Loss of Muscle Performance in Seniors: Changes to the Dynamic Muscle Structure and Muscle Gearing

by

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Abstract

Muscle structure changes with ageing in a manner that can alter its contractile mechanics, resulting in a reduction in strength and mobility. Fascicles within a muscle can shorten at slower velocities than the muscle belly, in a process known as belly gearing. Belly gearing allows the fascicles to produce a greater force when they contract. However, it may be compromised when we age.

The gastrocnemii muscles in the calf were imaged in young adults and seniors using ultrasound. Their muscle structures were compared during standing and during ankle extensions.

The overall gearing of a muscle-tendon-unit was largely determined by the belly gearing, but its variability was driven by changes in tendon stretch. The decrease in performance and power output in seniors may be associated with reduced gearing due to atrophy of the muscles.

Keywords: sarcopenia; muscle-gearing; muscle-bulging; ultrasound; atrophy; elderly

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List of Abbreviations

AGR	architectural gearing ratio
ANCOVA	analysis of covariance
BBS	berg’s balance scale
BMI	body mass index
CSA	cross-sectional area
GLM	general linear model
isoT	isotonic contractions (constant torque)
isoV	isovelocity contractions (constant angular velocity)
LG	lateral gastrocnemius
MG	medial gastrocnemius
MMSE	mini-mental state exam
MRI	magnetic resonance imaging
MTU	muscle-tendon unit
MVC	maximum voluntary contraction
PCSA	physiological cross-sectional area
ROM	range of motion
s.e.m.	standard error of mean
SD	standard deviation
TUG	timed-up and go test
2D	two dimensional
3D	three dimensional

List of Symbols

G_b	gearing of the muscle belly
G_{mtu}	gearing of the muscle-tendon unit
G_t	gearing of the tendon
L_b	length of the muscle belly
L_f	length of the muscle fascicle
L_t	thickness of the muscle belly
V_0	maximum unloaded shortening velocity of the muscle fascicles
V_b	shortening velocity of the muscle belly
$V_{b,max}$	maximum shortening velocity of the muscle belly
V_f	shortening velocity of the fascicles
$V_{f,max}$	maximum shortening velocity of the fascicles
V_{mtu}	shortening velocity of the muscle-tendon unit
V_t	shortening velocity of the tendon
β	pennation angle of the muscle fascicles
β_1	angle of incidence of fascicle to the superficial aponeurosis
β_2	angle of incidence of fascicle to the deep aponeurosis
ΔL_t	change in muscle belly thickness
$\Delta\beta$	change in pennation angle

1. Introduction

Muscle performance depends on the geometrical arrangement of fascicles (bundles of muscle fibres) and the contribution of connective tissue external and internal to the muscle belly. In the muscle belly, the fascicles can be arranged in parallel or at an angle to the line of action of the muscle. The angle at which the fascicles are oriented is known as the pennation angle. The muscles that contain fascicles with pennation angles greater than 0° are known as pennate muscles. During concentric contractions of the pennate muscle, the muscle belly shortens, the pennation angle increases and the belly thickness changes as a muscle produces force and power. When the fascicles are arranged in parallel, the shortening velocity of the fascicles is same as that of the belly. However, the shortening velocity of fascicles in pennate muscles is the product of the belly shortening velocity and the cosine of the pennation angle. Therefore, fascicles within a pennate muscle shorten at a slower velocity than that of the muscle belly, in a process called belly gearing. The overall muscle-tendon unit gearing is expressed as the product of the gearing of muscle belly and the gearing due to tendon stretch. Previous studies have shown that gearing is variable and increases at higher velocities. Changes in the muscle activation levels and force development have been suggested to affect tendon gearing and thus muscle-tendon unit gearing. However, it is important to explore the role of belly gearing as a part of muscle-tendon unit gearing. Also, the associations of belly gearing with the structural aspects of muscle and movement performance are important facets that need to be studied.

Changes in muscle structure due to ageing occur in a process known as sarcopenia which is the progressive loss of muscle mass and strength. The changes in muscle structure can alter muscle mechanics during contraction and contribute to the reduction in strength and limit mobility in seniors. Static changes in muscle structure with ageing have been observed previously, however, dynamic muscle behaviour has not been studied in the past. This study was performed to investigate the effect of sarcopenia on muscle fascicle length, pennation and belly thickness in a contracting muscle during isovelocity movements. Belly gearing may be affected by atrophy and so was also tested in seniors.

1.1. Fascicle lengths, angles and rotations

In pennate muscles, fascicles are arranged at an angle to the force-generating axis known as the pennation angle (Fukunaga et al., 1997, Kawakami et al., 1998, Narici et al., 1996). This arrangement influences functional performance (Gans, 1982, Gans & De Vree, 1987, Zajac, 1989, Maganaris et al., 2003) and determines the role-specific behaviour of pennate muscles (Burkholder et al., 1994, Lieber & Friden, 2000). There are many ways of calculating pennation angle: (1) the angle between the fascicle and the superficial aponeurosis (Maganaris et al., 1998), or (2) the fascicle and the deep aponeurosis (Fukunaga et al., 1997, Kawakami et al., 1998), or (3) the mean of these two measures (Klimstra et al., 2007, Narici et al., 1996). In the mid-belly region where the aponeuroses run parallel to each other, these measurements would give similar results (Wakeling et al., 2011). In man, pennation angles at rest and at maximal voluntary contraction (MVC) are approximately 29º and 51º for the medial gastrocnemius (MG) and 14º and 25º for the lateral gastrocnemius (LG) (Kawakami et al., 1998). However, they also vary depending on the knee and ankle joint angle (Kawakami et al., 1998, Maganaris et al., 1998).

The influential role of pennation on functional performance includes its relation to the intensity of muscle contraction (Maganaris et al., 1998, Maganaris et al., 2003, Narici et al., 1996), muscle fascicle lengths (Maganaris et al., 1998, Manal et al., 2006), belly thickness (Maganaris et al., 1998, Manal et al., 2006), and muscle gearing (Brainerd & Azizi, 2005). A pennate arrangement allows for greater number of fascicles to be packed in parallel in a given volume of muscle (Alexander, 1969) which increases the physiological cross sectional area of the muscle (PCSA). The PCSA of a muscle is directly proportional to the maximum force generated by the muscle (Narici et al., 1992, Fukunaga et al., 1996, Maganaris et al., 2001). In two dimensional muscle models, the component of fascicle force acting in the direction of line of action of muscles is a function of cosine of the pennation angle (Gans, 1982, Maganaris et al., 2003). Thus, the force output of a muscle is related to the pennation angle (Powell et al., 1984) and to the

PCSA (Lieber & Friden, 2000). During contraction, as fascicles shorten they get wider and must rotate to higher pennation in order to fit within the muscle belly. Higher fascicle pennation allows for greater fascicle rotations (Fukunaga et al., 1997, Kawakami et al., 1998, Muhl, 1982) and thus a greater decrease in fascicle lengths (Maganaris et al., 1998, Zuurbier & Huijing, 1992) as compared to fascicles with relatively lesser fascicle pennation, or to a non-pennate muscle that have no fascicle rotations.

The role-specific behaviour of pennate muscles can be described by a few examples. First, vastus lateralis and soleus have shorter fascicles with higher pennation angles to attain greater forces and postural control (Blazevich, 2006, Friedrich & Brand, 1990), generally at a shorter range of motion (Lieber & Friden, 2000). Contrastingly, adductor magnus and longus have longer fascicles and smaller pennation angles to generate forces at relatively wider range of motion at the hip joint (Blazevich, 2006). Muscles that attach to long tendons, such as the lateral gastrocnemius, have larger pennation angles to produce sufficient force during walking, running and to allow an optimal level of elongation and recoil of the tendon (Hof, 2003). This contrasting behaviour of dynamic musculature may make it challenging to formulate an ideal muscle design (Blazevich, 2006). Nevertheless, it highlights the importance of distinct fascicle geometries in the locomotion system.

1.2. Belly thickness and bulging

During shortening, the fascicle length decreases as it contracts, however, there is an increase in its diameter. This increases the cross sectional area (CSA) of muscle fascicles and thus the muscle PCSA (sum of the CSAs of all the fascicles in a muscle, Lieber & Friden, 2000). In order to conserve volume (Baskin & Paolini, 1967), this changes the thickness of muscle belly (Azizi et al., 2008, Maganaris et al., 1998, Wakeling et al., 2011). Thickness change can occur in two main ways: (1) by increase in thickness parallel to the fascicle planes called muscle bulging (Kawakami et al., 1998, Wakeling et al., 2011), or (2) increase in width perpendicular to the fascicle planes. Belly bulging was observed in the LG during isometric contractions (Maganaris et al., 1998), isotonic contractions (Azizi et al., 2008) and during cycling (Wakeling et al., 2011).

However, researchers in past have found no muscle bulging in the MG (Narici et al., 1996, Azizi et al., 2008, Wakeling et al., 2011).

The exact direction of change in thickness is constrained by connective tissue such as the superficial aponeurosis, deep aponeurosis and tendon. The pressures exerted by these surrounding tissues on the muscle belly specifically in the deeper layers (Sejersted et al., 1984) and by the internal tendon in bipennate muscles (van Leewen & Spoor, 1992) may also affect bulging. These tissues may stretch in proportion to internal pressure which is proportional to contractile force (Petrofsky & Hendershot, 1984) and this may modulate the direction of dynamic changes occurring in the muscle such as muscle bulging. For example, in a salamander model during isotonic contractions (Azizi et al., 2002), the mediolateral bulging was limited by surrounding tissues and redirected to dorsoventral direction. Therefore, it is possible that in order to maintain a constant volume (Baskin & Paolini, 1967), the MG increases in width if it does not bulge. A two-dimensional view of fascicle architecture such as in the study in chapter 2, determines changes in belly thickness and length. A three-dimensional view of muscle would determine changes to the muscle belly both parallel and perpendicular to the fascicle plane and thus account for the changes in width along with the changes to the length and thickness of the muscle belly.

Muscle belly thickness has been found to be sensitive to relative torque levels of muscles during experimental (Maganaris et al., 1998, Wakeling et al., 2011) and modelling studies (Azizi et al, 2008, van Leeuwen & Spoor, 1992). These changes were muscle specific with no change in MG thickness but a 41% increase in LG thickness observed during isometric contractions (Maganaris et al., 1998). Similarly, Wakeling and colleagues (2011) observed muscle bulging for LG and soleus with increasing crank torque but no change in MG thickness with torque during cycling. This again leads to the suggestion that MG would have changed in width as it shortens and this theory could be tested using three-dimensional ultrasound or MRI imaging.

1.3. Muscle gearing

Gearing mechanisms in the moving joints are similar to the mechanical gearing in automobiles (Carrier et al., 1994). Both, skeletal gearing and mechanical gearing, allow its system to operate at a peak power while the system has a limited speed range (Azizi et al., 2008, Carrier et al., 1994). In humans, the ankle extensor performance was enhanced by dynamic skeletal gearing during acceleration tasks and during running (Carrier et al., 1994). This skeletal gearing was taken as the ratio of ground force moment arm and muscle force moment arm, and influenced the ratio of muscle force to output force or muscle velocity to output velocity (Biewener et al., 2004, Carrier et al., 1994). A similar mechanism to skeletal gearing is seen in the muscles which in place of the moment arm, is affected by the contraction of the muscle belly and compliance of the in-series elastic elements of the tendons (Wakeling et al., 2011).

Overall gearing of a muscle called muscle-tendon unit (MTU) gearing G_{mtu}, is a product of two components: belly gearing G_{b} and tendon gearing G_{t} (Wakeling et al., 2011). Belly gearing is the ratio of the shortening velocity of the muscle belly V_{b} to the shortening velocity of the fascicles V_{f} (Azizi et al., 2008, Wakeling et al., 2011, Zuurbier & Huijing, 1992) and tendon gearing is the ratio of the shortening velocity of the MTU V_{mtu} to the shortening velocity of the muscle belly V_{b} (Wakeling et al., 2011). The MTU, belly and tendon gearing can be explained by the following formulae:

$$G_{\mathrm{mtu}} = G_{\mathrm{b}} \text{ x } G_{\mathrm{t}} \tag{1}$$

$$G_{\mathrm{b}} = V_{\mathrm{b}} / V_{\mathrm{f}} \tag{2}$$

$$G_{\mathrm{t}} = V_{\mathrm{mtu}} / V_{\mathrm{b}} \tag{3}$$

Replacing G_{b} and G_{t} in (1) from (2) and (3), we get:

$$G_{\mathrm{mtu}} = V_{\mathrm{mtu}} / V_{\mathrm{f}}$$

Muscle gearing was observed in the white musculature of fish (Alexander, 1969, Wakeling & Johnston, 1999) and in the axial muscles of salamanders (Azizi et al., 2002) during swimming, in the MG of rat during isokinetic contractions (Zuurbier & Huijing, 1992), in the LG muscle of turkey during isotonic contractions (Brainerd & Azizi, 2005, Azizi et al., 2008) and in the triceps surae in man during cycling (Wakeling et al., 2011).

Belly gearing is sensitive to muscle structure as the fascicles aligned at higher pennation angle at rest increase gearing during contraction (Azizi et al., 2002). In addition, muscle bulging allows for more changes in pennation (Wakeling et al., 2011) and thus, results in higher gearing. Since the structure of various muscles is different, gearing is also muscle specific (Wakeling et al., 2011). For example, belly gearing is higher for MG than the LG as MG is a thicker muscle with a higher pennation angle. Tendon gearing is sensitive to the compliance and stretch of the in-series elastic elements of the tendons as the tendon stretch can uncouple elongations of the MTU and the muscle belly. Tendon gearing depends on the stretch in the tendon that in turn is affected by muscle force and thus muscle activation (Wakeling et al., 2011). Therefore, the MTU gearing can vary with the type of muscle contractions or activation levels due to its tendon gearing component (Wakeling et al., 2011).

Muscle modelling studies proposed that gearing maybe related to muscle thickness, fibre rotation, force output and velocity changes and so gearing could change with given tasks (Wakeling et al., 2011). In mammalian skeletal muscle, there is a mix of fibre-types lying adjacently within the muscle belly. During fast contractions, the slow fibres may approach their maximum shortening velocity which may render the slow fibres incompetent for high speed contractions by producing less force output. This is because faster velocity of contraction yields the fibres mechanically incompetent by developing less force (Hill, 1938). Thus, the force a muscle generates decreases at high shortening velocities and may become zero at maximum unloaded shortening velocity, which also diminishes the power. A mechanism such as gearing (Azizi et al., 2008, Wakeling et al., 2011) would slow the speeds of the slow fibres so that force does not diminish when the slow fibres are approaching their maximum shortening speed. Thus, the structural properties of muscles such as fascicle rotations and belly bulging and the elastic properties of the tendon can vary the shortening velocities of the muscle fascicles and allow them to operate at different force levels to produce high power (Azizi et al., 2008, Wakeling et al., 2011).

1.4. Structural responses to ageing and training

Sarcopenia leads to muscle atrophy causing changes in the muscle size, connective tissues and structure (Kubo et al., 2003, Lauretani et al., 2003, Maganaris, 2002, Morse et al., 2005, Narici et al., 2003, Narici & Maganaris, 2006). There is significant loss of muscle mass which is predominantly due to the loss in number of fibres and reduction in their size, specifically the fast fibres (fast fibres area loss ranges from 20% to 50%, Lee et al., 2006, Lexell et al., 1993). Loss in muscle mass contributes to smaller muscle PCSA due to smaller fibre CSA and PCSA has a direct relation with force generation (Narici et al., 1992, Fukunaga et al., 1996, Maganaris et al., 2001). Consequently, there is a deterioration of force or muscle strength and power output with ageing (Lauretani et al., 2003, Lindle et al., 1997, Morse et al., 2005). One of the reasons for power deterioration is the decreased size of the muscle and smaller PCSA (Doherty et al., 2003, Morse et al., 2005), however, a lower muscle gearing possibly due to lower pennations and thickness found in the passive musculature in seniors (Morse et al., 2005) may be a causal factor. This is due to the fact that a lower static pennation found in seniors will decrease the rotation of fascicles during contraction. However, dynamic changes in muscles and muscle gearing have never been tested in past and need to be investigated to establish another potential reason for force and power deterioration.

Muscle power reflects the ability to generate work per unit of time which can be related to force or muscle strength (Bean et al., 2003). Power output plays an important role in the activities of daily living when rapid generation of force is required, when there is an urgent need to balance after a perturbation or when a task is time-dependant (Bean et al., 2003). In fact, power output of a muscle is the most important determinate of physical performance in older adults (Bean et al., 2003) and has a relatively greater decline than force with ageing (Bean et al., 2002, Foldavari et al., 1999, Lauretani et al., 2003, Suzuki et al., 2001). It is important to minimize reductions in muscle strength and particularly power as we age.

