tropocollagen molecule. Tropocollagen molecules possess a characteristic banding pattern, due to individual collagen molecules being quarterly staggered in orientation (Kannus, 2000). Tropocollagen molecules are initially stabilised with weak polar, hydrophobic and non-covalent interactions. At this stage, tropocollagen molecules are very reactive and undergo spontaneous fibrillogenesis to form the larger collagen fibre structures (Ottani *et al.*, 2002). However, the maturation of collagen results in the spontaneous conversion of the previously immature cross links formed by lysyl oxidase. Two pathways of crosslinking exist within fibrillar tendon, one based upon the lysine-derived aldehyde and the second on hydroxylysine-derived aldehyde. The adult cross-link of the latter involves 3-hydroxypyridinium residues, of which two forms have been identified. The more abundant of the two is named hydroxylysyl pyridinoline (HP) which embodies three hydroxylysines; whereas the less abundant form, lysyl pyridinoline (LP),

embodies two hydroxylysines and one lysine (Eyre, Paz and Gallop, 1984). These intermolecular cross-links are essential for providing stability and strength within the fibrils, whereby like AGEs, an increase in cross-links results in a stiffer tendon.

The above explains the process of collagen synthesis and the construction of fibrils. However, in order for turnover of the tissue, old or damaged tissue must be broken down and replaced. Collagen degradation is primarily instigated through extracellular matrix metalloproteinases (MMPs) (Kjær, 2004). MMP1 and MMP2 are believed to be primarily involved in the degradation of type 1 and type 3 collagen; both of which are abundant in tendon tissue. MMPs are additionally regulated through tissue inhibitors of matrix metalloproteinases (TIMPs) which, as their name suggests, inhibit the actions of MMPs. Thus, the degree or amount of overall tendon turnover is the direct result of the balance between synthesis and breakdown. However, the rate of turnover within tendon tissue is a topic of much debate. There are of course multiple ways to indirectly measure rates of synthesis or breakdown (Langberg, Skovgaard, Karamouzis, *et al.*, 1999; Langberg, Rosendal and Kjaer, 2001). But the most common way to measure tendon turnover is through the utilisation of protein tracers such as ^{13}C

proline (Miller *et al.*, 2005). Basal fractional synthesis rates (FSR) have been reported as low as 0.19%.day^{-1} (Nielsen *et al.*, 2014), or as high as 1.68%.day^{-1} (Nielsen *et al.*, 2011), interestingly these values (of a 10-fold difference) are presented by the same author. However, the choice of amino acid tracer used in each study differed, ^{13}C-phenylalanine and ^{13}C-proline respectively. In theory, the choice of AA tracer should not impact the measured FSR, instead it is likely that there may be analytical or modelling issues which may explain these differences. Nonetheless, the value of 1.68%.day^{-1} is similar to previous research studies which utilised ^{13}C-proline, reporting approximate values of: 1.1%day^{-1} (Miller *et al.*, 2005) and 1.2%day^{-1} (de Boer, Selby, *et al.*, 2007; Doessing *et al.*, 2010; Dideriksen *et al.*, 2013).

1.2.4 Mechano-transduction in the tendon

The way in which mechanical stimuli is sensed and transmitted into cellular signals remains one of the largest debates of tendon research. However, as collagen synthesis occurs within the tendon tenocyte, it is logical to suggest that the tenocyte plays an integral part in transduction of mechanical strain to biomolecular signals. Tenocytes are located around collagen

fibres (Wang, 2006), however they do not exist in isolation; instead they form longitudinal rows between the fibre bundles (Ralphs, Waggett and Benjamin, 2002). Moreover, tenocytes form a cellular network, connected to one another by gap junctions, as shown by the presence of both connexin32 and connexin43 (Benjamin, Kaiser and Milz, 2008) , in addition to cell-cell adhesion via adherens junctions (Ralphs, Waggett and Benjamin, 2002). Thus, these cell to cell connections allow signals to 'flow' through the full network of cells enabling co-ordinated response, such as increased collagen synthesis or structural remodelling.

For mechanical strain to be transferred into gene expression or biochemical signals, the tenocyte must be connected to the ECM in a manner which would allow the transmission of force, but also the detection of strain. The major transmembrane protein contained within the matrix contact is the heterodimeric receptor, integrin (Chiquet *et al.*, 2009). There are a number of matrix contacts that exist, the focal complex, the focal adhesion and the fibrillar adhesion, all of which contain integrins. The two forms of integrins located within the tenocyte are α5β1 and αvβ3, which attach to vitronectin and fibronectin upon the ECM respectively (Chiquet *et al.*, 2009). In order for

positional deformation to be transmitted into biochemical signalling, the integrin receptors require the function of intracellular adaptor molecules, which connect the integrin receptor to the actin cytoskeleton. Activation of the integrin receptor results in the initiation of intracellular signalling cascades such as the MAPK pathway or via NFκB (Chiquet, Sarasa and Fluck, 2003), consequently resulting in the production of transcription factors. This is the most direct mechanism in mechano-transduction, however in-direct pathways exist such as stretch activated Ca^{2+} channels and the auto- and paracrine release of growth factors (Sarasa-Renedo and Chiquet, 2005). It is highly probable, that mechano-transduction involves a combination of multiple pathways, rather than solely relying upon one or another.

1.2.4.1 Role of GH and IGF-1 in the turnover of tendon collagen

One of the most abundantly researched systems is the paracrine and autocrine release of growth factors resulting in induction of gene transcription and consequently protein synthesis (Chiquet, Sarasa and Fluck, 2003; Sarasa-Renedo and Chiquet, 2005). While the tendon is exposed to strain in any and every

movement, exercise provides a large additional bout of strain. Thus, in order to investigate how mechano-transduction occurs in tendon, a multitude of research has explored the concept of exercise induced release of growth factors, primarily the GH/IGF-1 axis.

An immobilization and rehabilitation study observed the effect of GH injections on the structural response of tendon as well as mRNA expression. It was demonstrated that following two weeks of immobilization the control group showed no change in IGF, COL1A1 or COL3A1 mRNA expression, whereas all three were significantly elevated in the GH group (a P. Boesen *et al.*, 2013). This indicates that GH has the potential to not only increase IGF-1, but also result in the increase of COL1A1/3A1 mRNA expression, potentially denoting collagen synthesis. Moreover, GH injections prevented the decrease in stiffness and Young's modulus seen in the control group. Further, while the stimulating effect of GH is shown to result in increases of COL1AL mRNA (Doessing *et al.*, 2010; a P. Boesen *et al.*, 2013), in support of this, FSR values have been shown to be simultaneously elevated (Doessing *et al.*, 2010). While local injection of GH in the tendon is shown to result in eventual collagen synthesis, it is important to consider whether GH has

a direct effect, or whether it is the action of the elevated downstream effector, IGF-1. It has been demonstrated that local injections of IGF-1 resulted in a similar increase of collagen FSR as GH (Hansen *et al.*, 2013; Nielsen *et al.*, 2014). Not all research has been able to correlate increases of IGF to increased FSR rates (Miller *et al.*, 2005). *Miller et al* demonstrated increases of FSR at 72hrs post exercise in the tendon, nevertheless it lacked a significant increase in micro dialysate IGF-1 levels. However, micro dialysate was only obtained at rest and 72hrs post exercise, and was not acquired at 6, 24 or 48hrs, whereby FSR rose and peaked at 24hrs, whereas at 72hrs the FSR values were beginning to decrease. It is therefore possible that IGF release occurs soon after the exercise and induces an increase of FSR before dropping back down to basal levels, while FSR remains elevated. In support of this concept, micro dialysate obtained from achilles tendon has shown raised IGF-1 levels immediately after exercise (Olesen *et al.*, 2007). In addition, collagen synthesis as measured by PICP in tissue micro dialysate didn't become increased until 72hrs where IGF-1 levels had reverted to basal, showing that increased collagen synthesis is not an immediate response. Collectively, these studies demonstrate that local injections of GH or IGF result in increased collagen synthesis, and that

increases of IGF can be seen immediately after exercise. This suggests that mechanical strain may be transduced into increased collagen synthesis via the actions of these growth factors. One final point of interest was alluded to with the study completed by *Doessing et al, 2010.* As previously stated, the study demonstrated that GH injections resulted in both increase COL1A1 mRNA expression and increases in FSRs when measured at rest. However, the study then exercised the participants whereby both FSR and mRNA levels were obtained shortly after. It was observed that there was no additional increase in FSR, or increase in COL1A1 mRNA expression following an acute bout of exercise.

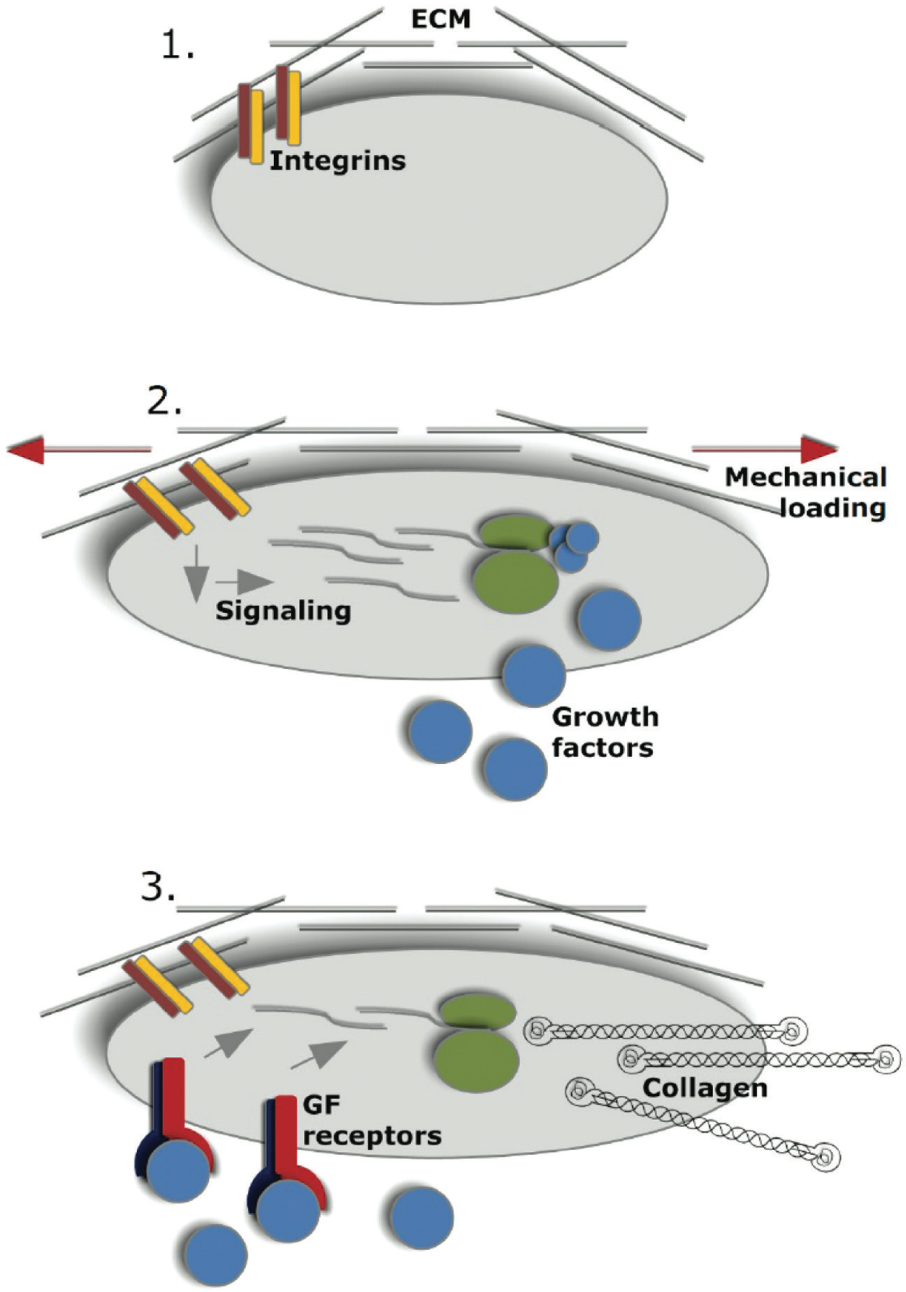


Figure 1.10: Possible mechanism for mechanotransduction, resulting in collagen synthesis: (1) Fibroblast connected to ECM via integrins. (2) Synthesis of growth factors induced by mechanical loading via altered intracellular signaling. (3) The autocrine/paracrine action of growth factors leading to increased collagen synthesis (Heinemeier, 2011)

Significantly if exercise, or more specifically mechanical strain, primarily acted via a different mechanism other than GH or IGF, then one may expect an additional increase in either FSR or mRNA expression. Nonetheless, while the GH injections increased both serum GH levels and tendon tissue mRNA levels of IGF-1, the concentrations of GH and consequent IGF-1 within the tendon were unknown, which of course raises an important issue. Though it is clear that the GH injection results in increased collagen synthesis, whether the increase of tendon IGF-1 is representative of a physiological exercise response is unclear. Moreover, if the IGF-1 levels exceed what would be expected in a physiological response, it may clarify why an exercise bout following the injections didn't elicit a further response. Of course, this has only considered the effect of IGF-1 and it is known that many other growth factors may be involved in mechano-transduction such as TGF-β-1, CTGF and IL-6 (Chiquet, Sarasa and Fluck, 2003; Kjær, 2004; Sarasa-Renedo and Chiquet, 2005; Chiquet *et al.*, 2009).

1.3 The Muscle-Tendon Unit (MTU)

The Muscle-tendon Unit (MTU) is comprised of three principle components; the muscle, the intramuscular connective tissue (IMCT), and the in-series tendon.

While the sarcomere is the contractile element of muscle responsible for producing force, the force must be transmitted via the intramuscular connective tissue (IMCT) and eventually onto the tendon, inducing locomotion. As outlined in subsection 1.1.1, muscle fibres have several layers of connective sheaths which envelop collections of fibres or fascicles. Individual muscle fibre bundles are surrounded by endomysium and many bundles are enclosed by perimysium. The perimysium merges into the epimysium at the muscle surface (Purslow, 2010). The epimysium is continuous with tendon tissue enabling the direct transmission of force from the IMCT to epitenon and hence to the subsequent bone.

Nonetheless, there are two pathways in which the force may be transmitted through muscle. Transmission may be longitudinal along the length of the muscle fibre, or it may be lateral, across one muscle fibre into the neighbouring IMCT network (Huijing, 1999; Zhang and Gao, 2014), allowing for comprehensive force transmission through the IMCT system onto the tendinous tissue. The integrity of the IMCT therefore is essential for effectual force transmission and should the properties decline there would be implications upon joint movement and overall functionality.

1.3.1 Functional implications of the MTU

The MTU comprises sites of both force production and force transmission. Therefore, it is apparent that properties of each component of the MTU can influence overall functionality through influencing both the force-length relationship (subsection 1.3.1.1) and the rate of torque development (subsection 1.3.1.2).

1.3.1.1 Force-Length relationship

As discussed in section 1.1.2.2, the length of the sarcomere dictates the degree of myosin/actin overlap and hence force output (Figure 1.3). However, there are other features within the MTU which may influence the operating length of the sarcomere. One property of the MTU that is believed to affect the operating range of the sarcomere, is the stiffness of the attached in-series tendon (Hawkins and Bey, 1997). If one was to consider a MTU which operated in the ascending limb of the F/L relationship whereby all variables, other than tendon stiffness, remained constant; then a decrease in tendon stiffness would increase the degree of myosin/actin overlap through a leftward shift of the F/L relation (Figure 1.12A). Whereas, an increase in tendon stiffness would result in a

rightward shift of the sarcomere in the F/L relation and consequently an increased sarcomere length (Figure 1.12B).

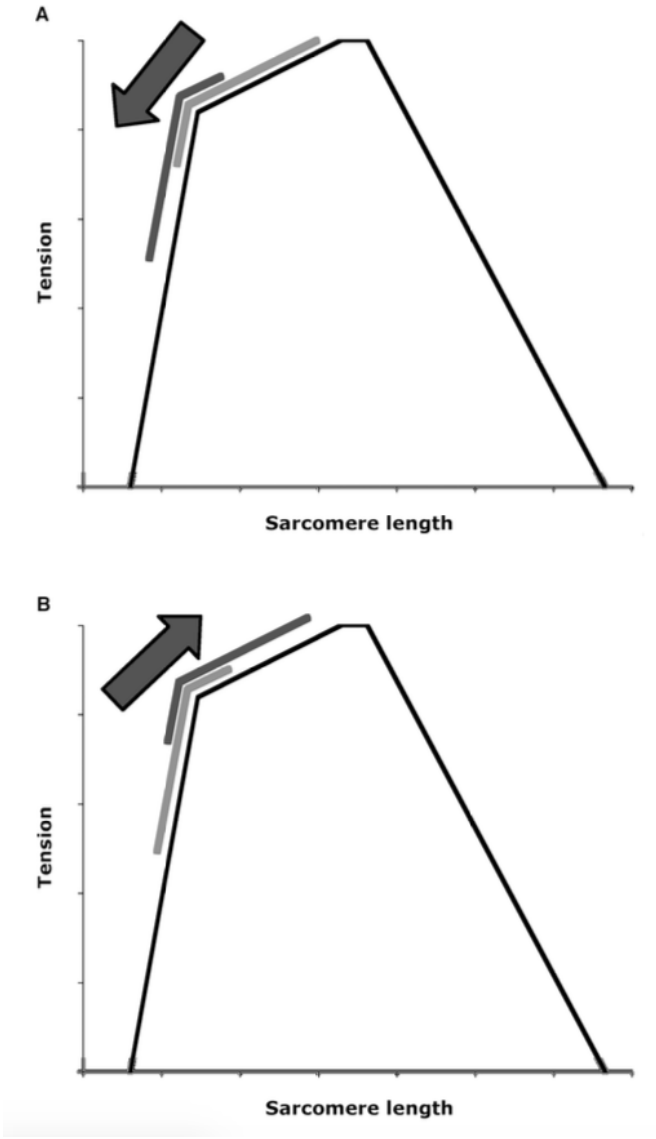


Figure 1.11: The proposed alterations in the F/L relationship in response to reduced tendon stiffness (A) or increased tendon stiffness (B) (Reeves, Narici and Maganaris, 2006).

In the scenario, whereby tendon stiffness is reduced, and hence a leftward shift occurs then this is shifting the muscle's

operating range away from the optimal region; thus reducing force production (Reeves, 2006). However, tendon stiffness is not the only property of the MTU that could affect the F/L relationship; the number of sarcomeres in series is also able to influence this relation. If fewer sarcomeres exist within a given fascicle length (FL), then the degree of shortening for each sarcomere will be relatively less (fig1.13A). Conversely, if there is a greater number of sarcomeres in series within the given FL, then each individual sarcomere must shorten relatively more for the muscle to shorten (fig1.13B). (Reeves, Narici and Maganaris, 2004b). In the first example, i.e. less sarcomeres and hence less shortening, one would observe a rightward shift of the sarcomere F/L relationship. Whereas, in the second example, one would observe a leftward shift of the sarcomere F/L relationship. Nonetheless, as with any scenario, one should consider the whole MTU rather than one individual component. This is such, that changes within the tendon (i.e. stiffness) and changes within the fascicle (i.e. number of sarcomeres in series) may act to cancel one another out; resulting in no alteration in the muscle's operating range and hence functional output.

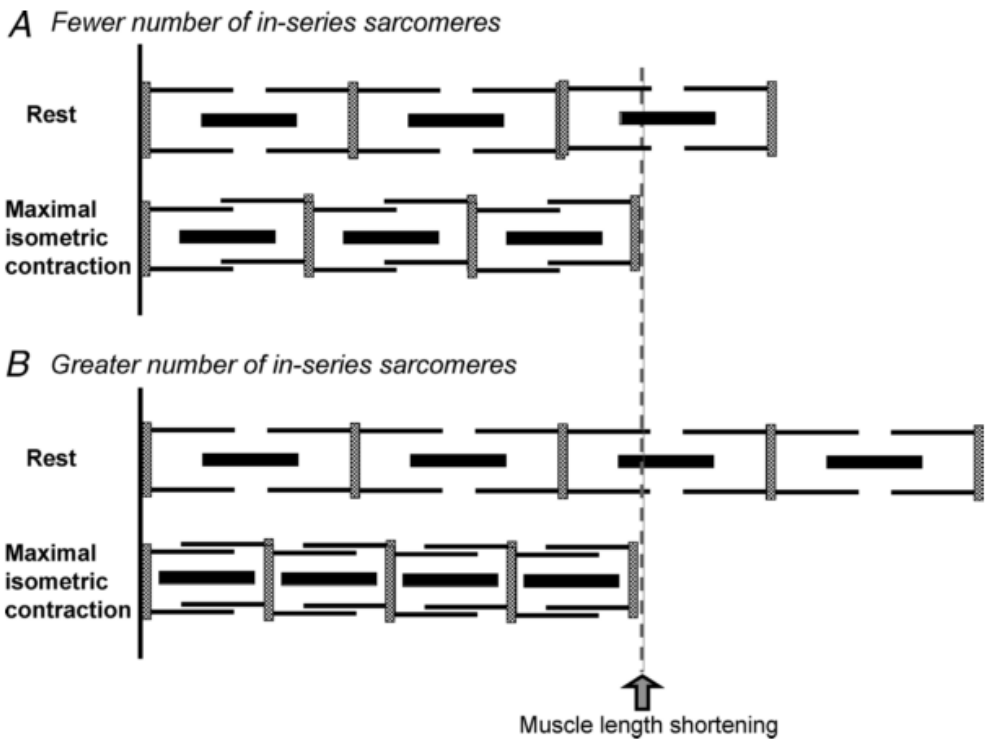


Fig 1.13: The influence of sarcomeres in series upon sarcomere length during contraction. (A) With fewer sarcomeres in series there is suitable overlap of myosin and actin, whereas (B) shows greater in series sarcomeres, and the resultant double overlap, and hence reduced force. Taken from (Reeves, 2004).

This can be observed within the elderly, it is believed they have reduced tendon stiffness (Onambele, Narici and Maganaris, 2006) and a loss of sarcomeres in-series (Narici *et al.*, 2003); therefore the first would cause a leftward shift and the second would cause a rightward shift of the muscle operating range. Thus, the resultant F/L relation and operating range of the

muscle would be unaltered, which has been observed by Reeves (Reeves, Narici and Maganaris, 2004b).

1.3.1.2 Rate of torque development (RTD)

As discussed at the beginning of this subsection, the maintenance of the MTU is essential for preserving functionality. The previous section discussed how properties of the MTU can affect the production of force and the muscle operating range. However, another aspect which is also reliant on a healthy MTU, is the rate at which force is produced and transmitted. While absolute force production is essential for undertaking daily activities, if one considers correcting a trip and hence preventing a fall, the rate of force development and transmission is equally as important as absolute force production. In order to counteract a trip, force production must occur extremely rapidly (<200ms) (Pijnappels, Bobbert and Van Dieën, 2005).

RTD is defined as the slope of the torque-time curve obtained during an isometric contraction (Andersen and Aagaard, 2006). Thus, to alter RTD, there must be a change in absolute force or in relative time taken to reach said force. There are many factors originating from the MTU, which are known to influence

RTD (Bojsen-Møller *et al.*, 2005; Gerstner *et al.*, 2017). From a muscular aspect, muscle size, myosin heavy chain (MHC) composition and maximal strength are all known to influence RTD (HÄkkinen and Komi, 1986; Harridge *et al.*, 1996; Van Dieën, Pijnappels and Bobbert, 2005; Gerstner *et al.*, 2017). One should also recognize neuronal factors such as motor unit firing frequency (Aagaard *et al.*, 2002) and rate of EMG rise (Pijnappels, Bobbert and Van Dieën, 2005), as these both influence the RTD. Moreover, it has long been suggested that connective tissue stiffness can influence RTD (Wilkie, 1949); stiffer connective tissue will require less time to be stretched and hence increase RTD. This concept is further supported with work by Bojsen-Møller in which they observed correlation between RTD and stiffness of the aponeurosis complex (Bojsen-Møller *et al.*, 2005). While stiffer connective tissue will reduce the time for force transmission, as mentioned in 1.3.1.1, tendon stiffness may also influence absolute force production.

Therefore, multiple components of the MTU (muscle, IMCT and the in-series tendon) influence the rate of force production and transmission and hence funtionality.

1.4 Adaptation of the MTU

As the MTU is responsible for the production and transmission of force, it is logical to suggest that changes in activity, through exercise, age or inactivity, can influence the overall functionality of the MTU. The functionality of the MTU relies upon the integrity of all components; thus, exercise and ageing may influence factors originating from both muscle and/or tendon. Therefore, the following subsections 1.4.1 and 1.4.2 will discuss the effect of exercise and ageing on the MTU, respectively.

1.4.1 MTU response to exercise

Through exercise, there is an increase in activity over what is habitual achieved. Consequently, the MTU is often required to produce more force. Thus, as activity is increased and the amount of force is either greater or more frequent, the MTU must be able to withstand and transmit this greater amount of overall strain through the connective tissue. Within this subsection the way in which the tendon, muscle and IMCT (respectively) adapt to an increased demand will be discussed.

1.4.1.1 Tendon responses to exercise

Tendons have been historically considered to be a relatively inert tissue, however it is now accepted that tendon tissue respond to increases in mechanical loading (Svensson *et al.*, 2016). Tendinous tissue has been demonstrated to adapt to mechanical stimuli in a multitude of studies. These adaptations include increases in tendon stiffness (Reeves, Narici and Maganaris, 2004b; Couppé *et al.*, 2008), increases in Young's modulus (Malliaras, Kamal, *et al.*, 2013), reduced hysteresis (Reeves, Narici and Maganaris, 2003), hypertrophic response in the form of increased tendon CSA (Kongsgaard *et al.*, 2007), increased acute collagen type 1 synthesis (Miller *et al.*, 2005) and increased cross linking (Couppé *et al.*, 2014). All of these modifications result in altered mechanical, structural and metabolic properties of the tendon tissue. However these alterations are not seen consistently in the literature, with many studies demonstrating a lack of response (K Kubo *et al.*, 2003; Kongsgaard *et al.*, 2007; Grosset *et al.*, 2014; Kubo *et al.*, 2017). This subsection will be further split into topics to address biomechanical changes, structural alterations and finally acute responses to exercise.

1.4.1.2 Impact of exercise and training on tendon biomechanical properties

Mechanical adaptation is defined as alterations in the biomechanical properties of the tendon tissue such as: tendon stiffness, stress, strain, Young's modulus or hysteresis. Tendon stiffness is perhaps one of the most important aspects to consider, as it is shown to increase following a large variety of training stimuli (Reeves, Maganaris and Narici, 2003; Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009a, 2011; Arampatzis *et al.*, 2010; Malliaras, Kamal, *et al.*, 2013; Grosset *et al.*, 2014; Kubo, Ishigaki and Ikebukuro, 2017); demonstrating that the tendon is very responsive to exercise. Moreover individuals who habitually load one extremity more than the other, through sports such as badminton or fencing, demonstrate an increased stiffness in the lead extremity (Couppé *et al.*, 2008). This fact demonstrates that tendon stiffness is highly responsive to the strain transmitted through the tissue. While individuals who play either badminton or fencing are still utilising both legs during their sport (i.e. to say both patellar tendons are being stretched), more strain is put through the 'leading' leg, resulting in a stiffer tendon. One limitation associated with measures of tendon stiffness is that it doesn't take into consideration the dimensions such as CSA and

length of the tissue. However even once this limitation was corrected, in the form of Young's Modulus, the same conclusions within each study remained.

However other studies have found that this change in stiffness in response to exercise doesn't always occur (Rosager *et al.*, 2002; K Kubo *et al.*, 2003; Kongsgaard *et al.*, 2007; Kubo *et al.*, 2007, 2017; Fouré, Nordez and Cornu, 2010; Boesen *et al.*, 2014). To understand why these adaptations are not seen in every circumstance, it is essential to probe further into the experimental protocols applied. Each of these research studies implemented a low intensity exercise-training programme in contrast to higher intensity strength training protocols, which demonstrated changes in tendon stiffness. Kubo et al, in 2003 implemented body weighted squat, Fouré et al in 2010, Kubo in 2003, 2007 and 2017 studied the effect of plyometric exercise and Rosager in 2002 studied long distance running. In fact, the study completed by Kubo in 2007, directly compared the effect of high intensity RE (80% 1RM) and plyometric training. As previously dicussed, no change was observed in those completing plyometric exercise, but a positive change was observed in the RE group. Therefore, these studies suggest that either the intensity of training dictates the degree of mechanical

adaptation, or that, perhaps it is the overall cumulative (volume of) exposure to strain which results in a positive change in tendon stiffness, whereby a dose-response scenario occurs. Thus, a higher intensity of training would provide a greater amount of strain in a shorter period. However, the second possibility still relies upon the fact that the strain applied through the exercise is sufficient. It has been shown that long distance running has no affect upon the stiffness of the tendon when compared to age matched, untrained controls (Rosager *et al.*, 2002). Thus, although the cumulative strain is relatively high in long distance training, the strain applied in every stride or training session is still relatively low. Additionally, Kongsgaard investigated the effect of high load (70% 1RM) versus low load, matched for total work (Kongsgaard *et al.*, 2007). Within the high load group, individuals completed 10 sets of 8 repetitions, whereas the low load group completed 10 sets of 32 repetitions, utilising a load so that the total work, or strain applied to the tendon, was equal between the two groups. Thus, the cumulative strain applied was equal, but the intensity used was either high or low. They found that tendon stiffness was elevated post intervention in the high load group only. This concept of sufficient force (or strain) being applied, is further supported by the fact that tendon stiffness undergoes the

greatest degree of adaptation within the higher force region of the force-elongation curve (Reeves, Maganaris and Narici, 2003). Moreover, it is also important to consider the length of time which the tendon is placed under strain during each contraction. Arampatzis and colleagues in 2010 investigated the effects of strain duration upon the adaptations of tendon stiffness (Arampatzis *et al.*, 2010). It was observed that a higher strain duration per contraction resulted in superior changes in tendon stiffness. Therefore, taken collectively it suggests that both intensity of training is important, as is the cumulative strain. Firstly, the training must first be over a specific intensity threshold, but secondly the cumulative strain must then be sufficient.

Significantly, several studies have attempted to clarify whether exercise intensity is a true influential factor in regards to the degree of tendon adaptation (Arampatzis, Karamanidis and Albracht, 2007; Malliaras, Kamal, *et al.*, 2013; Grosset *et al.*, 2014). Both Arampatzis and Grosset demonstrated that low levels of strain (or low training intensity) had no effect upon tendon mechanical properties, whereas their higher strain (or high training intensity) groups demonstrated positive changes in stiffness and or Young's Modulus. Malliaras and colleagues

however, investigated standard load ECC training (80% of CON-ECC 1RM) and high load ECC training (80% of ECC 1RM) as opposed to comparing to a much lower training intensity. They observed that both intensities caused an increase in tendon stiffness and Young's modulus, however the higher intensity group responded greater (+84.3% vs +59.4% and +87.3% vs +59.4% for stiffness and Young's Modulus respectively). However, while the former two studies (Arampatzis 2007 and Grosset 2014) focussed purely upon the level of intensity, Malliaras also investigated the difference of training modalities (i.e. CON vs ECC) on patellar tendon stiffness. In addition to the standard ECC and high load ECC groups, Malliaras also included standard CON training (80% of CON 1RM). Interestingly, the CON group demonstrated higher levels of tendon stiffness adaptation than low load ECC (80.7% vs 59.4%). Nonetheless, high load ECC had the largest change (+84.3%) and was the only group to have higher values than controls post study. One large limitation of this study remains that contractions were not pure CON or ECC. Concentric groups were instructed to use two legs to lower the weight, and eccentric groups to use two legs to raise the weight, before unilaterally raising or lowering the weight respectively. A recent publication by the Kubo group, also investigated the difference in CON and ECC training in

regards to patellar tendon stiffness. In contrast to Malliaras, they observed that only CON training and not ECC resulted in an increase in tendon stiffness following 12 weeks of unilateral training (Kubo *et al.*, 2017). The study utilised similar training loads to that of Malliaras, i.e. 80% of CON-ECC 1RM for both training modalities, thus making the ECC group of Kubo comparable to the lower intensity ECC group of Malliaras. However, this may present some errors as to truly investigate the effect of contraction mode, one should use 80% of CON 1RM for CON training and 80% of ECC 1RM for ECC training (as is with Malliaras and the high intensity ECC group). Nonetheless, these contradicting data only cloud the water. It remains to be truly answered as to whether contraction type differentially influences tendon biomechanics. Additionally, while it is relatively well established that low loads (<40%) do not induce changes, and high loads (>75% 1RM) do; whether submaximal loads (i.e. 60% 1RM) induce positive changes in tendon biomechanics still needs to be addressed.

