Differential behaviour of the medial and lateral heads of gastrocnemius during plantarflexion: the effect of calcaneal inversion and eversion

Susann Wolfram

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Health Exercise & Active Living Research Centre
Department of Exercise and Sport Science
Manchester Metropolitan University

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Ich schlief und träumte, das Leben wär‘ Freude.

Ich erwachte und sah, das Leben war Pflicht.

Ich handelte, und siehe, die Pflicht war Freude.

I slept and dreamt that life was joy.

I awoke and saw that life was service.

I acted and behold, service was joy.

Rabindranath Tagore

Declaration

I declare that no material within the current thesis has been submitted for any other academic award. Furthermore, I declare the current thesis complies with the Institutional Code of Practice and Research Degree Regulations. Work from the current thesis that has been published or presented elsewhere is attached in its original format at the end of the thesis.

Signed:

Miss Susann Wolfram

Conference presentations

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Abstract

Gastrocnemius medialis (GM) and lateralis (GL) have been considered as plantarflexors of the talocrural joint but also contribute to inversion and eversion of the calcaneus at the subtalar joint. The contractile behaviour has predominately been investigated for GM and findings have been generalised to both gastrocnemii. However, differences in their morphology and, possibly, function have been reported. The purpose of this thesis was to investigate differential contractile behaviour of GM and GL and the effect of inversion/eversion of the calcaneus. The effect of calcaneal inversion/eversion due to standing posture and due to positioning was investigated.

A new imaging-based method to assess calcaneal inversion/eversion during standing was developed. Using this method, the validity of the clinically applied method could not be established. Achilles tendon moment arm is unaffected by calcaneal inversion/eversion posture and position. An exploratory investigation into the application of the centre-of-rotation method to determine inversion/eversion Achilles tendon moment arms was also undertaken. Contractile behaviour of GM and GL was unaffected by calcaneal inversion and eversion but fascicle length, pennation angle and tendon length differed between GM and GL. A comparison of GM and GL showed that fascicle behaviour is similar between them but GM tendon elongation and strain exceeded GL tendon elongation and strain, especially at higher contraction levels. The contraction levels at which tendon strain differences occur are dependent on the amount of calcaneal inversion/eversion position.

GM and GL differ significantly in their anatomical composition. Adaptations of GM and GL to calcaneal inversion/eversion appear to occur at the tendon level rather than the fascicle level. Given the differential function of GM and GL reported in the literature and the findings of this thesis, it is suggested that GM and GL could be referred to as two separate muscles instead of two heads of the same muscle. It is, furthermore, suggested that GM and GL are not bi-articular but tri-articular due to their actions at the subtalar joint.

Abbreviations

ACSA	Anatomical cross-sectional area
AT	Achilles tendon
COR	Centre of rotation
F_{AT}	Achilles tendon force
GL	Gastrocnemius lateralis
GM	Gastrocnemius medialis
ICC	Intra-class correlation
M_{INV}	Inversion moment
MRI	Magnetic resonance imaging
MR	Magnetic resonance
MTJ	Muscle-tendon junction
MTU	Muscle-tendon unit
MVC	Maximum voluntary contraction
PCSA	Physiological cross-sectional area
PT	Patellar tendon
RCSP	Relaxed calcaneal stance position
STJ	Subtalar joint

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Chapter One: Introduction to gastrocnemius muscle-tendon unit function and the subtalar joint