Training and muscle hypertrophy have been associated with positive effects to muscle performance and muscle strength which are related to the force and power output of muscles. The structural changes to a hypertrophied muscle are largely opposite to the changes that occur with atrophy. Muscle volume increases due to fascicle hypertrophy as the connective tissues in the fascicles increase and fascicles attain larger CSAs (Kawakami et al., 1995). A higher fascicle CSA leads to greater muscle PCSA (Kawakami et al., 1995, Narici et al., 1989, Aagaard et al., 2001) and allows for more contractile material that can be attached to the tendon (Alexander & Vernon, 1975, Kawakami et al., 1995). Greater fascicle pennations are found in hypertrophied muscles which are related to the increase in fascicle CSA, the overall muscle PCSA and muscle volume with training (Kawakami et al., 1995). Researchers also found a positive correlation between increases in pennation angles and increased belly thickness with training (Narici et al., 1989, Kawakami et al., 1995). Greater pennation angles typically allow greater fascicle rotations (Brainerd & Azizi, 2005, Fukunaga et al., 1997, Kawakami et al., 1998). Increased rotations leads to greater muscle gearing (Wakeling et al., 2011) as it lowers the shortening velocity of the fascicles in comparison to the shortening velocity of the muscle-tendon unit. Thus, hypertrophy changes the structural properties of a muscle resulting in higher pennation angles (Kawakami et al., 1993) and greater muscle belly thickness at rest (Kawakami et al., 1993, Duclay et al., 2009).

Training also affects the tissues surrounding the muscle belly including the tendon and aponeurosis. Tendons and aponeurosis interact with the muscle during shortening (Kawakami et al., 1998, Muramatsu et al., 2001). When the muscle develops force, the tendinous tissue elongates (Alexander & Vernon, 1975) and stores elastic energy (Alexander, 1984, Alexander & Vernon, 1975). This elastic energy is consequently converted into kinetic energy upon recoil of the tendon (Alexander & Vernon, 1975). The mechanical properties of tendon influence the muscles to utilize the elastic energy (Wilson et al., 1994). Isometric training (Kubo et al., 2001) and strength training (Narici et al., 1996) leads to stiffer tendons. Tendons with increased stiffness aid greater initial rate of force generation (Wilson et al., 1994) during concentric and isometric contractions. There is a decreased time to peak torque (Narici et al., 1996) and greater force development (Wilson et al., 1994), due to the decreased electromechanical delay and faster transmission of muscle force to the bone (Kubo et al., 2001, Wilson et al., 1994). Overall, the static geometry of the muscle changes, the tendons get stiffer and the muscle-tendon unit generates greater forces and power output with hypertrophy (Narici et al., 1989, Maganaris et al., 1998, Kubo et al., 2001).

Training induced improvements in seniors have been previously reported. Specifically, muscle structure changes (Morse et al., 2007, Reeves et al., 2009) with significant increase in fascicle pennation and belly thickness occurring as a response to conventional physical training (Morse et al., 2007), resistance training (eccentric and concentric) or eccentric-only training (Reeves et al., 2009). Isometric training in older adults increases the tendon stiffness and consequently the muscle force or strength (Kubo et al., 2001). Thus, atrophic processes in the muscle structure (Lauretani et al., 2003, Morse et al., 2005) or the tendinous tissues (Child et al., 2010) affect the functional performance of muscle-tendon units and it is important to reverse any structural factors that may affect muscle strength and cause instability.

1.5. Purpose and hypotheses

The ankle plantarflexors, Medial Gastrocnemius (MG) and Lateral Gastrocnemius (LG) were tested for these studies as these perform differently from each other. In the first study, that is described in chapter 2, the primary goal was to establish the fundamental principles of muscle gearing and the structural and functional factors that influence its development. The two gastrocnemii of twenty young adults (20-40 years) were tested during isovelocity and isotonic contractions on an ankle dynamometer. Ultrasound images of both muscles were collected during contractions and were later digitized. Gearing was also predicted using a two-dimensional panel model of these muscles.

It was hypothesised that a higher gearing would occur at higher velocities during isovelocity and isotonic contractions for the experimental and model tests in both muscles. The changes in activation levels in the isotonic tests for the experimental and model data in both muscles would affect the tendon stretch and thus the tendon gearing, but this could only be tested in the model study. It was hypothesised that the tendon gearing would vary with the activation level in the model study.

In the second study, that is described in chapter 3, the primary goal was to perform a detailed muscle structural and functional comparison between young adults and seniors to determine differences in muscle structure and links to possible causes of deterioration in performance with ageing. The MG and the LG were tested using ultrasound from nine seniors (70-85 years) in addition to the ten young adults tested during isovelocity movements in the first study. The muscle structures were imaged during standing and during maximal plantarflexion at four constant velocities (150, 120, 90 and 45 ^{o} s^{-1}) on an ankle dynamometer and torque and ankle position were recorded during contractions.

It was hypothesised that the muscles in seniors would be thinner with smaller fascicle lengths and lower pennation angles during standing. During isovelocity shortening, the changes in belly thickness and fascicle pennation would be smaller along with smaller belly gearing for seniors at all four ankle velocity conditions. Additionally, the ankle torque and power output would be lower for seniors.

2. Muscle gearing in young adults

This study has been submitted as a manuscript for publication in a journal under the title "Muscle gearing during isotonic and isovelocity movements in the ankle plantarflexors" authored by Avleen Randhawa, Meghan E. Jackman and James M. Wakeling. It comprises of three sets of data. The isovelocity data that were collected by Avleen Randhawa, the isotonic data that were collected by Meghan E. Jackman and the model which was simulated by Dr. James M. Wakeling. Statistical analysis and manuscript preparation were both done by Avleen Randhawa.

2.1. Introduction

The force that a muscle-tendon unit develops during contractions depends on the forces of the individual muscle fibres and their geometrical arrangement within the muscle belly. Muscle fibre force is modulated by the velocity at which the fibres shorten, with lower forces occurring at faster velocities (Hill, 1938). For each fibre there is a maximum unloaded shortening velocity V_0 at which the fibre can no-longer develop any active force. It has been shown in fish that the anatomically distinct regions of fast and slow fibres differ in their gearing, and for maximal burst swimming performance the slow fibres may shorten at velocities that exceed V_0, and so are mechanically incompetent for aiding the swimming motion (Rome et al., 1988). In mammalian skeletal muscle there is commonly a mix of muscle fibre-types lying adjacently within the muscle belly. It has been argued that in man it may be unlikely that any contraction would be fast enough to render the slow fibres mechanically incompetent due to excessive shortening velocity (Bottinelli & Reggiani, 2000). However, such arguments were based on the assumption that contractions would occur at velocities that maximize the power output from the muscle. Measurements of muscle fibre strain rates during fast contractions have shown that the strain-rates can approach (and possibly exceed) V_0 for the slow fibres (Wakeling et al., 2006). Any mechanism within a muscle belly that can appropriately gear the fibres to shorten at a lower velocity during high-speed movements may thus increase their potential for generating force.

Gearing is the change in velocity between the muscle fibres and the muscle-tendon unit, and may occur due to mechanisms within the muscle belly, or due to the compliance and stretch of the tendons. In a mechanical system gearing is typically considered as a direct inverse relation between force and displacement. However, this consideration cannot be applied to muscle due to the non-linear force velocity relation within the muscle fibres (Hill, 1938), and the compliance of the in-series elastic elements of the tendons. Muscle gearing should thus be considered as a means to vary the shortening velocities of the muscle fibres, allowing them to operate at different force levels when they are activated.

Muscle-tendon gearing G_{mtu} is the ratio of the muscle-tendon unit velocity to the fascicle velocity. G_{mtu} can be expressed as the product of two factors: (a) G_{b} which is the ratio of the muscle belly velocity to the fascicle velocity, and (b) the tendon gearing G_{t} which is the ratio of the muscle-tendon unit velocity to the belly velocity (Wakeling et al., 2011). The gearing can vary, and has been shown to increase at higher velocities for the gastrocnemii in both turkeys (Azizi et al., 2008) and in man (Wakeling et al., 2011). Higher gearing at higher speeds makes mechanical sense, due to the fact that the muscle fibre force development would get compromised if their shortening velocity becomes too great. We have previously suggested that changes in the activation levels and force development of the muscle affect the tendon stretch and thus G_{t} (Wakeling et al., 2011). In this study we explore the factors that may contribute to variable gearing within the muscle belly.

During concentric contractions the muscle fibres shorten. In order to maintain their volume, this results in an increase in the cross-sectional area, or broadening of the fibres (Azizi et al., 2008). Muscles usually have their fibres set at a pennation angle that deviates from the line of action of the muscle belly (Zuurbier & Huijing, 1992). As pennate muscle fibres broaden during contraction they must rotate (Maganaris et al., 1998), and thus increase their pennation, in order to fit within the spatial constraints of the muscle belly. The fibre shortening acts to pull the aponeuroses together and thus reduce the muscle thickness, however, the fibre rotation acts to increase the muscle thickness. The actual changes in muscle thickness are thus a balance between the muscle contraction, and the architectural and geometrical constraints within the muscle. Major factors that influence the belly gearing are the changes in belly thickness and pennation angle (Azizi et al., 2008, Wakeling et al., 2011), and thus G_b should be expected to vary between muscles with different pennation and modes of bulging.

The shape of the muscle belly, and the orientation and curvature of the muscle fibres additionally varies with changes in the intramuscular pressure (Otten & Hulliger, 1995, van Leeuwen & Spoor, 1992). The intramuscular pressure increases during contraction as the muscle becomes more active, and so it should be expected that the muscle fascicle curvature and geometry is influenced by the activation level of the muscle. Changes in G_b due to the activation of the fibres may thus be an important factor that contributes to variable G_{mtu} in muscles. Within the gastrocnemii, the balance of activation between different regions of each muscle may vary in a task-dependent manner (Wakeling & Horn 2009), and regional heterogeneity in activation also results in changes to the internal geometry (Otten & Hulliger 1995) and thus presumably the gearing within the muscle belly.

The purpose of this study was to examine the factors that influence the belly gearing G_b in the gastrocnemii in man. Two series of tests were performed: the first was a series of isovelocity plantarflexion contractions where the muscle was maximally activated, and thus regional variation of activation across the muscle could be ignored. The second test involved cyclic contractions in which the plantarflexion phase was isotonic where the activation level could be changed, thus allowing the effect of submaximal activations on G_b to be tested. A simple planar muscle model was also used to identify the role of geometric parameters on the gearing, and conversely to highlight areas where more detailed structural knowledge will be necessary to explain the variation in muscle gearing. We expected that higher gearing would occur at higher velocities, and that the changes in activation levels in the isotonic tests would affect the tendon stretch and thus the tendon gearing.

2.2. Methods

2.2.1. Participants

Twenty young physically active male volunteers, ten for the isovelocity (isoV) group (age: 27.8 ± 4.4 yr, height: 180.9 ± 7.1 m, body mass: 77.7 ± 15.3 kg; means ± SD) and ten for the isotonic (isoT) group (age: 27.5 ± 5.4 yr, height: 178.5 ± 4.4 m, body mass: 74 ± 11.1 kg) without any subjective evidence of musculoskeletal disease or injury, participated in the study. The institutional research ethics committee approved this study, and participants provided informed consent.

2.2.2. Muscle contractions

Ankle plantarflexor movements were tested on a dynamometer (System 3, Biodex, New York, USA). The chair and footplate of the dynamometer were adjusted so that the longitudinal axis of the tibia was parallel to the ground. The medial gastrocnemius, MG, and lateral gastrocnemius, LG, were tested on opposite legs in order to minimize fatigue of muscles due to repetitive ankle extension movement. The participants had a mean knee angle of 140.7 ± 4.0°. The foot and the knee of the testing leg were securely fixed with both straps and mechanical stops in order to minimize heel-lift during maximal plantarflexion. Ankle torque, angle and angular velocity from the dynamometer were recorded at 1 kHz using a 16-bit data-acquisition system (USB-6229; National Instruments, Austin, TX, USA).

The isoV group was tested during isovelocity contractions where maximal-effort plantar-flexion movements were performed at constant ankle velocities of 150, 120, 90, and 45° s^{-1}. Each contraction started from a position of maximal dorsiflexion. Six trials were performed at each ankle velocity, with a 5-10 s period of rest between contractions. The conditions were tested in descending order of velocity.

The isoT group of participants was tested during cyclic isotonic contractions. The dorsiflexor torque of the ankle was set to 10 N m, and the plantarflexor torque was tested at 10, 35 or 60 N m. The subjects were instructed to match both the plantarflexion and dorsiflexion to the time of a metronome and to reach full dorsiflexion and plantarflexion at the end of each contraction. Contractions were tested at cycle frequencies of 0.33, 0.67

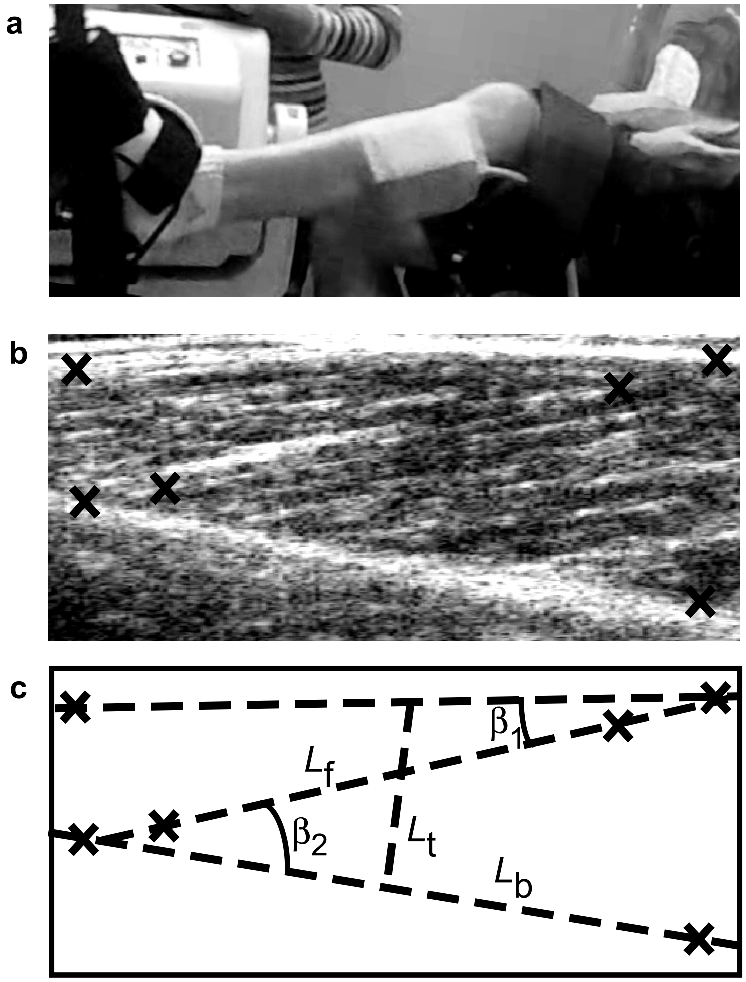
and 1.00 Hz. Six combinations of plantarflexor torque and cycle frequency were tested (10@0.33, 10@0.67, 10@1.00, 35@0.33, 35@0.67 and 60@0.33; N m@Hz). Five cyclic contractions were performed for each of the six conditions for each muscle. A rest period of 60s was provided between trials. The conditions were performed in random order. Subjects were given visual feedback of ankle position in order to achieve constant velocity.

2.2.3. Geometrical properties of the muscles

Geometrical properties of the muscle were imaged using B-mode ultrasound during the contractions (60mm linear ultrasound probe; Echoblaster 128, Telemed, Lithuania). The ultrasound probe was aligned to image a fascicle plane within the belly region of the muscle (Figure 2.1a,b) and was secured in place using a flexible support and medical stretch-adhesive tape to ensure the same region of the belly was imaged for each trial. Images were acquired at 50 Hz, and synchronized to the torque and position data from the dynamometer.

Twenty-five evenly spaced ultrasound images were selected for each contraction, and manually digitized (Figure 2.1b,c) (ImageJ software, NIH, Maryland, USA). Previous studies have measured pennation angle as the angle between the fascicle and the superficial aponeurosis (Maganaris et al., 1998) or the deep aponeurosis (Fukunaga et al., 1997, Kawakami et al., 1998), or the mean of these two measures (Klimstra et al., 2007, Narici et al., 1996). In this study the pennation was calculated as the mean of the angles that the fascicle intersected with the superficial and deep aponeuroses, so that this same angle could be used in the calculation of the minimum belly thickness that could be measured through the centre of the fascicle. Fascicle length L_f was the length of linear line passing through the digitized fascicle points and the superficial and deep aponeuroses (Figure 2.1c). Pennation angle β was calculated as mean of the angles β_1 and β_2, made by the fascicle and the superficial and deep aponeuroses (Figure 2.1c). The muscle belly thickness L_t was the shortest distance from superficial to deep aponeurosis through the centre of the measured fascicle (L_f Sin β) (Figure 2.1c). The 'projected belly length' L_b was taken as the length of the fascicle projected onto the central axis of the muscle, and calculated as L_f Cos β (Figure 2.1c). These measured muscle parameters were interpolated to 25 evenly

Figure 2.1 Picture of the testing equipment, ultrasound image of a muscle and illustration of its strucutral parameters.