Another measure of tendon biomechanical properties is the elastic hysteresis. This mechanical property has been shown to decrease following both strength training and plyometric training (Reeves, Narici and Maganaris, 2003; Fouré, Nordez

and Cornu, 2010). This would act as a benefit to individuals as less energy is dissipated within the tendon and consequently increases the store of elastic energy. However, this particular property of tendon tissue is more appropriate for energy storing tendons such as the Achilles tendon as opposed to the large force transmitting tendons such as the patellar (Kjær, 2004).

1.4.2 Impact of exercise and training on tendon structure

As demonstrated above, there is a large body of research which have shown a positive change in tendon biomechanics in response to exercise. However, it is also important to consider whether any hypertrophy of the tendon has occurred. From a functional point of view, a greater tendon CSA would facilitate a reduction in stress, as loading would be distributed over a larger surface area and thus in theory this could protect against strain injury (Magnusson and Kjaer, 2003; Magnusson *et al.*, 2003). Nonetheless, whether the tendon responds to a relatively short training regime in a hypertrophic fashion is somewhat debated.

Many studies demonstrate that RE intervention is incapable of inducing any changes in tendon CSA (Reeves, Maganaris and

Narici, 2003; Maganaris, Narici and Reeves, 2004; Malliaras, Barton, *et al.*, 2013; Grosset *et al.*, 2014). Conversely, other intervention studies have demonstrated that the tendon does indeed undergo hypertrophy in response to RE (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009a; a P. Boesen *et al.*, 2013). It is therefore confounding as to why some of the above resistance training studies altered tendon CSA, while others did not. These observations should be irrespective of total strain exposure as they were all operating at similar levels of intensity (70-80% 1RM) and for similar durations (9-14wks). The difference in results between these studies could be due to the methods in which the measurement for tendon CSA was obtained. Those who did not observe changes in CSA averaged CSA across multiple locations (proximal, mid point and distal); thus, taking into consideration regional differences. Whereas, the studies which found changes in tendon CSA, recorded individual values from one location. This is significant, as it has been observed that tendon hypertrophy occurs with regional specificity (Kongsgaard *et al.*, 2007; Couppé *et al.*, 2008; Seynnes *et al.*, 2009a). In particular, it was noted that the largest adaptation in tendon CSA occurred within the proximal location, with similar adaptation seen at the distal insertion. However, the hypertrophic response was absent within the mid tendon

location. It is speculated that adaptation occurs within these insertion locations due to the increased local compressive loads that they experienced (Kongsgaard *et al.*, 2007).

While the effect of RE on tendon CSA is unclear, there is a large body of evidence which has investigated the effects of habitual activity. It is very strongly indicated that habitual loading induces tendon tissue hypertrophy (via measured CSA) (Rosager *et al.*, 2002; Magnusson and Kjaer, 2003; Kongsgaard *et al.*, 2005; Couppé *et al.*, 2008, 2014). Within these studies it is shown that continual and repetitive low intensity straining of tendon tissue through sports such as running, badminton or fencing, induces an increase in tendon CSA. This concept of habitual loading suggests that to induce tendon hypertrophy with lower intensity exercise, there must be a repetitive and continual strain of the tendon tissue over many years, even decades. Research by Hansen in 2003 observed that 34 weeks of habitual running was insufficient to alter tendon CSA (Hansen *et al.*, 2003), further supporting the concept that if the loading is of low intensity, it must occur for a sufficient length of time. Couppé and co-workers presented further support of this idea in 2014. It was demonstrated that master athletes (MA) (those who had been running for an average of 28 years), had a higher

PT tendon CSA than age matched controls (Couppé *et al.*, 2014). Moreover, the MA tendon CSA was higher than younger athletes (YA) with 6 years of running experience and younger age matched controls. Additionally, there was no significant difference between the tendon CSA of YA and aged match controls. Taken collectively this demonstrates that alterations in tendon CSA using a repetitively low strain exercise requires prolonged exposure (many years), to accumulate a total magnitude of strain.

However, in consideration of both structure and biomechanical changes, one point of interest emerges; while tendon CSA is often increased following habitual running, tendon stiffness can be unaltered (Rosager *et al.*, 2002). In fact, in runners, patellar tendon stiffness has been found to be lower (Kubo *et al.*, 2010) which differs to those who have engaged in RE, whereby stiffness is higher. Thus, perhaps the type of exercise, or more simplistically, the job of which the tendon is required to do dictates the observed adaptation. If the tendon is exposed to low but repetitive strain (as in running), then the change in CSA is of high importance; an increased CSA would reduce the risk of strain injuries often associated with repetitive sports (Magnusson and Kjaer, 2003; Magnusson *et al.*, 2003).

Whereas, in RE, the tendon is required to and transmit large amounts of force and hence is strained to a large degree. Thus, stiffness is of high importance to prevent rupture of the tendon. This idea of specific adaptation is also supported by the concept that tendons from different anatomical locations also demonstrate different biomechanical and structural properties (Kjær, 2004).

1.4.3 Response of the tendon to acute exercise: Metabolism and mRNA

Adaptations in tendon biomechanical and structural properties represent the accumulation of acute responses to individual training bouts over a chronic period. One acute response which is often focussed on within research is collagen synthesis. There are many analytical methods that may be implemented to assess the amount of collagen synthesis that occurs in response to exercise. The measure of PINP/PICP (Langberg, Skovgaard, Petersen, *et al.*, 1999; Langberg, Rosendal and Kjaer, 2001), changes in mRNA expression (Sullivan *et al.*, 2009; Heinemeier and Kjaer, 2011) and the application of labelled tracers (Babraj *et al.*, 2005; Miller *et al.*, 2005) all provide data to support conclusions of collagen synthesis; each of these techniques will be addressed in turn. PINP/PICP levels within the tendon tissue

can be obtained using micro dialysis. While this would allow one to speculate as to the amount of collagen turnover it remains an indirect measure. One assumes that the immature procollagen, from which PINP/PICP is cleaved, is subsequently incorporated into the tendon structure, which is not always the case. Nonetheless, multiple studies have demonstrated that exercise induces an increase in either PINP or PICP obtained from tendon micro dialysis (Langberg, Skovgaard, Karamouzis, *et al.*, 1999; Langberg, Rosendal and Kjaer, 2001; Olesen *et al.*, 2007; Christensen *et al.*, 2011). However, it should be reiterated that this technique indirectly measures collagen synthesis. A limitation of this it that the application of a microdialysis needle itself is able to induce an increase in PINP/PICP (Olesen *et al.*, 2007). Furthermore it has been shown that increases in PINP/PICP have been absent during measurable increases in fractional synthesis rate (FSR) as measured using labelled AA tracers (Miller *et al.*, 2005, 2007; Hansen, Kongsgaard, *et al.*, 2009).

The second widely used approach is measures of mRNA expression within tendon tissue. Similar to PINP/PICP, increased mRNA expression only indicates towards alterations occurring within the tissue and does not provide a direct

measure of protein synthesis. Nonetheless, mRNA helps identify the genetic drivers behind the biomechanical changes. It has been demonstrated that the levels of Col-1A mRNA expression do not increase following either 1hr of leg kicking (Heinemeier *et al.*, 2013) or with resistance training (Sullivan *et al.*, 2009). However, in contrast, there others present data to support an increase in Col-1A mRNA expression following acute exercise (Dideriksen *et al.*, 2013). While Dideriksen saw an increase in Col-1A mRNA expression no significant increase in FSR was observed. In order to establish concrete conclusions from increases in mRNA expression, one would also expect to observe an increase in FSR. Thus, it is possible to speculate as to which genes are controlling the regulation of collagen synthesis. Direct measures of collagen type 1 FSR may be obtained via the infusion of stable isotopically labelled tracers such as ^{13}C-proline. Unfortunately however, only one research study exists which demonstrates an increased tendon FSR in response to acute exercise (Miller *et al.*, 2005), while several others demonstrate no change (Miller *et al.*, 2007; Hansen, Miller, *et al.*, 2009; Dideriksen *et al.*, 2013). However, like measures of tendon CSA, it is essential to consider the location from which the tendon sample is obtained. Many of the studies which observed no change in mRNA expression were obtained

from a medial location (Hansen, Miller, *et al.*, 2009; Sullivan *et al.*, 2009; K M Heinemeier *et al.*, 2013). Moreover, it is known that core tendon tissue has very little turnover, as revealed by nuclear bomb ^{14}C-carbon labelling (Heinemeier *et al.*, 2013). The data regarding tendon synthesis in response to acute exercise is somewhat ambiguous. Thus, this remains one of the questions that needs to be addressed. Additionally, whether tendon FSRs are altered over a chronic exercise intervention is also unknown, i.e. does the tendon become accustomed to mechanical stimuli.

1.4.3.1 Muscular changes in response to exercise

Human skeletal muscle is renowned for its remarkable ability to rapidly adapt to increasing mechanical loads (Atherton and Smith, 2012). However, it is known that the type of contraction (CON vs ECC) may induce differential adaptation (Franchi, Reeves and Narici, 2017). Regardless of the contraction stimuli, one irrefutable adaptation to RE is muscle hypertrophy (Anthony J Blazevich *et al.*, 2007; Seynnes *et al.*, 2009b; Franchi *et al.*, 2014). This increase in muscle mass is often accompanied by changes in muscle function such as strength (Schneider and Bishop, 2003), MVC (Seynnes, deBoer and Narici, 2007) and RTD (Aagaard *et al.*, 2002). Significantly, the

way in which the new contractile tissue is added can alter the muscle architecture (Narici, Franchi and Maganaris, 2016). These changes are brought about by cumulative responses to each individual RE bout, which accumulate over time.

Muscle size and functional changes in response to exercise

Hypertrophy of skeletal muscle occurs in response to RE due to the chronic incidence of increases in muscle protein synthesis (MPS) (Narici *et al.*, 1996; Wilkinson *et al.*, 2014). The accretion of new contractile material generated from these individual peaks in MPS causes an increase in individual fibre dimensions, leading to changes in muscle CSA and eventually muscle volume (Ahtiainen, Pakarinen and Alen, 2003). There are many methods in which one can quantify muscle growth; individual fibre CSA, whole muscle CSA via MRI or CT scans or muscle thickness via ultrasonography.

Unfortunately, the time course of muscle hypertrophy in response to RE is not greatly understood. While earlier studies suggested hypertrophy occurred in later stages of RE intervention (Abe, Dehoyos and Pollock, 2000), there is growing evidence that hypertrophy can occur after as little as 3-4 weeks

of RE (Seynnes, deBoer and Narici, 2007; B. M. Baroni *et al.*, 2013; Brook *et al.*, 2015). Interestingly however, it has been shown that the level of hypertrophy appears to diminish over time (Ogasawara and Yasuda, 2013; Brook *et al.*, 2015); thus, perhaps suggesting that the majority of muscle hypertrophy happens in the relatively early stages.

Lengthening contractions have the potential to generate considerably more force than those generated in shortening. Thus, it has commonly been believed that as one could train with heavier weights, more hypertrophy could therefore be obtained (Roig *et al.*, 2009). However, when contraction phases are separated into CON only and ECC only training a clearer picture emerges; there is no difference between contraction modes in regards to overall hypertrophy. Many studies have shown that there is no difference in muscle thickness (Cadore *et al.*, 2014; Franchi *et al.*, 2015), CSA growth (Moore, Young and Phillips, 2012; Farup *et al.*, 2013) or in overall muscle volume (Anthony J Blazevich *et al.*, 2007; Franchi *et al.*, 2014).

Hypertrophy inherently leads to the possibility of higher force output as there are greater amounts of contractile material.

However, hypertrophy is only one factor contributing to force output; thus, strength increases are often greater than increases in muscle mass (Jones and Rutherford, 1987). Other factors that influence MVC include an increase in neural drive or changes in the F/L relationship due to muscle architecture or alterations in the stiffness of attached tendon. Interestingly, while RE is well reported to induced increases in MVC, ECC RE (when performed at higher intensities) stimulates greater strength gains (both CON and ECC strength) than CON RE (Roig *et al.*, 2009; Isner-Horobeti *et al.*, 2014; Baroni *et al.*, 2015).

While strength is an important functional characteristic in regards to RE, RTD is equally as important as it also has functional implications. RTD can be assessed over a variety of given time periods (Maffiuletti *et al.*, 2016), and thus is a measure of the rate at which force is produced over a given time. Therefore, alterations affecting either total force production or the speed of contraction will inherently increase or decrease the value of RTD. Many studies have shown RTD to be positively affected by RE (Aagaard *et al.*, 2002; Reeves, Narici and Maganaris, 2003; Suetta *et al.*, 2004; Caserotti *et al.*, 2008; Andersen *et al.*, 2009). This is perhaps unsurprising, as aforementioned RE can increase the force producing

capabilities and increase contraction velocity (through changes in fascicle length and changes in tendon stiffness).

Muscle architecture adaptation in response to exercise

Muscle architecture is known to influence the functional properties of the muscle, including force output and contractile velocity (Narici, 1999; Narici, Franchi and Maganaris, 2016). Significantly, it is well reported that muscle architecture adapts in response to mechanical loading through RE (Aagaard *et al.*, 2001; Blazevich *et al.*, 2003; Anthony J Blazevich *et al.*, 2007; Potier, Alexander and Seynnes, 2009; Franchi *et al.*, 2014; Ryan G. Timmins *et al.*, 2016). Conventional RE (consisting of both CON and ECC phases) has been shown to result in increases to fascicle length (FL) (Narici *et al.*, 1996; Alegre *et al.*, 2006) and also increases in pennation angle (PA) (Baroni *et al.*, 2015). However, there is a growing body of evidence to demonstrate that CON and ECC contractions result in distinctly different structural adaptations (Franchi, Reeves and Narici, 2017). Thus, like tendon biomechanics, it is important to consider each contraction mode (i.e. CON and ECC) as a single modality; in doing so, one may gain a more accurate understanding of architectural adaptation.

When considering ECC only RE, the main outcome is an increase in LF (Duclay *et al.*, 2009; Potier, Alexander and Seynnes, 2009; Bruno Manfredini Baroni *et al.*, 2013). It is believed that an increase of FL is the direct result of the addition of sarcomeres in series, at the ends of the fascicles. The addition of contractile material in this manner has been hypothesised as a protective mechanism against ECC induced muscle damage (Morgan and Talbot, 2002). One of the main alterations in response to conventional RE is an increase of PA. However, conventional training is predominately limited by the CON portion; unsurprisingly, CON only RE also results in a change positive change in PA (Reeves *et al.*, 2009). Increases in PA are the result of sarcomeres have been added in parallel. This is a strategy to pack more contractile elements within the muscle (Gans, 1982; Kawakami, Abe and Fukunaga, 1993).

While the above studies demonstrate, changes induced within these training modalities, it is of course more apt to directly compare CON only and ECC only within one study. Indeed, there are five studies which have directly compared the two contractions (Blazevich *et al.*, 2007; Franchi *et al.*, 2014, 2015; Baroni *et al.*, 2015; Timmins *et al.*, 2016). Of these five,

Blazevich in 2007 was the only study which found that there is no difference between the architectural responses from CON and ECC training. Whereas the other four, demonstrated that CON RE resulted in greater changes in PA compared to ECC RE. Additionally, they found that ECC RE induced greater changes in FL than either CON or conventional RE. However, it should be noted that the Blazevich study in 2007 did not match CON and ECC training for work, relative load or neural drive; thus, it is highly probable that the ECC RE was under loaded in comparison to CON. In conclusion of these data, it is suggested that skeletal muscle can respond to different mechanical stimuli through two distinct strategies for the addition of new sarcomeric material (i.e. sarcomeres added in series or in parallel) (Figure 1.14) (Narici, Franchi and Maganaris, 2016). Alterations in muscle architecture will inherently alter muscle performance (Franchi, Reeves and Narici, 2017), in particular influencing the optimal F/L relationship (as discussed in subsection 1.3.1.1). In addition to changes in the F/L relation, more sarcomeres in series would act to increases the velocity of contraction, whereas more sarcomeres in parallel is associated with increased force output. It has been suggested that differential muscle architecture adaptation occurs due to the stress of which each contraction induces. Whereby eccentric

contraction induces large degrees of stretch and mechanical stress, whereas adaptations seen in concentric or conventional training may be due to metabolic stress (N. D. Reeves *et al.*, 2009).

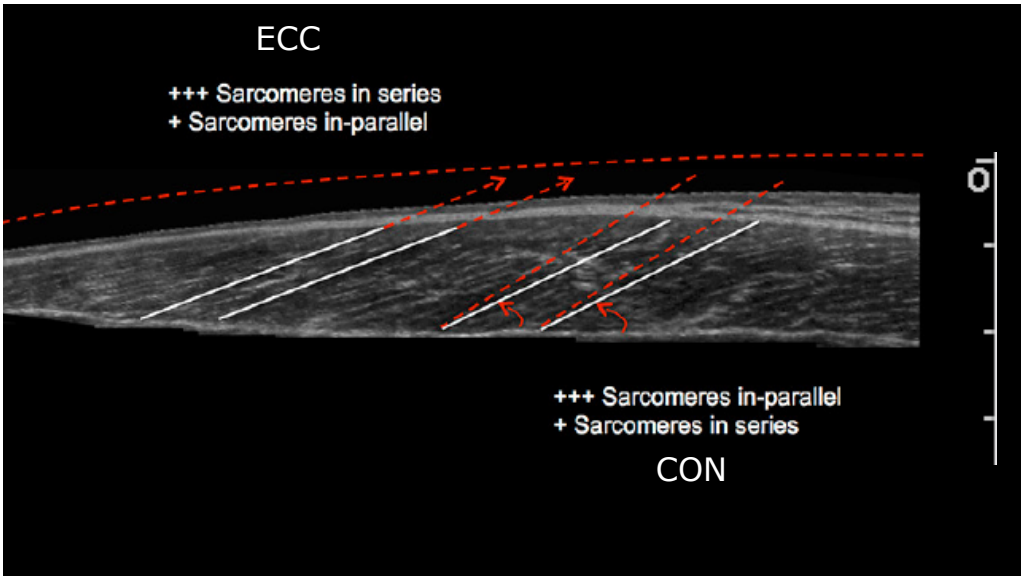


Figure 1.14: Illustration of where sarcomeres can be added with the fascicle, either in series or in parallel. ECC training predominately results in the addition of sarcomeres in series, whereas CON favours addition in parallel. Each dash on scale = 2cm (adapted from Franchi, Reeves and Narici, 2017).

Acute response to exercise: muscle protein metabolism

As aforementioned, whole muscle changes (such as muscle size, strength or architecture) occur due to the accumulation of acute responses to each exercise bout. Each time the muscle is exposed to RE, mixed and myofibrillar MPS is increased up to 2

or even 3 times that at rest (Phillips *et al.*, 1997; V Kumar *et al.*, 2009; Holm *et al.*, 2010). These increases in MPS can be seen after a large variety of exercise protocols, including 1hr of leg kicking (Miller *et al.*, 2005), CON only and ECC only resistance training (Cuthbertson *et al.*, 2006), or conventional RE (V Kumar *et al.*, 2009; Wilkinson *et al.*, 2014). These acute increases in MPS may be seen as early as 2 hours post exercise (V Kumar *et al.*, 2009; Kumar *et al.*, 2012) before soon retuning to baseline approximately 4 hours later.

Interestingly, there appears to be an intensity dependent relationship in regards to RE and MPS; such that MPS appears to peak with 60-90% 1RM RE (V Kumar *et al.*, 2009). However, it has also been shown that lower intensities of RE may elicit the same degree of MPS as high intensities but with the caveat that training occurs to volitional failure (Burd *et al.*, 2010). Burd and colleagues compared unilateral training whereby individuals performed RE at 90% 1RM to fatigue, 30% 1RM work-matched to 90%1RM group or at 30% 1RM to fatigue. They demonstrated that there was no difference in MPS responses between 90% 1RM to fatigue and 30% 1RM to fatigue (Burd *et al.*, 2010).

One may postulate that contraction mode may have differential effects on MPS. CON and ECC RE protocols have been shown to elicit similar increased in FSRs (Phillips *et al.*, 1997; Cuthbertson *et al.*, 2006; Franchi *et al.*, 2015). However, one study has been shown that ECC RE appears to stimulate increases of myofibrillar FSR at earlier time points than CON RE (Moore *et al.*, 2005). Moore and colleagues demonstrated that 4.5hrs post exercise, the ECC group showed superior increases of MPS in comparison to CON. This superior increase may then lead to greater increases in muscle mass, or a greater remodelling of the architecture (through changes in FL or PA).

1.4.3.2 Intermuscular connective tissue (IMCT)

It is essential that all components of the muscle-tendon complex respond to mechanical loading in synchronous fashion in order to maintain effectual biomechanical function. In a scenario whereby the amount of force the muscle is capable of producing increases, but the IMCT remains unaltered, it is clear potential issues may arise. Therefore, several studies have examined the rates of collagen synthesis before and after an acute exercise bout, establishing that collagen FSRs in muscle significantly increase (Miller *et al.*, 2005; Moore *et al.*, 2005;

Cuthbertson *et al.*, 2006). Interestingly two of which compared the effect of shortening and lengthening contraction upon collagen FSRs whereby it was shown that contraction type had no effect (Moore *et al.*, 2005) or that lengthening contractions stimulated a greater FSR response 3 hours post exercise (Cuthbertson *et al.*, 2006). Significantly, the latter also obtained FSR values at 6 hours post exercise, whereby no difference in contraction type was observed. It is postulated that due to the nature of eccentric contraction, sarcomeres inherently become stretched beyond the myofilament overlap (Whitehead *et al.*, 2001), which subsequently exposes the IMCT to greater tension. It is potentially the exposure to greater degrees of tension in eccentric contraction, which evokes a superior increase in FSR at the 3-hour time point. Regardless of this discrepancy it is clear that the FSR of muscle collagen becomes acutely increased following exercise. However, it remains that the effect chronic exercise imposes upon IMCT collagen FSRs requires elucidation.

1.4.4 MTU response to age

Ageing is well known to affect many aspects of the MTU in a negative fashion. However, whether these alterations are purely due to ageing or in fact a response to a decrease in habitual

activity is not known. While exercise exposes the MTU to a higher level of mechanical loading, it is believed that ageing may influence the underlying adaptive processes making individuals less responsive to RE.

1.4.4.1 Tendon biomechanical and structural changes in response to ageing

The biomechanical properties of tendon are known to influence overall functionality of the MTU, including force production, elastic energy return and electromechanical delay (Svensson *et al.*, 2016). Thus, alterations in tendon mechanics can affect functional outcomes such as balance and mobility which are known to be reduced in the elderly (Onambele, Narici and Maganaris, 2006). Further, tendon stiffness has an impact upon RTD (Wilkie, 1949), which itself is important for fall prevention (Cesar *et al.*, 2010). Therefore, any negative changes in tendon biomechanics would be detrimental to an elderly individual. In fact, the incidence of patella tendon strain injury is higher in older individuals (Jozsa *et al.*, 1991). Unfortunately, while the effects of exercise on tendon biomechanics are rather well researched, there remains a lack of knowledge concerning the influence of ageing per se (in humans).

However, many studies have utilised animal models to investigate the effect of ageing on tendon biomechanical properties. These studies demonstrated that as tendon tissue ages it may become stronger and stiffer (Shadwick, 1990; Nielsen, Skalicky and Viidik, 1998), weaker and more compliant (Vogel, 1978, 1983; Dressler *et al.*, 2002), or unchanged (Johnson *et al.*, 1994). While these models appear to contradict one another, it is possible that there is an explanation contained within the experimental design. It is apparent that the age of the animals involved vary somewhat, additionally in some cases they represent a mature tendon rather than that of an elderly animal.

In contrast to animal models, there is a significant lack of *in vivo* human studies that attempt to elucidate alterations in tendon biomechanical properties induced by ageing. Nonetheless, the few which exist have demonstrated that tendon stiffness may decrease in ageing (Onambele, Narici and Maganaris, 2006; Stenroth *et al.*, 2012) or remain unaltered (Carroll *et al.*, 2008; Couppé *et al.*, 2008, 2014). However, as they are obtained from different tendon tissue, the gastrocnemius (achilles) and patellar tendon respectively, it is

problematic to directly compare these results. Tendon tissue is known to vary in size, properties and adaptation based upon its location (Kjaer *et al.*, 2009) and therefore it is highly likely that the patellar tendon and Achilles tendon will age differently.

However, it must be reiterated that the tendon responds to mechanical stimuli through an increase in stiffness; even in older individuals (Reeves, Maganaris and Narici, 2003). Therefore, it is plausible that reductions in tendon stiffness may be due to a reduction in activity; something which is commonly observed in elderly populations (Taylor, 2014). This concept is further supported through many studies implementing immobilization and microgravity scenarios, whereby it has been demonstrated that PT stiffness decreases with 14 (de Boer, Maganaris, *et al.*, 2007; Couppé *et al.*, 2012; A. P. Boesen *et al.*, 2013) or 23 days of limb suspension (de Boer, Maganaris, *et al.*, 2007) and after 90 days of bed rest (Reeves *et al.*, 2005).

Tendon structural changes in response to ageing

Alterations to the structure will inherently affect tendon function. CSA is an important factor as it dictates the amount of stress placed through the tendon whereby a larger CSA would act to reduce stress (Stress = Force / CSA). Cross-sectional

studies in humans suggest that PT tendon CSA may be increased (Stenroth *et al.*, 2012) or unchanged with ageing (Carroll *et al.*, 2008; Couppé *et al.*, 2009, 2014). However, naturally alterations in CSA may be due to alterations in physical activity. Nonetheless the research carried out by Couppé in 2009 and 2014 matched their young and elderly group for activity levels. Unlike skeletal muscle (to be discussed in section 1.4.2.2), it appears that there is no signficant loss of tendon size in response to ageing.

Tendon biomolecular and metabolic changes in response to ageing

For alterations in tendon structure or biomechanics to occur, there must be changes of biomolecular origin (i.e. collagen content and crosslinking) which can be driven by changes in metabolism.

While tendon CSA appears to be unaffected by ageing, this does not necessarily mean that total collagen content is unchanged; instead the density of collagen may have been altered (i.e. less or more collagen within the same CSA). Nonetheless, the current human studies have proven inconclusive, demonstrating either a decrease (Couppé *et al.*, 2009), or no

change in collagen content (Couppé *et al.*, 2014). However, others have suggested that the techniques used to measure collagen content are unable to distinguish between free and incorporated collagen (Svensson *et al.*, 2016).

Within the tendon, there are two variations of crosslinking, one formed through enzymatic processes and those created by spontaneous non-enzymatic reactions. When considering enzymatic crosslinking in the tendon, the effect of age is inconsistent. The two Couppé papers discussed above also investigated the levels of HP and LP (which reflect enzymatic crosslinks) (Couppé *et al.*, 2009, 2014), whereby the 2009 study showed an increase in crosslinking, wheras in 2014 they observed no change. However, others suggested that the differences reported may only have modest signficance in relation to ageing (Svensson *et al.*, 2016). In contrast, it is believed that the non-enzymatic crosslinks formed through glycation have a more important role in an ageing tendon (Svensson *et al.*, 2016). The glycation of collagen molecules happens in a spontaneous and uncontrolled fashion whereby sugar molecules are continuously added to the collagen molecules; thus, resulting in the accumulation of AGEs throughout life.

One method of quantifying AGEs within the tendon is the measure of pentosidine, which is derived from lysine, arginine and ribose. Several studies have demonstrated that pentosidine concentrations within the patellar tendon increase with age (Couppé *et al.*, 2009, 2014). While the concentration of pentosidine is low within the tendon and is not believed to be the primary contributor to crosslinking (Avery and Bailey, 2005), it is accepted as a general biomarker for collagen glycosylation (Bailey, 2001). Thus, this suggests that the non-enzymatic glycosylation of collagen molecules increases with age. This accumulation of AGEs ultimately affects the functionality of the tendon (Bailey, 2001; Li *et al.*, 2013; Fessel *et al.*, 2014). Glycation has been reported to increase the distance between collagen molecules within the fibrils, thus affecting their molecular structure (James *et al.*, 1991). But also, AGEs affect the ability of the tendon fibrils to slide past one another during elongation.

As aforementioned, collagen content is believed to either decrease or remain unchanged with ageing; thus, if content is lower, one would also expect the FSR of collagen to be lower. Unfortunately, current literature that investigates the ageing

effect upon human tendon FSR is scarce. However, one study reported basal FSR values for both young and elderly (Nielsen *et al.*, 2014). They demonstrated that there was no difference between young and elderly FSR values, 0.21%.day^{-1} and 0.19%.day^{-1} respectively. A similar study also demonstrated equally low values of basal turnover in the elderly (Vestergaard *et al.*, 2012). Nonetheless, these values are significantly lower than those reported for young males elsewhere, with basal rates ranging between 1.08%.day^{-1} and 1.68%.day^{-1} (Miller *et al.*, 2005; de Boer, Selby, *et al.*, 2007; Doessing *et al.*, 2010; Nielsen *et al.*, 2011; Dideriksen *et al.*, 2013); hence this raises doubts to the fact that basal FSR is unchanged in ageing.

1.4.4.2 Muscle changes in response to ageing

While muscle is highly influenced by exercise, it is also susceptible to the ageing process. The term sarcopenia is used to define the geriatric condition typified by a significant and progressive loss of muscle mass (Rosenburg, 1989). However, aside of loss of tissue, the muscle is known to undergo a large number of changes which inherently affect the muscle function (Morley *et al.*, 2001). Dynapenia describes neuromuscular functional deficits in ageing (Clark and Manini, 2008). A multitude of factors are proposed to contribute towards the

development of both sarcopenia and dynapenia. Factors including muscle mass, muscle architecture, muscle composition and muscle protein turnover (Narici and Maffulli, 2010).

Muscle size and functional changes in response to ageing

One of the most apparent changes during ageing, is the progressive loss of muscle mass (Frontera *et al.*, 2006, 2008). In fact, from the age of 45, muscle mass declines approximately 0.5-1%, until they reach 70 years of age, whereby they will only possess around 80% of a 20-30 year old (Mitchell *et al.*, 2012). This loss of muscle mass is predominately contractile material, reflected by the reduced size and loss of type II fibers (Lexell, Taylor and Sjöström, 1988).

Inherently, following muscle atrophy and loss of contractile material, a reduction in strength is observed with ageing (Kritchevsky *et al.*, 2005). Interestingly, ECC strength appears to be maintained to some degreein old age (Mitchell *et al.*, 2012). However, the loss of muscle mass is not directly proportional to the loss of strength (Narici and Maffulli, 2010). In fact, loss of strength is faster and can be up to 2-5 times that

of muscle mass; strength loss can even occur without a loss of muscle mass (Goodpaster *et al.*, 2006; Delmonico *et al.*, 2009). There are of course many additional factors influencing loss of muscle strength in ageing. While there may not always be a measurable difference in muscle CSA, there may be a difference in muscle quality as reflected by the force per CSA. A reduced quality of muscle may be brought by increased fat and connective tissue infiltration between the individual muscle fibers (Delmonico *et al.*, 2009). While the contractile ability of the muscle relies upon contractile material, it also relies upon sufficient efferent nerve input.

The neuromuscular system undergoes several significant alterations with ageing, some of which result reduced muscle output, while others contribute towards muscle atrophy. During extension of the knee co-activation of the antagonistic flexor muscles occur to provide knee stability (Aagaard, Simonsen, Andersen, Magnusson, Bojsen-Møller, *et al.*, 2000). However, if co-activation becomes too large, it prevents effective operation of the knee extensors. The ageing process increases co-activation (Macaluso *et al.*, 2002), which inherently results in a reduction of muscle force. Additionally, there are alterations to the motor units. One of the largest causes of muscle fibre loss

is the progressive loss of motor units (Campbell, Mccomas and Petito, 1973; Faulkner *et al.*, 2007). It appears that this loss is predominantly fast twitch motor units, which results in the denervation of type II fibres. As type II fibres are known for their force producing capabilities, this may also explain a loss of muscle force. This denervation will precede one of two outcomes: atrophy of the fibres, or reinnervation from axonal sprouting of the remaining slow motor units. Although sprouting helps to maintain force production to some degree; it leads to the formation of giant motor units which will inevitably increase the innervation ratio. This reduction of neural drive is further demonstrated through a proposed reduction in voluntary activation capacity (Klass, Baudry and Duchateau, 2007).

Finally, it has also been shown that there is a slowing of the muscle contractile properties (Vandervoort and AA McComas, 1986; Baudry, Klass and Duchateau, 2005) with ageing. The combination of reduced force output and slower contractile properties, one would expect the RTD to be negatively affected in age. As expected, RTD has indeed been shown to decrease in ageing (Hakkinen *et al.*, 1998; Izquierdo *et al.*, 1999; Klass and Duchateau, 2008; Suetta *et al.*, 2009). RTD, as aforementioned is an essential characteristic in old age (Cesar *et al.*, 2010). In

order to counteract a trip and hence prevent a fall (more common in the elderly), force production must occur extremely rapidly (<200ms) (Pijnappels, Bobbert and Van Dieën, 2005).