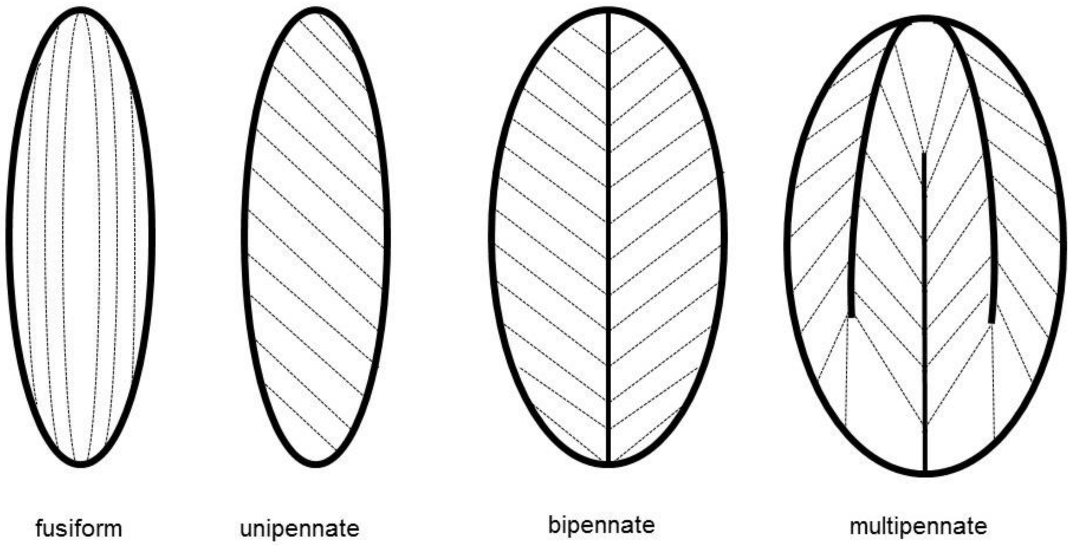
1.1 General muscle-tendon unit architecture and function of skeletal muscle

The skeletal musculature is an important part of the body and makes movement possible. The belly of a muscle consists of muscle fibres, which are bundled into fascicles and attach either directly to a tendon or to an aponeurosis. Via tendons and aponeuroses, muscle bellies are connected to bone to allow force transfer and, ultimately, the generation of joint moments and joint movement.

Muscle fascicles can be arranged in line with their tendons (fusiform) or at an angle (pennate). In pennate muscles, fascicles form an angle with an aponeurosis (pennation angle). The arrangement of muscle fascicles in pennate muscles can be unipennate, bipennate and multipennate (Fig. 1.1A). The force produced by a fascicle in fusiform muscles is directly transferred to the tendon (An et al., 1989). On the other hand, the force transfer from fascicles in pennate muscles to the tendon is dependent on the pennation angle (An et al., 1989) resulting in a smaller force at the tendon (Kawakami et al., 1998; Maganaris et al., 1998). This seems disadvantageous, but the advantage of a pennate fascicle arrangement lies in the amount of muscle fascicles that can be packed into a given volume. The larger the pennation angle, the more fascicles can be packed (Gans and Gaunt, 1991). Since the number of fascicles per volume is related to the force production capability of the muscle, highly pennate muscles are generally able to generate higher forces (Lieber and Fridén, 2000).

The size of a muscle and the arrangement of fascicles within it are related to the force generating capabilities of this muscle (Lieber and Fridén, 2000). The anatomical cross-sectional area (ACSA) represents the cross-sectional area of a muscle measured in the transverse plane. It is related to a muscle's volume and its length (Albracht et al., 2008). The physiological cross-sectional area (PCSA) is the cross-section through a muscle at a perpendicular angle to the muscle fibres. In fusiform muscles, the ACSA is the same as the PCSA. However, in pennate muscles the pennation angle must be taken into account when determining the PCSA (Fig. 1.1B). The PCSA is determined as the ACSA multiplied by the pennation angle and is directly proportional to the force producing capabilities of a muscle (Albracht et al., 2008). The larger the PCSA, the larger the maximum force the muscle can produce.