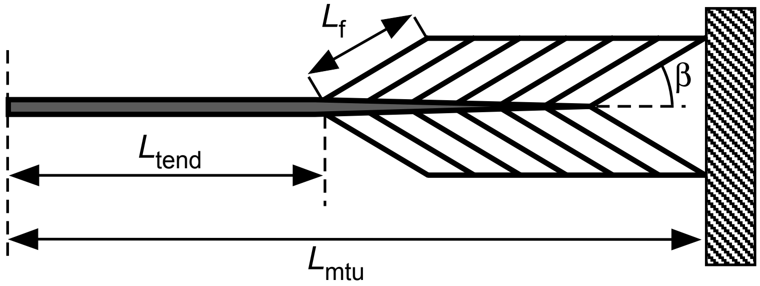
Picture shows a participant's foot in the ankle dynamometer and the ultrasound probe placed on Lateral Gastrocnemius (a). Ultrasound image of Lateral Gastrocnemius with scanning plane aligned to the muscle fascicle plane and digitized points represented by crosses on the superficial and deep aponeuroses, and a muscle fascicle (b). Superficial and deep aponeuroses, and a muscle fascicle are highlighted by black dashed lines (c). Belly length L_b, belly thickness L_t, fascicle length L_f and the angles that a fascicle makes with superficial aponeurosis β_1 and deep aponeurosis β_2 used to calculate fascicle pennation β are shown.

spaced values per plantarflexion cycle, and then calculated as the mean across all repeats for each condition. From these mean traces, the fascicle velocity V_f and belly velocity V_b were calculated as the first time-derivative of the changes in L_f and L_b, respectively. The maximum belly velocity $V_{b,max}$ was determined, as was the fascicle velocity at that same time $V_{f,max}$. The belly gearing G_b was calculated as $V_{b,max}/V_{f,max}$ (Wakeling et al., 2011).

2.2.4. Muscle model

Planar models of the muscle-tendon complex for the MG and LG were constructed based on Van Leeuwen's (1992) model (Figure 2.2). Subject-specific architectural parameters were based on ultrasound measurements from one subject during standing (Figure 2.2). The intrinsic physiological properties of the muscle fibres (active and passive force-length; force-velocity) and tendon followed those used in musculoskeletal modelling of the lower extremity (Delp et al., 2007). Aponeurosis compliance was assumed to equal the tendon compliance. Contractions were simulated for these models with prescribed activation levels and changes in MTU length. The models were excited with a bell-shaped activation function that began in advance of shortening at point 1 (Figure 2.3), and reached maximum activation midway through the contraction when the MTU velocity was at its maximum at point 4 (Figure 2.3)**.** The range of MTU lengths used in the models was set so that the calculated muscle fascicle lengths matched those from the subject. IsoV contractions were simulated for maximum activation by changing the contraction period, and this was tested between 0.5 to 2.0 s in six steps, spanning the experimental range that was 0.61 to 1.69 s. IsoT contractions were simulated by decreasing the maximum activation from 100 % to 78 % in six steps while the contraction period was increased from 0.5 to 2.0 s, and these parameters were set to result in the maximum predicted force remaining constant across the range of conditions. The maximum belly velocity $V_{b,max}$ was determined, as was the fascicle velocity $V_{f,max}$ at that same time (Figure 2.3c). The belly gearing G_b was calculated as $V_{b,max}/V_{f,max}$.

Figure 2.2 Illustration of a panel model for a muscle-tendon unit (van Leeuwen, 1992).

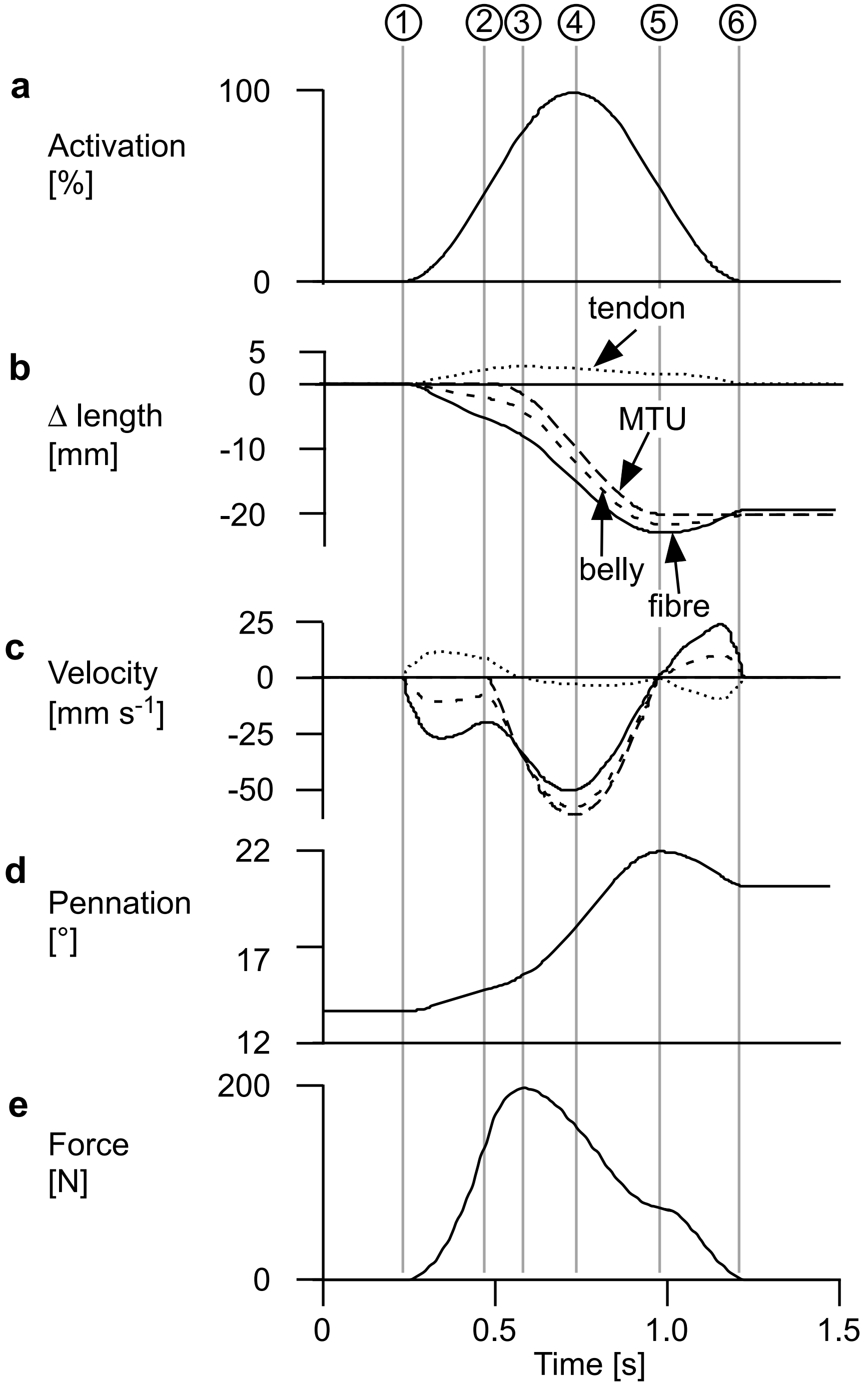


Architectural properties for muscle model

	Medial gastrocnemius	Lateral gastrocnemius
Fibre length, L_f (mm)	68.2	64.7
Tendon length, L_{tend} (mm)	160	160
Muscle-tendon unit length, L_{mtu} (mm)	430	430
Pennation angle, β (°)	11.8	14.6

The model was simulated using the architectural parameters determined for one participant.

Figure 2.3 Time-varying parameters for the model of the lateral gastrocnemius during contraction.



The velocities are shown for the muscle fibres, muscle belly, tendon and muscle-tendon unit. Note the smaller peak shortening (negative) velocity of the muscle fibres due to them being geared within the muscle belly. The marked times are discussed in the text.

2.2.5. Statistics

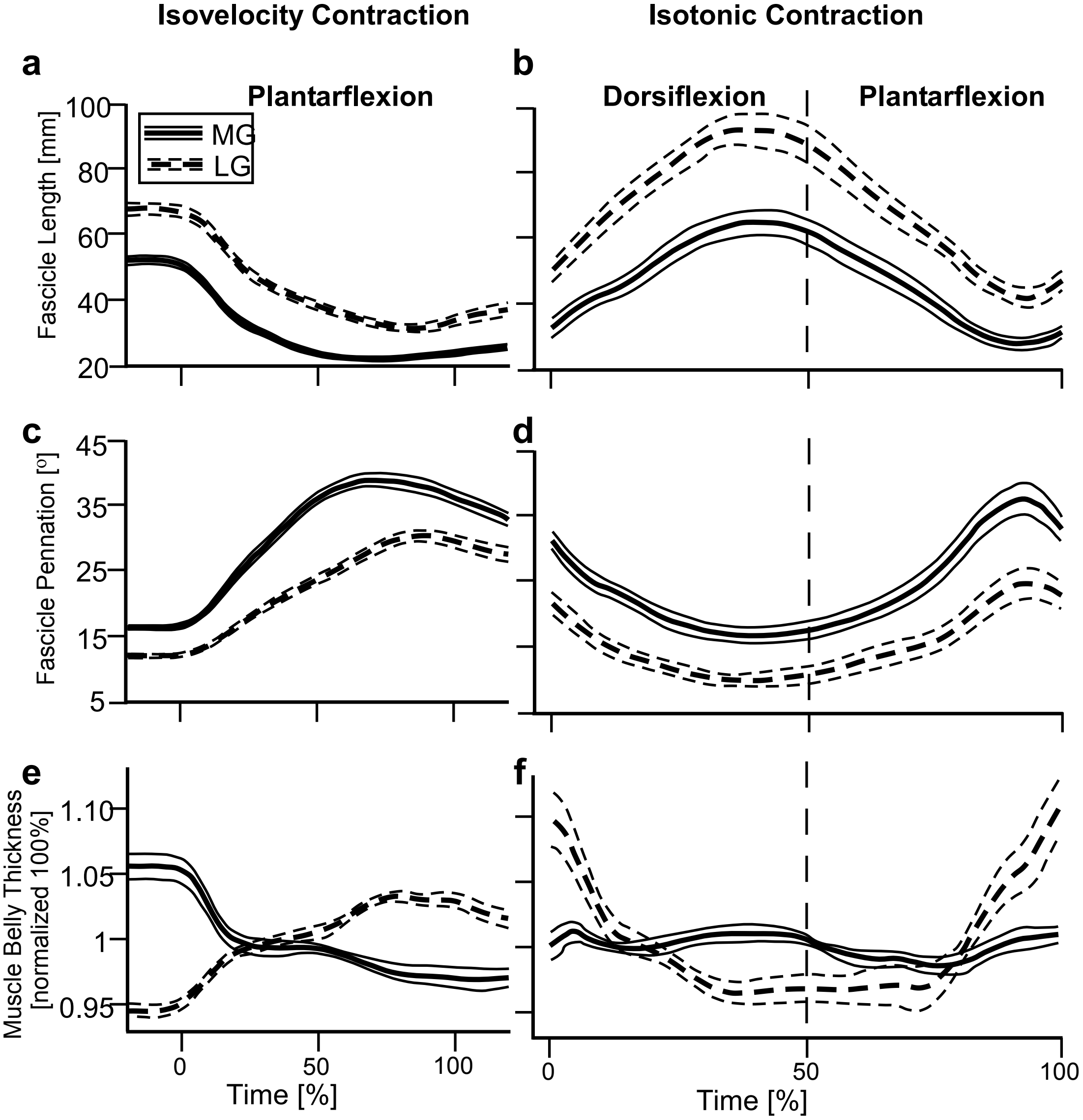
The effect of the measured kinematic parameters from the dynamometer on the muscle parameters was determined using general linear model analyses of covariance (ANCOVA; Minitab v16, Minitab Inc., State College, PA, USA). Separate tests were conducted for isoV and isoT, from experimental and model data. For experimental isoV, participant was a random factor and maximum angular velocity was a covariate. For experimental isoT, participant was a random factor and maximum angular velocity and maximum ankle torque were covariates. For model isoV, maximum MTU velocity was covariate. For model isoT, maximum MTU velocity and force were covariates. Initial tests found the muscle to be a significant factor, and so separate tests were evaluated for MG and LG. Effects were considered significant at $p < 0.05$. All results are presented as mean ± s.e.m..

2.3. Results

The initial architecture in the muscles, while the subjects were seated in the dynamometer before each contraction began were as follows: the MG had a pennation β of 16.12 ± 0.35 °, thickness L_t of 14.45 ± 0.38 mm, and fascicle lengths L_f of 52.98 ± 1.67 mm; the LG had β of 11.88 ± 0.39 °, L_t of 13.36 ± 0.43 mm, and L_f of 66.53 ± 2.19 mm. Matched pair t-tests showed that the initial values for β, L_t and L_f were significantly different between the MG and LG.

Belly gearing G_b, and changes in structural parameters including fascicle pennation and belly thickness for the given four isovelocity (isoV) and six isotonic (isoT) conditions were collected to determine the changes with contraction. During plantarflexion for both the isoV and isoT contractions of the MG and LG, the MTU length and belly length decreased. Within the muscle bellies, the fascicles shortened (Figure 2.4a,b) and increased in β (Figure 2.4c, d). For all tests there were significant changes in belly thickness ΔL_t (determined by t-test), and these changes were muscle specific. The MG thinned by 1.21 ± 0.25 mm during isoV and by 0.92 ± 0.05 mm during isoT contractions, and the LG got thicker by 1.72 ± 0.18 mm during isoV and by 2.41 ± 0.15 mm during isoT contractions (Figure 2.4e,f).

Figure 2.4 Time varying parameters from the experimental data are shown.



The changes for fascicle length, fascicle pennation and belly thickness during isovelocity contraction at $45^{o}\ s^{-1}$ (a,c,e) and isotonic contraction at 60N m@0.33Hz (b,d,f) for the medial gastrocnemius (solid) and the lateral gastrocnemius (dashed) are shown. Data are represented by mean ± SE.

The G_b from the experimental data for MG showed a general trend of being greater during lower velocity (low frequency) contractions for both isoV and isoT (Figure 2.5a,c). However, the velocity-dependent changes in LG were minor and insignificant for isoV (Figure 2.5b) but similar to MG during the isoT contractions (Figure 2.5d). The G_b was generally greater at higher ankle torques for the isoT contractions (Figure 2.5c,d). In all cases the G_b was significantly greater than 1 (1-sided t-test).

The muscle model showed similar features to the experimental data (Figure 2.3, 2.5 and 2.6) but additionally provided information on predicted tendon deformation and tendon gearing (Figure 2.6). Different stages of the contraction are identified in Figure 2.3. The activation increased from point 1 and resulted in an increase in muscle force that allowed the tendon to stretch and the belly and fibres to shorten. The MTU was held at a fixed length until point 2, and was then allowed to shorten. MTU shortening after point 2 resulted in an increase in the belly and fibres shortening velocities that in turn tended to temper the force due to the force-velocity effect; however, activation was still increasing after point 2 and resulted in a continued increase in force. The force reached its maximum at point 3, and the tendon stretch that was directly related to this force also reached a maximum at this point. Between points 3 and 4 the force decreased and thus the tendon started to return to its initial length. During this period the MTU length was still shortening but as the tendon length was increasing the shortening velocity of the belly and fascicles exceeded that of the MTU. At point 4 the fascicle shortening velocity was at its maximum, but this velocity was less than that for the muscle belly or the MTU, and thus the belly and tendon gearing were both greater than one. Between points 4 and 6 the activation level returned to zero, the MTU was brought to a fixed length that was shorter than its initial length and the muscle force returned to zero. In the period between points 5 and 6, the MTU length was fixed but the tendon was still shortening to reach to its initial length, and so in this phase the belly and fibre velocities were stretched to their final resting state.

During the MTU shortening phase in the muscle model (Figure 2.3) the β increased as the fascicles shortened. Both the modeled MG and LG reduced in thickness when they were active (Figure 2.3), and when the aponeurosis was under tension, but they returned to their initial thickness due to recoil of the aponeurosis length and the constant panel-area assumption of the model. The muscle model showed little

Figure 2.5 Bar graphs showing belly gearing for the medial and the lateral gastrocnemius testing data during isovelocity (a,b) and isotonic (c,d) contraction. Data are represented as mean + s.e.m.