Muscle architecture changes in response to ageing

Muscle architecture is pivotal when considering muscle function, as it is a main determinant of the length-force, and the force-velocity relationship (Lieber and Friden, 2000). Unsurprisingly, muscle architecture undergo negative alterations with ageing (Narici *et al.*, 2003; Narici and Maganaris, 2007). It has been shown that FL and PA are both reduced in ageing (K. Kubo *et al.*, 2003; Narici *et al.*, 2003; Morse *et al.*, 2005; Thom *et al.*, 2007; Stenroth *et al.*, 2012). In addition to the reduction in muscle fibre number (Lexell, Taylor and Sjöström, 1988), deterioration of architectural properties has a significant influence upon the functionality of muscle. A reduction in FL would mean a loss of sarcomeres in series; thus each sarcomere would experience a greater relative shortening in a short fibre than in a longer fibre (Narici, 1999). Consequently, the shortening velocity would be less in a short fibre than that of a longer fibre (Narici, 1999). Further, as mentioned in subsection 1.3.1.1, FL is also able to influence the F/L relationship and hence force output. In contrast, a reduction in PA would reflect

a loss of sarcomeres in parallel, thus reducing physiological cross sectional area (PCSA) and hence reduce force production (Narici and Maffulli, 2010). Therefore, it is clear to see why the reduction in either FL and PA would have functional implications in an aged population.

Muscle metabolism changes in response to ageing

Muscle mass is essentially controlled via the diurnal balance between MPS and muscle protein breakdown (MPB) (Phillips, Glover and Rennie, 2009; Atherton and Smith, 2012). Thus, for there to be muscle atrophy, an imbalance must occur, which is caused by an increase in MPB or a decrease in MPS or both; this results in a net breakdown of tissue. However, it has become apparent that neither basal MPS or MPB are altered with ageing in humans (Cuthbertson *et al.*, 2005; Katsanos *et al.*, 2006; Phillips, Glover and Rennie, 2009; V Kumar *et al.*, 2009). MPS and MPB are primarily regulated through two main sources of stimuli, food intake (in particular AAs) (Cuthbertson *et al.*, 2005; Katsanos *et al.*, 2006; Atherton *et al.*, 2010; Bukhari *et al.*, 2015) and physical exercise (V Kumar *et al.*, 2009; Vinod Kumar *et al.*, 2009; Atherton and Smith, 2012; Breen *et al.*, 2013). Both exercise and AA ingestion acutely stimulate MPS and if combined result in a positive net balance. However it is

believed that older individuals experience a blunted response to these anabolic stimuli, which has been termed 'anabolic resistance' (Cuthbertson *et al.*, 2005; Vinod Kumar *et al.*, 2009; Fry *et al.*, 2011; Wall *et al.*, 2015). Thus, while there is no difference in basal metabolism, elderly individuals inadequately stimulate MPS via AAs and/or exercise; consequently this results in a daily negative net balance. Over a prolonged period, the insufficient replenishment of lost muscle protein will result in overall atrophy of the muscle.

1.4.4.3 IMCT changes in response to ageing

Similar to tendon tissue, the collagen within the IMCT undergoes changes due to the natural ageing process, which as mentioned previously has negative effects upon force transmission. Animal models have demonstrated that the IMCT increases in stiffness with ageing (Reddy, 2004; Gao *et al.*, 2008), however the effect of ageing upon the human IMCT stiffness has not be investigated due technical and practical difficulties. Nonetheless, changes in crosslinking or more specifically the accumulation of AGE crosslinks in tissue with ageing in humans is well documented (Bailey, 2001; Haus *et al.*, 2007). Analogous to tendon tissue, this increase in glycosylated crosslinks would result in an increase of tissue

stiffness (Reddy, 2004). However, these crosslinks also alter the ability of collagen fibrils to slide past one another (Li *et al.*, 2013) affecting the biomechanics and hence have implications upon contraction and therefore locomotion in the elderly. Another method in which the IMCT may become stiffer is with an increase of collagen content due to an increase in basal collagen synthesis. However, it has been shown that collagen content within the muscle is unchanged with ageing (Babraj *et al.*, 2005; Haus *et al.*, 2007), it could therefore be logical to suggest that collagen synthesis remains unaltered with ageing. However, the fasted basal FSR of intramuscular collagen has been shown to increase with age (Babraj *et al.*, 2005), which doesn't appear to coincide with a constant collagen content. One conceivable explanation to this apparent confliction is an increase in collagen turnover. It is plausible that collagen synthesis increases in order to counteract an increase in intramuscular collagen breakdown, with the net collagen content remaining unaltered. However this remains speculative, as the rates of collagen breakdown within humans are unreported. Moreover, as the quantity of literature investigating human muscular collagen FSRs is negligible, it is difficult to make a firm conclusion on the effect of ageing on IMCT collagen synthesis rates.

1.5 Aims

The above sections review and critique the current literature concerning the MTU in respect to the effects of age and RE. While those discussed have built a solid foundation of understanding, there remains some essential questions which need to be addressed. Thus, the aim of this thesis is 4-fold. The first aim is to investigate whether there is an age-related change in patellar tendon stiffness and to what degree tendon stiffness influences RTD. In addition to tendon stiffness, chapter 3 will also investigate some of the main muscle influences upon RTD. The subsequent chapter, will elucidate whether the tendon in young males responds to submaximal RE, but also whether a different response is elicited from CON or ECC RE. Further, this chapter will investigate if there is a different temporal response of muscle and tendon in response to CON or ECC submaximal RE in young males. The penultimate chapter addresses the same questions as the former, except with older males. The final chapter will investigate the difference of young and old male's responses to submaximal RE, in regards to both muscle and tendon characteristics.

2 Chapter 2 – General methods

2.1 Participant recruitment

All participants underwent a full medical screening prior to enrolment, whereby those with any musculoskeletal, metabolic, respiratory, neurological or cardiovascular medical conditions were excluded from the study. None of the participants utilized within the study were deemed as frail, or had a history of falls. Participants were deemed healthy, and recreationally active, but did not take part in any formal resistance exercise training programme. All participants provided informed written consent to this study, which was approved by the University of Nottingham Ethics Committee and complied with the Declaration of Helsinki.

2.2 Study Design

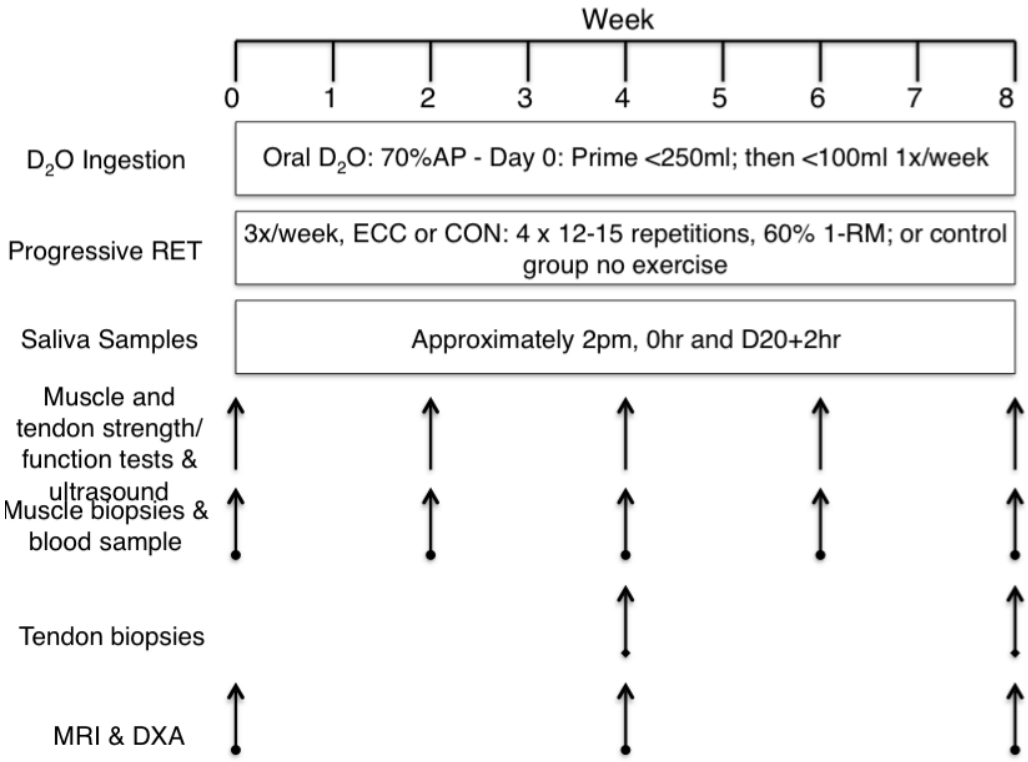


Figure 2.1: This schematic shows the full study design for each individual. However, for this thesis data is only taken from week 0, week 4 and week 8. Further, there is no inclusion of data obtained from the tissue biopsies.

2.3 Magnetic resonance imaging (MRI)

Muscle and patellar tendon (PT) dimensions, specifically cross sectional area (CSA), were obtained via a 3T MRI scanner, with slice thickness of 6mm and slice gap of 3mm. Transversal scans were performed with participants lying supine, with the knee

joint fixed at 180°. Peak quadriceps anatomical CSA (ACSA) was manually measured (Figure 2.2) using digital analysis software (OsiriX Lite 8, Pixmeo SARL). If a value was initially deemed to be the peak ACSA, 3 slices either side were checked to ensure accuracy. Vastus lateralis ACSA was also assessed in the same fashion as described above. Whole quadriceps volume was assessed utilising a proven method (Reeves, Narici and Maganaris, 2004a) of the truncated cone formula, shown below. Quad ACSA was assessed every 7 slices and inputted to the below truncated cone formula, whereby V = volume, d = distance between slices, a = first ACSA value, b = second ACSA value.

$$V = \frac{1}{3} \cdot d \cdot [a + \sqrt{(a \cdot b)} + b]$$

Equation 2.1: Truncated cone formula for the estimation of muscle volume

PT CSA was also manually assessed (Figure 2.3), whereby an average value was generated. The average value was based upon CSA measures along the full length (typically consisting of 8-10 separate slices) of the PT; hence compensating for spatial variations in PT CSA.

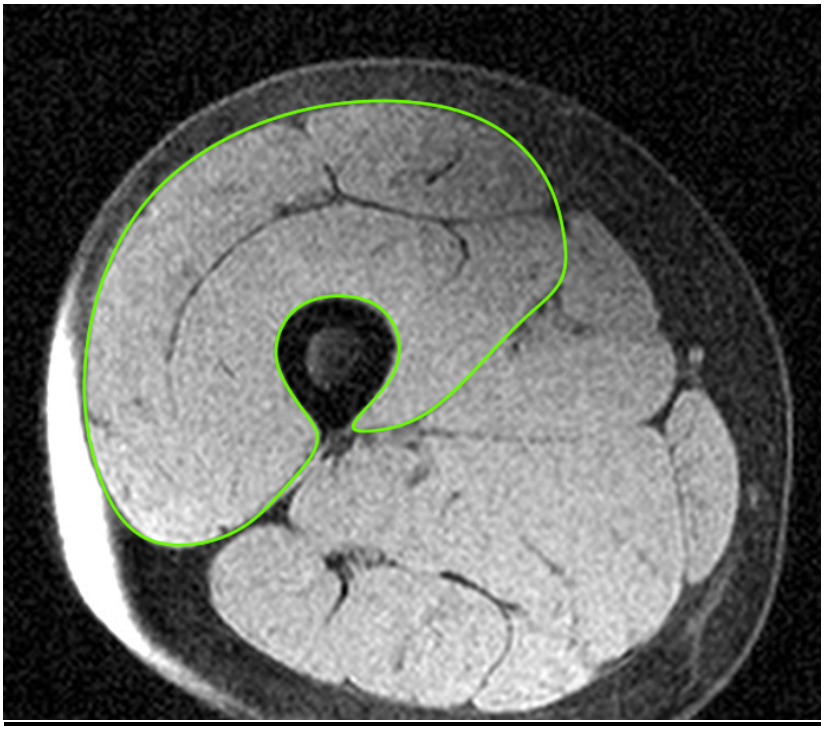


Figure 2.2: MRI image of thigh, with manual assessment of quadriceps ACSA highlighted (green line).

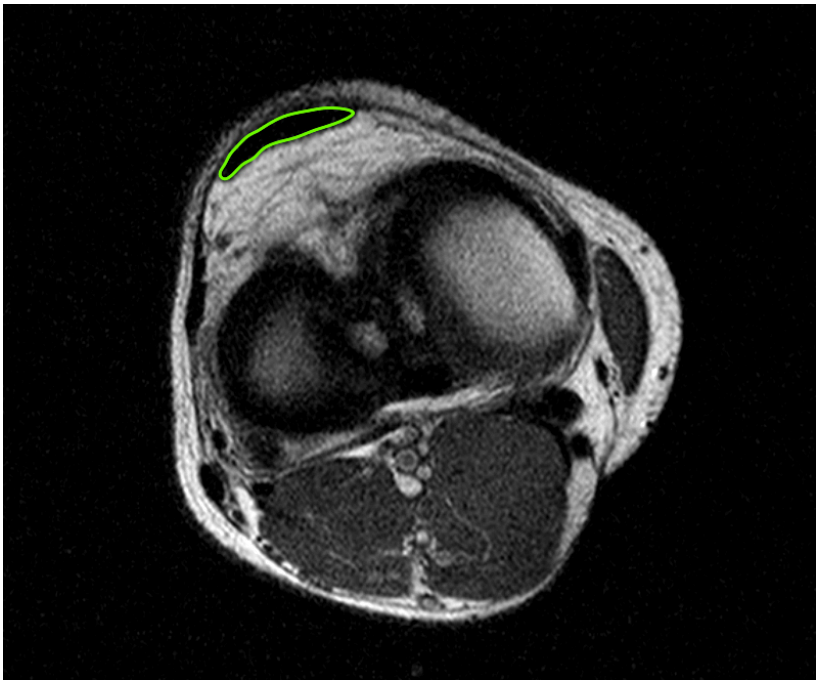


Figure 2.3: MRI image of knee, with manual assessment of PT CSA highlighted (green line).

2.4 Muscle architecture

VL FL was assessed through the acquisition and analysis of images using B-mode ultrasonography (Mylab 70, Esaote Biomedica) with a 100mm, 10-15MHz, linear array probe. Images were obtained while the participant was at rest, and lying supine upon a bed, in the same manner as previous work (Franchi *et al.*, 2015). Briefly, images were acquired from 50% of the VL length and midsagittal line of the muscle. The transducer was aligned to the fascicle plane allowing optimal capture of the fascicles (Reeves N *et al.*, 2009). Data was collected and analyzed by the same operator to remove any inter-operator error. Digital analysis of the images was completed using imageJ (ImageJ 1.50i), whereby the FL was directly measured wherever possible, or in the circumstance the fascicles extended beyond the visible field of view, linear extrapolation was applied (Franchi *et al.*, 2014).

2.5 Assessment of maximal voluntary contraction

Isometric knee extensor torque during maximal voluntary contraction (MVC) was assessed via dynamometry whereby participants were seated upon a rigid table top dynamometer where the knee joint angle was fixed to 90°, and full extension was equal to 0°. Participants were strapped tightly across the

thigh to reduce any unwanted movement of the hip joint, with their lower leg secured to a calibrated load cell via Velcro strapping. Force measurements were acquired and passed through an analogue to digital converter (BioPac MP150, BIOPAC Systems Inc) sampling at 500Hz and plotted using Acqknowledge software. Consequently, the torque values were calculated by multiplying the obtained force values by the external moment arm. The external moment arm was measured as the distance between the point of rotation within the knee joint and the midpoint of the Velcro strap attached to the ankle (0.39 – 0.40cm). Subjects were fully informed on the procedure prior to performance, where a total of 3 MVC attempts were completed, each separated by 30 seconds of rest. During all contractions, participants received loud verbal encouragement, which was consistent between participants. MVC was defined as the maximal torque value obtained over the 3 recorded attempts.

2.6 Calculation of specific tension

With both MVC and quadriceps ACSA ascertained, it is possible to calculate specific tension (Maganaris *et al.*, 2001). Specific tension refers to the amount of force normalised to the ACSA of the muscle. However, MVC must first be converted to total

quadricep force, using the following equations below, whereby F_2 = the force measured at the ankle, d_2 = external moment arm, d_1 = internal moment arm, obtained from Visser et al, 1990 (Visser *et al.*, 1990).

$$MVC\ (Torque) = F_2 \times d_2$$

$$Quadricep\ force\ (F_1) = \frac{F_2 \times d_2}{d_1}$$

Equation 2.2: Calculation of total quadriceps force

Following the calculation of quadriceps force, specific tension is calculated as below.

$$Specific\ tension = \frac{Quadricep\ force}{Peak\ quadricep\ ACSA}$$

Equation 2.3: Estimation of specific tension within the quadriceps

2.7 Rate of torque development (RTD)

It is possible to assess an individual's ability to produce an explosive and forceful contraction *in vivo*, by measuring contractile RTD. RTD is defined as the slope of the torque-time curve obtained during an isometric contraction (Andersen and Aagaard, 2006). RTD can be assessed over any time interval

that one chooses, however the traditional methods include 0-50ms, 0-100ms, 0-200ms (Maffiuletti *et al.*, 2016). However, it is also possible to evaluate the sequential RTD, i.e. 50-100, 100-200 (Maffiuletti *et al.*, 2016). A final way of representing RTD is the contractile impulse which is defined as the time-integrated force (Aagaard *et al.*, 2002; Behrens, Mau-Moeller and Bruhn, 2014; Maffiuletti *et al.*, 2016). While there are many differing ways in which to analyse RTD, the time interval selection would depend upon the other variables that are being investigated to ensure they are comparable. Investigating a variable which takes a little more time to reach a maximum, e.g. maximal voluntary contraction (Andersen and Aagaard, 2006) or the full stretch of a tendon (Quinlan *et al.*, 2017), then one might use the 0-200ms time interval. Conversely, if one was investigating a multitude of neuronal aspects, then perhaps an early time interval such as 0-50ms or 0-100ms would be more suitable due to the time course of neuronal responses. The other important consideration in regards to RTD, is whether to normalise the raw RTD values or not. Traditionally either MVC or quadriceps anatomical cross sectional area (ACSA) are used to make RTD values comparable (Maffiuletti *et al.*, 2016). Normalisation of the data allows the investigator to directly compare values between groups (e.g. young vs old, trained vs

untrained etc) and analyse whether one group can produce and transmit force more rapidly.

For the acquisition of quadriceps femoris contractile RTD, participants were seated upon the rigid tabletop dynamometer used for acquisition of MVC. The VL of the right leg was prepared for EMG in the manner stated below. Participants were strapped tightly across the thigh, with their lower leg secured to a calibrated load cell via Velcro strapping. Participants were instructed to produce a rapid and powerful contraction of the knee extensors, with emphasis upon speed. Subjects performed a trial repetition for familiarization, before completing three maximal isometric contractions, performed as fast as possible, each separated by 30 seconds of rest. Absolute RTD (aRTD) was defined as the change in force over a given period. We assessed RTD over the first 200ms of the maximal isometric contraction, whereby the onset of contraction was deemed as the point at which torque had risen 4Nm above baseline. We also obtained torque values at 50ms and 100ms to generate an averaged curve of torque over the initial 200ms of contraction. RTD values were consequently normalized (nRTD) for peak quadriceps anatomical CSA (ACSA). The time to peak EMG (TTPE) was calculated as the time taken from EMG onset to 90% of peak

EMG amplitude during the maximal isometric contraction (Pijnappels, Bobbert and Van Dieën, 2005).

2.8 Muscle activation capacity

Volitional muscle activation of the quadriceps was assessed using the interpolated twitch technique. Individuals produced a maximal knee extension at 90° knee joint angle, during which individuals received electrical stimulation using a stimulator device (DS7AH, dual-high voltage stimulator, Digitimer Ltd, Welwyn Garden City, Hertfordshire, United Kingdom). The stimulus was applied via two pads, which were placed proximally and distally to the quadriceps. The stimulation consisted of two supra-maximal single pulses, one delivered when a plateau was reached during contraction, a second (potentiated twitch) was applied at rest 1-second post-contraction. The electrical current required for each individual's supra-maximal stimulation was obtained prior to assessment. The supra-maximal current was acquired through monitoring force output during a succession of stimuli with increasing current. At the point whereby no additional force was observed for a further increase in current, the current was recorded and utilized during assessment. Muscle activation was consequently calculated via an equation previously used (Allen, Gandevia and

Mckenzie, 1995), which is listed below; whereby T_1 is the evoked interpolated twitch torque and T_2 is the resting control twitch torque.

$$Activation\ Capacity = \left(1 - \left(\frac{T_1}{T_2}\right)\right) x\ 100$$

Equation 2.4: Estimation of quadriceps activation capacity.

The maximal first derivative (dF/dt) of the resting control twitch (T_2) was also obtained as a marker of contractile speed.

2.9 Electromyography

Electromyography (EMG) was acquired from the belly of both the vastus lateralis (VL) and the biceps femoris (BF) muscles of the right lower limb using surface EMG electrodes. The belly of the VL was obtained by measuring the distance between the greater trochanter and the mid point of the patellar on the lateral side; electrodes were placed 2/3rds distally of this distance. The BF belly was obtained by marking 2/3rds of the distance between the posterior sacrum and the posterior of the knee joint. The skin was thoroughly prepared thoroughly, firstly via the removal of hair followed by light abrasion of the skin before being cleaned with 0.1% w/w alcohol wipes. Raw EMG signals were sampled at 2.0kHz and digitized with an analogue

to digital converter (BioPac MP150, BIOPAC Systems Inc) filtered and amplified. The root mean square (RMS) was calculated through an Acqknowledge software function (Acqknowledge 4.2.0, BIOPAC systems Inc), where the integral was calculated using a 30ms time-window.

2.10 Estimation of antagonist co-activation

Co-activation of the quadriceps and the antagonistic hamstring occurs during leg extension. It is believed that co-activation is a method which results in joint stabilisation (Hagood *et al.*, 1990). It can be calculated through comparing EMG activation of both the agonist and antagonist muscle during maximal contraction. Specifically, the area under the RMS EMG curve for agonist and antagonist muscles are calculated 250ms about the peak torque for two MVC attempts; thus, generating an average value. Consequently, the value of co-activation can be calculated utilising the equation below.

$$Co - activation\ (\%) = \left(\frac{AUC\ of\ antagonist}{AUC\ of\ agonist}\right) \times 100$$

Equation 2.5: Calculation of bicep femoris co-activation.

2.11 Tendon biomechanical properties

Measures of tendon biomechanical properties (tendon stiffness and Young's modulus) were obtained *in vivo* during voluntary isometric ramped contractions at 90° knee joint angle over a 5 second period. PT length (L_0) and hence elongation was obtained by sagittal-plane real time B-mode ultrasonography with a 10cm linear-array probe. A smaller probe is unable to observe both ends of the tendon in one video. The use a skin maker which would produce a trackable shadow on the scan, or stitch two separate videos together (Reeves, Narici and Maganaris, 2003) is more cumbersome and these methods are susceptible to an additional source of error, which is avoided using a 10cm probe.

L_0 was defined as the distance between the apex of the patella and the first tuberosity of the tibia. PT elongation was calculated through the use of a semi-automated pixel tracking software (Tracker v4.95, OpenPhysics). The software allows an individual to dictate a collection of pixels to be tracked throughout a video

(Figure 2.4), the accuracy and stringency of the software can also be altered through the 'evolution rate' and 'automark' (Figure 2.5). which was ran in triplicate and the average pixel movement was used for further analysis. Moreover, to prevent an overestimation of tendon elongation due to translation of the tibia, a calibrated goniometer was attached to the lateral side of the tested knee. In order to calculate PT stiffness, true knee extensor torque was calculated as the sum of the measured knee extension torque and the antagonistic torque, estimated through the RMS of the EMG acquired from the Biceps Femoris (BF). Consequently the PT force was calculated by dividing the true knee extensor torque by the estimated patellar tendon moment arm (equation below) (Visser *et al.*, 1990). Force-elongation data were fitted with a second order polynomial curve, which allowed the assessment of PT stiffness, defined as the gradient of the force-elongation curve over the final 10% of maximal force (Reeves, Maganaris and Narici, 2003). Tendon CSA (cm^2) was assessed along the full resting length of the tendon in the axial plane through MRI as described above, with the average value being presented as PT CSA. Young's modulus was calculated (equation below) as the change in stress (force/CSA) divided by the change in strain (L_0 / Elongation).

$$PT\ force = \frac{(Ext\ force + antagonist\ force) \times Ext\ moment\ arm}{Estimated\ internal\ PT\ moment\ arm}$$

$$PT\ stiffness = \frac{\Delta\ PT\ Force}{\Delta\ Elongation}$$

Equation 2.6: Calculation of PT stiffness

$$Young's\ Modulus = \frac{\Delta\ (PT\ Force\ \div PT\ CSA)}{\Delta\ (L_0\ \div Elongation)}$$

Equation 2.7: Calculation of PT Young's modulus

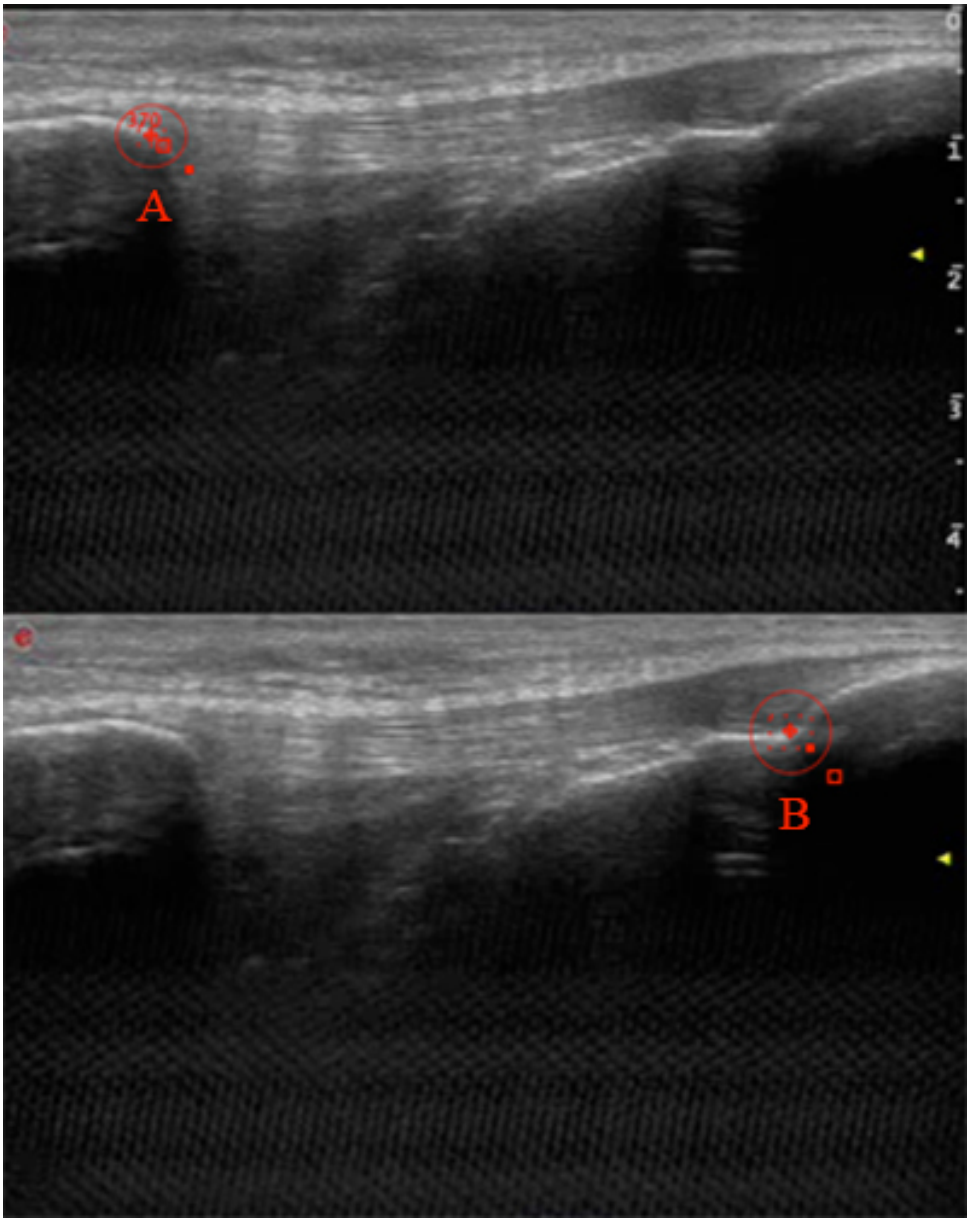


Figure 2.4: Demonstration of the templates set at the beginning of the ramped contraction at A) the apex of the patella, and B) first tuberosity of the tibia. The software tracks this collection of pixels and thus can generate two sets of co-ordinates, which in turn can generate a value for length. Each mark on scales represents 1cm.

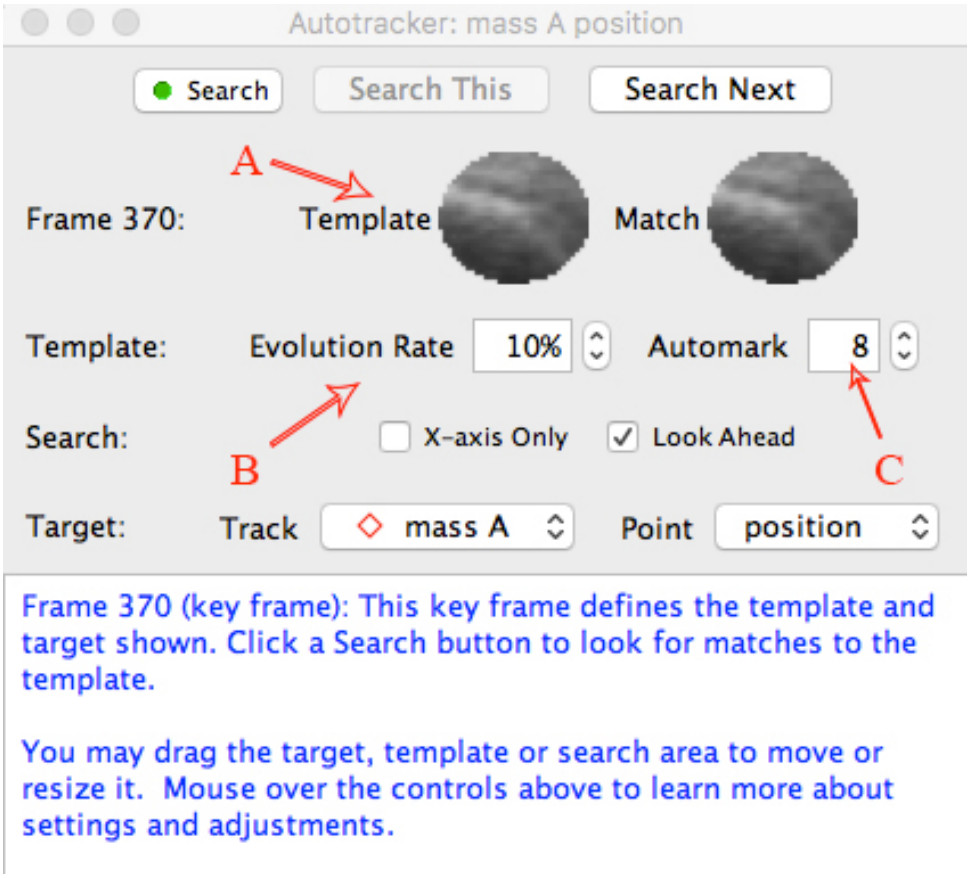


Figure 2.5: The set up used on Tracker v4.95, OpenPhysics. A) Shows the pixel template that was tracked. B) Shows 'evolution rate', which dictates the amount (%) the template image will adapt during the video clip, to ensure accuracy. C) 'Automark' is an inbuilt feature to indicate likeness of the potential image to the template. The higher the value is, the more stringent the software is at accepting a potential match, i.e. the higher the threshold is for acceptance.

2.12 1 – RM assessment

Due to the nature of the adapted leg press machine, we were limited with the amount of weight that could be utilised for safety reasons; which in turn provided some challenges in regards to the 1-RM assessment. While participants were not and hadn't been part of a regular strength training programme, they were still recreationally active. Prior to the study commencing, we trialled several young individuals, who easily carried out a single bilateral ECC contraction with the maximum weight; in fact some even managed this weight for 10 repetitions. As we required a way to obtain 1RM utilising the same method for all individuals to ensure same exposure (young and old, weak and strong), this presented a problem. Thus, we decided that for the 0-4week period, all participants would acquire a bilateral CON 10RM, which was consequently converted to an estimated CON 1RM using the Lander method (Lander, 1984).. Whereas, for the 4-8week period, all participants acquired a unilateral CON 1RM directly. If the individual was in the ECC group, this CON 1RM would be multiplied by the CON:ECC ratio obtained through neuronal matching (described below). As CON strength is inherently lower than ECC, this would reduce the required weight for assessment. Further naturally the weight utilised a 10RM would

be lower than that of a 1RM. Thus, this would make it plausible to utilise the leg press throughout the study for all individuals; without the risk of any the participant's CON 10RM assessments requiring more weight than what was available.