A



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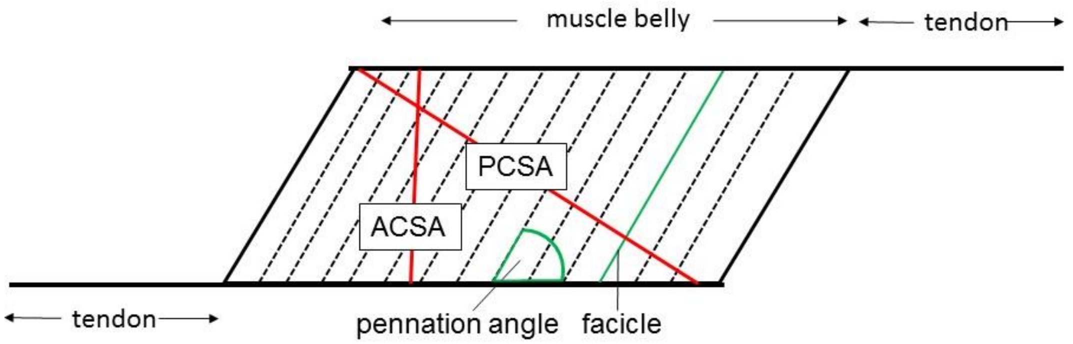


Figure 1.1. Schematic depiction of muscle fascicle arrangements within a muscle belly (A) and a longitudinal cross-section through a MTU showing fascicles, pennation angle, anatomical cross-sectional area and physiological cross-sectional area (B).

During contraction of a pennate muscle, fascicles shorten and rotate accompanied by an increase in pennation angle (Maganaris et al., 1998). The amount of fascicle shortening and fascicle shortening velocity is determined by the number of sarcomeres arranged in series within a muscle fibre. Longer fascicles contain more sarcomeres in series and, therefore, exhibit greater shortening capacities and higher maximum shortening velocities (Lieber and Fridén, 2000; Wickiewicz et al., 1984). The amount of fascicle rotation has been found to be enhanced during low contraction levels compared to higher contractions levels (Wakeling et al., 2011; Azizi et al., 2008). Hence, the velocity output of a muscle is greater during low-force contractions, while force output is favoured for contractions at high intensities.

Tendons attach to the muscle bellies and then connect to the bones to convert muscle force into joint moment. They are viscoelastic structures that lengthen when force is applied; for example, during an isometric contraction (Ito et al., 1998). The change in tendon length with respect to its initial length is called strain. The higher the force applied to a tendon, the greater is the strain the tendon experiences (Lieber et al., 1991). When a tendon is lengthened beyond its slack length, it will begin to resist elongation and exert a passive force. The increase in tendon force per unit of length change is called stiffness. Stiffness is a measure of the mechanical properties of a tendon and also increases with an increase in tendon force (Lieber et al., 1991).

Due to their viscoelastic nature, tendons can store elastic energy when lengthened and return it upon shortening (Cavagna et al., 1965). Tendon lengthening followed by shortening has been observed during numerous tasks and was termed the stretch-shortening cycle (SSC) (Komi, 2000). During SSC tasks, tendinous tissue is stretched during active contraction of the muscle belly, which is subsequently followed by shortening of the tendinous tissue (elastic recoil) and concentric contraction of the muscle belly. The ability of a tendon to store and return elastic energy is an important characteristic of tendons known to reduce the metabolic cost of muscle contraction during locomotion (Roberts et al., 1997) and to enhance performance (Kawakami et al., 2002). Muscles with stiffer tendons usually have long fascicles, such as the biceps brachii, while muscles with more compliant tendons, such as the gastrocnemius, usually have short fascicles and are involved in load bearing tasks (Biewener and Roberts, 2000).

1.2 Overview of gastrocnemius muscle-tendon unit anatomy and function

1.2.1 Anatomy of the gastrocnemius muscle-tendon unit

GM and GL form two of the three heads of the triceps surae. They are located at the posterior aspect of the lower leg and together with soleus as the third head constitute the main plantarflexor muscle group of the talocrural joint. GM and GL have their origin at the medial and lateral condyles of the femur, respectively, and conjoin with soleus into the AT, which then inserts on the posterior part of the calcaneus (Fig. 1.2).