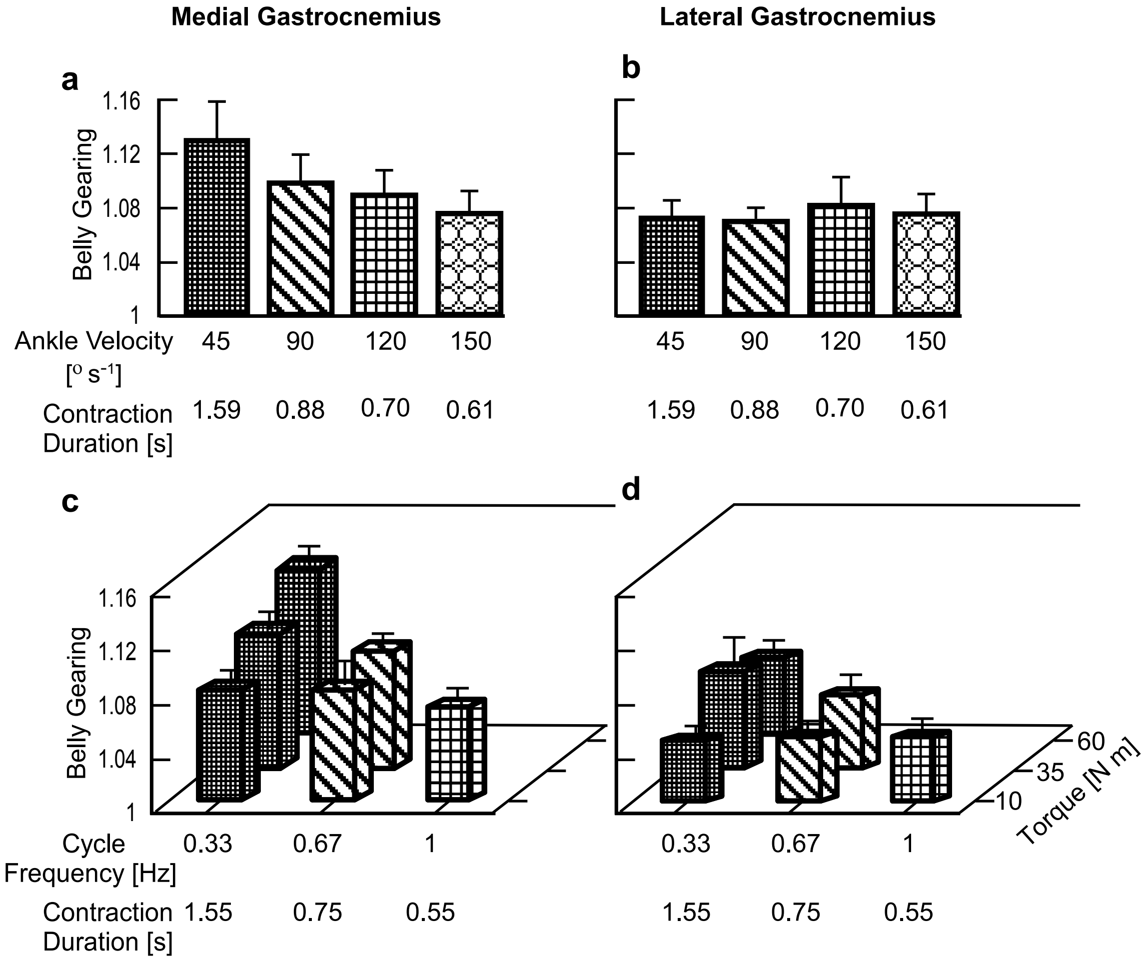
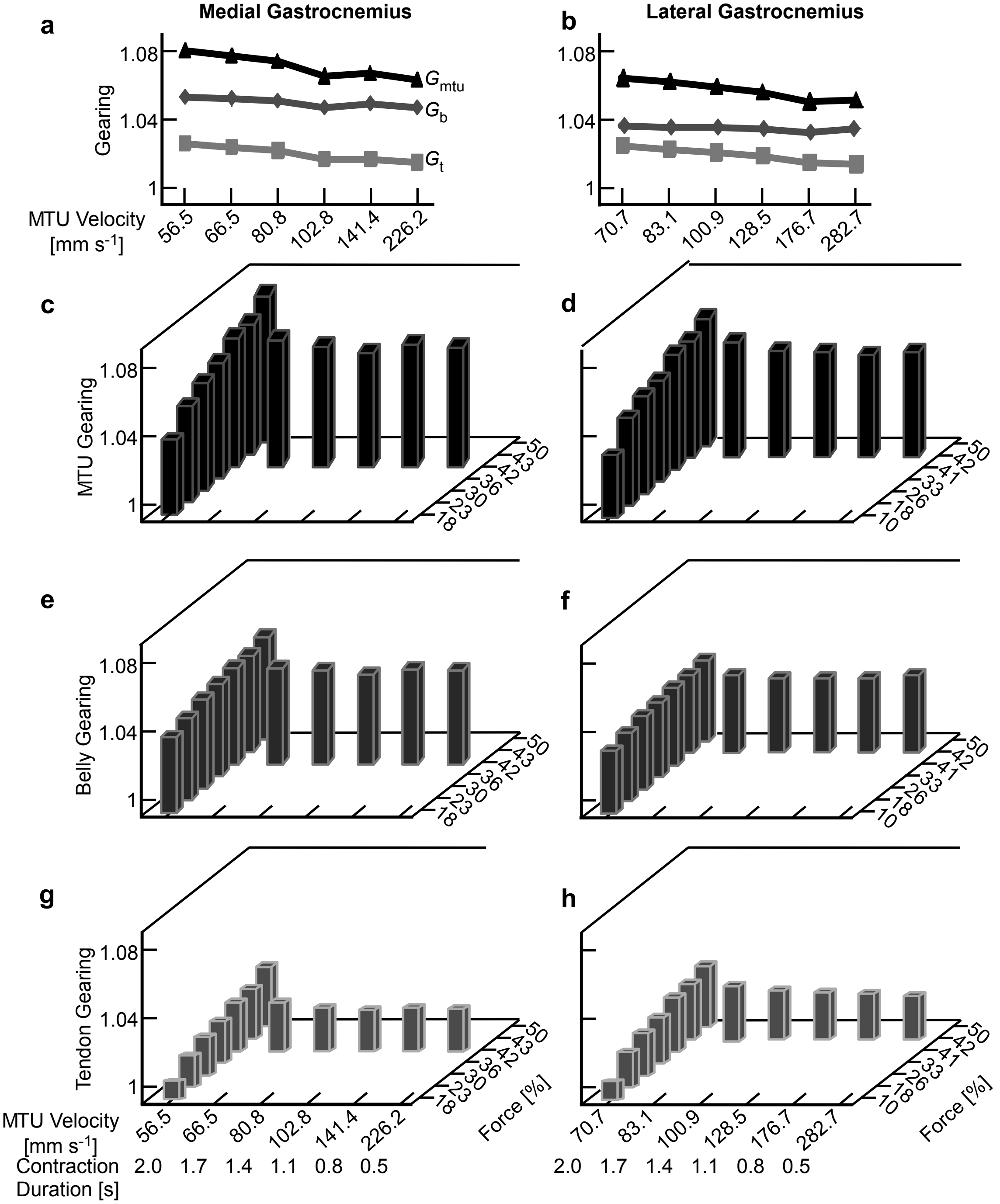


Figure 2.6 Graphs showing the gearing components from the 2D panel model.



Isovelocity results (a,b) and isotonic results (c-h) are shown for the medial gastrocnemius (a,c,e,g) and the lateral gastrocnemius (b,d,f,h). The model is detailed in Fig. 2. Data are shown for MTU gearing G_{mtu} in black, belly gearing G_b in dark grey and tendon gearing G_t in light grey. Force is normalized to maximum isometric force.

change in G_b for the modelled isoV tests (Figure 2.6a,b). The model results for both the MG and LG were similar to the experimental data for the isoT contractions with the G_b increasing at higher forces (Figure 2.6e,f). For both the isoV and isoT tests, G_b was greater than G_t (Figure 2.6a,b,e,f), and G_t showed a greater change with changes in force than with MTU velocity (Figure 2.6a,b,g,h).

The ANCOVA results from both muscles (MG and LG) and both the experimental data and the model are shown in Table 2.1. The associations between gearing, pennation and thickness with ankle torque or ankle velocity are reported. Both experimental and model data, during isoV and isoT, showed similar trends of G_b, G_t and G_{mtu}. During isoT, there was a general effect of significant increases in gearing with torque (or modeled force) and a decrease in gearing with velocity. Similarly, in general the gearing was lower at higher velocities during the isoV contractions. Fascicle rotations $\Delta\beta$ decreased with increases in velocity for both the MG and LG. There were no significant associations between ankle torque or velocity on the changes in muscle thickness for either muscle.

2.4. Discussion

2.4.1. Structural basis of muscle belly gearing

During these plantarflexion contractions of the MG and LG, the muscle-tendon unit (MTU) lengths increased as the ankle joint extended and this concurred with shortening of the muscle bellies, shortening of the muscle fascicles and rotation of those fascicles to greater pennation angles. The rotations of the fascicles during shortening allowed them to shorten at a lower rate than the muscle belly, which is the belly gearing G_b. The G_b has previously been described in the axial muscles of fish and the salamander (Alexander 1969, Azizi et al., 2002, Wakeling & Johnston 1999), and in the ankle plantarflexors of the turkey (termed the architectural gearing ratio, or AGR: Azizi et al., 2008) and man (Wakeling et al., 2011). G_b forms part of the muscle-tendon unit gearing, G_{mtu}, that is the ratio of the MTU shortening velocity to the fascicle shortening velocity (Wakeling et al., 2011, Zuurbier & Huijing, 1992). G_{mtu} has functional significance as it allows the fascicle shortening velocity to be reduced for fast

Table 2.1 Results from ANCOVA for medial gastrocnemius (MG) and lateral gastrocnemius (LG) using experimental data (Exp) and model (Model) data during isovelocity (isoV) and isotonic (isoT) are shown.

Muscle	Response factor	IsoV (Exp)	IsoT (Exp)	IsoV (Model)	IsoT (Model)	IsoV (Model)	IsoT (Model)	IsoV (Model)	IsoT (Model)
		Belly Gearing G_b				*Tendon Gearing* G_t		*MTU Gearing* G_{mtu}	
MG	Torque/Force		↑*		↑*†		↑†		↑†
	Velocity	↓	↓	–	–	↓‡	↓‡	↓‡	–
LG	Torque/Force		–		↑†		↑†		↑†
	Velocity	–	–	–	–	↓‡	↓‡	↓‡	↓
		Change in Fascicle Pennation ($\Delta\beta$)							
MG	Torque		↑						
	Velocity	–	↓‡						
LG	Torque		–						
	Velocity	–	↓‡						
		Change in Belly Thickness (ΔL_t)							
MG	Torque		–						
	Velocity	–	–						
LG	Torque		–						
	Velocity	–	–						

Key:
↑ significantly positive relation
↓ significantly negative relation
– not significant
* similar results of testing and model for belly gearing, in a column
† similar results of torque in MG & LG for a structural parameter, in a row
‡ similar results of velocity in MG & LG for a structural parameter in a row

Arrows show where the three structural parameters (belly gearing, fascicle pennation and belly thickness change for testing, and belly gearing, tendon gearing and MTU gearing change for model) were significantly related to torque/force and velocity ($p < 0.05$). A dash is shown where the factor was tested, but there was no significant effect.

movements, thus giving the fascicles a greater force-producing potential due to the force-velocity relationship during contraction (Hill, 1938). Changes in gearing with different dynamic tasks (contracting against high loads or at fast velocities; Azizi et al., 2008, Wakeling et al., 2011) have been described, and in particular we have suggested that activation levels may contribute to gearing during natural behaviours (Wakeling et al., 2011). The purpose of this study was to investigate some of the contractile parameters that may influence gearing.

The two gastrocnemii perform a common action of knee flexion and ankle plantarflexion; however, they have different muscle geometries both at rest and during shortening. Our study shows that at rest MG has greater muscle thickness L_t, pennation β and shorter fascicle length, L_f than LG (Figure 2.4) and this was consistent with previous reports (Kawakami et al., 1998). These initial factors influence the dynamic muscle behaviour and thus may influence task specific performance. A greater initial β allows for greater fascicle rotations, $\Delta\beta$, as the fascicles shorten (Brainerd & Azizi 2005, Fukunaga et al., 1997, Kawakami et al., 1998) thus resulting in $G_b > 1$. By contrast, parallel fibred muscles cannot experience rotations of the fascicles within the muscle belly during contraction, and thus are constrained to having $G_b \approx 1$ (Azizi et al., 2002, Brainerd & Azizi, 2005, Wakeling et al., 2011). It is important to note that the belly gearing values observed in our study for both muscles were significantly greater than 1. Interestingly, the greater G_b for the MG that we observed in this study (Figure 2.5) corresponded with its greater initial β, giving support to the idea that muscles with greater pennation will have greater gearing.

The fascicle length depends on both β and L_t (Azizi et al., 2002, Wakeling et al., 2011), and it has previously been shown that there is no unique combination of β and L_t that occurs within the gastrocnemii during cycling (Wakeling et al., 2011). The isovelocity and isotonic tests in this study showed that ΔL_t was not sensitive to either ankle velocity or torque (Table 2.1), although it has been shown to be sensitive to both pedal cadence and crank torque during a cycling task (Wakeling et al., 2011). By contrast, the $\Delta\beta$ was sensitive to both ankle velocity and torque during the isotonic contractions (Table 2.1; Figure 2.5). The results from this study indicate that $\Delta\beta$ may be more important to gearing than changes in the muscle thickness. However, both ΔL_t and $\Delta\beta$ do change in response to the mechanical demands of the movement task, and indeed these changes are distinct between the MG and LG (Table 2.1; Wakeling et al., 2011). Differences in the gearing behaviour between the MG and LG thus result from differences in both their resting architecture, and in how their geometries change during contraction.

The volume of a muscle belly can be assumed to remain nearly constant during contraction (Baskin & Paolini, 1967). As the muscle belly shortens it must thus increase in cross-sectional area in order to maintain constant volume. Increases in cross-sectional area can occur in both thickness and width. In this study we have defined the thickness as the distance between the aponeuroses as seen in the ultrasound images, when the images were scanned along the fascicle planes, and thus the width is the direction perpendicular to these planes. Increases in muscle thickness have previously been shown in the LG during contraction (Azizi et al., 2008, Maganaris et al., 1998, Wakeling et al., 2011) but not in the MG (Azizi et al., 2008, Narici et al., 1996, Wakeling et al., 2011). Similar changes were observed in this study where L_t increased for LG during plantarflexion in both isoV and isoT (Figure 2.4e,f). Contrastingly, L_t decreased for MG during the plantarflexion phase of both the isoV and isoT contractions. In cases where the belly thickness decreases for the MG during belly shortening, then the muscle width must have increased. In the LG, where the belly shortened and L_t increases, the width could increase or decrease, depending on the magnitude of the other displacements. It is worth noting that for contractions that are isometric for the joint angles, there would still be belly shortening due to compliance and stretch in the tendon and this must result in an increase in cross-sectional area. Observations that the MG thickness remains unchanged during isometric contractions (Maganaris et al., 1998, Narici et al., 1996) can thus still be interpreted as the MG increasing in width. Therefore, there are distinctions in the way in which the MG and LG muscle bellies bulge in different directions, and this bulging is a factor in ΔL_t and thus gearing. The mechanisms driving the differential bulging between MG and LG are not yet known, however understanding the 3D aspect of muscle bulging will be an important part of identifying the mechanisms of muscle gearing.

2.4.2. Belly gearing during ankle plantarflexion

The belly gearing was affected by both torque and velocity, particularly for the MG (Table 2.1; Figure 2.5). As the muscle fibres shorten they must increase in cross-sectional area to maintain their individual fibre volumes. These increases in girth may occur in the directions of either the muscle thickness or width, and this directionality will be constrained by a number of parameters that include the intramuscular pressure and fascicle geometry, the stiffness of surrounding tissues such as aponeuroses, and pressure from external tissues. Intramuscular pressures, that may be related to muscle bulging, increase with muscle forces (Aratow et al., 1993, Sejersted et al., 1984) and in turn the muscle force depends on both the activation level and shortening velocity. The isotonic tests were developed to test the contractions across a range of activation levels, muscle forces, and shortening velocities. It was thus expected that changes in the internal geometry and gearing would vary with both joint torque and velocity, and this was indeed the case (Table 2.1; Figure 2.5). However, surprisingly, the result that higher G_b concurs with low velocity and high torque is different from results reported for cycling.

Differences between the magnitudes of G_b reported here and from a previous study (Wakeling et al., 2011) may reflect differences in the methodological approach. In our previous cycling study (Wakeling et al., 2011) it was assumed that the fascicle and belly lengths oscillated in a sinusoidal fashion, and were in phase. By contrast, in this current study we have removed these assumptions and calculated instantaneous gearings. It is not possible to consider the mean gearing over a complete contraction cycle because when the fascicle velocity approaches zero the gearing would approach infinity. Therefore, we have presented the gearing that occurred when the velocity was at a maximum, and this has functional relevance because this is when the gearing would be most important to reduce the rate of fascicle shortening. The current submaximal isotonic results support the concept that variable gearing can occur at the level of both G_t and G_b (Wakeling et al., 2011). However, the decrease in gearing at higher velocities came as a surprise and was different from previous results in both the turkey and man (Azizi et al., 2008, Wakeling et al., 2011). However, our current findings were consistent across two separate experiments and modelling (Figure 2.5 and 2.6). It has previously been suggested that the gearing depends on the activation profiles and thus the coordination between the stretch-shortening cycle and the muscle excitations (Wakeling et al., 2011). During cycling, the gastrocnemius muscles have functions of providing joint stability and force transmission (Neptune et al., 2000, van Ingen Schenau et al., 1992), which are different from the assumed function of power generation for the plantarflexion tests in this study. It is likely that as the functional task of a muscle changes, it will be activated differently and its role in muscle coordination may change. These changes would result in an altered gearing.

2.4.3. Tendon gearing predicted during modelled contractions

The muscle-tendon unit gearing G_{mtu} is the product of G_b and G_t. Tendon gearing G_t depends on the stretch in the tendon that in turn is affected by muscle force and thus muscle activation (Wakeling et al., 2011). The experimental measures of gearing in this study only allowed G_b to be calculated, and so the muscle model was used to also predict the effect of the contractile parameters on G_t. The muscles were modelled as 2D panels with constant area. The aponeurosis compliance allowed the aponeurosis to stretch during contraction, and this translated to a decrease in thickness during the contraction due to the constant area assumption (Figure 2.3e). This capability to change thickness during contraction will affect the fibre rotations and thus gearing in a manner that may mimic the physiologic state. However, two features of the model did not reflect the complex nature of the muscle bulging: firstly, the muscle thickness returned to its initial state after the contraction despite the length being shorter, and secondly the modelled MG and LG both changed thickness in the same manner because the differential changes in muscle width were not captured by the model.