2.13 Neuronal matching

When one is comparing the effects of CON and ECC training upon variables, there is one essential experimental consideration that needs to be addressed. Due to the physiological nature of the two contractions, an individual undertaking ECC training would firstly be utilising a heavier load than those in the CON group, but more significantly would be doing so with less metabolic and neuronal demand. Thus, it is arguable that as less lean muscle is utilised to produce force during ECC contractions (compared to CON for an equivalent load), you may observe a differential response to CON for purely this reason only; opposed to the mechanical differences in the mode of contractions themselves. Therefore, one should match CON and ECC training to either the total work-load (Anthony J Blazevich *et al.*, 2007), or for neural drive (Franchi *et al.*, 2014). However, as our study separated individuals into two separate training groups (as opposed to using one limb per training modality) the former method of matching is a little more challenging. Therefore, we instead decided to use a method

based upon neural drive, which we believed to be more suitable.

During the baseline 10-RM assessment (described above), we utilised electromyography (EMG) upon the right VL (set up is described in 'Electromyography' section below) to record each concentric contraction; the root mean square (RMS) of the raw EMG (described below) was used calculated. Following 10-RM assessment, and hence acquisition of maximal concentric load, the RMS EMG was used to calculate the area under the curve (AUC) over the whole 3 seconds of all 10 contractions, with the mean average consequently calculated. This averaged value provided us a measure of the neural drive during CON contraction, to which we needed to match to that of ECC contraction (only if the individual was in the ECC group). Previous work by the group had investigated the CON:ECC ratio for training loads (Franchi *et al.*, 2014), where it was found the ratio was between 1.21 and 1.29. Thus, we multiplied the CON 10RM weight by 1.3 to form an estimated ECC 10RM weight, which was consequently used to perform 10 ECC contractions where EMG of the right VL was recorded. The same calculation of neural drive was completed, and the values compared to that of the 10RM CON; whereby similar neural drive was deemed to occur when there was less than 10% difference between the

two values. If a greater difference than 10% was observed, the ratio was therefore incorrect and a new estimated weight must be calculated; depending whether this value was too high or low, a higher or lower ratio would be attempted. The schematic below demonstrates this process (Figure 2.6). Once the neural drive values matched, the ratio used was recorded. Further, individuals now had a training load for CON and ECC contractions that would, in theory, recruit a similar degree of muscle mass due to neural drive. It is with this principle that we believe, even though they are in separate groups, those training CON and ECC would be training with the same relative neural drive, and hence degree of muscle recruitment.

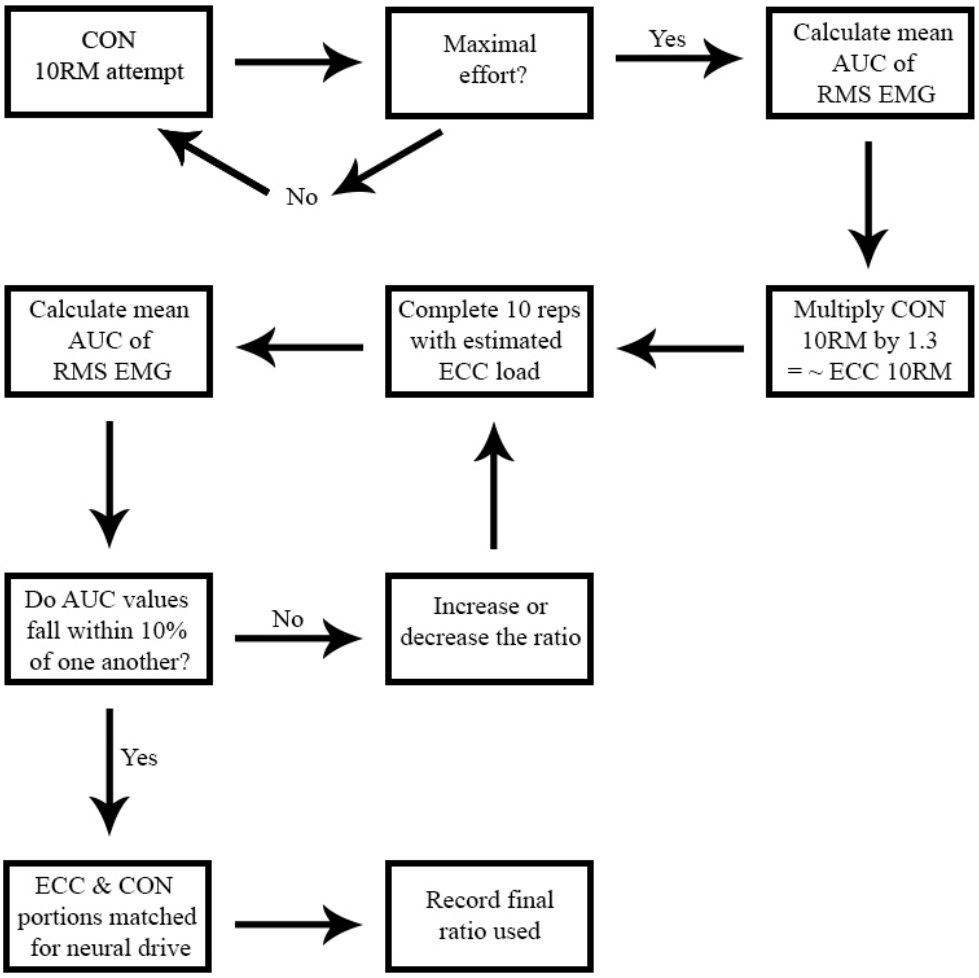


Figure 2.6: Process of neuronal matching for ECC training.

2.14 Resistance training

Recruited volunteers were consequently split into one of three groups; ECC only training, CON only training, or a control (Xtrl) group with no training. Individuals were kept within their age groups, hence forming young or old concentric (YC, OC respectively), young or old eccentric (YE, OE respectively), and young or old control (YX, OX respectively). Those in the Xtrl group carried on with their habitual behaviours, without altering

their activity level, significantly they did not take part in any structured RE programme.

The training consisted of 4 sets of 12-15 repetitions, with an intensity of 60% 1-RM, which was reassessed every 2 weeks (method described below). Training occurred 3 times a week for a total of 8 weeks; whereby 0-4 weeks entailed bilateral training, whereas 4-8 weeks was unilateral (right leg). The reasoning behind the change from bilateral to unilateral is due to an additional component of the study. Tendon biopsies were obtained at week 4 and week 8 (data not included); due to the risk of injury associated with training after the tissue biopsy, we altered to unilateral training with the non-biopsied leg.

Training took place on a specialised Technogym leg press machine which was modified with an electric engine. The electric engine powered a winch, which attached to the chair of the leg press via a strong steel cable. The winch allowed for the true isolation of CON and ECC portions during training. In such, for ECC training, the winch would pull the chair backwards until the individual was at near full extension, hence removing the CON portion of the lift. The weight would then be transferred

onto to the individual, allowing them to then complete the ECC portion by returning themselves to the starting position over a 3 second period. Conversely, for CON training, the individual would push themselves out over a 3 second period, with the winch then taking the weight off the individual at full extension, before lowering them back to the starting position, hence removing the ECC portion. During CON training, the individuals were asked to remove their legs from the press position when the winch was lowering the chair to remove any additional passive ECC stretch of the muscle

3 Chapter 3 – Muscle and tendon contributions to the reduced rate of torque development in healthy older males

3.1 Abstract

Background: The ability to rapidly generate and transfer muscle force is essential for effective corrective movements in order to prevent a fall. The aim of this study was to establish the muscle and tendon contributions to differences in rate of

torque development (RTD) between younger (YM) and older males (OM).

Method: 28 young males (23.9 yrs ± 1.1) and 22 old males (68.5 yrs ± 0.5) were recruited for assessment of Quadriceps Anatomical CSA (ACSA), maximal voluntary contraction (MVC), rate of torque development (RTD) and tendon biomechanical properties. Activation capacity (AC), maximal muscle twitch df/dt) and time to peak EMG amplitude (TTPE) were also assessed.

Results: Absolute RTD (aRTD) was lower in OM (577.5±34.6 Nm/s vs 881.7±45.6 Nm/s, $p<0.0001$). RTD remained lower in OM following normalization (nRTD) for muscle ACSA (9.93±0.7 $Nm/s/cm^2$ vs 11.9±0.6 $Nm/s/cm^2$, $p<0.05$). Maximal muscle twitch df/dt (1086 $Nm \bullet s^{-1}$ vs 2209 $Nm \bullet s^{-1}$, $p<0.0001$), TTPE (109.2±8.6ms vs 154.6±16.6ms, $p<0.05$) and AC (75.8±1.5 % vs 80.1±0.9 %, $P<0.01$) were all affected in OM. Tendon stiffness was found to be lower in OM (1222±78.4 N/mm vs 1771±154.1 N/mm, $p<0.004$). nRTD was significantly correlated with tendon stiffness ($R^2=0.15$).

Conclusion: These observations provide evidence that in absolute terms, a lower RTD in the elderly is caused by slower muscle contraction speeds, slower TTPE, reduced ACSA, reduced MVC and a decrease in tendon stiffness. Once the RTD

is normalized to quadriceps ACSA, only MVC and tendon stiffness remain influential. This strongly reinforces the importance of both muscle and tendon characteristics when considering RTD.

Published in Journal of Gerontology on 2nd August 2017.

DOI: https://doi.org/10.1093/gerona/glx149

3.2 Introduction

Loss of muscle mass and strength (Narici and Maffulli, 2010; Mitchell *et al.*, 2012) together with an increased incidence of falls (Skelton, 2001) are common outcomes of an ageing neuromuscular system. While absolute force production is essential for daily activities such as rising from a chair or bath, carrying groceries or stair negotiation, the ability to generate force rapidly is equally important. In order to counteract a trip and hence prevent a fall, force production must occur extremely

rapidly (<200ms) (Pijnappels, Bobbert and Van Dieën, 2005). Therefore, not only maximal torque production but also a rapid rate of torque development (RTD) have been identified as key performance characteristics in elderly individuals (Cesar *et al.*, 2010); significantly, RTD has been shown to decrease in ageing (Hakkinen *et al.*, 1998; Izquierdo *et al.*, 1999; Klass and Duchateau, 2008; Suetta *et al.*, 2009).

It is possible to assess an individual's ability to produce an explosive and forceful contraction *in vivo*, by measuring contractile RTD (Aagaard *et al.*, 2002). RTD is defined as the slope of the torque-time curve obtained during an isometric contraction and can be assessed over varying time intervals (Andersen and Aagaard, 2006). One factor that may influence RTD, first hypothesized by Wilkie (Wilkie, 1949), is the compliance of the attached tendon, which provides a mechanical link between muscle and joint. As such, a more extensible or compliant tendon, will take a longer period to stretch and thus reduce the RTD. It has previously been shown that RTD is significantly correlated to the stiffness of the m. vastus lateralis tendon-aponeurosis complex (Bojsen-Møller *et al.*, 2005), further suggesting that connective tissue may play an important role in explosive strength development. Thus any

changes to the tendon tissue in response to ageing, could potentially alter the RTD. Unfortunately, conclusive evidence for changes in tendon tissue with ageing remains elusive. Measurements of tendon biomechanical properties, including tendon stiffness and Young's modulus have long been acquired *in vivo* via real time ultrasonography (Fukunaga *et al.*, 1996; Maganaris and Paul, 1999) and provide important information on the biomechanical integrity of the tendon. Only a few studies have applied this technique to investigate the effect of ageing; some studies reported that tendon stiffness does not alter during the ageing process (Carroll *et al.*, 2008; Couppé *et al.*, 2009), while others demonstrated that tendon stiffness decreases with age (Onambele, Narici and Maganaris, 2006). The latter study investigated the gastrocnemius tendon while the former studies were performed on the patellar tendon, pointing to possible tendon-specific differences. Unfortunately, it is difficult to compare tendon tissues from differing anatomical locations as both their properties and consequential adaptations are known to vary, presumably to fulfill its specific requirement (Kjaer *et al.*, 2009). It is therefore plausible, that some tendinous tissue may age differently, generating these contradictory conclusions.

Tendinous tissue can also impose an influence upon the force-producing capabilities of the attached muscle. The stiffness of the tendon can influence the force-length relationship of its respective muscle, particularly at the extremes of the muscle range of motion (Hawkins and Bey, 1997), underpinned by a change in myofilament overlap in the sarcomeres of the muscle. When considering the effect of tendon stiffness on muscle and sarcomere length and consequent contractile force in different muscle-tendon units (MTUs), differences in tendon length and muscle length between MTUs should be considered, as for given tendon elongation will have to be shared by more in-series sarcomeres in longer muscles. One way of assessing differences in whole MTU stiffness between different MTUs is by comparing muscle fascicle: tendon length ratios between MTUs (Zajac, 1989; Garner and Pandy, 2003; O'brien *et al.*, 2010).

Significantly though, tendon stiffness is of course not the only influential factor when considering RTD; there are many characteristics of the muscle-tendon complex, which play a role (Bojsen-Møller *et al.*, 2005; Gerstner *et al.*, 2017). Muscle size, myosin heavy chain (MHC) composition, motor unit firing frequency, rate of EMG rise and maximal strength are all known to influence RTD (HÄkkinen and Komi, 1986; Harridge *et al.*,

1996; Aagaard *et al.*, 2002; Pijnappels, Bobbert and Van Dieën, 2005; Van Dieën, Pijnappels and Bobbert, 2005; Maffiuletti *et al.*, 2016; Gerstner *et al.*, 2017). Therefore, when investigating RTD, it seems imperative to consider all possible determinants including muscular, neural and tendinous.

Therefore, the purpose of this study was first, to establish whether patellar tendon stiffness undergoes any age-related changes. Secondly, we wanted to establish all potential muscle and tendon factors affecting RTD and to what extent they do so.

3.3 Methods

Participants

We recruited 50 healthy, recreationally active individuals, 28 of which were younger males (YM, 23.9 yrs ± 1.1) and 22 older males (OM, 68.5 yrs ± 0.5). There were no differences in height (177 ± 1.3 vs 177 ± 1.3), mass (75.9 ± 3.2 kg vs 78.5 kg ± 2.3) or body mass index (BMI, 24.2 ± 0.8 vs 24.9 ± 0.6) between the YM and OM respectively. All participants underwent a full medical screening prior to enrolment, whereby those with any musculoskeletal, metabolic, respiratory, neurological or cardiovascular medical conditions were excluded from the

study. None of the participants utilized in this study were deemed as frail, or had a history of falls. All participants provided informed written consent to this study, which was approved by the University of Nottingham Ethics Committee and complied with the Declaration of Helsinki.

Statistical analyses

All data is presented as mean ± SEM. Differences between the two groups (YM vs OM) were analyzed using unpaired two tailed t-tests (level of significance was set at $P<0.05$). Linear regression analyses were assessed through Pearson's correlation coefficient (level of significance was set at $P<0.05$), similarly correlation matrices were also conducted in this fashion.

Assessments

The specific details for each technique and assessment are included within the general methods section (Section 2). However, the following were assessed within this study: MVC, RTD and nRTD, AC, TTPE, maximal df/dt, muscle architecture including pennation angle and fascicle length, peak quad ACSA via MRI and tendon biomechanical properties including stiffness and Young's modulus.

3.4 Results

OM demonstrated a lower isometric MVC (159±6.8 Nm vs. 220±10 Nm, P<0.0001) and a reduced quadriceps ACSA (59.1±2.5 cm^2 vs 79.4±2.9 cm^2, P<0.0001) compared to YM (Fig 1). Quadriceps voluntary activation (AC) during maximal leg extension was significantly lower in OM than in YM (75.8±1.5 % vs 80.1±0.9 %, P<0.01), and the muscle twitch maximal df/dt (1086 $Nm•s^{-1}$ vs 2209 $Nm•s^{-1}$, p<0.0001) was also lower in OM (Fig 1). Also, TTPE was significantly slower in OM than in YM (154.6±16.6ms vs. 109.2±8.6ms, p<0.05) (Figure 3.1). Absolute RTD (aRTD), assessed over the first 200ms of a maximal isometric contraction, was significantly reduced in OM (Fig 2) (577±34$Nm•s^{-1}$ vs. 881±46$Nm•s^{-1}$, P<0.0001).

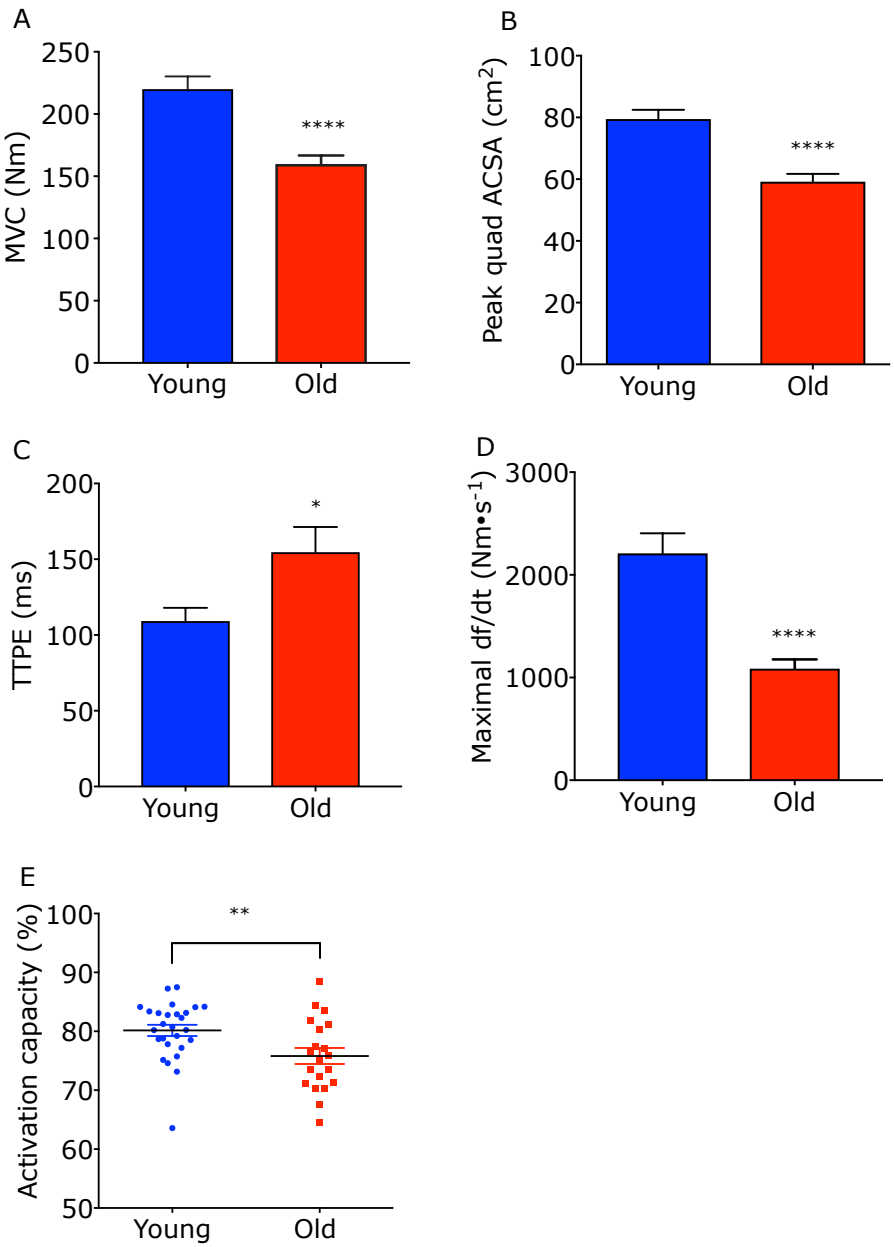


Figure 3.1: Age related difference in MVC (A), Peak Quadriceps CSA (B), TTPE (C), Maximal twitch df/dt (D) and Activation capacity (E), p values denoted by * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

The age-related reduction in RTD was maintained after normalization of RTD to peak ACSA (nRTD) (Figure 3.2) (11.9±0.64 $Nm•s^{-1}•cm^2$ vs. 9.93±0.77$Nm•s^{-1}•cm^2$, P<0.05).

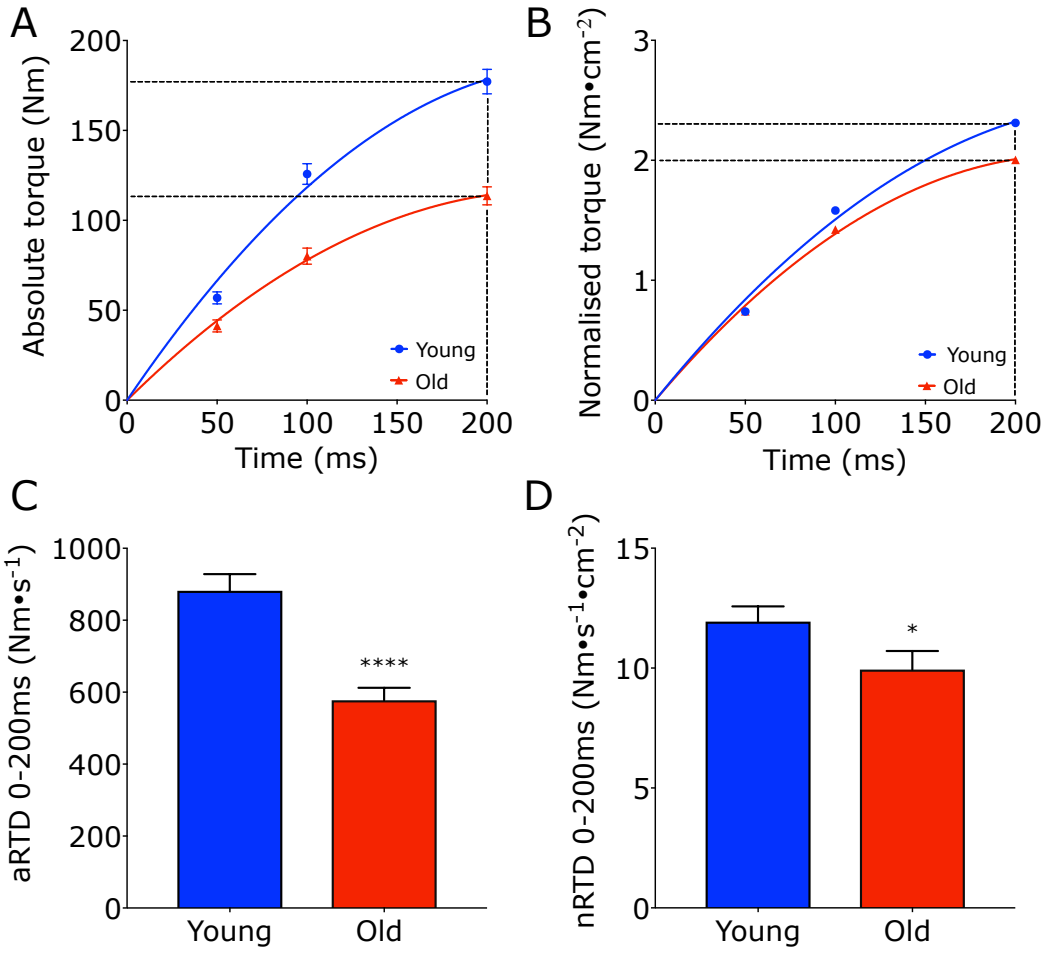


Figure 3.2: Graphs depict the average raw torque (A) and normalized torque (B) values obtained at 50, 100 and 200ms during rapid isometric contraction. Age related difference in aRTD (C) and nRTD (D), p values denoted by * p<0.05, **** p<0.0001.

Patellar tendon CSA (86.6.1±2.6 mm^2 vs 83.8±2.7 mm^2 for YM and OM respectively, P>0.05) and length (50.8±0.9 mm vs 51.9±0.8 mm for YM and OM respectively, P>0.05) were found to be unaffected by age. Patellar tendon stiffness assessed over the maximal 10% of the force elongation curve was found to be lower in OM than YM (Figure 3.3) (1222±78 $N•mm^{-1}$ vs 1771±154 $N•mm^{-1}$, P<0.01). Young's modulus was also lower in OM than YM (Figure 3.3) (0.74±0.04 GPa vs 1.0±0.09 GPa, P<0.05). No differences in either fascicle length (YM: 85±1.8 mm vs OM: 82.7±3 mm, P>0.05) or in resting fascicle: tendon length ratio (YM: 1.69±0.04 vs OM: 1.58±0.05, P>0.05) were found between groups.

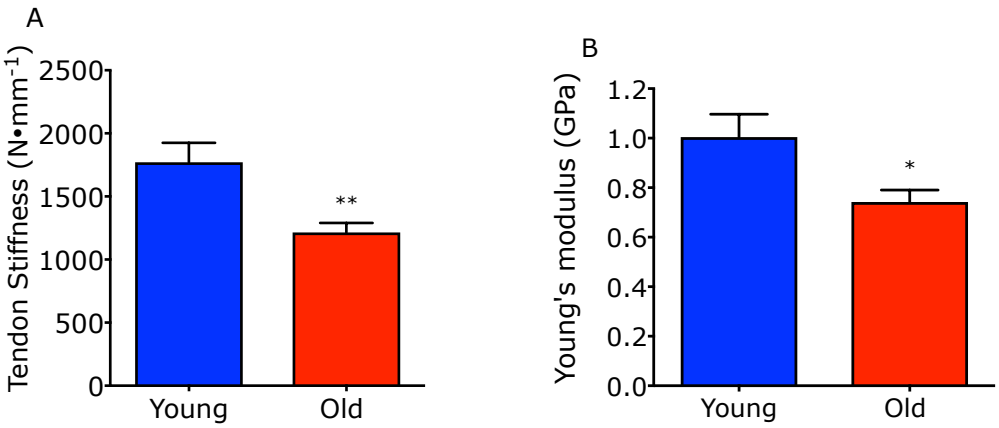


Figure 3.3: Age related difference in patellar tendon stiffness (A) and Young's Modulus (B), p values denoted by * p<0.05, ** p<0.01.

aRTD correlated (Fig 3.4 and table 3.1) to MVC (R^2=0.80, P<0.0001), CSA (R^2=0.36, P<0.0001), tendon stiffness (R^2=0.28, P<0.0001),TTPE (R^2=0.11, P<0.05), and muscle twitch maximal df/dt (R^2=0.11, P<0.05). nRTD remained correlated with tendon stiffness (Figure 3.4 and table 3.2) (R^2=0.15, P<0.01).

	Stiffness	**MVC**	**CSA**	**TTPE**	**Max df/dt**	**FL**
Stiffness	1	.605*** p<0.001	.583*** p<0.001	-.209 NS	.247 NS	.165 NS
MVC	.605*** p<0.001	1	.703** p<0.001	-.122 NS	.347* p<0.05	.082 NS
CSA	.583*** p<0.001	.703** p<0.001	1	-.122 NS	.587 p<0.001	.018 NS
TTPE	-.209 NS	-.224 NS	-.122 NS	1	-.231 NS	.183 NS
Max df/dt	.247 NS	.347* p<0.05	.587*** p<0.001	-.231 NS	1	-.029 NS
FL	.165 NS	.082 NS	.018 NS	.183 NS	-.029 NS	1

Table 3.1: Correlation matrix for all independent variables with r values displayed, p values denoted by * p<0.05, ** p<0.01 and *** p<0.001.

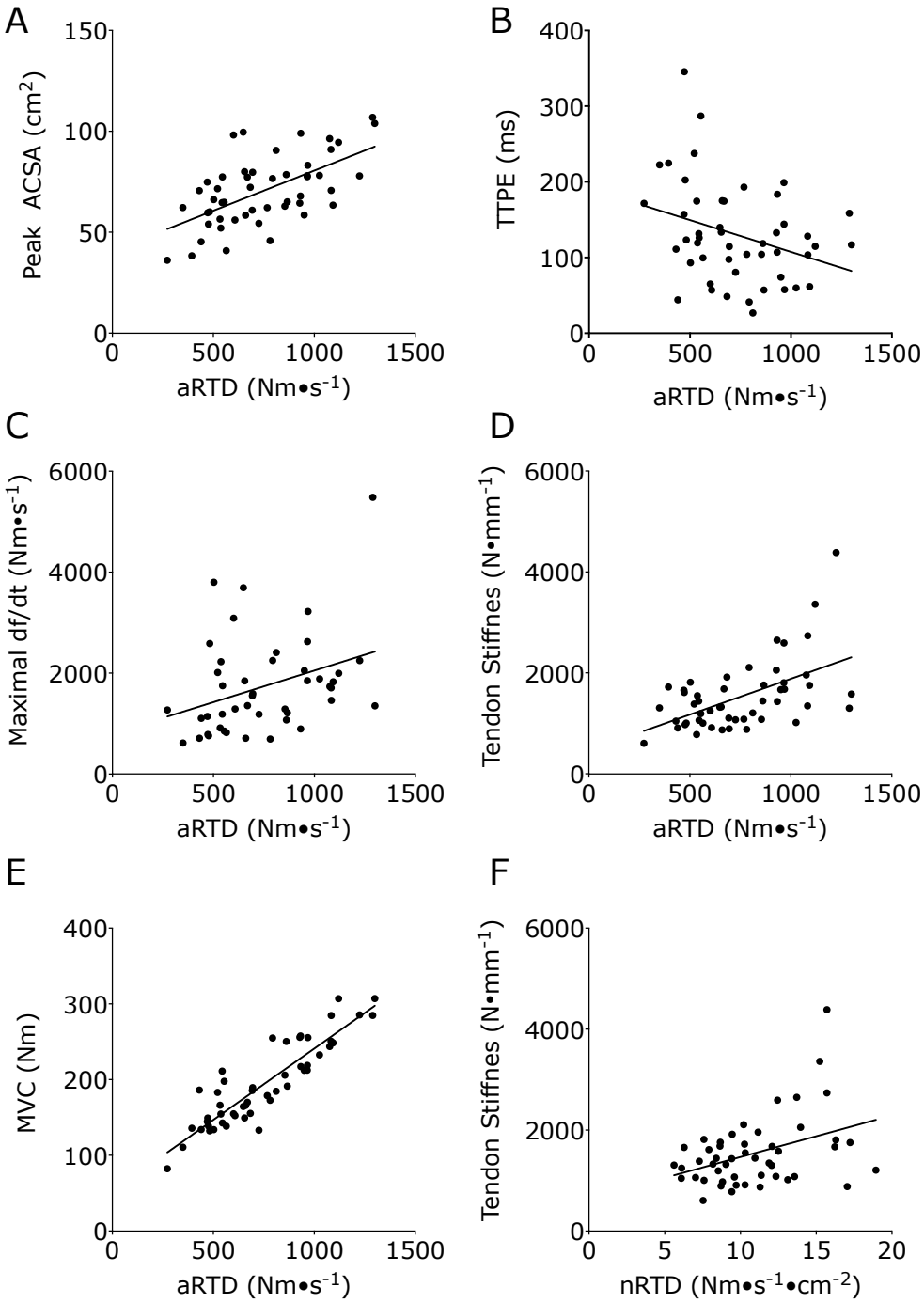


Figure 3.4: Graphs A-E demonstrate correlation between aRTD and peak quadriceps ACSA (A), TTPE (B), maximal twitch df/dt (C), tendon stiffness (D) and MVC Graph (E). The correlation between nRTD and tendon stiffness is also shown (F).

	Stiffness	**MVC**	**CSA**	**TTPE**	**Max df/dt**	**FL**
aRTD	.534*** p<0.001	.895*** p<0.001	.600*** p<0.001	-.320* p<0.05	.345* p<0.05	.144 NS
nRTD	.402** p<0.01	N/A	N/A	-.264 NS	.147 NS	.184 NS

Table 3.2: Correlation matrix for aRTD and nRTD with r values displayed, p values denoted by * p<0.05, ** p<0.01 and *** p<0.001.

3.5 Discussion

This study aimed to identify the origin of age-related differences in the rate of torque development in healthy older individuals. Our findings show that significant differences in the rate of torque development exist between young and older males both in terms of absolute RTD (aRTD) and RTD normalized (nRTD) for muscle size, thus accounting for age-related loss in muscle mass (sarcopenia). Notably, this study provides evidence that the observed decrease in RTD is accounted by, 1) lower tendon stiffness, 2) slower neuromuscular activation and 3) reduced ACSA and thus force output.

The results herein clearly demonstrate that at the maximal 10% of the force-elongation curve, the calculated patellar tendon stiffness was significantly lower in OM than YM. Moreover, Young's modulus was also found to be significantly lower, an expected result, as no tendon dimensional differences were observed. Thus, these observations suggest that these biomechanical changes cannot be explained by differences in tendon dimensions per se but are likely due to alterations in material properties. The lack of differences in tendon dimensions between groups explains why tendon stiffness was not different or affected RTD. Tendon tissue material alterations may include changes in tendon hydration status, glycosamine concentration, changes in elastin content, changes in cross-linking, a change in overall collagen content or a reduction in collagen fibril diameter (Narici and Maganaris, 2007; Couppé *et al.*, 2009). However, the latter two may be excluded, since previous work has demonstrated that there is no change in tendon collagen content with ageing (Couppé *et al.*, 2014) and one would assume a reduction in tendon CSA would follow a reduction in fibril diameter, which was not detected herein. To thoroughly investigate the potential drivers behind a change in tendon stiffness, biochemical analysis of the tendon tissue would need to be performed.