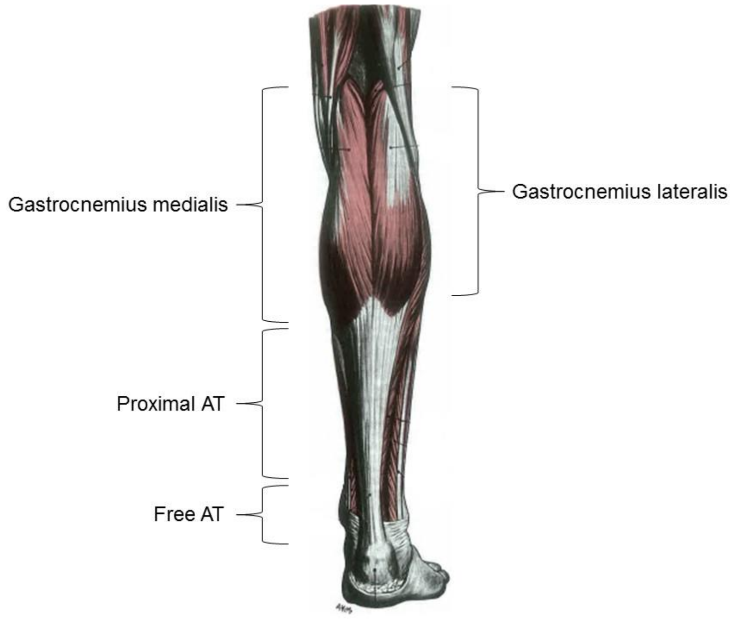


Figure 1.2. Posterior view of the right lower leg showing GM, GL, the proximal AT and the free AT (adapted from Gray's Anatomy).

GL is the smallest of the triceps surae muscles occupying approximately 15% of the triceps surae volume and has a PCSA of 26 cm^2, while GM occupies approximately 30% with a PCSA of 49 cm^2 (Kinugasa et al., 2005; Morse et al., 2005; Fukunaga et al., 1996). Furthermore, the muscle belly of GM is reported to be significantly longer than the GL muscle belly (Antonios and Adds, 2008) resulting in a shorter GM tendon length (Morrison et al., 2015). GM and GL are both unipennate muscles with muscle fascicles oriented at an angle to their tendon. Fascicle length and pennation angle for GM has been reported to be between 44 and 52 mm and between 16° and 24°, respectively (Héroux et al., 2016; Chow et al., 2000; Kawakami et al., 1998; Maganaris et al., 1998; Narici et al., 1996). Fascicle length and pennation angle for GL has been reported to be between 44 and 74 mm and between 11° and 13°, respectively (Héroux et al., 2016; Chow et al., 2000; Kawakami et al., 1998; Maganaris et al., 1998). The fibre type composition was found to be similar between GM and GL with both muscles containing about 50% fast twitch fibres (Edgerton et al., 1975).

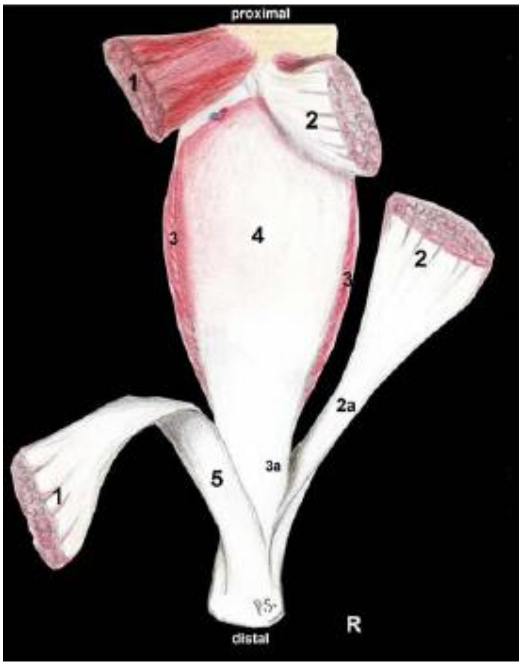


Figure 1.3. Compartments of the AT corresponding to the three individual muscles of the triceps surae muscle group. (1) gastrocnemius medialis, (2) gastrocnemius lateralis, (2a) tendinous fibres from gastrocnemius lateralis, (3) soleus, (3a) tendinous fibres from soleus, (4) aponeurosis of soleus, (5) tendinous fibres from gastrocnemius medialis (Szaro et al., 2009).