The model predicted higher belly gearings than tendon gearings and additionally, there was a smaller variation in G_b than in G_t (Figure 2.6) in a manner consistent with our previous cycling studies (Wakeling et al., 2011). The isoT simulations predicted that G_t would vary more with changes in muscle force (driven by the different activation levels) than with velocity (Figure 2.6g,h). Thus the tendon gearing does vary with the muscle coordination, as previously predicted (Wakeling et al., 2011). The general model results indicate that the level of the G_{mtu} is largely determined by the belly gearing, and the variability in G_{mtu} is largely determined by the tendon gearing, and these general patterns are consistent with the gearing components reported for cycling (Wakeling et al., 2011).

Structurally, the modelled MG and LG were much more similar to each other than the experimentally measured values. The model MG and LG did not capture all the features of ΔL_t and thus it was not surprising that differences in actual muscle behaviour were not reflected (Figure 2.3e). Some of this 3D complexity again directs to incorporating 3D methods for studying fascicle architecture. Since the contraction and rotation of the fascicles are affected by the intramuscular pressures, muscle bulging and thus the constraints of the elastic aponeurosis and tendon (Herbert & Gandevia, 1995, Kawakami et al., 1998), the influence of these tissues on the muscle shape and internal geometry cannot be ignored.

2.5. Conclusions

During plantarflexion contractions, the fascicles of the MG and LG muscles are geared to contract at lower velocities than the muscle-tendon unit. The gearing is dependent on the levels of muscle activation, and thus the coordination of activation relative to movement during a stretch-shortening cycle. The majority of the muscle-tendon-unit gearing is due to the fascicle rotations within the muscle belly, but varying tendon stretch during contractions results to variability in the muscle-tendon-unit gearing. Fascicle shortening depends on both fascicle rotations and changes in thickness of the muscle. During plantarflexion the MG decreases in thickness whilst the LG increases in thickness, with presumably different extents of changes in muscle width. This 3D complexity in the changes in muscle shape undoubtedly contributes to the muscle gearing and the mechanisms behind this warrant further investigation.

3. Muscle structure and muscle gearing in seniors

This study has been submitted as a manuscript for publication in a journal under the title "Changes in muscle structure in seniors may contribute to a loss in contractile performance" authored by Avleen Randhawa and James M. Wakeling. All data collection, analysis and manuscript preparation were done by Avleen Randhawa.

3.1. Introduction

Skeletal muscles play a key role in body movements and locomotion. During concentric contractions the muscle belly shortens and its thickness changes as the fascicles rotate and increase in pennation angle. Muscle performance depends on the geometrical arrangement of these fascicles and the contribution of the connective tissue both external and internal to the muscle belly. In addition, the obliqueness of fascicles, and their ability to rotate within the belly, allows them to be geared to slower speeds of contraction than the muscle belly, and this gives them higher force potentials. Fascicle rotations and gearing thus enhance the overall functional performance of a muscle. As we age, physiological changes occur in the muscles that may limit the activities of daily life (Doherty, 2003). Sarcopenia is an age related loss of skeletal muscle mass (Rosenberg, 1989) and strength (Bemben et al., 1991), and this atrophy occurs with reductions in size, strength and power output (Lauretani et al., 2003, Lindle et al., 1997). Sarcopenia is prevalent in 30% of those over 60 years, mainly involves wasting of type II fibres (Lexell, 1993), and is associated with a reduction in mobility.

Muscle power output is a more important determinate of physical performance than impairments in strength for older adults (Bean et al., 2003). Force and power deterioration has been observed in seniors between 75-85 years and it was found that reduction in power (60%) was more pronounced than muscle strength (knee-extension torque, 43.4%) in seniors between 75-85 years compared to young adults between 20-29 years (Laurentani et al., 2003). The force generated by a muscle is a function of its shortening velocity (Hill, 1938) and so the slower shortening velocities of pennate muscle fascicles can result in greater muscle force and thus joint torques during contraction. This advantageous effect may be lost in an ageing muscle due to changes in structural properties controlling the shortening velocity of muscle fascicles such as pennation angle, muscle thickness and fascicle rotations. One of the reasons for a greater loss in power in seniors may be due to the predominant atrophy of fast muscle fibres (Lang et al., 2010, Lexell, 1993) that would otherwise have produced greater power during shortening.

When muscle shortens, it must bulge or increase in cross sectional area (CSA) to conserve volume (Baskin & Paolini, 1967). Muscle bulging occurs by increases in thickness (parallel to fascicle planes) or increases in width (perpendicular to fascicle planes). The exact direction of this bulging is constrained by surrounding connective tissues such as superficial aponeurosis, deep aponeurosis and the tendon. In mammalian muscle, the pennation angle varies between 0° to 30° at rest (Zuurbier & Huijing, 1993) and may increase to approximately 70° in the medial gastrocnemius in man with maximal contraction (Kawakami et al., 1998). The pennation angle is also associated with the muscle fascicle length and belly thickness (Maganaris et al., 1998, Manal et al., 2006, Wakeling et al., 2011). During contraction the increases in muscle thickness allow the fascicles to rotate to greater pennation, which results in them gearing down to lower velocities. Thicker muscles, with greater pennation, would typically be able to allow greater fascicle rotations and gearing. Muscle hypertrophy changes the structural properties of a muscle with increases in pennation angle (Kawakami et al., 2000a), CSA (Aagaard et al., 2001) and thickness (Duclay et al., 2009) and so it may be expected that hypertrophied muscle would operate with a higher gearing. Conversely, the muscle atrophy that occurs with sarcopenia may result in a decreased gearing and thus decreased performance during dynamic contractions, but this idea remains untested.

Changes in muscle structure with sarcopenia have previously been reported for seniors during static muscle tests using ultrasound (Narici et al., 2003) and MRI (Morse et al., 2005). It has also been shown that connective tissue properties change with ageing (Maganaris, 2002, Narici & Maganaris, 2006). These studies reported that as muscle size reduces, the fascicles become shorter and less pennate. Specifically, pennation angles reduce between by 10-16% in medial gastrocnemius and by 18% in lateral gastrocnemius. Additionally, fascicle lengths in the medial gastrocnemius were also reduced by 13-16% in seniors. Cross sectional area was reduced to 74% in muscles of individuals above 75 years as compared to those between 20-40 years (Lauretani et al., 2003) along with significant reductions in muscle volume. Additionally, however, a consideration of the relation between structural changes and the reduction in performance in the elderly has never been conducted.

As a muscle undergoes sarcopenia the changes to its structure should affect its contractile performance. The main goal of this study was to investigate associations between the structural characteristics of a muscle and its functional output that lead to limitation in performance in seniors. We hypothesized that changes in fascicle rotations and muscle thickness in seniors would lead to a reduction in the belly gearing with a subsequent reduction in the power output during dynamic contractions.

3.2. Methods

3.2.1. Participants

Ten young-adults (Y) (males, age: 27.8 (4.4) yr, height: 180.9 (7.1) cm, body mass: 77.7 (15.3) kg; means (SD)) and nine seniors (S) (males, age: 75.5 (4.0) yr, height: 176.0 (6.0) cm, body mass: 83.5 (8.3) kg) were tested. Both groups included physically active participants without any subjective evidence of an ongoing musculoskeletal disease or injury. The institutional research ethics committee approved this study, and participants provided informed consent.

The seniors were recruited from a local confederation centre with prior physical and functional assessments. The inclusion criteria for seniors were individuals who were active, mobile and capable of walking on level ground and up and down the stairs. The exclusion criteria were individuals with on-going serious cardiovascular problems (recent heart attack, stroke, or chest pains), musculoskeletal injuries (fracture or injury to lower body in past one year), neurological disorders (Parkinson, Multiple sclerosis, Alzheimer’s, Huntington’s, Polio) or individuals who had undergone surgical procedures within one year prior to testing. The assessment of seniors included a medical screening questionnaire which included general questions on senior’s overall health. Some standardized and validated tests were performed prior to testing to analyze the cognitive health of seniors through mini-mental state exam (MMSE) (Woodford, 2007) and to assess the functional ability using timed up and go test (TUG) and Berg’s balance scale (BBS) tests (Steffen et al., 2002).

3.2.2. Isovelocity testing protocol

Ankle plantarflexor movements were tested on a dynamometer (System 3, Biodex, New York, USA). The medial gastrocnemius, MG, and lateral gastrocnemius, LG, were tested on opposite legs in order to minimize fatigue of muscles due to repetitive ankle extension movements. The Y group had a mean knee angle of 140.7 (s.e.m. 4.0) degrees and the S group had a mean knee angle of 138.2 (s.e.m. 4.1) degrees. Ankle torque, angle and angular velocity from the dynamometer were recorded at 1 kHz using a 16-bit data-acquisition system (USB-6229; National Instruments, Austin, TX, USA). Participants performed maximal-effort plantar-flexion movements from maximum dorsiflexion to maximum plantarflexion at constant ankle velocities of 150, 120, 90, and 45° s^{-1}. Six trials were performed at each ankle velocity, with a 5-10 s period of rest between contractions.

3.2.3. Structural properties of muscle

Geometrical properties of the muscle were imaged using B-mode ultrasound during standing and during contractions (60mm linear ultrasound probe; Echoblaster 128, Telemed, Lithuania). The ultrasound probe was aligned to image a fascicle plane within the belly region of the muscle (Figure 2.1a), and secured in place using a flexible support and medical stretch-adhesive tape to ensure the same region of the belly was imaged for each trial. Ultrasound scans during standing were also recorded to acquire passive muscle images for determination of static structural parameters (fascicle length, pennation angle and muscle belly thickness). The maximum calf girth was also measured during standing. The ultrasound images were acquired at 50 Hz, and synchronized to the torque and position data during the dynamometer tests. One ultrasound image from standing and twenty-five evenly spaced images from each ankle extension were selected and manually digitized (Figure 2.1a, b) (ImageJ software, NIH, Maryland, USA).

Pennation angle β was calculated as the mean of the angles β_1 and β_2, made by the fascicle and the superficial and deep aponeuroses, respectively (Figure 2.1b). Fascicle length L_f was the length of linear line passing through the digitized fascicle points and the superficial and deep aponeuroses (Figure 2.1b). The muscle belly thickness L_t was the shortest distance from superficial to deep aponeuroses through the centre of the measured fascicle (L_f Sin β) (Figure 2.1b). The 'projected belly length' L_b was taken as the length of the fascicle projected onto the central axis of the muscle, and calculated as L_f Cos β (Figure 2.1b). For the ankle extension tests, these measured muscle parameters were interpolated to 25 evenly spaced values per plantarflexion movement, and then calculated as the mean across all repeats for each condition. From these mean traces, the fascicle velocity V_f and belly velocity V_b were calculated as the first time-derivative of the changes in L_f and L_b, respectively. The maximum belly velocity $V_{b,max}$ was determined, as was the fascicle velocity $V_{f,max}$ at that same time. The belly gearing G_b was calculated as $V_{b,max}/V_{f,max}$ (Wakeling et al., 2011).

3.2.4. Data analysis

Statistical differences in the body mass index (BMI) and the structural parameters during standing (including the calf girth) were tested using two-sample t-tests. During ankle extensions, the age effects on the measured kinematic parameters from the dynamometer (maximum torque and joint power) and the muscle structural parameters (changes in pennation, changes in belly thickness and belly gearing), were determined using GLM ANCOVA (Minitab v16, Minitab Inc., State College, PA). Age was a factor in the ANCOVA models for all the tests and the maximum angular velocity was a covariate. Initial tests found the structural parameters to be significantly different between muscles so separate tests were evaluated for MG and LG. GLM ANCOVA was also used to test whether the rates that the pennation and thickness changed for a given ankle extension were age dependant.

The mean reduction in ankle torque and power between the young and senior groups were calculated for each ankle velocity and the means were compared using a two-sample t-test. The mean pennation angles that occurred during plantarflexion were also compared between the two groups with a two-sample t-test. Statistical effects were considered significant at a level of $P<0.05$ and 95% CI. Results are reported as means (s.e.m.).

3.3. Results

The initial assessment tests performed by seniors were the mini-mental state exam (MMSE), timed up and go test (TUG) and the Berg's balance scale (BBS). All the values were within the normal ranges found in healthy elderly population (Steffen et al., 2002, Woodford, 2007) and have been given in Table 3.1 along with the BMI and maximum calf girth. Seniors had significantly higher BMI and smaller calf girths as compared to young adults.

During standing, there was no significant difference between the fascicle lengths L_f in the gastrocnemii in seniors and young adults. However, seniors had significantly lower β and thickness L_t (Figure 3.1). During isovelocity plantarflexion contractions of the MG and LG the muscle tendon unit length and belly length decreased for both groups. Within the muscle bellies, the fascicle lengths L_f shortened (Figure 3.2a, b) and the fascicles rotated to a higher β (Figure 3.2c, d). In MG, the thickness L_t decreased as the muscle shortened while it increased in LG (Figure 3.2e, f). Seniors had significantly lower ankle torques at all four ankle velocities for both muscles/ legs (Figure 3.3a and 3.4a). Consequently, the muscle power output in seniors was also lower (Table 3.1).

There was a significantly greater reduction in maximum power output than for maximum ankle torque in the seniors for these plantarflexion tests, and there was a trend for greater reduction in power output at higher velocities (Table 3.1).

Structural parameters were different between the seniors and young adults during contraction. There was a general trend of atrophy in seniors as they had thinner muscle bellies (Figure 3.3d and 3.4d). The initial pennation angles at the start of each plantarflexion movement were higher for seniors in MG (Figure 3.3c) but there was no

Table 3.1 Results from the assessment tests for seniors and from the data of the two groups. BMI, maximum calf girth, power output and torque at all four velocities is given.

Assessment tests for seniors

MMSE (score)	29 (1.0)
TUG (sec)	11 (1.0)
BBS (score)	54 (1.5)

Body mass index **(kg m^{-2})**

Seniors	Young adults
25.8 (1.4)	23.3 (3.9)

Maximum calf girth (mm)

Seniors	Young adults	Seniors	Young adults
Left leg		Right leg	
379 (16)	408 (36)	378 (15)	407 (35)

Maximum power output at four given velocities (W)

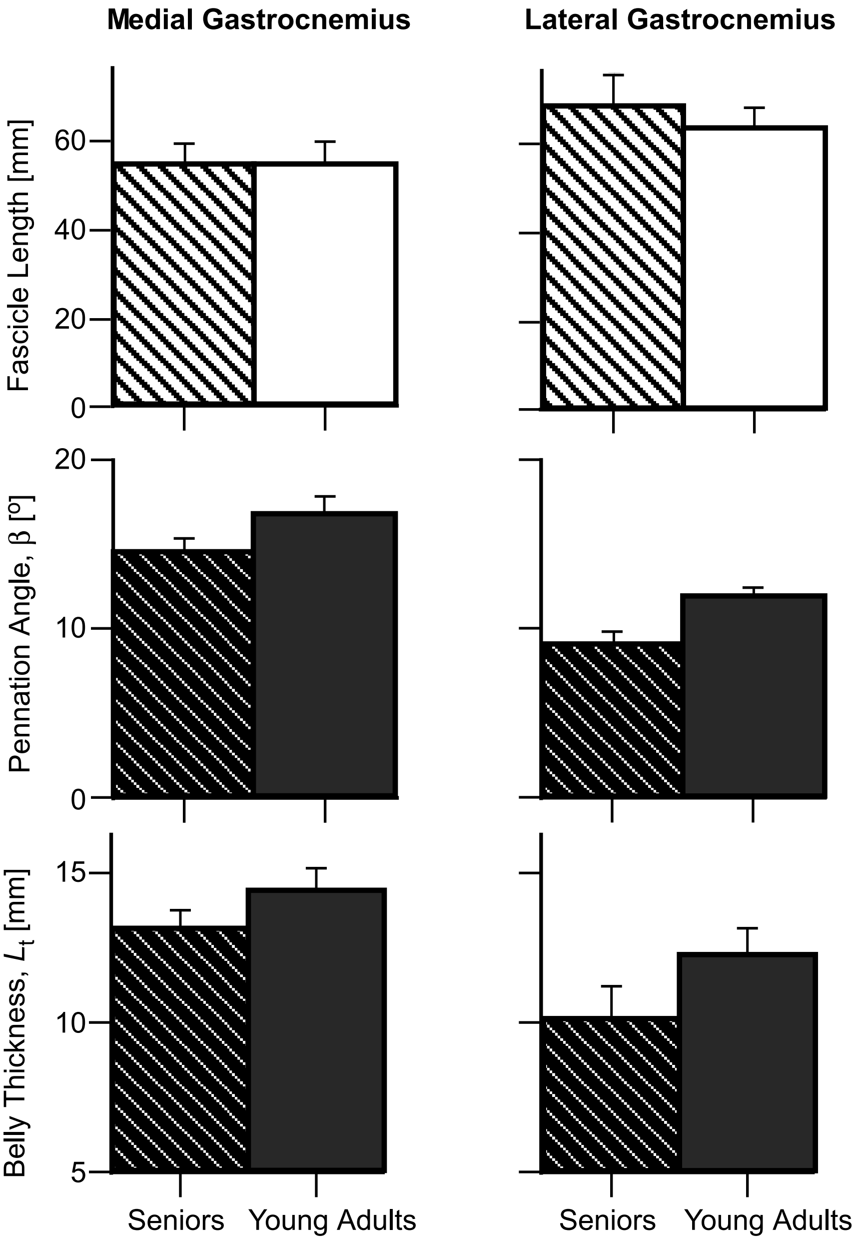
	Seniors	Young adults		Seniors	Young adults	
Velocity	**Medial gastrocnemius**		**% Reduction**	**Lateral gastrocnemius**		**% Reduction**
45 ° sec^{-1}	34.99 (4.12)	79.20 (6.61)	55.9%	40.45 (3.86)	76.36 (8.02)	47.0%
90 ° sec^{-1}	46.89 (8.83)	103.81 (9.41)	54.8%	47.41 (7.10)	102.28 (12.59)	53.6%
120 ° sec^{-1}	44.65 (10.99)	100.38 (12.57)	55.5%	36.50 (6.02)	109.64 (15.32)	66.7%
150 ° sec^{-1}	32.20 (8.75)	85.56 (11.27)	62.4%	26.44 (5.84)	84.19 (13.34)	68.6%