Nonetheless, our finding that patellar tendon stiffness is lower in OM is contradictory to previous work (Carroll *et al.*, 2008; Couppé *et al.*, 2009) therefore this is the first paper, to our knowledge, to demonstrate an age-related decline in patellar tendon stiffness. The two-aforementioned studies had smaller group sizes (n=6) and large variations in stiffness measuring, possibly limiting the detection of age-related differences. Large variations in tendon stiffness are often observed as adaptation is dependent upon total exposure to strain and hence levels of activity. The level of daily activity in the elderly varies considerably and may therefore produce a wide range of values, supporting the need for larger cohorts. Unfortunately, our study did not employ any measure of habitual activity and therefore it is plausible that our elderly group, despite being healthy and fully independent, were somewhat less active than the young.

Notably, our data illustrates that the aRTD of OM is significantly reduced in comparison to YM. As aforementioned RTD is a multi-factorial variable, however these data clearly shows that maximal force production has the greatest influence (R^2=0.52) when considering the absolute value of RTD. Inherently, there are additional neuromuscular factors which could contribute to

the age-related decrease in force output including a reduction in quadriceps peak ACSA and quadriceps AC, both of which were lower in OM. The data also demonstrates an increase in the twitch CT in OM, which is known to reflect a shift in the distribution of muscle fibre MHC-II isoforms (Harridge *et al.*, 1996). Our findings therefore suggest that one of the key-determinants of aRTD is the shortening velocity of the contractile component (Wilkie, 1949).

However, aRTD is also influenced by muscle force level of which one key factor is muscle size. Hence, if an individual is capable of larger force output, the value of RTD will inherently be higher simply because of allometric scaling. Accordingly, it is essential to normalize the absolute RTD value to muscle size to account for the influence of dimensional scaling on force production. Our findings show that following normalization, the age-related reduction in RTD is still present, indicating that RTD is not due simply to differences in muscle size. Significantly, after normalization to quadriceps ACSA, tendon stiffness still remains correlated to nRTD (R^2=0.16). This is an important finding as it demonstrates that even when the main muscular and neuronal factors are accounted for, tendon stiffness may still influence the rate of force development. This has important implications

for exercise interventions designed for the elderly. While it is very well known that muscle weakness in the elderly can be partially prevented through resistive exercise training, it is also known that tendon tissue adapts to exercise (Reeves, Narici and Maganaris, 2003; Kjaer *et al.*, 2009; Malliaras, Kamal, *et al.*, 2013). Therefore, it is essential that these interventions should also target the tendon tissue to induce some restoration of tendon stiffness. Furthermore, the data presented herein was acquired from healthy/non-frail individuals and thus one would assume these deficits would be even greater in those deemed frail; thus, further emphasising the need to appropriately recondition both muscle and tendon in old age. While these alterations wouldn't reduce the likelihood of tripping, the alterations would however, increase the individual's ability to counteracting the trip and hence reduce either the incidence or severity of the fall.

Unfortunately, this study has the limitation of lacking force-velocity measures. Nonetheless, given the close correlation between contraction time and MHC-II (Harridge *et al.*, 1996), the observed decrease in maximal df/dt in the OM participants suggests that velocity of contraction would be lower in these subjects. Conversely, a decreased contraction time is reflective

of an increased velocity of contraction (Bárány, 1967; Spector *et al.*, 1980). In addition, we also note that the data is cross-sectional in nature and inherently longitudinal data would be more apt in addressing the questions herein.

In conclusion, we have shown that a reduction in absolute and normalized knee extensor RTD with ageing is strongly influenced by an ageing-induced reduction in overall strength, but also in part due to a decrease in patellar tendon stiffness. Our study demonstrates evidence of a role of tendon stiffness and multiple neuromuscular factors in the decrease of RTD in older individuals.

4 Chapter 4 – MTU response to 8 weeks of submaximal RE in young males: The effect of CON vs ECC.

4.1 Abstract

Background: An effective MTU requires both the muscle and tendon to remain healthy and respond to mechanical loading in a synergistic manner. The aim of this study was to investigate the temporal response of both muscle and tendon to a submaximal RE protocol, consisting of either CON only or ECC only training.

Method: 20 young males (24.2 ± 1.3 yrs) were recruited for a submaximal training study and randomly assigned to a CON only or ECC only training group. Training consisted of 4 sets of 15 reps (60%1RM) 3 times a week on a modified leg press for 8 weeks. MVC, quadriceps volume, AC, RTD (0-200ms), pennation angle and fascicle length were chosen as the primary outcome muscle measures. Tendon stiffness and Young's modulus were assessed for tendon biomechanical properties. These assessments were carried out at week 4 and week 8.

Results: CON and ECC resulted in equal changes in MVC, quadriceps volume and AC by week 4 (CON:+11%, +2.8% and

+4.5%; ECC:+11%, +3.1% and +7.8% respectively) and week 8 (CON:+12%, +3.5% and +7.7%; ECC:+13%, +6.5% and +7.8% respectively). Pennation angle changed more in CON than ECC (Week 4: +3.7% vs +1.5% Week 8: +5.7% vs +2.4%). Whereas fascicle length change was greater in ECC (Week4: +0.9% vs +5.6% vs Week 8: +1.5% vs +7.1%). Tendon modulus was significantly increased from baseline for both CON and ECC at week 4 (+43% and +72% respectively) and 8 weeks (+44% and +79% respectively), with no statistical difference between contraction type.

Conclusion: The majority of tendon biomechanical alterations occur within the first 4 weeks of a RE intervention, regardless of contraction type. Similarly, muscle hypertrophy and strength gain were independent of contraction type. However, CON and ECC RE resulted in distinctly different architectural remodelling of the muscle. Interestingly, the tendon responses appeared greater than the muscle response to this submaximal training paradigm; albeit with a similar temporal pattern in the young i.e. early (4-week) changes which appeared to plateau and did not change further.

4.2 Introduction

While skeletal muscle is the site of force production; the tendon acts as the mechanical link between the muscle and the corresponding bone. As such the patellar tendon connects the quadriceps to the tibia, enabling leg extension. The primary role of the patellar tendon is force transmission which is unlike other tendons, such as the Achilles tendon, which also play a key in the storing and release of kinetic energy. Nonetheless, the patellar tendon has a key role in not only locomotion, but in rapid contraction of the leg extensors. Thus, the effective production and efficient transmission of force relies upon all components of the MTU (muscle and tendon) being optimal and working coordinately.

It is very well known that skeletal muscle responds to alterations in mechanical load (Atherton and Smith, 2012), whether it be an increase through exercise (hypertrophy) or a decrease through reduced contractile activity (atrophy). When mechanical loading is increased through RE, a number of changes are observed; they include increased strength (Schneider and Bishop, 2003; Seynnes, Boer and Narici, 2007), increased muscle mass (A. J. Blazevich *et al.*, 2007; Franchi *et al.*, 2014) and increased RTD (Aagaard *et al.*, 2002). Traditional RE typically entails the progressive overload of a

specific movement, consisting of both CON and ECC phases; whereby fascicles shorten in the CON phase, and lengthen in the ECC phase. ECC contractions are known to produce considerably more force than either CON contractions or even isometric contractions (Aagaard, Simonsen, Andersen, Magnusson, Halkjaer-Kristensen, *et al.*, 2000). However, during traditional RE, the eccentric phase is commonly under stimulated as the training weight is often based upon CON strength assessment. Significantly, when separating training modes into CON or ECC only, differential responses can be observed with regards to muscle architecture (Franchi *et al.*, 2014, 2015) and strength (Roig *et al.*, 2009; Baroni *et al.*, 2015).

As aforementioned the maintenance of an effective MTU relies upon both muscle and tendon remaining in optimal condition. Therefore, if any changes occur i.e. hypertrophy or atrophy in the muscle tissue, one would expect alterations to the in-series tendon, thus maintaining/coordinating the functionality of the MTU. Tendon tissue is known to respond to increases in mechanical loading (Reeves, Narici and Maganaris, 2003; Couppé *et al.*, 2008; Malliaras, Kamal, *et al.*, 2013). The tendon primarily responds by altering the biomechanical properties, such as stiffness and Young's modulus. Additionally, if the

mechanical loading is sufficient (i.e. total exposure), then changes in tendon dimensions (either through thickening or lengthening) may also occur (Kongsgaard *et al.*, 2007). The most common measures of tendon adaptation to training are tendon stiffness and/or Young's modulus, which both reflect the compliance and hence integrity of the tissue. As tendon tissue is hypothesised to respond to the amount of overall strain, one would postulate that ECC RE would elicit greater or perhaps quicker responses than CON or traditional RE. However, recent work suggests this may not be the case; instead it shows that there are no overall differences between CON and ECC training in terms of tendon biomechanics (Malliaras, Kamal, *et al.*, 2013). Nonetheless, a very recent publication by the Kubo research group, demonstrated that changes in patellar tendon stiffness only occur in response to CON training (Kubo *et al.*, 2017). Significantly, in these studies they were unable to isolate 'true' ECC only training, since the group training eccentrically, also included a CON contraction component; this may impact the conclusion and result in the lack of observable modality specific differences. Additionally, many studies that have investigated tendon response to RE only compared variables at baseline and the final time point (often 6-12 weeks). Therefore, the observed biomechanical modifications may occur earlier and any mode specific differences be missed.

Further to this, many studies focus upon only on muscle or tendon, thus whether one tissue responds quicker or to a greater degree than the other remains unknown.

Finally, the large majority of previous studies have either utilised high intensity RE (75-80% 1RM) (Reeves, Narici and Maganaris, 2003; Kongsgaard *et al.*, 2007; Malliaras, Barton, *et al.*, 2013), bodyweight (Kubo *et al.*, 2007) or plyometric training (Fouré, Nordez and Cornu, 2010; Kubo, Ishigaki and Ikebukuro, 2017); whereby positive changes were only observed with heavy RE. While the bodyweight and plyometric exercises pose a lower risk of injury than the heavy RE, the intensity was not sufficient to alter the tendon tissue biomechanics. However, whether a medium intensity RE regime (i.e. 60% of 1-RM) would induce adaptations in tendon is still to be investigated.

Thus, this study aims to investigate several outcomes. Firstly, we wish to investigate whether the tendon undergoes any biomechanical changes in response to a submaximal RE (60% 1-RM), with the additional aim of exploring whether CON or ECC specific training results in differential response. Moreover, we also aim to study the time-course and extent of tendon

adaptation and whether this response differs to that of muscle, and whether the two tissues alter in synergy.

4.3 Methods

Participant recruitment

A total of 20 healthy, recreationally active individuals, young males (YM, 24.2 ± 1.3 yrs) were recruited. These individuals were consequently randomly assigned to either the CON or ECC RE group. There were no differences in height (175 ± 1.3 vs 176 ± 2.2 cm), mass (74.2 ± 4.4 kg vs 73.0 ± 6.1 kg) or body mass index (BMI, 24.1 ± 1.2 vs 23.3 ± 1.4) between CON and ECC subjects respectively.

Statistical analyses

All data is presented as mean ± SEM. Time dependent changes were assessed using Bonferroni's two-way ANOVA with repeated measures, carried out upon the raw data values. Similarly, differences between ECC and CON RE, percentage change values (in respect to baseline), were also assessed with the above statistical approach. The significance level was set at $p=0.05$.

Assessments

The specific details for each technique and assessment are included within the general methods section (Section 2).

However, only the following muscle parameters were assessed within this study: MVC, RTD and nRTD, AC, co-activation, specific tension, muscle architecture including pennation angle and fascicle length by ultrasound, peak quad ACSA, peak VL ACSA and muscle volume via MRI. Tendon biomechanical properties assessed included stiffness and Young's modulus.

4.4 Results

Muscle size and function

MVC was significantly increased from baseline in the CON group by week 4 (222.1 ± 12.7Nm vs 245.3 ± 11.6Nm, $p<0.001$) and week 8 (222.1 ± 12.7Nm vs 248 ±13Nm, $p<0.001$). Similarly, MVC was also significantly higher than baseline after ECC RE at 4 (224.6 ± 18.4Nm vs 249.5 ± 20.9Nm, $p<0.001$) and 8 weeks (224.6 ± 18.4Nm vs 252.9 ± 20.5Nm, $p<0.0001$). There was no difference between contraction type at either time point (Figure 4.1).

Peak VL ACSA was found to be significantly increased from baseline by week 4 in both CON (Wk0: 27.0 ± 1.6cm^2 vs Wk4 29.0 ± 2.1cm^2, $p<0.01$) and ECC (Wk0: 28.9 ± 2.0cm^2 vs Wk4: 30.9 ± 1.7cm^2, $p<0.01$). Additionally, peak VL ACSA was still elevated compared to baseline at week 8 for both CON (Wk0: 27.0 ± 1.6cm^2 vs Wk8: 30.6 ± 2.1cm^2, $p<0.0001$) and ECC

(Wk0: 28.9 ± 2.0cm^2 vs Wk8: 32.2 ± 1.9cm^2, $p<0.0001$), (Figure 4.2, Table 4.2). Like VL ACSA, peak quadriceps ACSA showed a similar trend with increases from baseline at week 4 in CON (Wk0: 71.6 ± 4.2 vs Wk4: 77.0 ± 4.4, $p<0.0001$) and at week 8 in both CON and ECC (CON: Wk0: 71.6 ± 4.2cm^2 vs Wk8: 76.6 ± 4.2, $p<0.001$ and ECC: Wk0: 79.4 ± 5.3 vs Wk8: 84.3 ± 5.6, $p<0.001$) (Figure 4.2, Table 4.2). Total quadriceps muscle volume was found to be significantly increased by 4 weeks (Wk0: 2026 ± 107.6cm^3 vs Wk4: 2077 ± 109cm^3, $p<0.001$) and 8 weeks of CON training (Wk0: 2026 ± 107.6cm^3 vs Wk8: 2093 ± 110 cm^3, $p<0.0001$) (Figure 4.2). Similarly, quadriceps volume was significantly increased by ECC training by week 4 (Wk0: 2143 ± 138cm^3 vs Wk4: 2209 ± 140cm^3, $p<0.0001$) and week 8 (Wk0: 2143 ± 138cm^3 vs Wk8: 2243 ± 145cm^3, $p<0.0001$). Further, there was a significant increase from week 4 to week 8 in ECC (Wk4: 2209 ± 140cm^3 vs Wk8: 2243 ± 145cm^3, $p<0.05$), (Figure 4.2). There was no difference between contraction type at either time point.

AC was found to be significantly higher after 4 weeks of CON (Wk0: 84 ± 1.7% vs Wk4: 80.3 ± 1.3%, $p<0.01$) and ECC RE (Wk0: 87.1 ± 1.1% vs Wk4: 80.9 ± 1.4%, $p<0.01$). AC was also higher than baseline after 8 weeks of both CON (Wk0: 86.4 ±

0.9% vs Wk8: 80.3 ± 1.3%, p<0.001) and ECC (Wk4: 86 ± 1.5% vs Wk8: 80.9 ± 1.4%, p<0.01) RE (Figure 4.2).

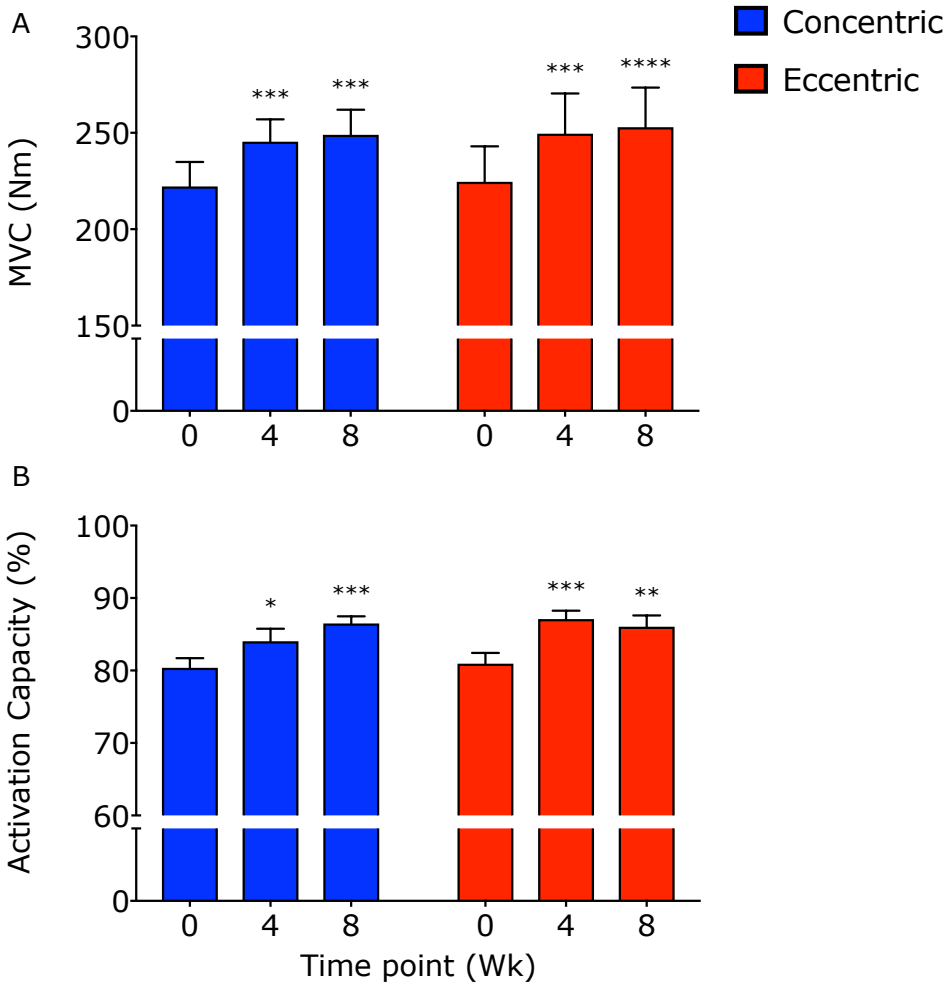


Figure 4.1: Time dependent changes in MVC (A) and AC (B) in response to CON (blue) and ECC (red) RE. Significant difference from baseline, or otherwise indicated, defined by * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.0001.

The measured RTD (0-200ms) was unaltered by either CON or ECC training at either time point (CON: 872 ± 60 Nm•s^{-1} vs Wk4: 847 ± 77 Nm•s^{-1} vs Wk8: 891 ± 64 Nm•s^{-1}, ECC: Wk0:

831 ± 98 Nm•s^{-1} vs Wk4: 819 ± 69 Nm•s^{-1} vs Wk8: 858 ± 85 Nm•s^{-1} for week 0 vs 4 vs 8 respectively). nRTD (normalised to peak quad CSA) was similarly unaffected by either CON (Wk0: 12.2 ±0.7Nm•s^{-1}•cm^{-2} vs Wk4: 10.3 ± 0.9Nm•s^{-1}•cm^{2} vs Wk8: 11.6 ± 0.6Nm•s^{-1}•cm^{-2}) or by ECC training (Wk0: 10.6 ± 1.2Nm•s^{-1}•cm^{-2} vs Wk4: 10.6 ± 1.1Nm•s^{-1}•cm^{-2} vs Wk8: 10.6 ± 1.4Nm•s^{-1}•cm^{-2})

Specific tension was unchanged from baseline to week 4 or week 8 in response to either CON (Wk0: 79.3 ± 3.9N•cm^{-2} vs Wk4: 82.0 ± 4.7N•cm^{-2} vs Wk8: 83.5 ± 4.9N•cm^{-2}) or ECC RE (Wk0: 72.9 ± 5.8N•cm^{-2} vs Wk4: 77.4 ± 6.1N•cm^{-2} vs Wk8: 77.2 ± 6.1N•cm^{-2}).

Further, co-activation of the antagonist was unaltered in either CON (Wk0: 12.6 ± 2.5% vs Wk4: 13.3 ± 1.9% vs Wk8: 16.1 ± 2.6%) or ECC RE (Wk0: 16.6 ± 3.1% vs Wk4: 14.7 ± 1.9% vs Wk8: 19.2 ± 3.9%)

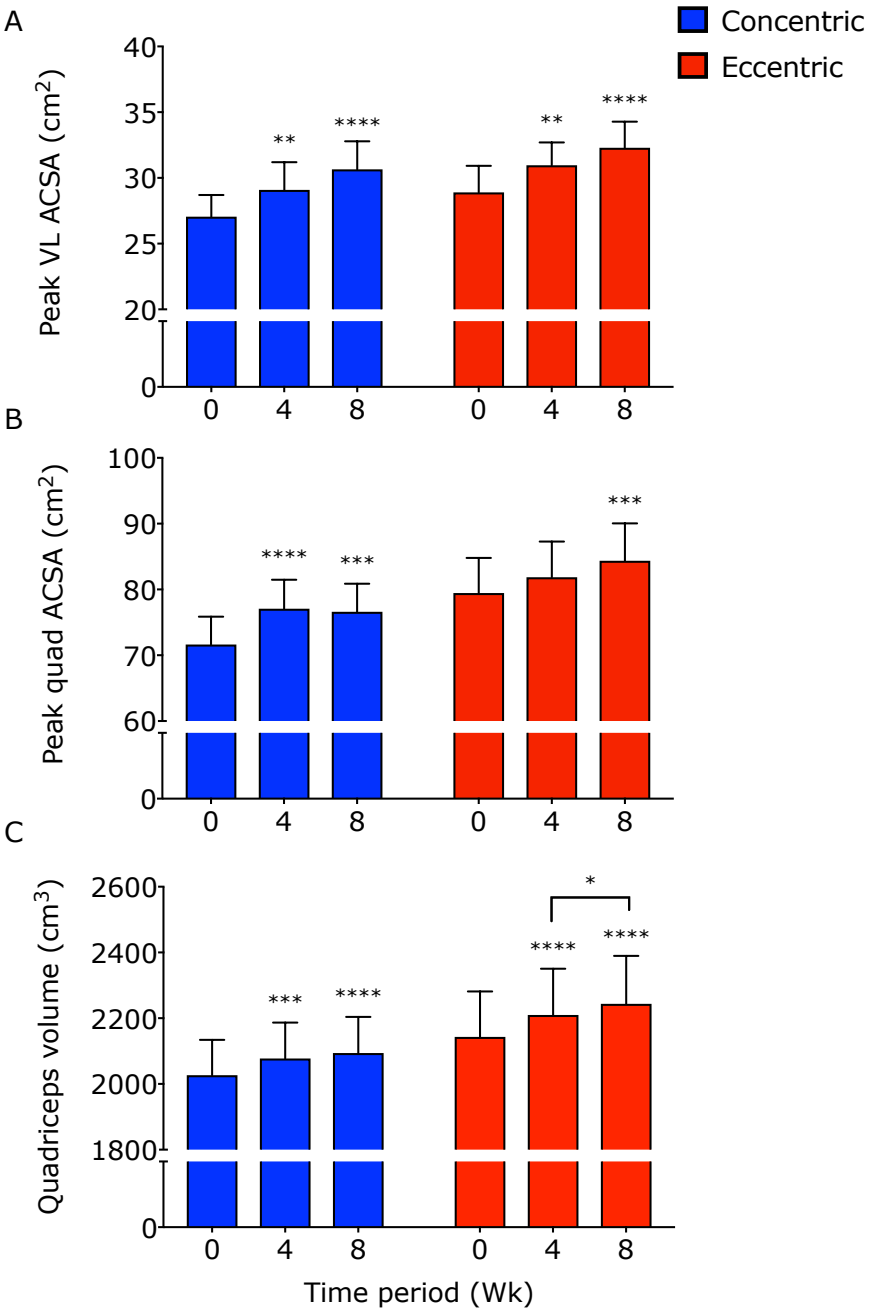


Figure 4.2: Time dependent changes in muscle size; peak VL ACSA (A), quadriceps peak ACSA (B), quadriceps volume (C) in response to CON (blue) and ECC (red) RE in young males. Significant difference from baseline, or otherwise indicated, defined by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$.

Muscle architecture

VL pennation angle was significantly increased in response to CON RE by week 4 (Wk0: 17.4 ± 0.5° vs Wk4: 18.1 ± 0.6°, $p<0.0001$) and by week 8 (Wk0: 17.4 ± 0.5° vs Wk8: 18.4 ± 0.6°, $p<0.0001$) compared to baseline, with an additional increase from week 4 to week 8 (Wk4: 17.4 ± 0.5° vs Wk8: 18.4 ± 0.6°, $p<0.01$). Pennation angle also increased in response to ECC training by week 8 (Wk0: 17.7 ± 0.8° vs Wk8: 18.1 ± 0.7°, $p<0.01$) (Figure 4.3A). There was a significant difference between CON and ECC in regards to percentage change in pennation angle from baseline at week 4 (CON:3.7% ± 0.4% vs ECC: 1.5 ± 0.6%, $p<0.05$) and week 8 (CON:5.7 ± 0.8% vs ECC: 2.4 ± 0.5%, $p<0.05$), (Figure 4.3C). Fascicle length was significantly increased from baseline in response to CON RE by week 8 (Wk0: 8.65 ± 0.3cm vs Wk8: 8.79 ± 0.3cm, $p<0.05$). However, fascicle length was significantly increased from baseline at both week 4 (Wk0: 8.34 ± 0.2 vs Wk4: 8.80 ± 0.2, $p<0.0001$) and week 8 (Wk0: 8.34 ± 0.2 vs Wk8: 8.92 ± 0.2, $p<0.0001$) in response to ECC RE. There was an additional increase from week 4 to week 8 (Wk4: 8.80 ± 0.2 vs Wk8: 8.92 ± 0.2, $p<0.05$), (Figure 4.3B). There was a significant difference between CON and ECC in regards to percentage change in fascicle length from baseline at week 4 (CON:0.9%

± 0.5% vs ECC: 5.6 ± 0.6%, p<0.0001) and week 8 (CON:1.5 ± 0.6% vs ECC: 7.1 ± 0.7%, p<0.0001), (Figure 4.3D).

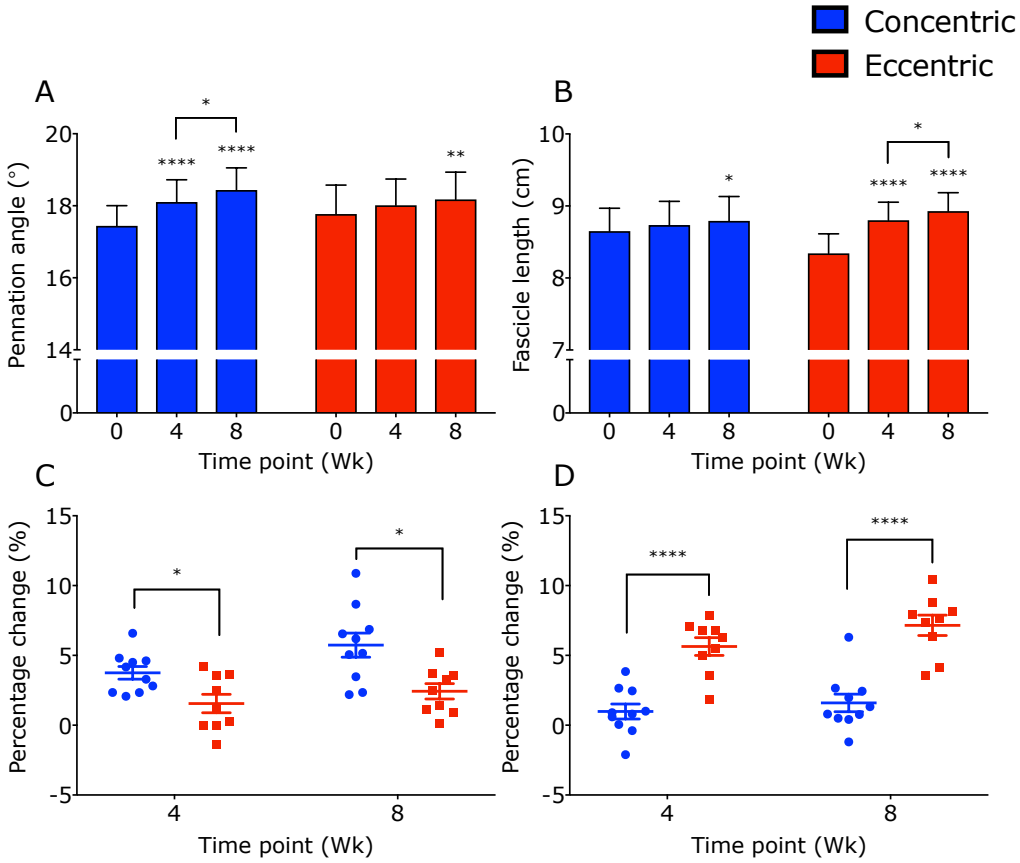


Figure 4.3: Time dependent responses in pennation angle (A), fascicle length (B) in response to CON (blue) and ECC (red) RE. Additionally, (C) and (D) reflect percentage change from baseline. Significant difference from baseline, or otherwise indicated, defined by * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.0001.

Tendon biomechanics

Tendon stiffness was found to be significantly different after 8 weeks of CON (Wk0: 1740 N•mm^{-1} ± 140 vs Wk8: 2421 N•mm^{-1} ± 204, p< 0.01) and ECC RE (Wk0: 1665 ± 195N•mm^{-1} vs Wk8: 2567 ± 226 N•mm^{-1} p<0.001). However, tendon stiffness was also found to be significantly altered after 4 weeks of ECC RE (Wk0: 1665 ± 195N•mm^{-1} vs Wk4: 2385 ± 248 N•mm^{-1} p<0.01). There were no differences in response to either ECC or CON RE at either time point (Figure 4.4). Tendon dimensions, L_0 and CSA, were unaltered at any time point for either training modality. Young's modulus followed a similar trend as tendon stiffness; whereby CON RE also caused significant increases by week 4 (Wk0: 1.20 ± 0.11GPa vs Wk4: 1.57 ± 0.15GPa, p<0.05) and week 8 (Wk0: 1.20 ± 0.11GPa vs Wk8: 1.67 ± 0.19GPa, p<0.01). Additionally, ECC RE caused an increase by week 4 (Wk0: 0.87 ± 0.11GPa vs Wk4: 1.30 ± 0.14GPa, p<0.01) and by week 8 (Wk0: 0.87 ± 0.11GPa vs Wk8: 1.34 ± 0.15GPa, p<0.001), (Figure 4.4).