Muscle fibres of GM and GL merge into the proximal AT, which is joined by the soleus to form the free AT distally. The AT is fan shaped with a smaller cross-sectional area at the point of transition from the free tendon to the proximal tendon. The cross-sectional area increases in distal direction to an almost oval shape near the insertion at the calcaneal bone (Finni et al., 2003).

The AT is not a homogenous structure, but consists of compartments each corresponding to one of the three heads of the triceps surae (Szaro et al., 2009). In the proximal portion of the AT, distal to the muscle-tendon junction (MTJ) of GM and GL, the tendinous fibres of GM and GL are parallel before joining into the posterior aspect of the free AT, while soleus joins into the central and anterior portion (Fig. 1.3). Approximately 44% of the cross-sectional area (CSA) of the free AT is made up of fibres from GL, while GM and soleus each occupy approximately 28% (Pekala et al., 2017). From the point of merging into the free AT, the collagenous fibres show an internal twist, which is counter-clockwise for the right leg and clockwise for the left leg (Szaro et al., 2009). Specifically, at the calcaneus, fibres of GM insert in a more lateral location, fibres of GL insert in an anterior location, and fibres of soleus insert into a more medial location (Edama et al., 2016), but the amount of twist was found to differ between individuals (Fig. 1.4A) (Edama et al., 2015). In a recent study, Pękala et al. (2017) confirmed inter-individual variations in the twist of the AT portions and they furthermore described that the portions themselves exhibit twisting (Fig. 1.4B).