Maximum ankle torque at four given velocities (N m)

	Seniors	Young adults		Seniors	Young adults	
Velocity	**Medial gastrocnemius**		**% Reduction**	**Lateral gastrocnemius**		**% Reduction**
45 ° sec^{-1}	44.12 (5.07)	98.78 (8.03)	55.3%	51.30 (4.83)	95.66 (10.13)	46.4%
90 ° sec^{-1}	31.96 (5.09)	65.94 (5.86)	51.5%	32.54 (3.81)	64.96 (7.96)	50.0%
120 ° sec^{-1}	24.88 (4.41)	47.65 (5.76)	47.8%	24.01 (2.60)	51.90 (7.02)	53.7%
150 ° sec^{-1}	18.19 (3.11)	34.02 (3.78)	46.5%	22.44 (2.64)	32.87 (4.78)	31.7%

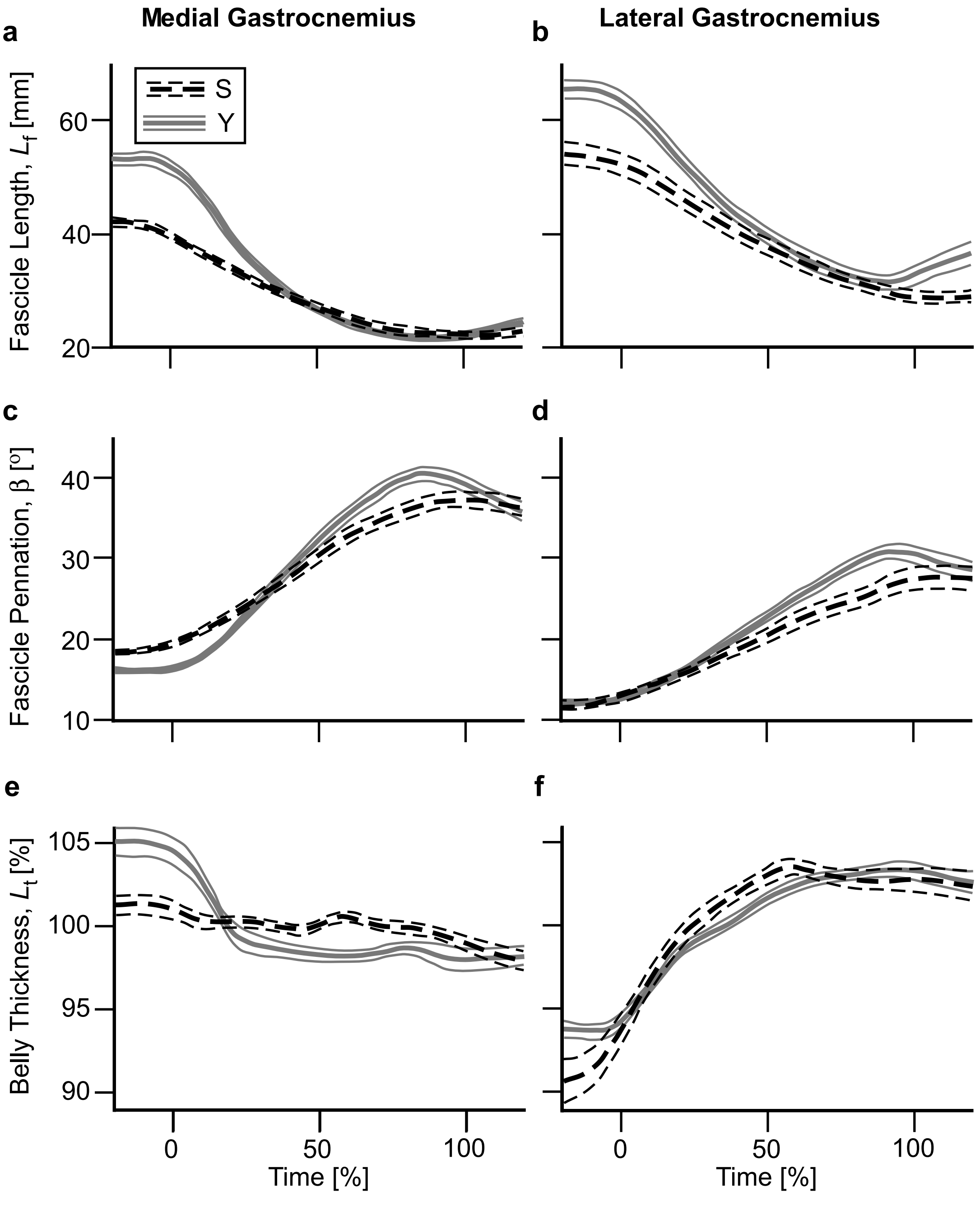
Data were represented as mean (SD) for assessment tests, BMI and maximum calf girth, and as mean (s.e.m.) for maximum power output and maximum ankle torque.

Figure 3.1 Mean and s.e.m. of structural parameters for both muscles during standing in seniors and young adults.



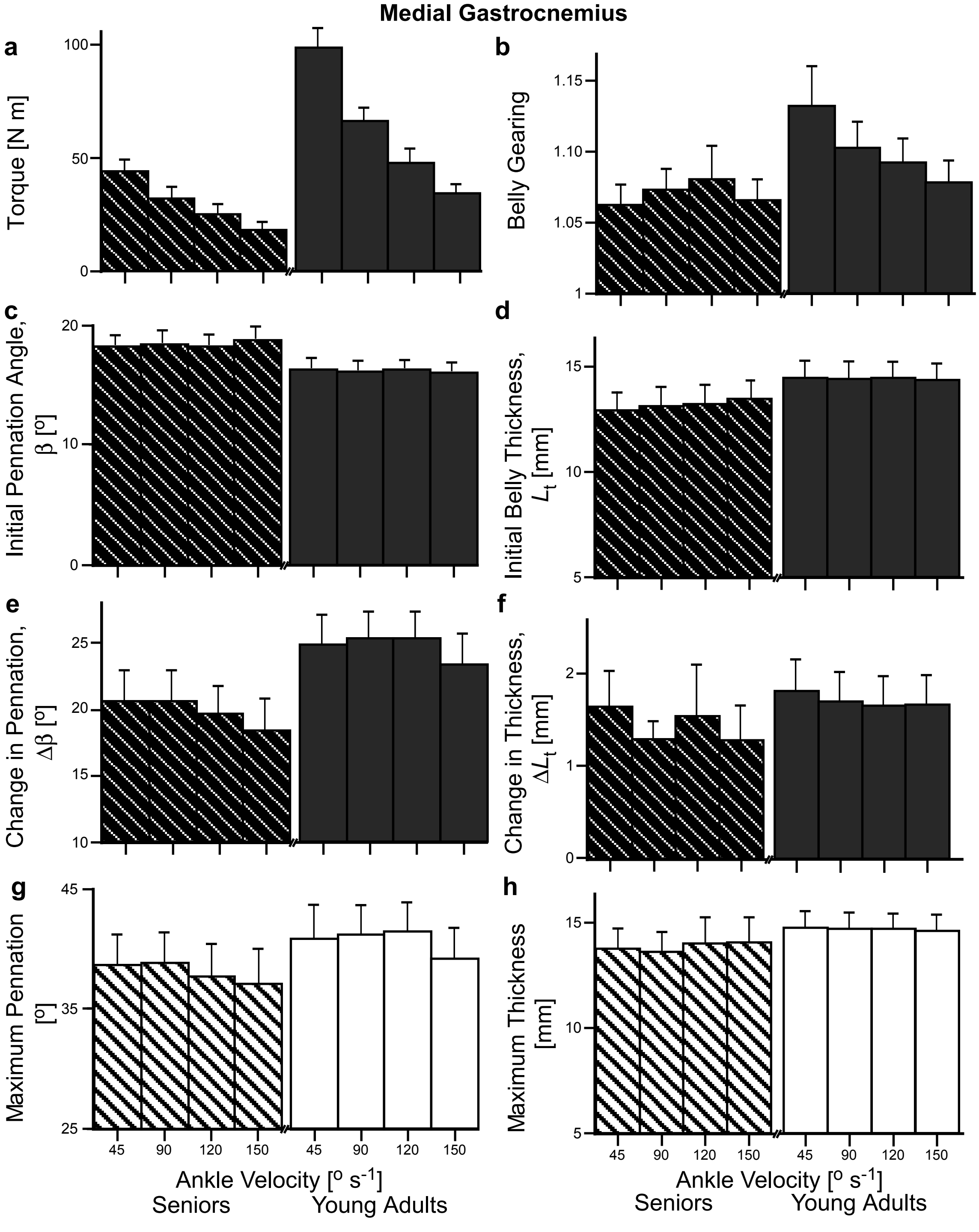
The values for fascicle length, pennation angle and belly thickness are shown. Seniors are represented as dashed bars and young adults as solid bars. Significant effect of age (*P*<0.05, 95% CI) is represented by grey bars and insignificant effect is shown by unshaded bars.

Figure 3.2 Time varying structural parameters are shown during isovelocity contractions at 90^{o} s^{-1} in both groups.



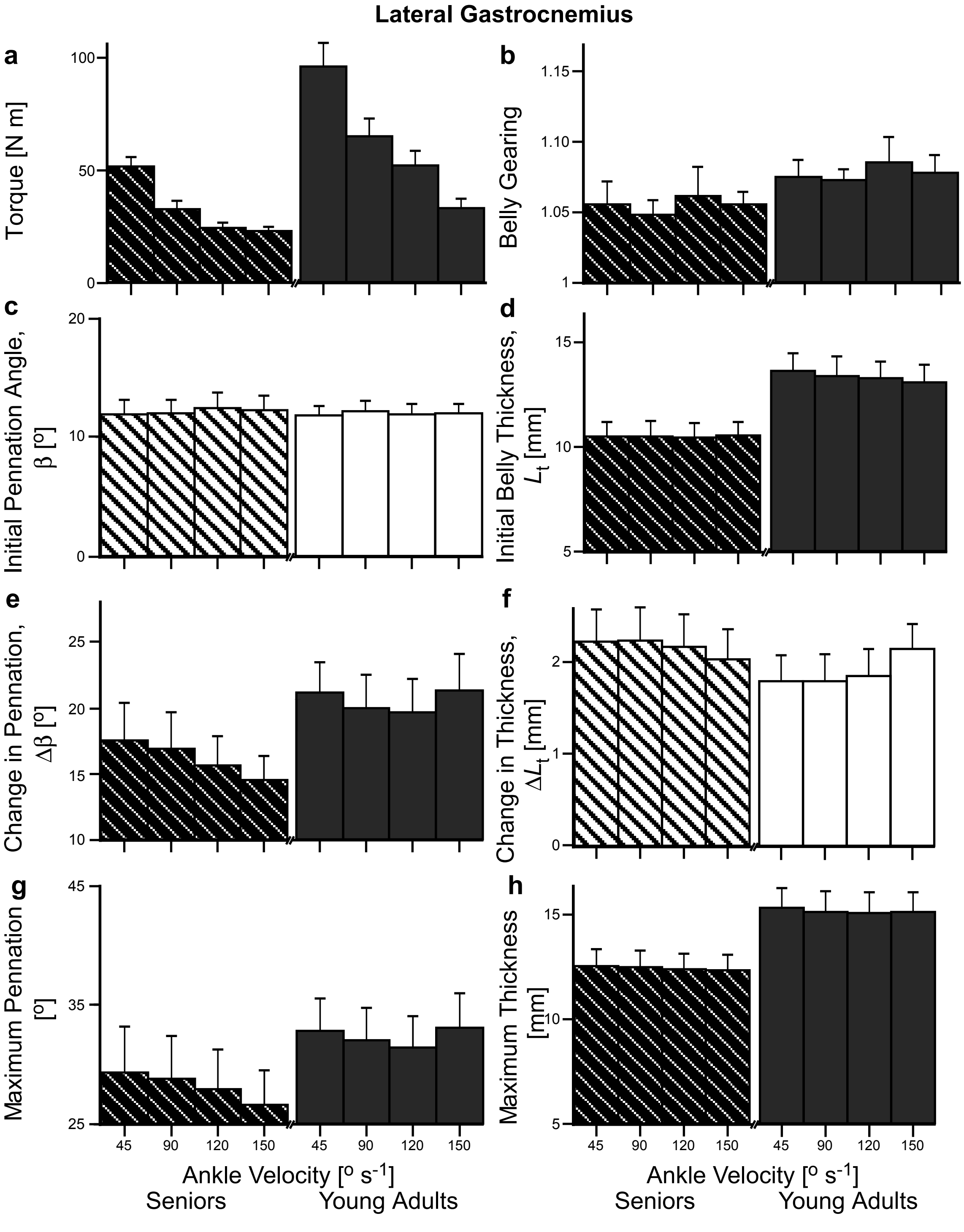
The changes for fascicle length, fascicle pennation and belly thickness for MG (a,c,e) and LG (b,d,f) are shown for the seniors group (S) as dashed black lines and for the young adults group (Y) as solid grey lines. Data are represented by mean and s.e.m..

Figure 3.3 Mean and s.e.m. of various parameters at the four ankle velocites in medial gastrocnemius for seniors and young adults.



The values for torque (a), belly gearing (b), pennation angle (c,e,g) and belly thickness (d,f,h) are shown. Seniors are represented as dashed bars and young adults as solid bars. Significant effect of age (*P*<0.05, 95% CI) is represented by grey bars and insignificant effect is shown by unshaded bars.

Figure 3.4 Mean and s.e.m. of various parameters at the four ankle velocites in lateral gastrocnemius for seniors and young adults.



The values for torque (a), belly gearing (b), pennation angle (c,e,g) and belly thickness (d,f,h) are shown. Seniors are represented as dashed bars and young adults as solid bars. Significant effect of age (*P*<0.05, 95% CI) is represented by grey bars and insignificant effect is shown by unshaded bars.

significant age effect for this initial pennation in LG (Figure 3.4c). As the muscles shortened, the MG and LG in seniors had smaller fascicle rotations (Figure 3.3e and 3.4e) with a lower change in thickness in the MG but not LG (Figure 3.3f and 3.4f). Additionally, the LG in seniors reached a lower maximum pennation and thickness compared to the young adults (Figure 3.4g, h). The mean pennation angles that occurred during plantarflexion were not significantly different between the two groups. The gastrocnemii of seniors attained lower belly gearing than the young adults for both muscles during all ankle velocity conditions (Figure 3.3b and 3.4b).

The range of motion (ROM) from maximum dorsiflexion (negative value) to maximum plantarflexion (positive value) on the dynamometer for both groups was determined by the initial and final ankle positions. The mean ROM for MG was -1.39 (1.61) to 38.93 (1.07) in seniors and -19.53 (1.66) to 48.07 (2.73) in young adults, and for LG it was 1.35 (2.37) to 38.77 (0.90) in seniors and -21.63 (1.40) to 47.78 (1.35) in young adults, mean (s.e.m.). The rate of change of thickness for a given change in ankle angle was higher for seniors in LG but not in MG. The rate of change of pennation for a given change in ankle angle was not significantly different for the two groups for MG and LG.

3.4. Discussion

Static muscle geometry is an important determinant of muscle dynamics during motion. The size of the muscles, arrangement of fascicles and their lengths help define the functional role that a muscle may play (Burkholder et al., 1994, Lieber & Friden, 2000). For instance, the muscles of lower limb such as vastus lateralis and soleus have shorter fascicles with higher pennation angles to attain greater forces and postural control, respectively (Friedrich & Brand, 1990). Contrastingly, adductor magnus and longus with their longer fascicles and smaller pennation angles perform high velocity movements at wider range of motion at the hip joint (Blazevich, 2006). Structural parameters also aid in assessing the health of a muscle after periods of disuse. According to previous studies on the medial gastrocnemius (Narici & Ceretelli, 1998) and vastus lateralis (Bleakney & Maffulli, 2002), fascicles become shorter and have lower pennation angles with disuse atrophy and with immobilization after intramedullary nailing of the femur. Effects of ageing on muscle structure are similar to atrophy of a muscle (Morse et al., 2005) causing reductions in its size.

The standing measures in our study showed that muscles of seniors were thinner than young adults (belly thickness decreased by 9.0% in MG and by 17.7% in LG); the fascicle pennation decreased by 13.5% in MG and by 23.8% in LG; however, there was no age effect on the fascicle length (Figure 3.1). Interestingly, during a MRI study in the elderly (Morse et al., 2005), the fascicle lengths did not change in LG but decreased in MG in contrast to this current study where the fascicle lengths during standing were unaffected by age for both muscles. Differences between these results may be attributed to structural differences between the two gastrocnemii and differences in the position of limb during the two studies (supine in MRI study versus standing in our study).