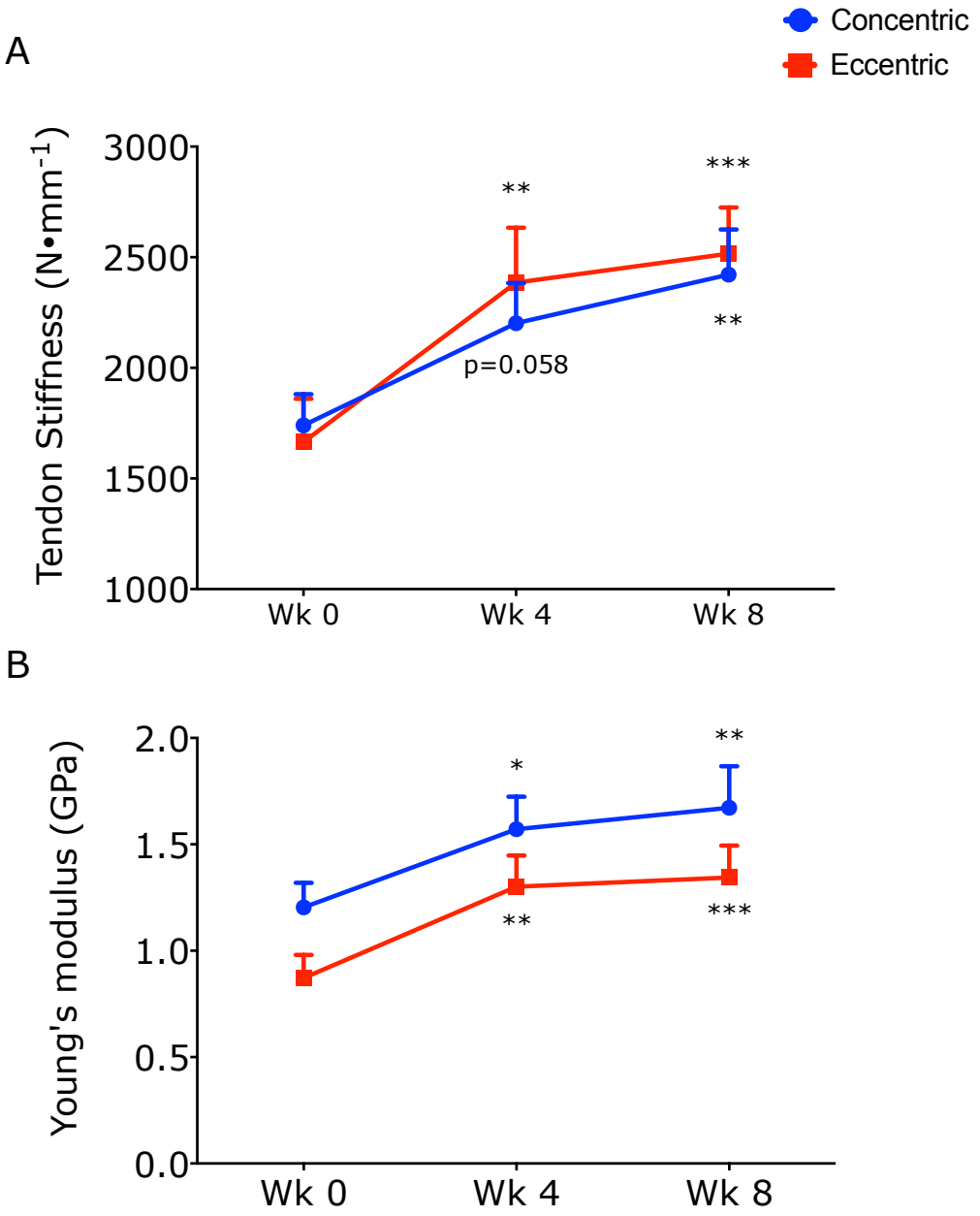


Figure 4.4: Time dependent changes in tendon stiffness (A) and Young's modulus (B) in response to CON (blue) and ECC (red) training in young males. Significant difference from baseline defined by * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

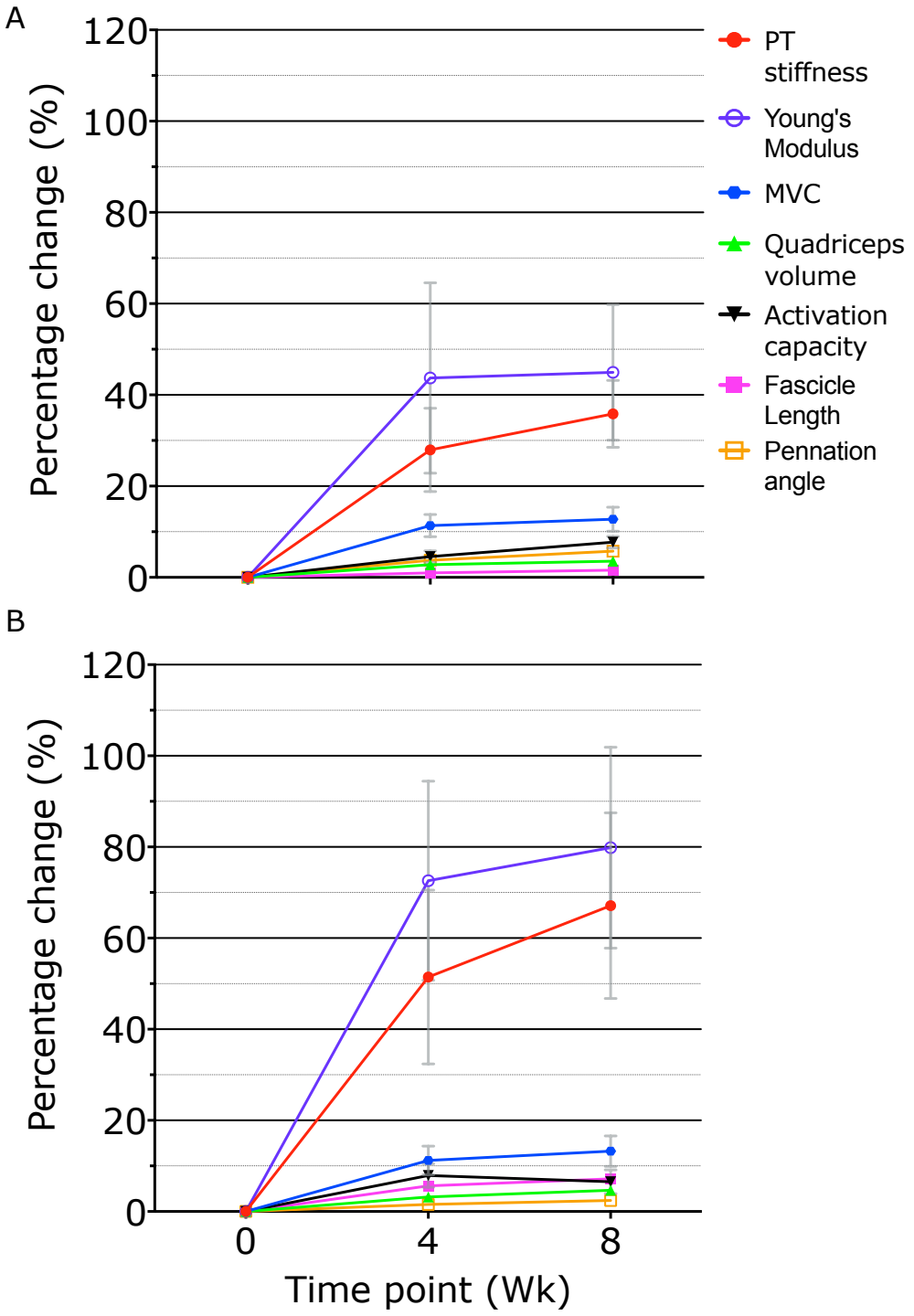


Figure 4.5: Summary of the temporal percentage change for all muscle and tendon variables in response to CON (A) or ECC (B) RE in young males.

4.5 Discussion

This study primarily aimed to investigate whether patellar tendon would respond to submaximal RE, and whether the response would differ between CON and ECC RE. Further to this, we wished to investigate whether the muscle and tendon would respond synergistically. The data herein clearly illustrates that the tendon responds robustly to submaximal RE, regardless of the specific contraction mode involved. Similarly, increases to muscle size and maximal strength were independent of contraction type; although hypertrophy occurred via two distinctly different architectural remodelling processes i.e. changes in pennation angle or fibre length, as has been observed previously at higher intensities (Franchi *et al.*, 2014, 2015). However, a major finding of this study is that while the muscle and tendon appear to adapt in a similar temporal fashion, the magnitude of tendon adaptation appears to be greater than that of the muscle, at least in percentage terms. Nonetheless, it is difficult to compare the two tissues, as a 100% change in one measure, may not be as influential as a 10% in another.

Our data clearly shows that PT stiffness (assessed over the maximal 10% of the force-elongation curve) is highly responsive to submaximal RE, with changes of 35% and 67%

by week 8 for CON and ECC RE respectively. Further to this, Young's modulus increased with changes of 43% and 44% for CON and 72% and 79% for ECC RE, at week 4 and week 8 respectively. As no changes in PT CSA or length were found, it is therefore entirely consistent that Young's modulus increased in line with stiffness changes. Further, this change in Young's modulus demonstrates that the alterations in PT biomechanical properties are due to changes in the material properties of the tendon. As aforementioned, tendon tissue material alterations may include changes in tendon hydration status, glycosamine concentration, changes in elastin content and/or changes in cross-linking (Narici and Maganaris, 2007; Couppé *et al.*, 2009). Further studies are underway to investigate biochemical changes in tendon. Most likely, it is a combination of these factors as opposed to one single contributor. Nonetheless, an interestingly, the large majority of biomechanical changes within tendon occur in the first 4 weeks, after this point our data suggests that the response may begin to plateau in the young.

Interestingly, the changes in modulus by week 8 herein (CON: +44%, ECC: +79%) are similar what has been reported after 12 weeks in a high intensity study (CON: 70%, ECC:84%) (Malliaras, Kamal, *et al.*, 2013); albeit their CON response was

26% higher than what we observed. However, the Malliaras study didn't utilise 'true' CON or ECC contractions; the CON group utilised one leg to raise the weight, and two to lower. Thus, while the additional ECC stimulation may have been minimal, over a prolonged period such as 12 weeks, this may have resulted in a slightly larger stimulus in modulus than the CON alone. Additionally, the Malliaras study took place over 12 weeks compared to 8 weeks herein, which may explain a slightly higher modulus change. Nonetheless, even though Malliaras utilised a high intensity training (~80% 1RM), the changes in PT modulus are comparable to our data at a much lower intensity; which may have additional benefits in elderly or frail subjects. Therefore, this suggests that intensity may not be the main driving factor behind tendon biomechanical changes; in such that once a specific threshold of strain or intensity is surpassed, the adaptations will occur, though this remains to be fully tested. This concept is supported by the notion that bodyweight, low strain plyometric training (Fouré, Nordez and Cornu, 2010; Kubo, Ishigaki and Ikebukuro, 2017), and low intensity RE (40% 1RM) (Grosset *et al.*, 2014) do not alter tendon biomechanics; whereas higher intensity RE and even submaximal RE, as shown herein, does. Our data also demonstrates that there is no difference in the young in the responses to CON and ECC. However, young's modulus

changed by 43% and 44% in CON, whereas ECC changed by 72% and 79% for week 4 and 8 respectively. Therefore, there is approximately a 30% difference between the two contraction types at both time points, although this wasn't statistically significant, perhaps due to limited sample number or shorter duration of training.

Following 8 weeks of submaximal CON or ECC RE, peak VL ACSA, peak quadriceps ACSA and total quadriceps muscle volume were found to be significantly increased from baseline, clearly demonstrating muscle hypertrophy. Although, it was also noted that there were no differences, in the percentage change from baseline, between CON and ECC RE. This is not the first time it has been shown that CON and ECC illicit similar hypertrophic responses in terms of ACSA (A. J. Blazevich *et al.*, 2007; Farup *et al.*, 2013) and muscle volume (Franchi *et al.*, 2014). Surprisingly, at week 4, an increase in quadriceps ACSA was absent in the ECC group despite increases to both VL ACSA and whole quadriceps volume; whereas, the CON group had changes in all measures. However, it has previously been shown that regional/spatial hypertrophy occurs specifically in response to CON or ECC RE (Franchi *et al.*, 2014), wherein, ECC RE resulted in greater distal hypertrophy compared to CON which resulted in greater mid-belly hypertrophy. This study

measured peak ACSA, at around 50-60% of the muscle length, i.e. mid belly. Thus, in the CON group, we are likely to detect an increase in peak ACSA, however, as ECC RE predominantly result in distal hypertrophy, we may not observe a change in ACSA.

Nonetheless, while similar levels of overall muscle hypertrophy occurred by week 8 (i.e. quadriceps volume); it is likely that the pattern and spatial arrangement of structural remodelling, by which this hypertrophy was achieved, differs between contraction type. This is supported by changes in fascicle length and pennation angle observed herein. CON training elicits a significant increase in pennation angle (as early as 4 weeks), with a smaller increase in fascicle length. Whereas, the opposite occurs in ECC training whereby remodelling occurs via increases to fascicle length, and small changes to pennation angle. Thus, this suggests that the hypertrophy observed in CON RE, occurs predominately via the addition of sarcomeres in parallel and hence increased pennation angle. Whereas in contrast, in response to ECC RE, the new sarcomeres are placed in series; hence increased fascicle length. This data is of course not the first to demonstrate this contraction specific structural remodelling (N. D. Reeves *et al.*, 2009; Franchi *et al.*, 2014, 2015). However, these studies all utilised a higher intensity of

training (~70-80% 1-RM), thus it is interesting to note that the contraction specific remodelling of muscle still occurs with a submaximal training programme. Therefore, taken collectively this implies that the absolute intensity of training doesn't appear to dictate architectural adaptation; instead, differential architectural remodelling occurs due to the specific mechanical stress that is placed upon the muscle fascicles in each mode of contraction i.e. shortening contractions vs lengthening contractions.

This data also demonstrates that rapid force production (i.e. RTD) is unaffected by either submaximal CON or ECC RE. In contrast, previous research has shown RE can positively affect RTD (Aagaard *et al.*, 2002; Caserotti *et al.*, 2008), although this is not always the case (Hakkinen *et al.*, 1998). However, those who observed changes in RTD utilised not only high intensity training (75-80% 1RM), but they also trained with 'maximal intentional acceleration of the load'. In contrast, the training method utilised herein was considerably slower, with each contraction lasting 3 seconds. Thus, perhaps it is unsurprising that this training paradigm did not induce changes in RTD.

While both tendon and muscle appeared to adapt to both CON and ECC training regimes, we wanted to investigate whether the magnitude or temporal aspect of these responses differed between the tissues. We found that the tendon appears to respond more readily than all of the muscle measures in both the CON and ECC groups (Figure 4.5). This is, to the best of my knowledge, the first time that this difference in degree of response has been reported. Therefore, as the tendon is known to influence many factors such as force production (Reeves, Narici and Maganaris, 2006) and RTD (Quinlan *et al.*, 2017), it is important that any research study investigating muscular functionality also considers changes in the corresponding tendon. Nonetheless, while the magnitude of responses are more readily observed within the tendon, it appears that the overall temporal pattern of responses is similar between the two tissues. All the muscle measures (apart than RTD) are maximally increased by week 4, as is tendon modulus. Thus, it appears all components of the MTU adapt simultaneously, which enables the MTU to co-ordinately maintain its efficacy and functionality.

In conclusion, these data clearly show that tendon biomechanical properties are substantially improved by submaximal (60% 1-RM) RE in young males, regardless of

contraction mode. Additionally, this response primarily occurred within the first 4 weeks of intervention, before plateauing at week 8. While CON and ECC resulted in similar increases in muscle strength and size, we demonstrate that CON and ECC RE result in distinctly different architectural remodelling of the muscle. Finally, the most significant conclusion is that while the muscle showed a mild response (changes to MVC, muscle volume, AC and architecture) to this submaximal RE; the response of the tendon biomechanics was greater (~4-6 times that of muscle). However, while the responses were different in terms of magnitude, they followed a similar temporal pattern, presumably to coordinate the changes and maintain the efficacy of the MTU.

5 Chapter 5 – MTU response to 8 weeks of submaximal RE in older males: The effect of CON vs ECC.

5.1 Abstract

Background: Many aspects of the MTU become negatively affected in healthy ageing, including loss of muscle mass, strength and tendon biomechanical properties, which consequently affects functionality. However, it is known that the MTU in older people still responds RE training. The aim of this study was to investigate the temporal response of both muscle and tendon to a submaximal RE protocol, consisting of either CON only or ECC only training in older male healthy volunteers.

Method: 18 older males (68.2 ± 2.4 yrs) were recruited for a submaximal training study and randomly assigned to a CON only or ECC only training group. Training consisted of 4 sets of 15 reps (60% of 1RM) 3 times a week on a modified leg press for 8 weeks. MVC, quadriceps volume, AC, RTD (0-200ms), pennation angle and fascicle length were chosen as primary muscle outcome measures. Tendon stiffness and Young's modulus were assessed for changes in tendon biomechanical properties. These assessments were carried out at weeks 0, 4 and 8.

Results: CON and ECC resulted in similar changes in MVC, quadriceps volume and AC by week 8 (CON:+20%, +2.9% and +6.3%; ECC:+16%, +4.2% and +10.2% respectively). However, ECC resulted in greater muscle hypertrophy at week 4 compared to CON (+2.8% vs +1.0%). Pennation angle changed more in CON than ECC (Week 4: +3.6% vs +1.8% Week 8: +5.7% vs +2.0%). Whereas fascicle length change was greater in ECC (Week4: +1.2% vs +3.8% vs Week 8: +0.8% vs +5.5%). Tendon modulus was significantly increased from baseline for both CON and ECC at week 4 (+41% and +45% respectively) and further increased at 8 weeks (+85% and +90% respectively), with no difference between contraction type.

Conclusion: There were no significant differences between CON and ECC in terms of overall hypertrophy, muscle strength or tendon modulus at 8 weeks. However, our data suggests that with submaximal RE, ECC results in greater early hypertrophy, and this was achieved via distinctly different architectural remodelling of the muscle. Significantly, it appears that the tendon responds far more than the muscle in response to a submaximal training paradigm (i.e. 60% of

1RM); albeit with a similar temporal pattern, presumably to maintain the functionality of the MTU.

5.2 Introduction

During the natural ageing process, there is a significant loss of both muscle mass and strength (Narici and Maffulli, 2010; Mitchell *et al.*, 2012), which inherently leads to an increased incidence of falls (Skelton, 2001). While the loss of muscle mass is defined as sarcopenia, dynapenia instead refers to the age-related loss of strength and ultimately function (Clark and Manini, 2008, 2012). Dynapenia is multi-factorial, including loss of muscle mass, reduced neural drive, alterations in muscle architecture and muscle composition (Narici and Maffulli, 2010; Clark and Manini, 2012). In addition to this, the tendon becomes more compliant with ageing (Onambele, Narici and Maganaris, 2006; Quinlan *et al.*, 2017). It is also known that tendon stiffness can influence force production (Reeves, Narici and Maganaris, 2006) and also RTD (Quinlan *et al.*, 2017), thus ageing of the tendon may also contribute towards dynapenia.

While both muscular and tendinous properties are negatively affected with age, they can be, at least partially, restored through the implementation of a RE regime. High intensity (75-80% 1RM) RE programmes employed in the elderly result in

increases in strength (Reeves, Narici and Maganaris, 2004b; Caserotti *et al.*, 2008), muscle mass (Reeves, Narici and Maganaris, 2004a; Onambélé-Pearson, Breen and Stewart, 2010), RTD (Suetta *et al.*, 2004; Caserotti *et al.*, 2008), neural drive (Scaglioni *et al.*, 2002; Morse *et al.*, 2007), changes in muscle architecture (pennation angle and fibre length) (Reeves, Narici and Maganaris, 2004a, 2004b) and patellar tendon biomechanics (Reeves, Narici and Maganaris, 2003; Grosset *et al.*, 2014). However, if one could achieve similar benefits in the elderly utilising lower intensity RE, then it should be considered for multiple reasons. Lighter loads inherently pose less of an injury risk for the elderly or frail individual, reducing both potential muscle damage and placing less metabolic demand on the muscle. It has been suggested that the maximal response to RE in the elderly, in terms of MPS, can be achieved with training at 60% 1RM (V Kumar *et al.*, 2009) in comparison to 75 and 90%. Further, many studies have shown that low intensity training (40-50% 1RM) can still achieve strength gains in the elderly (Vincent *et al.*, 2002; Seynnes *et al.*, 2004). However, it is unknown whether the tendon would also respond at this submaximal intensity in older individuals. Nonetheless, these results provide rationale for utilising a lower intensity of training in the elderly.

The studies discussed above implemented conventional RE, which is underpinned primarily by the CON component of contraction, thus, the ECC component will inherently be under loaded. We wished to study the impact of purely ECC contractions, since when ECC contractions are isolated it is possible to see differential adaptations in the elderly, specifically in terms of muscle architecture (N. D. Reeves *et al.*, 2009). It was demonstrated that conventional training (dominated by the CON portion) elicited significant changes in pennation angle, which was greater than ECC. Whereas, ECC training elicited robust changes in fascicle length, greater than with CON. Thus, indicating that the divergent architectural remodelling of CON and ECC training is still present within elderly muscle. Consequently, these distinct remodelling mechanisms can influence the F/L relation in differing ways. Likewise, the tendon is also postulated to influence the F/L relation (Reeves, 2006). While it has been shown in the young there is either no difference in response to CON or ECC (Malliaras, Kamal, *et al.*, 2013), or perhaps a slight increase with CON (Kubo *et al.*, 2017); it is unknown which response occurs in the elderly. While CON and ECC contractions differ in terms of mechanics, they also differ with regard to the metabolic demand placed upon the muscle. ECC contractions place lower metabolic demands upon muscle compared to CON

(Bigland-Ritchie and Woods, 1976; Lombardi and Piazzesi, 1990; Jones, Round and de Haan, 2004). Further it has also been reported that the perception of effort during ECC RE is lower than in conventional training in an elderly population (Reeves N *et al.*, 2009). Moreover, as ECC contractions can produce larger amounts of force, it is possible for individuals to lift a heavier load during RE. Interestingly, it has been shown that ECC strength is somewhat preserved in the elderly (Roig *et al.*, 2010). Thus, ECC contractions provide an attractive form of training for elderly, and possibly frail subjects, to improve muscle mass and function at a lower weight, due to their lower metabolic demand and higher force outputs per unit weight.

The maintenance of an effective MTU relies upon both the condition of both muscle and tendon. Thus, it is essential to understand the temporal pattern of any structural or biomechanical changes in response to RE for both tissues in the elderly as this may have significant implications for the design and implementation of appropriate rehabilitation programmes.

Thus, this study aims to investigate several outcomes. Firstly, we wish to investigate whether tendon in the elderly undergoes structural (thickness or length) and/or biomechanical changes (stiffness and youngs modulus) in response to a submaximal

RE (60% 1-RM) programme. Additionally, we wish to explore whether CON or ECC provide a better counter measure for the ageing tendon and muscle. Finally, we also aim to study the time-course of adaptation for both tissues, and whether the response differs between muscle and tendon or whether the tissues adapt in a synergistic fashion.

5.3 Methods

Participant recruitment

A total of 18 healthy, recreationally active individuals, old males (68.2 ± 2.4 yrs) were recruited. These individuals were consequently randomly assigned to either the CON or ECC RE group. There were no differences in height (175 ± 3.3 vs 176 ± 5.8 cm), mass (79.2 ± 8.5 kg vs 76.8 ± 10.4 kg) or body mass index (BMI, 25.9 ± 2.2 vs 24.5 ± 2.7) between CON and ECC respectively. None of the participants utilized in this study were deemed as frail, or had a history of falls.

Statistical analyses

All data is presented as mean ± SEM. Time dependent changes were assessed using Bonferroni's two-way ANOVA with repeated measures, carried out upon the raw values. Similarly, differences between ECC and CON RE, percentage change

values (with respect to baseline), were also assessed with the above method. The significance level was set at p=0.05.

Assessments

The specific details for each technique and assessment are included within the general methods section (Section 2). However, the following were assessed within this study: MVC, RTD and nRTD, AC, specific tension, co-activation, muscle architecture including pennation angle and fascicle length by ultrasound, peak quad ACSA, peak VL ACSA and muscle volume via MRI. Tendon biomechanical properties including stiffness and Young's modulus were assessed by X & Y. Unfortunately one individual from each group was unable to access the MRI scanner due to previous history of working in metal industry without eye protection. Thus, for measures of via MRI, n=8 for both CON and ECC groups.

5.4 Results

Muscle size and function

MVC was unchanged at week 4 by either CON (Wk0: 152.4 ± 9.2Nm vs Wk4: 172.6 ± 10.9Nm) or ECC training (Wk0: 164.4 ± 10.9Nm vs Wk4: 187.4 ± 11.2Nm, p=0.0507). However, by week 8, MVC was increased from baseline in both the CON group (Wk0: 152.4 ± 9.2Nm vs Wk8: 186.5 ± 21.2Nm, P<0.01)

and ECC group (Wk0: 164.4 ± 10.9Nm vs Wk8: 187.7 ± 10.6Nm, $P<0.05$). There was no difference between contraction type at either time point (Figure 5.1).

AC was significantly increased at week 4 (Wk0: 75.6 ± 2.8% vs Wk4: 78.7%, $P<0.05$) and week 8 (Wk0: 75.6 ± 2.8% vs Wk8: 80.2 ± 2.5%, $P<0.01$) in the CON group. AC was also found to be significantly higher than baseline following 4 (Wk0: 75.7 ± 1.7% vs Wk4: 80.7 ± 2.4%, $P<0.001$) and 8 weeks of ECC RE (Wk0: 75.7 ± 1.7% vs Wk8: 83.1 ± 0.8%, $P<0.0001$) (Figure 5.1). There were no additional changes between week 4 and 8, or any difference between contraction type at either time point. Co-activation of the antagonist (Bicep femoris) was unchanged in either group at any time point for CON (Wk0: 20.6 ± 6.1% vs Wk4: 18.9 ± 4.7% vs Wk8: 23 ± 5.4%) or for ECC: (Wk0: 16.6 ± 3.6% vs Wk4: 16.06 ± 1.3% vs Wk8: 21.5 ± 3.7%) (Figure 5.1).

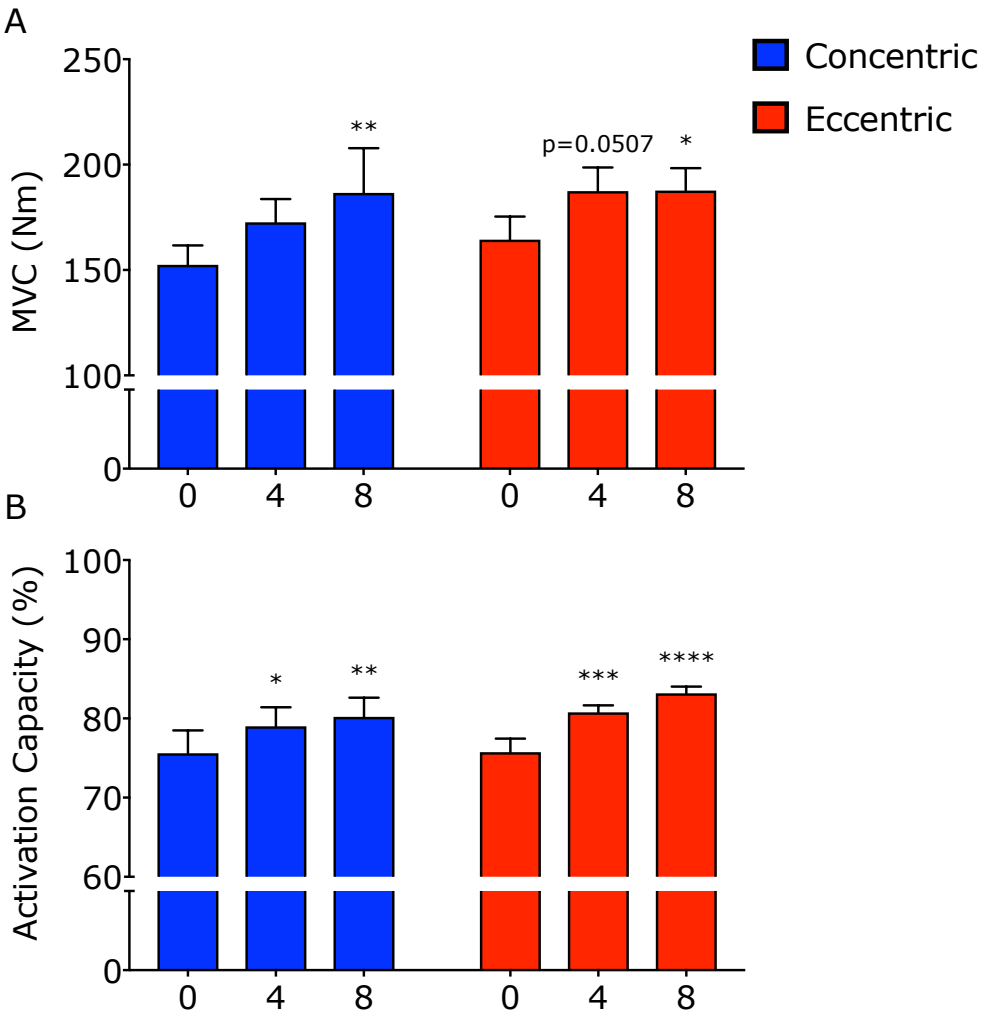


Figure 5.1: Time dependent changes in MVC (A) and AC (B) in response to CON (blue) and ECC (red) training in older males. Significant difference from baseline indicated by * P<0.05, ** P<0.01, *** P<0.001 and **** P<0.0001.

Peak ACSA of the VL was elevated compared to baseline at week 8 for both CON (Wk0: 23.3 ± 1.3cm^2 vs Wk8: 25.5 ± 1.5cm^2, P<0.001) and ECC (Wk0: 24.7 ± 1.6cm^2 vs Wk8: 26.6 ± 1.7cm^2, P<0.01), (Figure 5.2). Peak quadriceps ACSA, was found to be increased from baseline in both CON and ECC at

both week 4 (CON: Wk0: 62.0 ± 2.4 vs Wk4: 65.7 ± 2.4, P<0.01 and ECC: Wk0: 62.5 ± 3.7 vs Wk4: 66.3 ± 3.8, P<0.001) and week 8 (CON: Wk0: 62.0 ± 2.4 vs Wk8: 66.8 ±2.6, P<0.001 and ECC: Wk0: 62.5 ± 3.7 vs Wk8: 67.2 ± 4.0, P<0.0001). Quadriceps volume was found to be significantly increased by 4 weeks (Wk0: 1688 ± 93.7cm^3 vs Wk4: 1709 ± 91cm^3, P<0.05) and 8 weeks of CON training (Wk0: 1688 ± 93.7cm^3 vs Wk8: 1735 ± 90.9cm^3, P<0.0001), (Figure 5.2). Additionally, there was a further increase from week 4 to week 8 (Wk4: 1709 ± 91cm^3 vs Wk8: 1735 ± 90.9cm^3, P<0.01). There was a similar trend with ECC training; quadriceps volume significantly increased by week 4 (Wk0: 1706 ± 104cm^3 vs Wk4: 1751 ± 103cm^3, P<0.0001) and by week 8 (Wk0: 1706 ± 104cm^3 vs Wk8: 1777 ± 109cm^3, P<0.0001). Further, there was also a significant increase in ECC from week 4 to week 8 (Wk4: 1751± 103cm^3 vs Wk8: 1777 ± 109cm^3, P<0.01), (Figure 5.2).

The measured aRTD (0-200ms) was unaltered by either CON (Wk0: 515 ± 37 Nm•s^{-1} vs Wk4: 525 ± 41 Nm•s^{-1} vs Wk8: 529 ± 31 Nm•s^{-1}) or ECC RE (Wk0: 578 ±51 Nm•s^{-1} vs Wk4: 592 ± 41 Nm•s^{-1} vs Wk8: 614 ± 38 Nm•s^{-1}). Similarly, nRTD (normalised to peak quad CSA) was also unaltered by either training modality at any time point (CON: Wk0: 8.43 ±0.6Nm•s^{-1}•cm^2 vs Wk4: 8.48 ±0.9Nm•s^{-1}•cm^2 vs Wk8: 8.14 ± 0.6Nm•s^{-1}

^{1}•cm^{2}; ECC Wk0: 10.2 ± 1.1Nm•s^{-1}•cm^{2} vs Wk4: 8.3 ± 0.4Nm•s^{-1}•cm^{2} vs Wk8: 8.7 ±0.6Nm•s^{-1}•cm^{2})

In response to CON RE, specific tension was unchanged from baseline to week 4 (Wk0: 62.5± 3.9N•cm^{-2} vs Wk4: 68.0 ± 4.5N•cm^{-2}) but significantly increased at week 8 (Wk0: 62.5± 3.9N•cm^{-2} vs Wk8: 71.6 ± 7.6N•cm^{-2}, P<0.05) (Figure 5.3). However, in response to ECC RE, specific tension was unaltered at any time point (Wk0: 68.8 ± 4.3N•cm^{-2} vs Wk4: 72.5 ± 5.1N•cm^{-2} vs Wk8: 71.7 ± 4.5N•cm^{-2}).

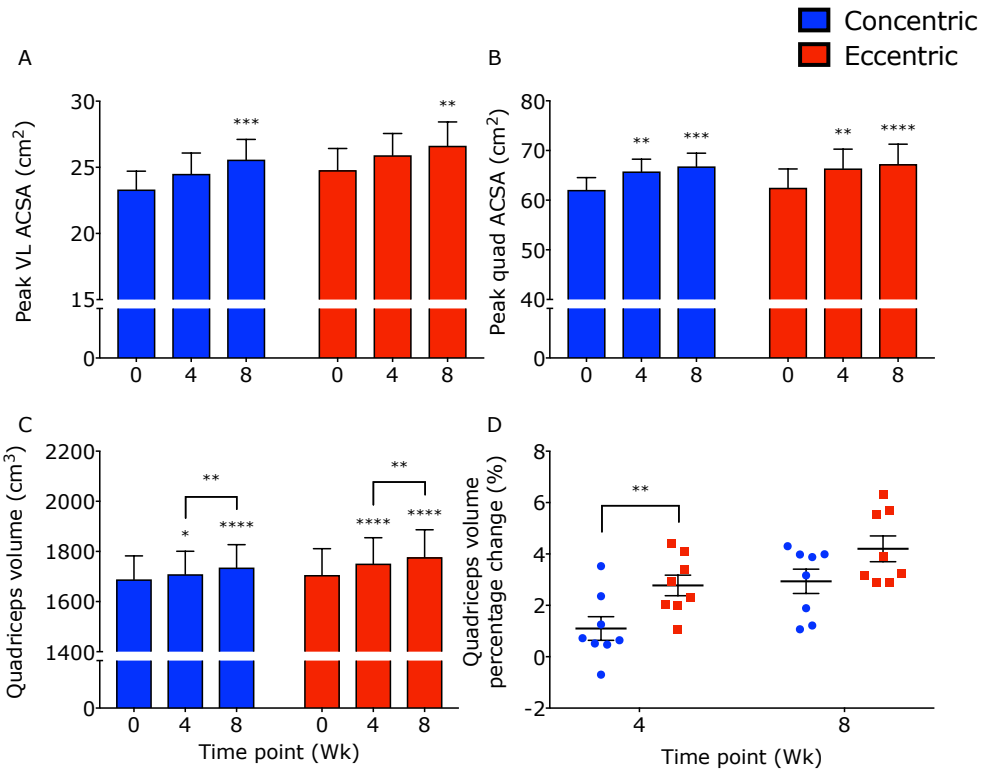


Figure 5.2: Time dependent changes in muscle size; peak VL ACSA (A), quadriceps peak ACSA (B), quadriceps volume (c)

and the percentage change in volume (D) in response to CON (blue) and ECC (red) RE in older males. Significant difference from baseline, or otherwise indicated, defined by * P<0.05, ** P<0.01, *** P<0.001 and **** P<0.0001.

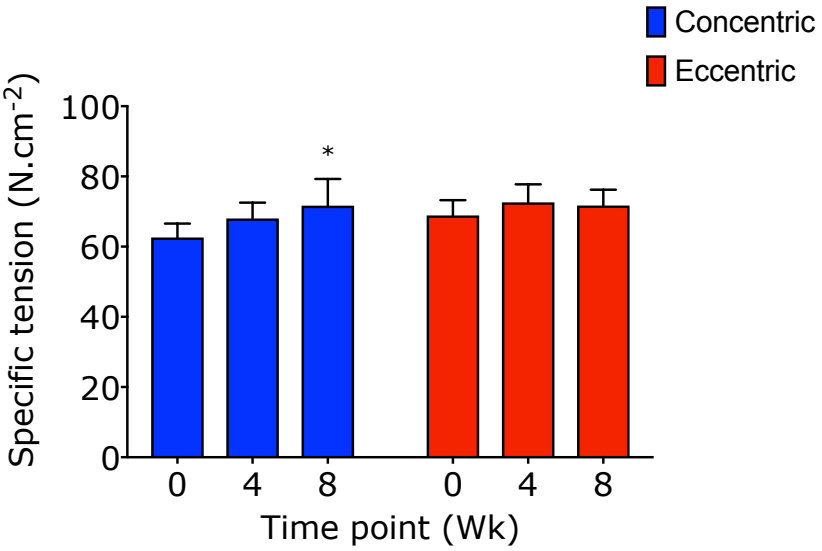


Figure 5.3: Time dependent changes in specific tension in response to CON (blue) or ECC (red) RE in old males.

Muscle architecture

Pennation angle was found to be significantly increased from baseline in response to CON training at week 4 (Wk0: 15.30 ± 0.8° vs Wk4: 15.83 ± 0.8° , *P*<0.0001) and by week 8 (Wk0: 15.30 ± 0.8° vs Wk8: 16.15 ± 0.8°,P<0.0001). There was also a significant increase from week 4 to week 8 in response to CON training (Wk4: 15.83 ± 0.8° vs Wk8: 16.15 ± 0.8°, P<0.001). Similarly, pennation angle increased from baseline in response to ECC RE by week 4 (Wk0: 14.38 ± 1.1° vs Wk4:

14.61 ± 1.0°, P<0.05) and week 8 (Wk0: 14.38 ± 1.1° vs Wk8: 14.64 ± 1.0°, P<0.01), (Figure 5.4A). It was also found that there was a significant difference between CON and ECC, in regards to the percent change in pennation angle at week 8 (CON: 5.7 ± 0.7% vs ECC: 2.0 ± 0.5%, P<0.001), (Figure 5.4C). Fascicle length was entirely unchanged in response to CON RE; whereas it was significantly increased from baseline in the ECC group at week 4 (Wk0: 8.41± 0.3cm vs Wk4: 8.74± 0.3cm, P<0.0001) and week 8 (Wk0: 8.41± 0.3cm vs Wk8: 8.87± 0.3cm, P<0.0001), (Figure 5.4B). There was also an additional increase from ECC week 4 to week 8 (Wk4: 8.74± 0.3cm vs Wk8: 8.87± 0.3cm, P<0.01). Finally, there was also a significant higher percentage increase to ECC than CON RE at both week 4 (ECC: 3.8 ± 0.4% vs CON: 1.2 ± 0.4%, P<0.0001) and week 8 (ECC: 5.5 ± 0.6 vs CON: 0.87 ± 0.5, P<0.0001) in respect to fascicle length (Figure 5.4D).

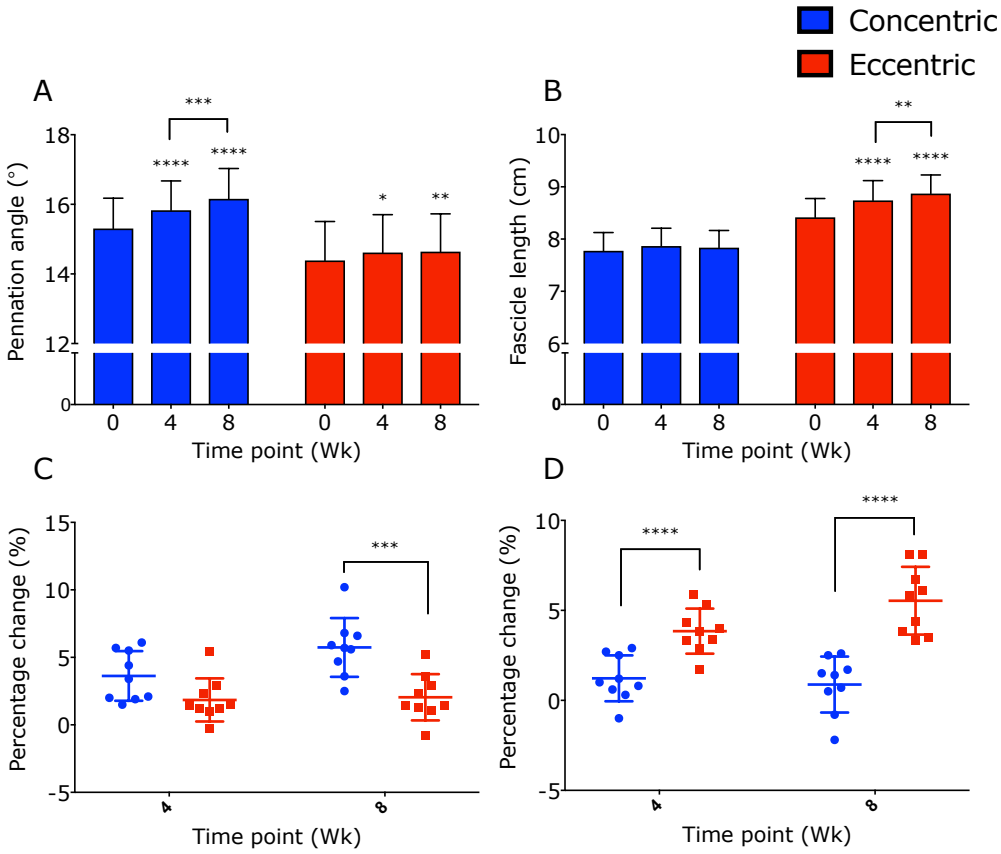


Figure 5.4: Time dependent responses in pennation angle (A), fascicle length (B) in response to CON (blue) and ECC (red) RE. Additionally, (C) and (D) reflect percentage change for pennation angle and fascicle length from baseline respectively. Significant difference from baseline, or otherwise indicated, defined by * P<0.05, ** P<0.01, *** P<0.001 and **** P<0.0001.

Tendon biomechanics

Tendon stiffness was positively affected after 4 (Wk0: 1316± 131N•mm^{-1} vs Wk4: 1882 ± 239N•mm^{-1}, P<0.01) and 8 weeks (Wk0: 1316± 131N•mm^{-}1 vs Wk8: 2391 ± 263N•mm^{-1},

P<0.0001) of CON RE. There was also a detectable increase from week 4 and week 8 (Wk4: 1882 ± 239N•mm^{-1} vs Wk8: 2391 ± 263N•mm^{-1}, P<0.05), (Figure 5.5A). PT stiffness was also found to be significantly higher than baseline after both 4 weeks (Wk0: 1203 ± 84N•mm^{-}1 vs Wk4: 1823 ± 193N•mm^{-1}, P<0.01) and 8 weeks (Wk0: 1203 ± 84N•mm^{-}1 vs Wk8: 2297 ± 154N•mm^{-1}, P<0.0001) in the ECC group (Figure 5.5A). There was also an increase from week 4 to week 8 (Wk4: 1823 ± 193N•mm^{-1} vs Wk8: 2297 ± 154N•mm^{-1}, P<0.01), (Figure 5.5A). There were no alterations in tendon dimensions (L_0 or CSA) with either training modalities at either time point. Therefore, Young's modulus followed a similar trend to tendon stiffness; whereby it increased in response to CON training by week 4 (Wk0: 0.75GPa ± 0.06 vs Wk4: 1.08 ± 0.17, P<0.05) and week 8 (Wk0: 0.75GPa ± 0.06 vs Wk8: 1.39 ± 0.22, P<0.0001), (Figure 5.5B). Similarly, Young's modulus increased in the ECC group by week 4 (Wk0: 0.68 ± 0.04 vs Wk4: 1.02 ± 0.11, P<0.05) and week 8 (Wk0: 0.68 ± 0.04 vs Wk8: 1.28 ± 0.1 vs, P<0.001), (Figure 5.5B).

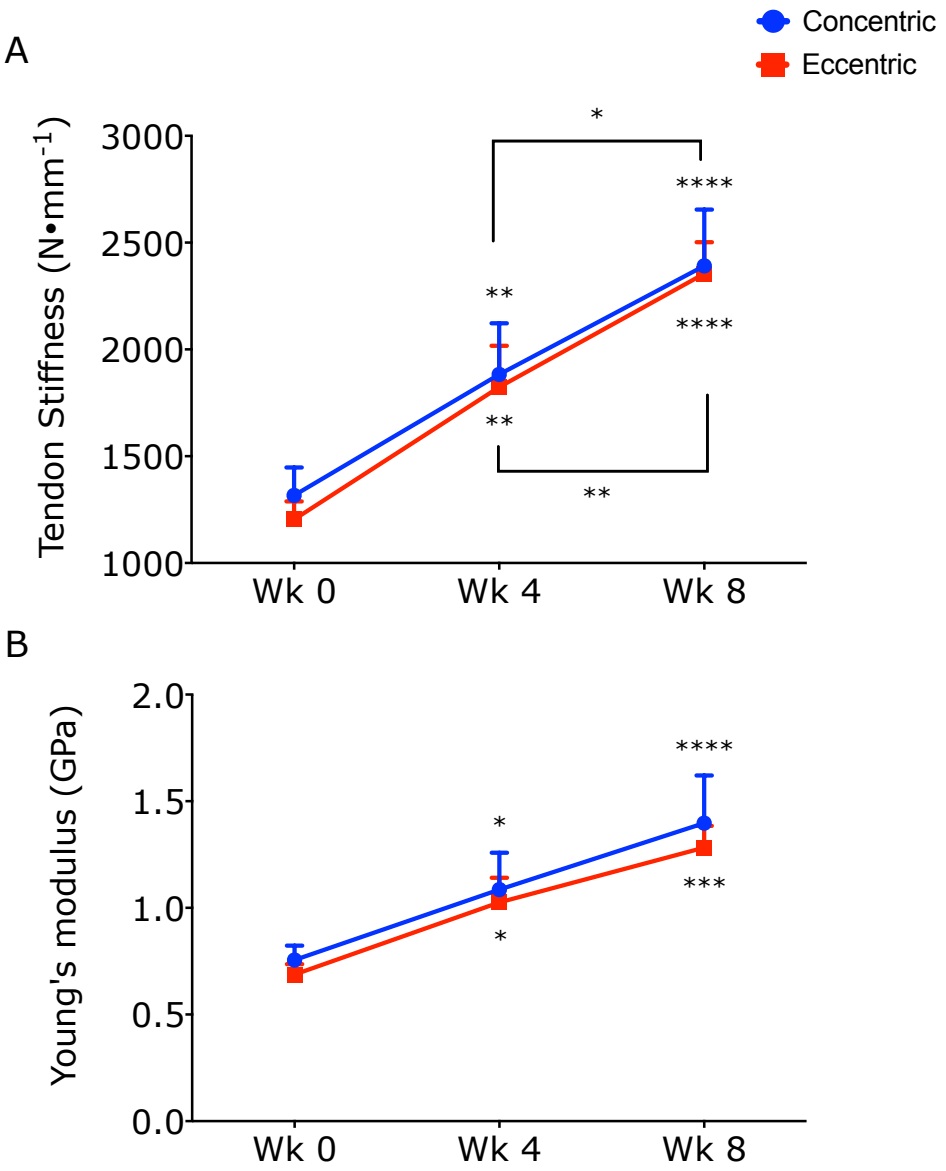


Figure 5.5: Time dependent changes in tendon stiffness (A) and Young's modulus (B) in response to CON (blue) and ECC (red) training in old males. Significant difference from baseline, or otherwise indicated, defined by * P<0.05, ** P<0.01, *** P<0.001 and **** P<0.0001

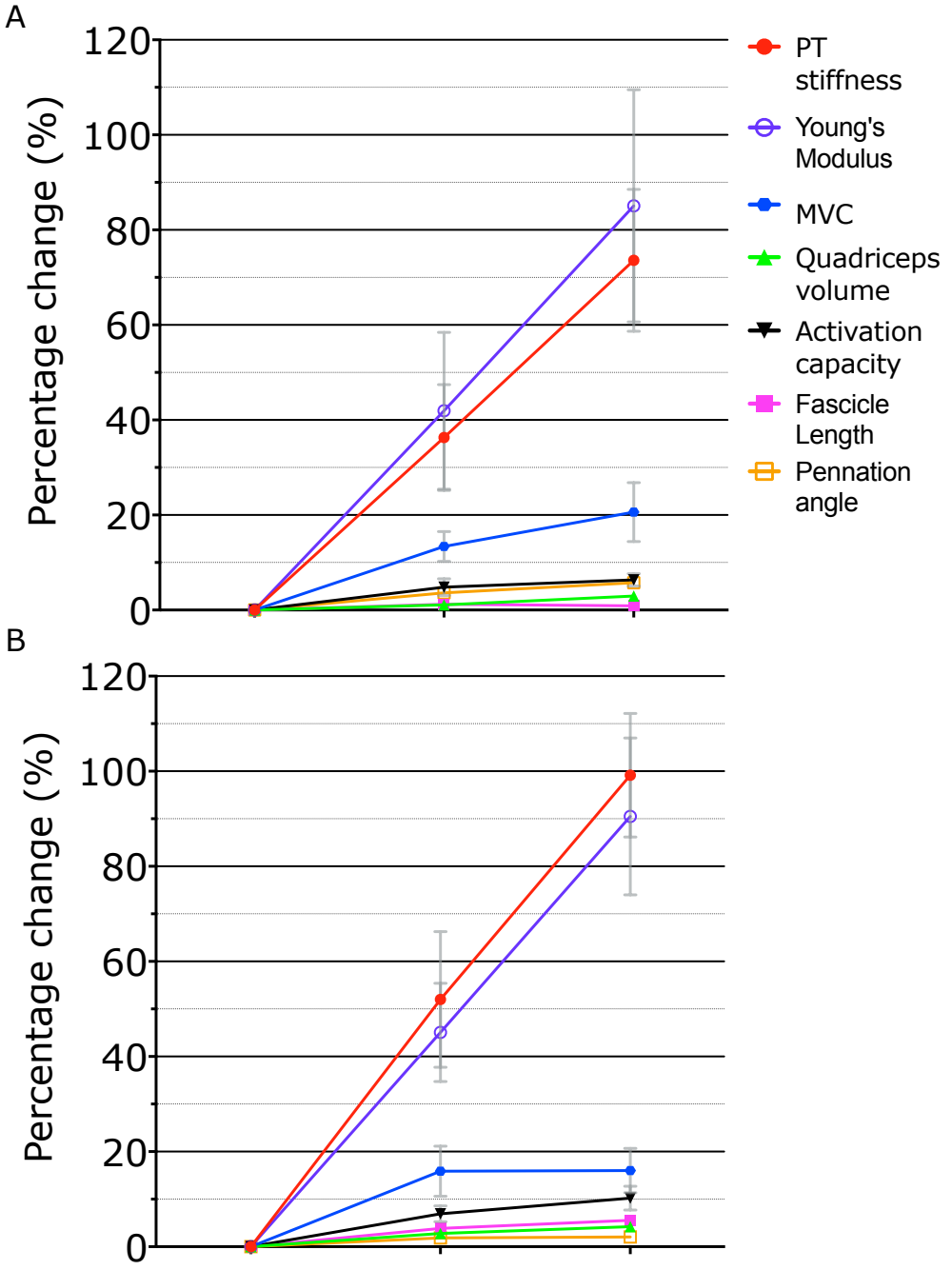


Figure 5.6: Summary of the temporal percentage change for all muscle and tendon variables in response to CON (A) or ECC (B) RE in old males.

5.5 Discussion

This study aimed to investigate whether the tendon would respond to submaximal RE in old males and whether the response would differ between CON and ECC RE. Further to this, we wished to investigate whether the muscle and tendon would respond in a coordinated and synergistic manner. The data herein clearly illustrates that an older tendon remains remarkably responsive to submaximal RE, regardless of the specific contraction type. Further, it also appears that both muscle hypertrophy and strength gains are achieved independent of contraction type. Nonetheless, spatial architectural remodelling is distinctly different in each contraction group. Significantly, the data clearly shows that while the muscle and tendon appear to adapt in similar temporal fashion, the apparent magnitude of the measures of tendon adaptation are numerically greater, and seem more robust than the functional and structural adaptations of the muscle.

Our data clearly shows that an older tendon is still highly responsive to submaximal RE, with changes in PT stiffness (assessed over the maximal 10% of the force-elongation curve)

of 36% and 51% by week 4 and 73% and 99% by week 8 for CON and ECC RE respectively. Interestingly, there are additional increases in stiffness from week 4 to week 8 in both CON and ECC groups; this suggests that the response of tendon stiffness in elderly subjects continues to increase for up to at least 8 weeks. Further studies are warranted to determine if this effect has plateaud at this point or if it would continue to rise, albeit at a slower rate. We also found no changes to PT CSA or PT length in response to either contraction type, therefore, unsurprisingly, Young's modulus followed a similar trend to PT stiffness, with changes of 41% and 45% by week 4 and 85% and 90% by week 8 for CON and ECC respectively. As discussed in the previous chapter, changes in tendon modulus indicate that alterations in PT biomechanical properties are due to alterations in the material properties and not tendon dimensions.

Significantly, we found no difference in the rate of change of stiffness or modulus between the CON or ECC group. Thus, this suggests that within the elderly, over this time period (8w) the tendon doesn't appear to distinguish between contraction type and will simply respond to the increased strain that it is exposed to; providing the strain stimulus is sufficient. This concept that sufficient strain is required to induce tendon

adaptation is further supported by previous research in the elderly. It has previously been shown that the tendon responds to high intensity (75-80% 1RM) RE through changes in both PT stiffness and modulus (Reeves, Maganaris and Narici, 2003; Onambele-pearson and Pearson, 2012; Grosset *et al.*, 2014). However, these changes are absent in response to low loading (40% 1RM) (Kongsgaard *et al.*, 2007; Grosset *et al.*, 2014). Collectively, this data suggests that there may be a strain threshold which requires to be surpassed before any adaptations to the biomechanical properties of tendon occurs (over 40%, but up to 60%). However, this strain threshold could be due to either the intensity of training, or perhaps the cumulative strain, i.e. total volume of strain, akin to time under tension effects in muscle (Burd *et al.*, 2012). Data from Kongsgaard's study suggests that the intensity of the training is possibly more critical (Kongsgaard *et al.*, 2007). They utilised one heavy training group (70% x 8 reps, 10 sets), and one light training group that was matched for total training volume (10 sets of 36 reps with weight which equalled total work in heavy group), thus total strain. It was found that only the heavy RE group had changes in tendon stiffness, whereas the light RE did not. Thus, although the total cumulative strain placed upon the tendon was the same, the intensity at which it was loaded upon the tendon was differed. Perhaps sufficient intensity 'switches

on' the responsiveness of the tendon, and then the overall strain dictates the amount or speed of tendon adaptation, similar to the stimulation of MPS observed in response to intensity (>60%-90%) (V Kumar *et al.*, 2009) although increasing volume also increased MPS response at lower intensities (Kumar *et al.*, 2012).

There was no difference in the response of CON or ECC groups in terms of tendon biomechanical properties in the elderly subjects. Likewise, within the muscle, many of the structural and functional data also showed that CON and ECC groups showed similar responses. MVC, AC, quad ACSA and VL ACSA were all equally increased in both groups at both time points. However, the increase in quadriceps volume at week 4 was greater in ECC than in CON (CON: +1.0% ECC: +2.7%); thereby, suggesting quicker hypertrophic responses i.e. within a short period (4 weeks) in the ECC group. There are several possible explanations for this difference, including differences in muscle activation, total work done or total time under tension. However, this study matched the training loads for neural activation. In matching for neural activation, we ensured that both training groups were upon the same F/V curve; such that both CON and ECC groups were training at the same relative intensity. However, while the intensity was matched,

due to the nature of ECC contractions, the total work or exposure to total tension would be higher than in CON RE (Katz, 1939) and it is known that mechanical tension influences the amount of hypertrophy (Fry, 2004). Further, previous investigations found that the force-time interval was strongly correlated to p38 and m-TOR (Rahnert and Burkholder, 2013) and that MAPK activation is quantitatively linked to tension (Martineau *et al.*, 2001). It is through this increased level of signalling that would result in greater degrees of hypertrophy. Thus, it is likely that the increased exposure to tension in ECC RE resulted in significantly greater levels of hypertrophy by week 4. Nonetheless, by week 8 the overall change in muscle volume compared to baseline was equal (CON: +2.9%, ECC: +4.2%).

While the overall degree of hypertrophy (quadriceps volume) is similar between CON and ECC at week 8, it is likely that the spatial structural remodelling, via insertion of new contractile units, occurs via two distinctly different pathways (Franchi, Reeves and Narici, 2017). As in the previous chapter, CON RE predominantly resulted in the increase of pennation angle; whereas, ECC RE resulted in changes to the fascicle length. These specific architectural alterations were found to be significantly different between the groups (i.e. pennation angle

CON>ECC, fascicle length ECC>CON). This architectural remodelling suggests that new contractile material is added in parallel in response to CON RE and in series in response to ECC RE in the elderly. While this specific remodelling has previously been reported to occur in the elderly (N. D. Reeves *et al.*, 2009); it has not been demonstrated with submaximal training. Thus, taken collectively with increases in strength and quadriceps hypertrophy, these data provide strong evidence for the use of submaximal training for rehabilitation or the application of RE within elderly cohorts.

We report an increase in specific tension at week 8 in the CON (+14%) but not ECC group. While it is not surprising that there in an increase following CON RE, it is puzzling that this did not occur in response to ECC RE, which had been shown to occur previously (Brandenburg and Docherty, 2002). There are multiple ways in which specific tension may be altered; (1) changes in whole muscle activation, (2) changes in co-activation of the antagonist or (3) changes in the muscle architecture, in particular pennation angle. However, in both CON and ECC, we see increases in AC, and no changes in co-activation; with no difference between the contraction types. Thus, this would not explain why specific tension increases in CON only. However, while pennation angle increases in both

groups, it occurs to a greater extent in the CON group at week 8. Increases in pennation angle reflect the addition of sarcomeres in parallel, thus increasing the force output for the same relative area which in turn would increase specific tension. As the increase in pennation angle is lower in the ECC group, this may not have been sufficient to result in increased specific tension.

RTD is known to be lower in old age (Klass and Duchateau, 2008; Couppé *et al.*, 2009) and low RTD has been associated with an increased incidence of falls (Cesar *et al.*, 2010); thus it would be beneficial to increase the RTD in an older population. We observed increases to both MVC and tendon stiffness, both of which influence RTD (Quinlan *et al.*, 2017), and so would expect to observe an increase in RTD. However, contrary to this, we found that both aRTD and nRTD remained unaltered by either CON or ECC RE at submaximal intensity. This lack of change is likely due to the nature of the training protocol, specifically the contraction profile. The RE applied herein required individuals to carry out each contraction over an elongated 3 second period, the aim being to increase time under tension, maximise load and consequently muscle hypertrophy. It is thought that changes in RTD are more

prominent when contractions occur with 'maximal intentional acceleration of the load' (Caserotti *et al.*, 2008).

Submaximal CON and ECC RE has been proven capable of inducing positive changes in many variables of the MTU in an older population, including tendon modulus, muscle size, strength and muscle architecture, albeit without changes to RTD. However, for the MTU to remain functional and effective the adaptations of each main component should occur in coordinated and synergistic fashion. It is of interest that, as with the young volunteers (Chapter 4), the elderly tendon responds more robustly than any of the muscle measures, at least in terms of percentage change from baseline (Figure 5.6). Tendon modulus increased by +41% in CON and +45% in ECC by week 4, whereas MVC had only changed by +13% and +15% and volume changed by +1% and +2.7% for CON and ECC respectively. As tendon is the structural bridge between muscle and bone, its main purpose is to transmit force onto the joint. However, when connected to large force producing muscles such as the quadriceps, if the tendon is insufficiently stiff, it is prone to rupture. Thus, it is possible that the tendon responds significantly greater than muscle so that it is prepared for the increased force production, whilst limiting the risk of injury. Nonetheless, while the degree of response may differ, it

appears that both muscle and tendon respond with a similar temporal profile; with changes occurring early i.e. in both tissues by week 4, and that this coordinated temporal response maintains the efficacy of the MTU during a RE training.

In conclusion, these data clearly show that tendon biomechanical properties are substantially changed by submaximal (60% 1-RM) RE in old males, irrespective of contraction type. Further, the submaximal RE induced hypertrophy and increased maximal strength in both CON and ECC, while RTD was unaffected. However, we demonstrate that CON and ECC RE result in distinctly different architectural remodelling of the muscle. It was also found that ECC RE resulted in greater levels of early hypertrophy compared to CON. Thus, as ECC contractions impose less metabolic demand upon the muscle than CON, this study provides evidence that submaximal ECC training provides an attractive alternative, but highly specific intervention protocol in an elderly or frail population. Finally, we also found that while the muscle showed a mild response (changes to MVC, muscle volume, AC and architecture) to this submaximal RE; the response of the tendon was considerably more. Nonetheless, our data suggests that both muscle and tendon follow similar temporal responses, presumably to maintain the efficacy of the MTU.

6 Chapter 6 – Do young and older males respond differently to 8 weeks of either CON or ECC submaximal RE?

6.1 Abstract

Background: There is a loss of muscle mass and strength in response to healthy ageing. While OM still respond to submaximal RE (as shown in chapter 5), it is suggested that the anabolic response to RE is dampened. Thus, this chapter will investigate whether the response in YM is greater than OM.

Method: 20 YM (24.2 ± 1.3 years) and 18 OM (68.2 ± 2.4 yrs) were recruited for a submaximal training study and randomly assigned to a CON only or ECC only training group. These individuals are the same that were utilised within chapters 4 and 5. The average percentage increase for MVC, VL ACSA, quadriceps ACSA, quadriceps volume, AC, pennation angle, fascicle length, PT stiffness and Young's modulus at both week 4 and week 8 were calculated.

Results: Hypertrophy and strength gain were equal between YM and OM, in both contraction types. Similarly, AC, and fascicle length changes were also comparably increased in YM

and OM. However, it appeared that ECC RE resulted in greater changes to pennation angle in OM than YM (+2.4 ± 0.5% vs +5.7 ± 0.7%, $p<0.05$).

Conclusion: It appears that both YM and OM respond comparably to submaximal CON or ECC, with similar changes to maximal strength, AC and muscle size. However, it is possible that the tendon of OM respond more so than YM, although this is likely due to their lower starting points. Nonetheless, our data suggests that the tendon of OM is restored to that of the YM.

6.2 Introduction

Chapters 4 and 5 have explored the response of the MTU to CON and ECC submaximal RE, in YM and OM, respectively. It was conclusively found that by week 8 many measures of the MTU were positively increased in both YM and OM. The alterations included VL and quadriceps hypertrophy, gains in strength, architectural remodelling and a stiffening of the PT. However, while the elderly adapt to this submaximal training, it is important to consider how complete this response was. Thus, this chapter will focus upon the comparison of YM and OM in terms of the percentage change in the measures discussed above.

Naturally as one ages, the musculoskeletal system experiences negative changes through the loss of muscle mass and a loss of strength (Narici and Maffulli, 2010; Mitchell *et al.*, 2012). Thus, RE intervention is an attractive option in an attempt to restore some of these lost characteristics and functions. However, within OM it is suggested that there is a dampened response to anabolic stimuli such as RE or AAs (Cuthbertson *et al.*, 2005; Vinod Kumar *et al.*, 2009; Fry *et al.*, 2011; Wall *et al.*, 2015). Therefore, it is believed that within an exercise intervention, OM would experience less adaptation to the RE than compared to YM; which has been shown to occur

previously (Brook *et al.*, 2016). Nonetheless, one strategy to overcome this anabolic resistance is potentially through an increased training volume (Kumar *et al.*, 2012). Further it has also been shown that, in term of MPS response, OM appear to peak around 60% 1RM, whereas YM are marginally higher (75%) (V Kumar *et al.*, 2009). Albeit, Kumar and others also showed that the MPS response in YM was always higher than that of OM with higher intensities (60%-90% 1RM).

While the intensity of training may illicit differential responses from YM and OM, it is also possible that CON or ECC RE is more preferential for one age group. In light of this, it has been reported that the perception of effort during ECC RE is lower than in conventional training in an elderly population (Reeves N *et al.*, 2009). Moreover, as ECC contractions can produce larger amounts of force, it is possible for individuals to utilise a heavier load during RE. As the elderly possess less muscle mass and have a reduction of strength, the ability to utilise heavier training loads with less metabolic demand is appealing. Further, as the OM commence the study with lower baseline values compared to YM (discussed in chapter 3), it is possible the ECC RE would have a greater effect in OM.

Therefore, the purpose of this chapter is to investigate whether YM and OM would respond similarly to the submaximal training. Further to this, whether either contraction type would be more beneficial in OM compared to YM.

6.3 Methods

Participants

The participants analysed within this chapter are the 20 YM (24.2 ± 1.3 years) and 18 OM (68.2 ± 2.4 years) discussed within the previous two chapters.

Statistical analyses

All data is presented as mean ± SEM. Differences between YM and OM in terms of percentage change (in respect to baseline), were assessed using Bonferroni's two-way ANOVA with repeated measures. The significance level was set at p=0.05.

6.4 Results

These data are taken from the previous two chapters (Chapter 4 and 5). However, herein the data will be displayed in CON young males (YM) vs old male (OM), and ECC YM vs OM in regards to the percentage change from baseline. As there are

two variables (age and contraction), the results will be split into concentric and eccentric.

Muscle size and function in response to CON

There was no difference in strength gain between YM and OM at week 4 (+11.3 ± 2.4% vs 13.3 ± 3.1%, respectively) or week 8 (+12.7 ± 2.6% vs +20.6 ± 6.2%, respectively) (Figure 6.1). The increase in activation capacity was also equal at week 4 and week 8 (YM: +4.5 ± 1.2% and +7.7 ± 1.2%; OM: +4.8 ± 1.7% and +6.3 ± 1.2%, for week 4 and 8 respectively), (Figure 6.1). The hypertrophic response to CON RE was equal between YM and OM with all measures of growth (Figure 6.2). VL ACSA increased equally at week 4 and week 8 (YM: +6.3 ± 1.6% and +8.6 ± 2.2%; OM: +4.5 ± 1.9% and 11.2 ± 2.1% for week 4 and week 8 respectively). Quadriceps ACSA showed the same, with no changes at week 4 or week 8 (YM: +8.0 ± 2.1% and +7.5 ± 2.6%; OM: +6.2 ± 2.3% and +7.7 ± 2.0%). Whole quadriceps volume also showed no significant difference between YM and OM at week 8 (+3.5 ± 0.6% vs 2.9 ± 0.4%, respectively), however there was a trend to suggest that young increased more at week 4 (+2.7 ± 0.2% vs +1.0 ± 0.4%, p=0.052) (Figure 6.2).