Previous reports have shown no differences in the BMI of seniors (65 to 85yr) and young adults (20 to 34 yr) (Lindle et al., 1997), however a decrease in calf muscle area may occur (Lauratani et al., 2003). We found a significant reduction in maximum calf girth, however, the senior group had a higher BMI. This may in part be explained by the increase in percentage body fat that can occur with ageing (Lindle et al., 1997).

The pennation angle and belly thickness were also recorded when participants were plantarflexing on the dynamometer. Similar to the standing results, the mean muscle belly thickness was significantly lower in seniors as compared to young adults (decrease by 8.6 % in MG and 21.5 % in LG, Figure 3.3d and 3.4d). However, the initial pennation angles (when the subjects were sitting with maximum dorsiflexion) were significantly higher in MG (13.8%) for seniors and with no significant difference in LG (Figure 3.3c and 3.4c) and the fascicles were significantly shorter in seniors (Table 3.1). This was surprising because the pennation was lower in seniors and the fascicle lengths were not different between the two groups during standing. This difference can be explained by the decrease in the ROM of various joints including ankle dorsiflexion movement that occurs with age (Vandervoort et al., 1992). Indeed, we observed differences in the initial ankle joint angles (about -2 ° in seniors and -20 ° in young adults), and these affect the muscle geometry (Fukunaga et al., 1997). Specifically, the fascicle pennation of MG and LG is lower when the ankle is in a dorsiflexed position and increases as the ankle moves towards plantarflexion (Kawakami et al., 1998). Thus, the higher initial pennation angles that were recorded for MG at maximum dorsiflexion for the seniors (on dynamometer) may reflect their inability to dorsiflex to the same extent as the younger group due to the less flexible muscle structures in seniors (Buckwalter et al., 1993). It should be remembered, however, that the ROM is compromised at extremes of flexion and extensions, and the mean pennation that was observed during the plantarflexion tests was not different between the groups.

In order to tease out the effect of the altered ROM, the rate that the pennation and thickness changed as a function of changes in ankle angle was also determined. The results showed that the these rates were similar for the two age groups for the pennation in both muscles and for the thickness changes in MG, confirming that ROM may have an important effect on these measures. It should be remembered, however, that the gearing measures were calculated at the moment of maximum velocity, that occurred mid-way through the ROM, and so the gearing measures would not be limited by the reduced ROM in the seniors group.

Changes in the static properties of ageing muscles have been reported previously (Kubo et al., 2003, Morse et al., 2005, Narici et al., 2003). However, the age-dependent differences in dynamic muscle geometries have never been measured. Since ageing changes the static properties of muscle, it should also be expected that it influences the dynamic changes to muscle structure. The fascicle rotations (change in pennation) during plantarflexion were smaller for both muscles in seniors and the change in belly thickness was less for MG however not for LG in seniors (Figure 3.3e, f and 3.4e, f). The fascicle rotations and changes in thickness that occur during muscle shortening contribute to higher belly gearing in muscles (Azizi et al., 2002, Wakeling et al., 2011). A functional benefit of gearing may be a greater force output at high velocities during isotonic movements (Azizi et al., 2002) leading to greater power production in a muscle. Thus, thinner muscles with decreased fascicle rotations may produce a significantly lower gearing and this was the case in our study in both muscles in seniors (decrease by 2.8% in MG and 2.0% in LG, Figure 3.3b and 3.4b). Therefore, in addition to changes in muscle structure, age-dependent changes to the muscle result in lower gearing of the fascicles within the muscle belly. Thus, a lower gearing may be a factor for the loss of ankle torque (Figure 3.3a and 3.4a) and a consequent reduction of power output (Table 3.1) during the faster contractions observed in this study.

Previous studies have shown improvements to muscle structure that result from specific training regimes in elderly populations (Morse et al., 2007, Reeves et al., 2009) with distinct adaptations in fascicle pennation, fascicle length and belly thickness occurring as a response to conventional physical training (Morse et al., 2007), resistance training (eccentric and concentric) or eccentric-only training (Reeves et al., 2009). Additionally, structural adaptations also depend on the duration of training (Blazevich et al., 2007) and may not occur if the training period is too short. In relation to the present research these training studies highlight two main points: (1) muscle in seniors responds to training with performance-enhancing changes in its static structure. Thus, it is possible that a muscle's dynamic structure would also benefit from training, resulting in a reversal of the ageing effect of reduced fascicle rotations (MG and LG, Figure 3.3e and 3.4e) and changes in belly thickness (MG, Figure 3.3f). (2) Structural changes in these studies were accompanied by significant improvements in generation of muscle torque. This suggests that the changed dynamic musculature may have an influential role during functional performance. More importantly, belly gearing is dependent on these structural changes including pennation and thickness, which change with ageing. Thus, it can be inferred that a lower gearing may play a role in the lower torque and power production observed in ageing muscles.

It is important to minimize reductions in muscle strength as we age because muscle strength can affect stability (Melzer et al., 2009) and instability can increase the risk of falls (Mechagni et al., 2000). The reduced belly gearing in seniors that was observed in this study may contribute to reduced power output making it more challenging for seniors to perform daily mobility tasks. There was a greater reduction in power output than in ankle torque in seniors and these results are in line with those stated previously (Laurentani et al., 2003). Thus, future research on the effects of training on belly gearing in seniors may be helpful to understand its influential role in the process of muscle ageing or anti-ageing.

3.5. Conclusion

In conclusion, we found that ageing affects the dynamic geometry of a muscle causing smaller fascicle rotations and lesser change in belly thickness. The ROM was reduced for the elderly group, and this affected the structural parameters that were measured at the extremes of the motion. However, the muscle belly gearing that occurred part-way through the ROM, and that occurred when the velocities were greatest was lower in the senior group compared to young adults. The reduced gearing would have contributed to the reduction in the force generation and power output from muscles in elderly. It is suggested that specific training regimes that positively affect the static structures of muscle may also result in improvements to the dynamic performance of muscle due to increases in the belly gearing.

4. Discussion

4.1. Static and dynamic behaviours of the gastrocnemii muscles

Skeletal muscles are an important entity of the locomotion system serving as motors, brakes, springs, and struts (Dickinson et al., 2000). These functional roles are greatly reflected in the distinct designs and structures of the muscles. Each muscle in the body has a peculiar architectural anatomy depending on its location and function. The two gastrocnemii, medial gastrocnemius (MG) and lateral gastrocnemius (LG) are muscles that have the role of plantarflexion at the ankle and flexion at the knee. In the past, these muscles have been tested during isometric contractions (Maganaris et al., 1998, Kawakami et al., 1998, Narcici et al., 1996), during functional tasks such as running (Lichtwark et al., 2007), during cycling (Wakeling & Horn, 2009) and commonly on a dynamometer in various rehabilitation facilities. Many studies have found differences in the architectural characteristics of the MG and LG between muscles or during different contractions. For example, the pennation angles of the MG are larger than the LG during rest (Wickiewicz et al., 1983, Manal, 2006) and thus MG experiences higher fascicle rotations during contraction (Brainerd & Azizi 2005, Fukunaga et al., 1997, Kawakami et al., 1998).

The present two studies found similar results to the previous studies that the MG had greater initial pennation than LG in both young adults and seniors. Consequently, fascicle rotations were higher in the MG. The LG belly bulged more during isotonic and isovelocity contractions in young adults and during isovelocity in seniors. Belly gearing is greater than one when the fascicles are oriented at an angle from the line of action of muscles and fascicle rotations during contraction are higher (Azizi et al., 2002, Brainerd & Azizi 2005, Wakeling et al., 2011). All the participants in both studies had belly gearing greater than one. Interestingly, the belly gearing was higher for MG owing to the initial higher fascicle pennations and greater fascicle rotations during contraction. An important observation from the isotonic contractions of the first study suggested that change in pennation (fascicle rotations) may contribute in the development of belly gearing more than the belly thickness. However, it has been suggested previously that there is no unique combination of fascicle rotations and change in belly thickness that may influence belly gearing (Wakeling et al., 2011). Thus, it will be interesting to measure changes in belly gearing when these structural parameters are selectively altered with internal compression to contractile components such as in vascular pathologies (varicose veins, compression syndrome) or with external compression such as bandages or tight clothing.

Previous studies on changes in muscle belly thickness reported that MG belly thickness does not change during a maximum voluntary contraction (MVC) (Maganaris et al., 1998) and during cycling (Wakeling et al., 2011). However, in this current study. there was a decrease in MG belly thickness in the young adults and seniors. This suggested that MG belly increased in width instead of thickness owing to the fact that the muscle volume remains constant during a contraction. It will be interesting to observe the three-dimensional changes in muscle structure to quantify the changes in muscle size in all three dimensions simultaneously. Additionally, for measurement of pennation angles in two-dimensions, it was assumed that the fascicles are straight; whereas in reality fascicles are curved and their curvature increases from rest to maximum voluntary contraction (Maganaris et al., 1998, Muramatsu et al., 2002). Under the assumption that fascicles are straight versus the fact that fascicles are curved, the estimated fascicle length in MG would be underestimated by approximately 6% (Muramatsu et al., 2002). This may have implications on the estimation of the shortening velocity of fascicles and thus the calculation of belly gearing. The extent to which a 6% lower fascicle length estimation affects the belly gearing would need further investigation. This can be studied through the comparison of belly gearing measurements in two-dimensional or even and three-dimensional views.

4.2. Belly gearing, tendon gearing and muscle-tendon unit gearing

The structural changes that geared the fascicles within the belly were sensitive to both the torque and the velocity. Surprisingly, however, there was a decrease in belly gearing at greater velocities which was not the case during cycling in man (Wakeling et al., 2011). This can be explained by two main points: (1) the activation profiles of muscles can vary the muscle behaviours by altering the coordination between stretch-shortening cycle and the muscle excitations (Wakeling et al., 2011). During cycling, the gastrocnemius muscles provide joint stability and force transmission (Neptune et al., 2000, van Ingen Schenau et al., 1992) while these muscles presumably generate power for the tests done in this study. The difference in activation profiles in the current study was reflected in the highly variable tendon gearing and somewhat variable belly gearing and similar features were reported in the cycling study. The variable belly gearing, albeit smaller in magnitude that the variations in tendon gearing, may also be a result of elastic compliance of th connective tissue within the muscle belly (Muramatsu et al., 2001). (2) Wakeling and colleagues (2011) measured gearing in the triceps surae during cycling which included the MG, LG and the soleus taken together. Interestingly, when the data from the cycling study were reanalyzed (personal observation) using belly gearing values of each muscle separately, the belly gearing of LG was greater at higher cadence but not affected by torque, however, soleus and MG belly gearings were not affected with cadence or torque. This again suggests that muscle activation during variety of given tasks can alter muscle behaviours and thus belly gearing in man.

During locomotion the muscles interact with surrounding tissues such as tendon (Kawakami et al., 1998, Muramatsu et al., 2001), aponeuroses (Muramatsu et al., 2002) and other muscles (Maas et al., 2003). In fact, the stretch to the tendons is affected by the force generation in the muscles and thus the muscle activation (Petrofsky & Hendershot, 1984, Wakeling et al., 2011). These interactions resulted in a variable tendon gearing in young adults during the plantarflexion tests and this variability was greater than in belly gearing. Since ageing can change the mechanical properties of tendons (Maganaris et al., 2001), it will be interesting to measure the extent of deterioration in tendon gearing or a possible reduction in variability of tendon gearing in seniors.

4.3. Ageing muscle structures and implications for training rehabilitation

Muscular ageing associated with marked loss of muscle mass is predominantly due to both a loss of muscle fibers and a reduction in their size, specifically of the fast fibers (fast fiber area loss ranges from 20% to 50%; Lee et al., 2006, Lexell et al., 1993). Loss in fascicle CSA leads to decrease in muscle PCSA which has a direct impact on force generation or muscle strength (Narici et al., 1992, Fukunaga et al., 1996, Maganaris et al., 2001). However, the decline in strength with ageing is greater than the reduction in muscle PCSA (15 to 20%) (Jubrias et al., 1997, Morse et al., 2005). Furthermore, the decline in the power output (60%) is greater than the muscle strength (40%) (Lauretani et al., 2003, Morse et al., 2005, Raj et al., 2010). Thus, the overall decrease in power output was not fully accounted for by the loss of muscle mass. The concomitant changes in muscle structure with ageing (Narici et al., 2003) can explain some aspects of loss in power output. This includes the changes in pennation and thickness at rest and the fascicle rotations and belly bulging during shortening. In addition to these factors, a lower belly gearing observed in seniors (2 to 3%) may also contribute to power deterioration. It is possible that the performance reduction caused by the change in gearing can be reversed with training or exercise in seniors, and this is an area that warrants further investigation.

Power deterioration contributes to slower walking speeds and increased risks of falls (Doherty, 2003, Raj et al., 2010), and affects activities of daily living greater than changes in strength alone (Bassey, 1997). It is important to note that muscle behaviours and roles may be different in experimental tests versus real life muscle activities. Thus, some studies have selectively investigated functional tasks such as stair climbing, rising from chair or walking (Bassey et al., 1992, Bean et al., 2002, Runge et al., 2004). The belly gearing was found to be sensitive to activation levels and may be mechanically beneficial during faster movements in seniors. This calls for analyzing the role of belly gearing during daily activities especially in seniors.

Training rehabilitation in elderly populations has shown positive changes to the muscle architecture. Increase in fascicle pennation (12%) at rest with a 12-month physical training programme (Morse et al., 2007) indicates that fascicle rotations would also be greater during movement. Since, greater fascicle rotations would develop higher gearing, it will be an interesting question to address. A higher power output observed with training in seniors (Newton et al., 1999) would be reflected by potential improvements in belly gearing along with the increase in PCSA. However, since structural changes may not occur initially to training (Blazevich et al., 2007, Rutherford & Jones, 1992), it is crucial to consider the nature and duration of training.

4.4. Conclusion

This thesis explains the relationships between structural changes such as fascicle rotations and changes in belly thickness to the muscle gearing. Specifically, the factors that contribute to variable belly gearing as previously suggested were addressed in details for their specific role in belly gearing mechanisms. Since the gastrocnemii muscles have different static and dynamic geometry, this helped explain that higher fascicle pennation angles such as in the medial gastrocnemius accounts for greater rotations which can either primarily or in conjunction with muscle bugling help develop higher belly gearing. However, belly bulging, which has previously been suggested as an important factor to belly gearing could be studied in more details using three-dimensional methods. This will allow us to include the width factor along with the thickness and length factor that can be measured using two-dimensional methods used in this study.

Muscle activation and coordination can alter muscle geometry and gearing which was the case observed in the isotonic contractions in this study. Thus, the mechanical demands and roles that a muscle plays during locomotion such as power generation during climbing the stairs versus stability during a precise task can have different implications on muscle gearing.

When ageing affects the structural parameters during rest it can be expected to also affect gearing during dynamic movements. In support of this hypothesis, the muscles in seniors had lesser fascicle rotations and belly thickness changes, which were evident in the reduced belly gearing in seniors. Since power output and forces are greatly affected by muscle structure, deteriorations in power were also observed. This forms an important base for future studies on muscle gearing in seniors and the benefits that physical activities and training regimes would have on dynamic muscle behaviours. It may be possible to prevent the functional loss that occurs with the age-related structural alterations if the decrease in belly gearing can be prevented, but this needs further investigation.

References

Aagaard, P., Andersen, J.L., Dyhre-Poulsen, P., Leffers, A.M., Wagner, A., & Magnusson, S.P. (2001). A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *Journal of Physiology*, 534, 613–623.

Alexander, R.M. (1969). The orientation of muscle fibres in the myomeres of fishes. *Journal of Marine Biological Association of the UK*, 49, 263-290.

Alexander, R.M., & Vernon, A. (1975). The dimensions of knee and ankle muscles and the forces they exert. *Journal Human Movement Studies,* 1, 115-123.

Alexander, R.M. (1984). Elastic energy stores in running vertebrates. *American Zoologist,* 24, 85–94.

Aratow, M., Ballard, R.E., Crenshaw, A.G., Styf, J., Watenpaugh, D.E., Kahan, N.J., & Hargens, A.R. (1993). Intramuscular pressure and electromyography as indexes of force during isokinetic exercise. *Journal of Applied Physiology,* 74, 2634 – 2640.

Azizi, E., Brainerd, E.L., & Roberts, T.J. (2008). Variable gearing in pennate muscles. *Proceedings of the National Academy of Science*, 105, 1745-1750.

Azizi, E., Gillis, G.B., & Brainerd, E.L., (2002). Morphology and mechanics of myosepta in a swimming salamander Siren lacertina. *Comparative Biochemistry and Physiology, A*, 133, 967-978.

Baskin, R.J., & Paolini, P.J. (1967). Volume change and pressure development in muscle during contraction. *American Journal of Physiology*, 213, 1025-1030.

Bassey, E.J., Fiatarone, M.A., O'Neill, E.F., Kelly, M., Evans, W.J., & Lipsitz, L.A. (1992). Leg extensor power and functional performance in very old men and women. *Clinical Science,* 82(3), 321–327.

Bean, J., Kiely, D.K., & Herman, S.(2002). The relationship between leg power and physical performance in mobility limited elders. *Journal of the American Geriatrics Society,* 50, 461–467.

Bean, J.F., Leveille, S.G., Kiely, D.K., Bandinelli, S., Guralnik, J.M., & Luigi, F. (2003). A comparison of leg power and leg strength within the InCHIANTI study: which influences mobility more? *Journal of Gerontology*, 58A, 728-733.

Bemben, M.G., Massey, B.H., Bemben, D.A., Misner, J.E., & Boileau, R.A. (1991). Isometric muscle force production as a function of age in healthy 20- to 74-yr-old men. *Medicine and Science in Sports and Exercise*, 23(11), 1302–1310.

Biewener, A. A., Farley, C. T., Roberts, T. J., & Temaner, M. (2004). Muscle mechanical advantage of human walking and running: implications for energy cost. *Journal of Applied Physiology*, 97, 2266-2274.

Blazevich, A.J. (2006). Effects of physical training and detraining, immobilisation, growth and aging on human fascicle geometry. *Sports Medicine,* 36(12), 1003–1017.

Blazevich, A.J., Gill, N.D., Deans, N., & Zhou, S. (2007). Lack of human muscle architectural adaptation after short-term strength training. *Muscle and Nerve*, 35, 78–86.

Bleakney, R., & Maffulli, N., (2002). Ultrasound changes to intramuscular architecture of the quadriceps following intramedullary nailing. *Journal of Sports Medical and Physical Fitness*, 42, 120–125.

Bottinelli, R., & Reggiani, C. (2000). Human skeletal muscle fibres: molecular and functional diversity. *Progress in Biophysics and Molecular Biology*, 73(2-4), 195-262.

Brainerd, E.L., & Azizi, E. (2005). Muscle fiber angle, segment bulging and architectural gear ratio in segmented musculature. *Journal of Experimental Biology*, 208(17), 3249-3261.

Buckwalter, J.A., Woo, S.L., Goldberg, V.M., Hadley, E.C., Booth, F., Oegema, T.R., & Eyre, D.R. (1993). Soft-tissue aging and musculoskeletal function. *Journal of Bone and Joint Surgery American,* 75(10), 1533–1548.

Burkholder, T.J., Fingado, B., Baron, S., & Lieber, R.L. (1994). Relationship between muscle fiber types and sizes and muscle architectural properties in the mouse hindlimb. *Journal of Morphology,* 221, 177-1790.

Carrier, D.R., Heglund, N.C., & Earls, K.D. (1994). Variable gearing during locomotion in the human musculoskeletal system. *Science*, 265, 651–653.

Child, A.L., Bryant, R.A., & Clark, K.M. (2010). Mechanical properties of the achilles tendon and aponeuroses are altered in athletes with achilles tendinopathy. *American Journal of Sports Medicine,* 38, 1885–1893.

Delp, S.L., Anderson, F.C., Arnold, A.S., Loan, P., Habib, A., John, C.T., Guesndelman, E., & Thelen, D.G. (2007). OpenSim: Open-Source Software to Create and Analyze Dynamic Simulations of Movement. *IEEE Transaction on Biomedical Engineering*, 54(11), 1940-1950.

Dickinson, M.H., Farley, C.T., Full, R.J., Koehl, M.A.R., Kram, R., & Lehman, S. (2000). How animals move: an integrative view. *Science*, 288, 100-106.

Doherty, T.J. (2003). Invited review: aging and sarcopenia. *Journal of Applied Physiology*, 95(4), 1717–1727.

Duclay, J., Martin, A., Duclay, A., Cometti, G., & Pousson, M. (2009). Behavior of fascicles and the myotendinous junction of human medial gastrocnemius following eccentric strength training. *Muscle and Nerve*, 39(6), 819–827.

Foldavari, M., Clark, M., & Laviolette, M.J.A. (1999). Association of muscle power with functional status in community dwelling elderly women. *Medicine and Science in Sports and Exercise*, 31, S378.

Friederich, J.A., & Brand, R.A. (1990). Muscle fiber architecture in the human lower limb. *Journal of Biomechanics,* 23, 91-95.

Fukunaga, T., Ichinose, Y., Ito, M., Kawakami, Y., & Fukashiro, S. (1997). Determination of fascicle length and pennation in a contracting human muscle in vivo. *Journal of Applied Physiology*, 82, 354-358.

Fukunaga, T., Roy, R.R., Shellock, F.G., Hodgson, J.A., & Edgerton, V.R. (1996). Specific tension of human plantar flexors and dorsiflexors. *Journal of Applied Physiology*, 80, 158–165.

Gans, C. (1982). Fiber architecture and muscle function. *Exercise and Sports Science Reviews*, 10, 160–207.

Gans, C., & de Vree, F. (1987). Functional bases of fiber length and angulation in muscle. *Journal of Morphology*, 192(l), 63-85.

Herbert, R.D., & Gandevia, S.C. (1995). Changes in pennation with joint angle and muscle torque: in vivo measurements in human brachialis muscle. *Journal of Physiology*, 484, 523–532.

Hill, A.V. (1938). The heat of shortening and the dynamic constant of muscle. *Proceedings of the Royal Society B*, 126, 136-195.

Hof, A.L. (2003). Muscle mechanics and neuromuscular control. *Journal of Biomechanics*, 36, 1031-1038.

Jubrias, S.A., Odderson, I.R., Esselman, P.C., & Conley, K.E. (1997). Decline in isokinetic force with age: muscle cross-sectional area and specific force. *Pflugers Archiv*, 434, 246–253.

Kawakami, Y., Abe, T., & Fukunaga, T. (1993). Muscle-fiber pennation angles are greater in hypertrophied than in normal muscles. *Journal of Applied Physiology*, 74, 2740–2744.

Kawakami, Y., Abe, T., & Kuno, S.Y. (1995). Training-induced changes in muscle architecture and specific tension. *European Journal of Applied Physiology,* 72, 37-43.

Kawakami, Y., Ichinose, Y., & Fukunaga, T. (1998). Architectural and functional features of human triceps surae muscles during contraction. *Journal of Applied Physiology,* 85, 398-404.

Klimstra, M., Dowling, J., Durkin, J.L., & MacDonald, M. (2007). The effect of ultrasound probe orientation on muscle architecture measurement. *Journal of Electromyography and Kinesiology*, 17, 504–514.

Kubo, K., Kanehisa, H., Kawakami, Y., & Fukunaga, T. (2001). Influence of static stretching on viscoelastic properties of human tendon structures in vivo. *Journal of Applied Physiology,* 90, 511–519.

Kubo, K., Kanehisa, H., Azuma, K., Ishizu, M., Kuno, S.Y., & Okada, M. (2003). Muscle architectural characteristics in women aged 20–79 years. *Medicine and Science in Sports and Exercise,* 35, 39–44.

Lang, T., Streeper, T., Cawthon, P., Baldwin, K., Taaffe, D.R., & Harris, T.B. (2010). Sarcopenia: etiology, clinical consequences, intervention, and assessment. *Osteoporosis International*, 21, 543–559.

Lauretani, F., Russo, C.R., Bandinelli, S., Bartali, B., Cavazzini, C., & Lorio, A.D. (2003). Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *Journal of Applied Physiology,* 95, 1851-1860.

Lee, W.S. (2006). Age-associated decrease of type IIA/B human skeletal muscle fibers. *Clinical Orthopaedics and Related Research*, 450, 231–237.

Lexell, J. (1993). Ageing and human muscle: observations from Sweden. *Canadian Journal of Applied Physiology,* 18, 2-18.

Lichtwark, G. A., Bougoulias, K., & Wilson, A. M. (2007). Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *Journal of Biomechanics*, 40, 157-164.

Lieber, R.L., & Friden, J. (2000). Functional and clinical significance of skeletal muscle architecture. *Muscle and Nerve*, 23, 1647-1666.

Lindle, R.S., Metter, E.J., Linch, N.A., Fleg, J.L., Fozard, J.L., & Tobin, J. (1997). Age and gender comparisons of muscle strength in 654 women and men aged 20-93 years. *Journal of Applied Physiology*, 83, 1581-1587.

Maas, R.T., Jaspers, G.C., & Baan, P.A. (2003). Huijing Myofascial force transmission between a single muscle head and adjacent tissues: length effects of head III of rat EDL. *Journal of Applied Physiology,* 95, 2004–2013.

Maganaris, C.N., Baltzopoulos, V., & Sargeant, A.J. (1998). In vivo measurements of the triceps surae complex architecture in man: implications for muscle function. *Journal of Physiology*, 512, 603-614.

Maganaris, C.N. (2002). Tensile properties of in vivo human tendinous tissue. *Journal of Biomechanics*, 35, 1019-1027.

Maganaris, C.N. (2003). Tendon conditioning: artefact or property? *Proceedings of the Royal Society of London B (Suppl)*, 270, S39–S42.

Maganaris, C.N., Baltzopoulos, V., Ball, D., & Sargeant, A.J. (2001). In vivo specific tension of human skeletal muscle. *Journal of Applied Physiology*, 90, 865–872.

Manal, K., Roberts, D.P., & Buchanan, T.S. (2006). Optimal pennation angle of the primary ankle dorsi and plantarflexors: variations with sex, contraction intensity and limb. *Journal of Applied Biomechanics*, 22, 255–263.

Mecagni, C., Smith, J.P., Roberts, K.E., & O'Sullivan, S.B. (2000). Balance and ankle range of motion in community-dwelling women aged 64–87 years: a correlational study. *Physical Therapy,* 80, 1004–1011.

Melzer, I., Benjuya, N., Kaplanski, J., & Alexander, N. (2009). Association between ankle muscle strength and limit of stability in older adults. *Age and Ageing*, 38, 119–123.

Morse, C.I., Thom, J.M., Birch, K.M., & Narici, M.V. (2005). Changes in triceps surae muscle architecture with sarcopenia. *Acta Physiologica Scandinavica,* 183, 291–298.

Morse, C.I., Thom, J.M., Mian, O.S., Birch, K.M., & Narici, M.V. (2007). Gastrocnemius specific force is increased in elderly males following a 12-month physical training programme. *European Journal of Applied Physiology,* 100, 563–570.

Muhl, Z.F. (1982). Active length-tension relation and the effect of muscle pinnation of fiber lengthening. *Journal of Morphology,* 173, 285-292.

Muramatsu, T., Muraoka, T., Takeshita, D., Kawakami, Y., & Fukunaga, T. (2002). In vivo mechanical properties of proximal and distal aponeuroses in human tibialis anterior muscle. *Cells Tissues and Organs,* 170, 162–169.

Muramatsu, T., Muraoka, T., Takeshita, D., Kawakami, Y., Hirano, Y., & Fukunaga, T. (2001). Mechanical properties of tendon and aponeuroses of human gastrocnemius muscle in vivo. *Journal of Applied Physiology,* 90, 1671–1678.

Narici, M.V., Binzoni, T., Hiltbrand, E., Fasel, J., Terrier, F., & Cerretelli, P. (1996). In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. *Journal of Physiology*, 496, 287-297.

Narici, M.V., Roi, G.S., Landoni, L., Minetti, A.E., & Cerretelli, P. (1989). Changes in forces, cross-sectional areas and neural activation during strength training and detraining of the human quadriceps. *European Journal of Applied Physiology,* 59, 310 –319.

Narici, M., & Cerretelli, P. (1998). Changes in human muscle architecture in disuse-atrophy evaluated by ultrasound imaging. *Journal of Gravitational Physiology,* 5, 73–74.

Narici, M., Landoni, L., & Minetti, A. E. (1992). Assessment of human knee extensor muscles stress from in vivo physiological cross-sectional area and strength measurements. *European Journal of Applied Physiology*, 65, 438-444.

Narici, M.V., & Maganaris, C.N. (2006). Adaptability of elderly human muscles and tendons to increased loading. *Journal of Anatomy,* 208, 433–443.

Narici, M.V., Maganaris, C.N., Reeves, N.D., & Capodaglio, P. (2003). Effect of aging on human muscle architecture. *Journal of Applied Physiology*, 95(6), 2229–2234.

Neptune, R.R., Kautz, S.A, & Zajac, F.E. (2000). Muscle contributions to specific biomechanical functions do not change in forward versus backward pedaling. *Journal of Biomechanics*, 33(2), 155-164.

Newton, R.U., Häkkinen, K., & Häkkinen, A. (1999). Mixed-methods resistance training increases power and strength of young and older men. *Medicine and Science in Sports and Exercise*, 34, 1367-75.

Otten, E., & Hulliger, M. (1995). A finite-elements approach to the study of functional architecture in skeletal muscle. *Zoology,* 98, 233-242.

Petrofsky, J. S., & Hendershot, D. M. (1984). The interrelationship between blood pressure, intramuscular pressure, and isometric endurance in fast and slow twitch skeletal muscle in the cat. *European Journal of Applied Physiology,* 53, 106-111.

Powell, P.L., Roy, R.R., Kanim, P., Bello, M.A., & Edgerton, V.R. (1984). Predictability of skeletal muscle tension from architectural determinations in guinea pig hindlimbs. *Journal of Applied Physiology*, 57, 1715–1721.

Raj, I.S., Bird, S.R., & Shield, A.J. (2010). Aging and the force-velocity relationship of muscles. *Experimental Gerontology*, 45, 81–90.

Reeves, N.D., Maganaris, C.N., Longo, S., & Narici, M.V. (2009). Differential adaptations to eccentric versus conventional resistance training in older humans. *Experimental Physiology,* 94(7), 825–833.

Rome, L.C., Funke, R.P., Alexander, R.M., Lutz, G.J., Aldridge, H., Scott, F., & Freadman, M. (1988). Why animals have different muscle fibre types. *Nature*, 335, 824-827.

Rosenberg, I.R. (1989). Summary comments. *American Journal of Clinical Nutrition*, 50, 1231–1233.

Runge, M., Rittweger, J., Russo, C.R., Schiessl, H., & Felsenberg, D. (2004). Is muscle power output a key factor in the age-related decline in physical performance? A comparison of muscle cross section, chair-rising test and jumping power. *Clinical Physiology and Functional Imaging,* 24(6), 335–340.

Rutherford, O.M., & Jones, D.A. (1992). Measurement of fibre pennation using ultrasound in the human quadriceps in vivo. *European Journal of Applied Physiology*, 65, 433-437.

Sejersted, O.M., Hargens, A.R., Kardel, K.R., Blom, P., Jensen, O., & Hermansen, L. (1984). Intramuscular fluid pressure during isometric contraction of human skeletal muscle. *Journal of Applied Physiology*, 56, 287–295.

Steffen, T.M., Hacker, T.A., & Mollinger, L. (2002). Age and gender-related test performance in community-dwelling elderly people: Six-minute walk test, berg balance scale, timed up & go test and gait speeds. *Physical Therapy,* 82, 128-137.

Suzuki, T., Bean, J, & Fielding, R. (2001). Muscle strength and power of the ankle plantar and dorsi flexors predict functional performance in community dwelling older women. *Journal of the American Geriatrics Society*, 49, 1161–1167.

van Ingen Schenau, G.J., Boots, P.J.M., de Groot, G., Snackers, R.J., & van Woensel, W.W.L.M. (1992). The constrained control of force and position in multi-joint movements. *Neuroscience*, 46(1), 197-207.

van Leeuwen, J.L. (1992). Muscle function in locomotion. In *Mechanics of animal locomotion* (ed. Alexander RMcN), 191-250. Springer, Berlin.

van Leeuwen, J.L., & Spoor, C.W. (1992). Modelling mechanically stable muscle architectures. *Philosophical Transactions of the Royal Society B*, 226, 275-292.

Vandervoort, A.A., Chesworth, B.M., Cunningham, D.A., Rechnitzer, P.A., Paterson, D.H., & Koval, J.J. (1992). An outcome measure to quantify passive stiffness of the ankle. *Canadian Journal of Public Health,* 83, suppl 2, S19 –S23.

Wakeling, J.M., Blake, O.M., Wong, I., Rana, M., & Lee, S.M. (2011). Movement mechanics as a determinate of muscle structure, recruitment and coordination. *Philosophical Transactions of the Royal Society B*, 366(1570), 1554-1564.

Wakeling, J.M., & Horn, T. (2009). Neuromechanics of muscle synergies during cycling. *Journal of Neurophysiology*, 101, 843-854.

Wakeling, J.M., & Johnston, I.A. (1999). White muscle strain in the common carp and red to white muscle gearing ratios in fish. *Journal of Experimental Biology*, 202, 521-528.

Wakeling, J.M., Uehli, K., & Rozitis, A.I. (2006). Muscle fibre recruitment can respond to the mechanics of the muscle contraction. *Journal of the Royal Society Interface*, 3, 533-544.

Wickiewicz, T.L., Roy, R.R., Powell, P.L., & Edgerton, V.R. (1983). Muscle architecture of the human lower limb. *Clinical Orthopaedics and Related Research*, 179, 275–283.

Wilson, G. J., Murphy, A. J., Pryor, J. F. (1994). Musculotendinous stiffness: its relationship to eccentric, isometric, and concentric performance. *Journal of Applied Physiology*, 76, 2714–2719.

Woodford, H., & George, J. (2007). Cognitive assessment in the elderly: a review of clinical methods. *Quarterly Journal of Medicine*, 100(8), 469-484.

Zajac, F.E. (1989). Muscle and tendon: properties, models, scaling, and application to biomechanics and motor control. *Critical Reviews in Biomedical Engineering*, 17, 359-411.

Zuurbier, C.J., & Huijing, P.A. (1992). Influence of muscle geometry on shortening speed of fibre, aponeuroses and muscle. *Journal of Biomechanics,* 25, 1017-1026.

Zuurbier, C.J., & Huijing, P.A. (1993). Changes in geometry of actively shortening unipennate rat gastrocnemius muscle. *Journal of Morphology,* 218, 167–180.