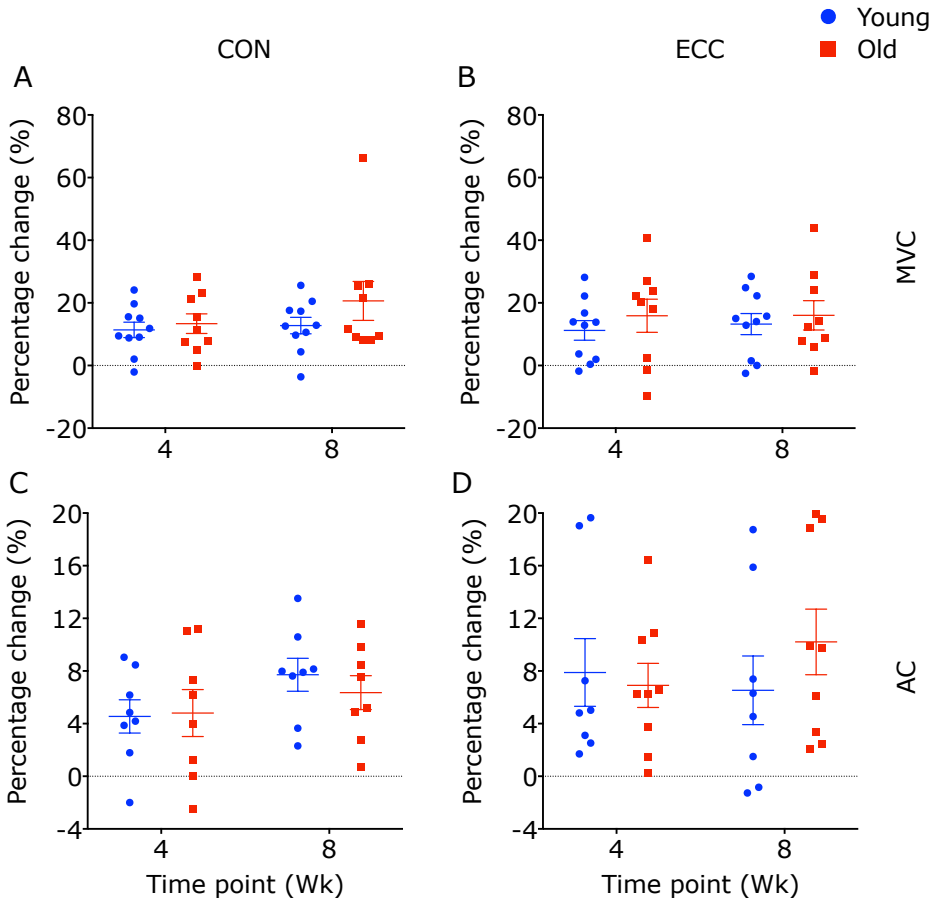


Figure 6.1: Percentage change from baseline in MVC (A: CON, B: ECC) and AC (C: CON, D: ECC), in YM (blue) and OM (red), at week 4 and week 8.

Muscle size and function in response to ECC

There was no difference in strength gain between YM and OM at week 4 (+11.2 ± 3.1% vs 15.8 ± 5.2%, respectively) or week 8 (+13.2 ± 3.3% vs +16.0 ± 4.6%, respectively) (Figure 6.1). The increase in activation capacity was also equal at week 4 and week 8 (YM: +7.8 ± 2.5% and +6.5 ± 2.6%; OM: +6.9 ± 1.6% and +10.2 ± 2.4%, for week 4 and 8 respectively), (Figure 6.1). The hypertrophic response to CON RE was equal

between YM and OM with all measures of growth (Figure 6.2). VL ACSA increased equally at week 4 and week 8 (YM: +7.1 ± 1.9% and +10.7 ± 1.8%; OM: +4.3 ± 1.8% and 6.8 ± 1.2% for week 4 and week 8 respectively). Quadriceps ACSA showed the same, with no changes at week 4 or week 8 (YM: +2.2 ± 1.0% and +5.9 ± 0.9%; OM: +6.3 ± 1.6% and +7.6 ± 1.6%). Whole quadriceps volume also showed no significant difference between YM and OM at week 4 (+3.1 ± 0.6% vs 2.7 ± 0.4%, respectively) or week 8 (+4.6 ± 0.7% vs 4.2 ± 0.5%, respectively), (Figure 6.2).

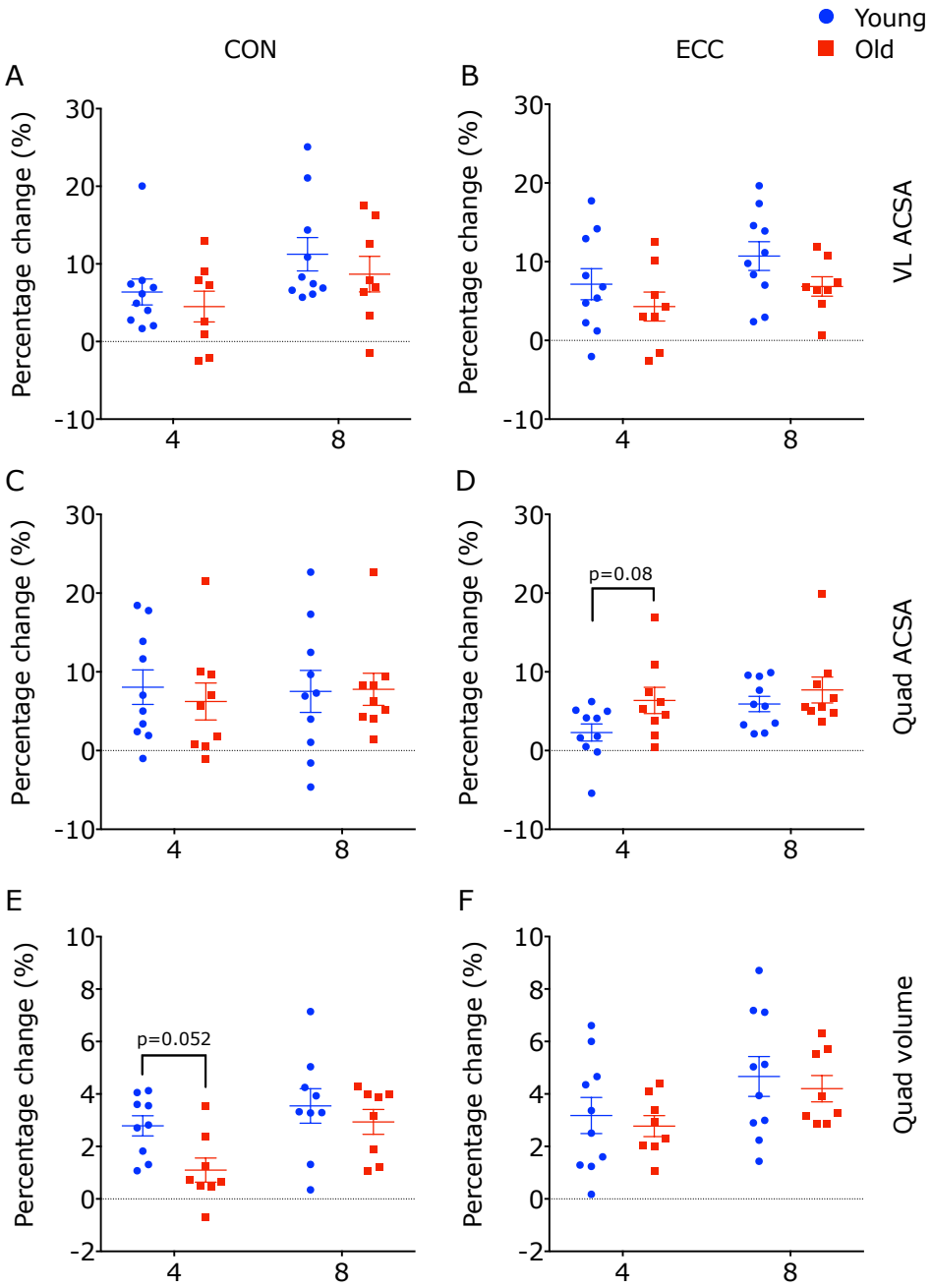


Figure 6.2: Percentage change from baseline in VL ACSA (A: CON, B: ECC), quadriceps ACSA (C: CON, D: ECC) and quadriceps volume (E: CON, F: ECC), in YM (blue) and OM (red) at week 4 and week8.

Muscle architecture in response to CON

Changes to muscle architecture were similar in both YM and OM. Pennation angle increased comparably at week 4 and week 8 (YM: +3.7 ± 0.4% and +5.7 ± 0.8%; OM: +3.6 ± 0.6% and +5.7 ± 0.6% for week 4 and week 8 respectively). Fascicle length changes were also equal at week 4 and week 8 (YM: +0.9 ± 0.5% and +1.5 ± 0.6%; OM: +1.2 ± 0.4 and +0.8 ± 0.5% for week 4 and week 8 respectively).

Muscle architecture in response to ECC

At week 4, there was a trend of greater increase in pennation angle with OM (1.5 ± 0.6% vs +3.6 ± 0.6%, $p=0.057$), however at week 8 this trend became significant (2.4 ± 0.5% vs 5.7 ± 0.7%, $p<0.05$). Fascicle length changes were also equal for YM and OM at week 4 (+5.6 ± 0.6% vs 3.8 ± 0.4%, $p=0.09$, respectively) and week 8 (+7.1 ± 0.7% vs +5.5 ± 0.6).

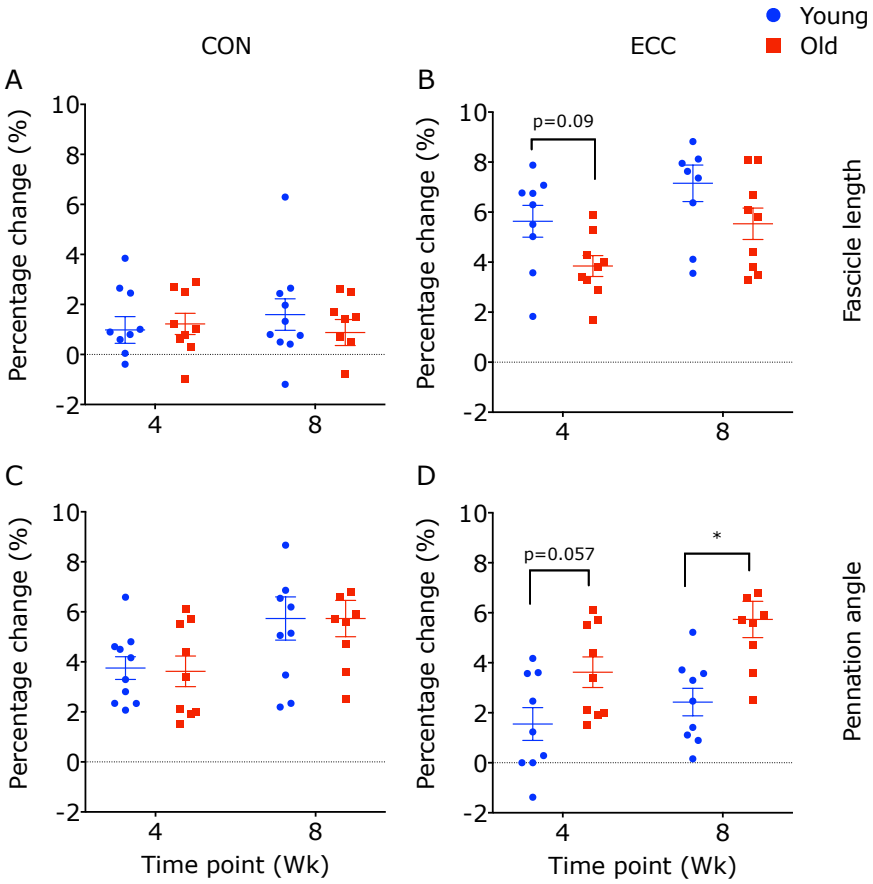


Figure 6.3: Percentage change from baseline in fascicle length (A: CON, B: ECC) and pennation angle (C: CON, D: ECC), in YM (blue) and OM (red), at week 4 and week8. Significance between age groups at time point, defined by * $p<0.05$.

Tendon biomechanics in response to CON

PT stiffness changed equally in YM and OM by week 4 (+29.9 ± 8.1% vs 40.8 ± 11.5%, respectively). However, at week 8 the percentage change was higher in OM (+36.3 ± 7.1% vs +82.7 ± 13.3, $p<0.01$). Young's modulus underwent similar changes at week 4 (YM: 33.4 ±10.6% vs OM: 40.8 ± 11.5%), with a

trend to suggest larger changes in OM at week 8 (+50.0 ± 12.8% vs 82.7 ± 13.3, p=0.074).

Tendon biomechanics in response to ECC

PT stiffness changed equally in YM and OM by week 4 (+34.2 ± 9.1% vs 53.0 ± 13%, respectively). However, at week 8 the percentage change was higher in OM (+50.9 ± 15% vs +100 ± 13, $p<0.05$). Young's modulus underwent similar changes in YM and OM at week 4 (YM: 43.0 ± 14% vs OM: 45.0 ± 10%, respectively) and week 8 (+58.5 ± 12% vs 90.4 ± 16%, respectively).

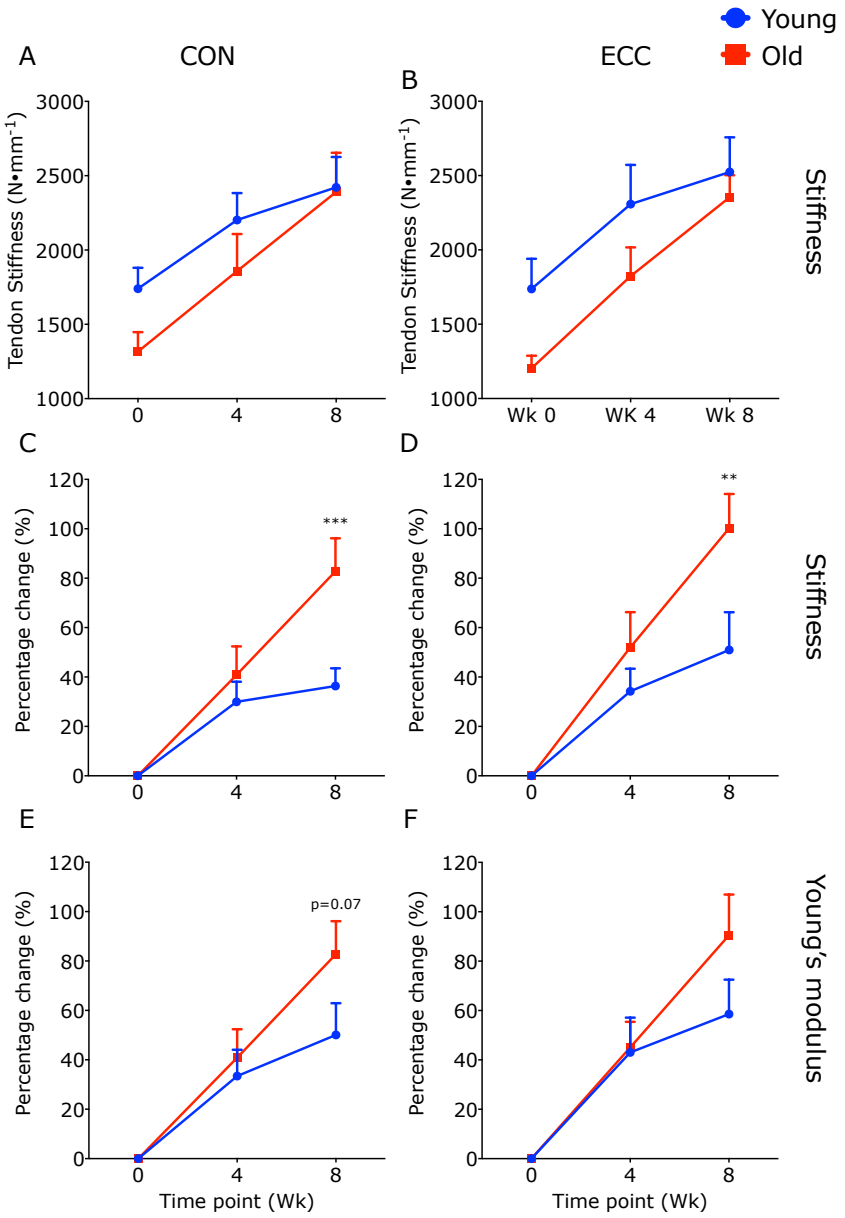


Figure 6.4: Raw values of tendon stiffness in response to CON (A) and ECC (B). Percentage change from baseline in PT stiffness (C: CON, D: ECC) and Young's modulus (E: CON, F: ECC), in YM (blue) and OM (red), at week 4 and week8. Significance between age groups at time point, defined by ** $p<0.01$, *** $p<0.001$.

6.5 Discussion

The main conclusion from the data herein, is that both YM and OM respond equally as well to submaximal RE training, in both CON and ECC groups. Hypertrophy occurs to a similar degree in both CON and ECC regardless of age, which concurs with strength gain. Nonetheless, the data suggests that the elderly tendon responds significantly more than the young counterpart at week 8, however this may be due to a lower baseline value in the elderly. Interestingly, it appears that the stiffness of the tendon in the elderly is nearly equal to that of the young post training, in both CON and ECC.

Muscle architecture adaptations were comparable between YM and OM in the CON training groups, with similar increases in pennation angle and fascicle length at both time points. However, in the ECC training groups, it was found that pennation angle was increased significantly more in OM than YM at week 8, with a trend also at week 4 (P=0.057). In the previous chapters it was discussed that CON and ECC result in two predominant paths of architectural remodelling, with changes in pennation angle and fascicle length dominating, respectively. However, in both YM and OM, we found significant increases in pennation angle also occurring in the ECC groups. Nonetheless, as mentioned this increase was greater in OM

than YM at week 8 (2.4% vs 5.7%). It is likely that OM are prone to greater increases due to their lower starting point (ECC, OM: 14.3 ± 1.2% vs YM: 17.7 ± 0.8%). Further, it is likely this lower initial baseline value may be due to a higher level of habitual activity within the YM group.

The data suggests that the tendon in OM responds greater than in YM after 8 weeks of training (Figure 6.4C & 6.4D), in both CON and ECC. However, if one considers the raw values (Figure 6.4A & 6.4B), it is possible to see that the elderly start at a lower baseline. In fact, in chapter 3, it was shown that OM have significantly lower tendon stiffness than YM (1222 ± 78N•mm^{-1} vs 1771 ± 154N•mm^{-1}, P<0.01). Therefore, it is possible that this difference in percentage change observed at week 8, is likely due to a larger scope for improvement in the OM tendon. This concept is perhaps further supported by the change in the raw values of tendon stiffness. Within the CON group, tendon stiffness finished with post intervention values of 2421 ± 204N•mm^{-1} for YM and 2391 ± 263N•mm^{-1} for OM. Similarly, tendon stiffness values post intervention in the ECC group were 2516 ± 208N•mm^{-1} for YM and 2354 ± 147N•mm^{-1} for OM. Thus, both YM and OM finish with similar tendon stiffness values within each training group and even between contraction types. Therefore, if PT stiffness was marginally

higher in the OM at baseline, then it is possible that the percentage change would have been equal. Nonetheless, there are two interesting concepts from within this data. Firstly, it shows that an elderly tendon can adapt equally as well as that of the young. But, secondly, and perhaps more importantly, it suggests that tendon stiffness can be restored to that of YM with as little as 8 weeks of submaximal training.

Muscle hypertrophy appeared to be equal in both OM and YM (Figure 6.2), regardless of the contraction type. Nonetheless, there is a trend ($p=0.052$) to suggest that CON RE may induce larger increase in muscle volume at the week 4-time point. Maximal strength was also found to increase similar in both YM and OM, irrespective of contraction mode. However, this is perhaps expected considering that both muscle size and AC increases similarly in both age groups. In consideration of the results, overall it appears that there is no difference in the muscle response of YM and OM to submaximal CON or ECC training. However, it is known that OM have a blunted anabolic response to both acute exercise and the ingestion of AAs compared to YM (Cuthbertson *et al.*, 2005; Vinod Kumar *et al.*, 2009; Fry *et al.*, 2011; Wall *et al.*, 2015). Over a prolonged period, is it believed that these dampened acute responses would consequently result in reduced adaptation within the

muscle. Thus, the results contained herein are perhaps conflicting. However, as the training was submaximal, it is likely that the response in YM was dampened compared to if a higher intensity (75-80% 1RM) was utilised. This explanation is supported by work completed by Kumar and colleagues in 2009. The study investigated the effect of exercise intensity upon the acute MPS response in both YM and OM. It was found that the MPS peak for OM occurred around 60% 1RM, whereas in young it appeared to happen at 75% 1RM (V Kumar *et al.*, 2009). Thus, with this data in mind, it is likely that our study would have been optimal for hypertrophy and architectural remodelling in the OM, but potentially suboptimal for the YM group. In line with this, when 75% 1RM has previously been implemented, YM had greater increases in MVC and VL muscle thickness than OM (Brook *et al.*, 2016).

In conclusion, it appears that the MTU of YM and OM respond equally as well as each other to this submaximal RE, regardless of contraction type. However, it is likely that the training intensity was suboptimal for the YM. Nonetheless, for the OM, submaximal RE provides a very suitable and successful exercise intervention for gains in strength, hypertrophy and stiffening of the tendon. Further, ECC RE appears to be slightly more advantageous in the elderly compared to the young.

7 Conclusions and future directions

This thesis primarily aimed at addressing the functional and biomechanical adaptations of the MTU in response to submaximal CON and ECC RE in both young and old males. However, it was first established that the elderly MTU was negatively affected in terms of RTD and PT stiffness. Further, OM had reduced muscle size, strength and activation capacity. The significant reduction in both aRTD and nRTD were mainly accounted for by reduced strength, but also partially due to a reduction in PT stiffness in ageing.

In response to submaximal CON or ECC RE, it was found that many muscle and tendon measures increased independent of contraction type, in both young and old. Interestingly, while overall hypertrophy (quadriceps volume at week 8) was equal between CON and ECC, it was found that both YM and OM responded to CON and ECC with differential architectural remodelling. It was found that in response to submaximal CON RE, increases in pennation angle superseded that which occurred in ECC. Whereas, in response to submaximal ECC RE, the increase to fascicle length was far greater than in CON. Naturally these architectural alterations will have implications upon both the F/L and F/V relation. Unfortunately, within the study design, we didn't assess muscle power, thus preventing

the estimation of muscle velocity. Therefore, we were unable to map any changes in the F/V relation within everyone. Further, as only one angle was utilised in the assessment of MVC, we also couldn't map changes in the F/L relation. Thus, while in theory the architectural alterations would result in these adaptations, we are unable to say whether in fact they did.

While muscle hypertrophy (muscle volume) occurred by week 4 in both CON and ECC for OM, the increase was significantly greater in ECC. These results suggest that ECC has greater early hypertrophy than CON. It is known that ECC contractions can produce greater force with less metabolic cost. Thus, it is for these reasons that submaximal RE appears to be a more appealing form of training in the elderly. Further, it appears that ECC RE is more beneficial in OM than YM. It was found that there were greater increases in pennation angle in OM compared to YM by week 8. Thus, this adds further weight to the implementation of ECC exercise in elderly populations.

The final finding from this thesis, is that while PT stiffness is significantly reduced in ageing, it can be almost fully restored to that of YM in response to either CON or ECC submaximal RE. Further, it appears that the tendon may respond more than the

muscle in response to training. However, it is difficult to compare the two tissues directly; a 10% change in one outcome may be more significant than a 90% change in another. Nonetheless, it appears that the two tissues respond with a similar temporal pattern, presumably to maintain the functionality of the MTU.

There are of course many ways in which these results will impact the current research field. One of the main findings is the importance of the tendon in both ageing and exercise. Unfortunately, many studies which investigate muscular adaptation to exercise or during rehabilitation, do not consider the in-series tendon. Until now, it has perhaps been considered that PT stiffness was unaffected in OM. However, as we know that the tendon can influence the F/L relation and RTD and that submaximal RE can improve the tendon; it is likely that some of the functional changes that occur in RE are caused through changes in tendon biomechanics. Further to this, our data also demonstrates that in young, most of the biomechanical changes occur in the first 4 weeks, whereas in the elderly it takes the full 8 weeks. Albeit, we do not know whether the tendon would continue to increase in stiffness should the RE have continued passed 8 weeks. Logically, the length of intervention would be based upon the baseline biomechanics;

in such that more time would be required for a lower starting point. Nonetheless, this provides some evidence for the programming of both RE intervention and rehabilitation.

The other important clinical finding is that OM respond to submaximal training. Inherently, this intensity of training imposes less metabolic demand and would naturally result in less potential muscle damage. Nonetheless, we demonstrated that the submaximal training (regardless of CON or ECC) could induce increases in MVC, muscle size and positive alterations to muscle architecture and tendon. Therefore, it shows that this form of training would be ideal for those in an elderly population or those with low metabolic output in the form of rehabilitation.

While this thesis answers some questions within the field, it inherently generates several more which require further investigation. One of the biggest responses to submaximal RE was from the tendon, with changes in modulus of up to +90%. Changes in modulus suggest that the alterations are not caused by dimensional changes, but instead in changes to the biomolecular properties. However, it is likely that a 90% increase in modulus is not matched by a 90% increase in crosslinking for example. Thus, with biomolecular analysis it would be possible to determine what magnitude of change

drives such a large increase in modulus. Further to this, assuming biopsied tissue was available, one would be able to investigate whether collagen synthesis was increased in response to a chronic RE programme. In addition to this, it would also be possible to investigate the genetic drive behind such changes, through analysis of RNA. However, thankfully this study also obtained tendon biopsy samples (not included in this thesis) at week 4 and week 8, thus making it possible for this analysis to be completed in the future. It is likely that there will be an increase in cross-linking. Previous studies have shown that with long exposure to running (5 years+), there is increased markers of cross links (Couppé *et al.*, 2014). Further, while it is unlikely that there would be an increase in basal collagen synthesis rates, the data will add more evidence to whether the tendon has high or low collagen turnover rates.

Our studies have demonstrated that the biomechanical response of tendon may begin to plateau in the young after 8 weeks, however in the elderly it appears to continue to increase. It is possible that there is an upper limit for stiffness and modulus adaptation, presumably there would be a point whereby the tendon would become too stiff. If the tendon became increasingly stiff, it would inherently influence the F/L relation, whereby force production would likely be reduced. As

suggested in chapter 5, while it is possible that the intensity of training 'switches on' the responsiveness of the tendon it may be the cumulative strain that dictates the degree of adaptation. Thus, by increasing the duration of the RE intervention, the tendon would inherently be exposed to more strain, potentially resulting in more adaptation. Nonetheless, it is also quite possible that the 'adaptation limit' had already been reached in response to this submaximal RE. Thus, it would be interesting to investigate whether the tendon continues to respond to submaximal training beyond 8 weeks.

8 Appendices

	YOUNG			OLD		
Variable	**Wk0**	**Wk4**	**Wk8**	**Wk0**	**Wk4**	**Wk 8**
MVC (Nm)	222 (12.7)	245 (11.6) ***	248 (13) ***	152 (9.2)	172 (10.9)	186 (21.2) **
Specific Tension ($N \bullet cm^{-2}$)	79.3 (3.9)	82.0 (4.7)	83.5 (4.9)	62.5 (3.9)	68.0 (4.5)	71.6 (7.6) *
AC (%)	80.3 (1.3)	84.0 (1.7) **	86.4 (0.9) ***	75.6 (2.8)	79.0 (2.4) *	80.2 (2.4) **
Co-activation (%)	12.6 (2.5)	13.3 (1.9)	16.1 (2.6)	20.6 (6.1)	18.9 (4.7)	23.0 (5.4)
aRTD ($Nm \bullet s^{-1)}$	872 (60)	847 (77	891 (64)	515 (37)	525 (41)	529 (31)
nRTD ($Nm \bullet s^{-1} \bullet cm^{-2)}$	12.2 (0.7)	10.3 (0.9)	11.6 (0.6)	8.43 (0.6)	8.48 (0.9)	8.25 (0.7)

Table 8.1: Muscle functional data at week 0, week4 and week 8 after CON RE, raw values and (SEM). Significant difference from baseline defined by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$.

	YOUNG			OLD		
Variable	**Wk0**	**Wk4**	**Wk8**	**Wk0**	**Wk4**	**Wk 8**
Peak VL ACSA (cm^2)	27.0 (1.6)	29.0 (2.1) **	30.6 (2.1) ****	23.3 (1.3)	24.5 (1.5)	25.5 (1.5) ***
Peak Quad ACSA (cm^2)	71.6 (4.2)	77.0 (4.4) ****	76.6 (4.2) ***	62.5 (3.7)	66.3 (3.8) **	67.2 (4.0) ****
Quad volume (cm^3)	2026 (107)	2077 (109) ***	2093 (110) ****	1688 (93)	1709 (91) *	1735 (90) ****, ##
VL Fascicle length (cm)	8.65 (0.3)	8.73 (0.3)	8.79 (0.3) *	7.76 (0.3)	7.86 (0.3)	7.83 (0.3)
VL Pennation angle (°)	17.4 (0.5)	18.1 (0.6) ****	18.4 (0.6) ****, #	15.3 (0.8)	15.8 (0.8) ****	16.1 (0.8) ****, ###

Table 8.2: Muscle allometric and architecture data at week 0, week4 and week 8 after CON RE, raw values and (SEM). Significant difference from baseline defined by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$. Significant difference between week 4 and week 8 defined by # $p<0.05$.

	YOUNG			OLD		
Variable	**Wk0**	**Wk4**	**Wk8**	**Wk0**	**Wk4**	**Wk 8**
PT Stiffness ($N \bullet mm^{-1}$)	1739 (140)	2202 (181) p=0.058	2421 (204) **	1316 (131)	1882 (239) **	2391 (263) ****, #
PT CSA (cm^2)	78.6 (2.5)	78.1 (2.4)	78.6 (2.7)	83.3 (4.0)	83.5 (4.1)	84.3 (4.3)
PT Young's Modulus (GPa)	1.20 (0.11)	1.57 (0.15) *	1.67 (0.19) **	0.75 (0.06)	1.08 (0.17) *	1.39 (0.22) ****

Table 8.3: Tendon biomechanical and structural data at week 0, week4 and week 8 after CON RE, raw values and (SEM). Significant difference from baseline defined by * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

	YOUNG			OLD		
Variable	Wk0	Wk4	Wk8	Wk0	Wk4	Wk8
MVC (Nm)	224 (18.4)	249 (20.9) ***	252 (20.5) ****	164 (10.9)	187 (11.2) p=.0507	187 (10.6) *
Specific Tension ($N \bullet cm^{-2}$)	72.9 (5.8)	77.4 (6.1)	77.2 (6.1)	68.2 (4.3)	72.5 (5.1)	71.7 (4.5)
AC (%)	80.9 (1.4)	87.1 (1.1) **	86 (1.5) **	75.7 (1.7)	80.7 (0.8) ***	83.1 (0.8) ****
Co-activation (%)	16.6 (3.1)	14.7 (1.9)	19.2 (3.9)	16.6 (3.6)	16.0 (1.3)	21.5 (3.7)
aRTD ($Nm \bullet s^{-1)}$	831 (98)	819 (69)	858 (85)	578 (51)	592 (41)	614 (38)
nRTD ($Nm \bullet s^{-1} \bullet cm^{-2)}$	10.6 (1.2)	10.6 (1.1)	10.6 (1.4)	9.37 (0.9)	8.35 (0.4)	8.76 (0.6)

Table 8.4: Muscle functional data at week 0, week4 and week 8 after either ECC RE, raw values and (SEM). Significant difference from baseline defined by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$.

	YOUNG			OLD		
Variable	Wk0	Wk4	Wk8	Wk0	Wk4	Wk8
Peak VL ACSA (n=8) (cm^2)	28.9 (2.0)	30.9 (1.7) **	32.2 (1.9) ****	24.7 (1.6)	25.9 (1.6)	26.6 (1.7) **
Peak Quad ACSA (n=8) (cm^2)	79.4 (5.3)	81.8 (5.4)	84.3 (5.6) ***	62.5 (3.7)	66.3 (3.8) **	67.2 (4.0) ****
Quad volume (n=8) (cm^3)	2143 (138)	2209 (140) ****	2243 (145) ****, #	1706 (104)	1751 (103) ****	1777 (109) ****, ##
VL Fascicle length (cm)	8.34 (0.2)	8.80 (0.2) ****	8.92 (0.2) ****, #	8.41 (0.3)	8.74 (0.3) ****	8.87 (0.3) ****, ##
VL Pennation angle (°)	17.1 (0.8)	18.0 (0.7) p=0.07	18.1 (0.7) **	14.3 (1.1)	14.6 (1.0) *	14.6 (1.0) **

Table 8.4: Muscle allometric and architecture data at week 0, week4 and week 8 after ECC RE, raw values and (SEM). Significant difference from baseline defined by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$. Significant difference between week 4 and week 8 defined by # $p<0.05$, ## $p<0.01$ and ### $p<0.001$

	YOUNG			OLD		
Variable	Wk0	Wk4	Wk8	Wk0	Wk4	Wk8
PT Stiffness ($N \bullet mm^{-1}$)	1664 (195)	2385 (248) **	2516 (208) ***	1203 (84)	1832 (193) **	2354 (147) ****, ##
PT CSA (cm^2)	89.8 (4.9)	89.2 (5.0)	88.8 (4.9)	87.5 (3.1)	86.8 (3.2)	87.3 (2.8)
PT Young's Modulus (GPa)	0.87 (0.10)	1.30 (0.14) **	1.34 (0.15) **	0.68 (0.04)	1.02 (0.11) *	1.28 (0.10) ***

Table 8.6: Tendon biomechanical and structural data at week 0, week4 and week 8 after ECC RE, raw values and (SEM). Significant difference from baseline defined by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$. Significant difference between week 4 and week 8 defined by # $p<0.05$ and ## $p<0.01$.

9 References

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