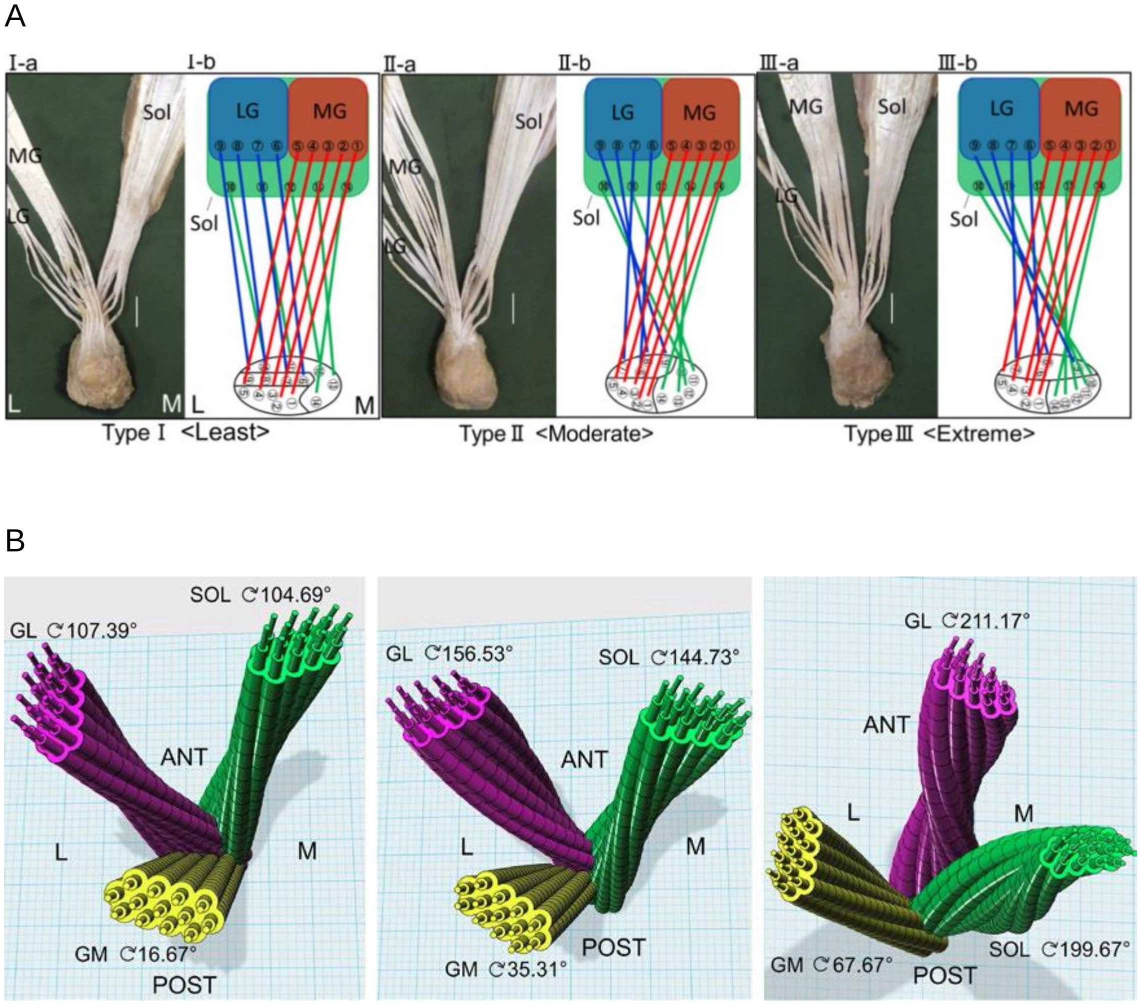


Figure 1.4. (A) Twist of tendinous fibres of the AT. Most ATs exhibit a small or moderate amount of twist. Only a small percentage was found to show an extreme twist (Edama et al., 2015). (B) Internal twist of AT portions corresponding to soleus (green), GM (yellow and GL (purple) (Pękala et al., 2017).

1.2.2 Function of the gastrocnemius muscle-tendon unit

Together with soleus, GM and GL are the primary plantarflexor muscles of the talocrural joint. As the muscle group contracts, it exerts a plantarflexion moment at the calcaneus which causes plantarflexion of the foot. The gastrocnemii also cross the knee joint, where they act as knee flexors. Through their plantarflexion action at the talocrural joint and flexion action of the knee, the gastrocnemii play an important role in providing propulsion during gait (Hamner et al., 2010; Novacheck, 1998) as well as jumping tasks (Fukashiro et al., 2005). During gait, the gastrocnemii utilise SSC action. Upon heel-strike and during the first part of the stance phase of walking, the GM MTU was found to lengthen, while muscle fascicles act mainly isometrically (Lichtwark et al., 2007; Ishikawa et al., 2005). Through lengthening of the MTU, elastic energy is stored in the tendon and returned through elastic recoil in the later stance phase, while fascicles shorten (Ishikawa et al., 2005). The ability to store and return elastic energy is an important characteristic of the gastrocnemius tendon, which provides a large proportion of the positive work required of the gastrocnemius for locomotion (Kurokawa et al., 2003). For example, Kawakami et al. (2002) determined that the AT contributes approximately 4.4 J of the 5.1 J required for a countermovement task.

The interaction of fascicles and tendon during movements is complex and task-dependent. Specifically, it was found during SSC tasks that the contribution of tendon length change to the overall length of the MTU increases with an increase in movement velocity or load (Sakuma et al., 2012; Fukashiro, 2006; Kawakami et al., 2002). A difference in muscle-tendon interaction during jumping tasks has also been found between GL and soleus (Farris et al., 2016).

1.2.3 Architectural and functional differences of the medial and lateral gastrocnemius

Given the importance of the gastrocnemius for movement, it has received a great deal of attention in scientific research. The majority of the literature, however, investigated the behaviour and mechanical properties of GM, and results are often generalised to both gastrocnemii (e.g. Albracht and Arampatzis, 2013; Arampatzis et al., 2006; Maganaris and Paul, 2002; Magnusson et al., 2001; Muramatsu et al., 2001). While some studies have reported differences in muscle architecture and MTU geometry of GM and GL (Héroux et al., 2016; Morrison et al., 2015), very little attention has been paid to possible differences in their mechanical properties and function. This seems logical since both muscles are plantarflexors, but their specific functions might differ. A few published studies report differential behaviour of GM and GL

(Wakeling, 2009b; Antonios and Adds, 2008; Higham et al., 2008; Lee and Piazza, 2008). Both muscles differ considerably in their size and force producing capabilities. GM is larger than GL, has a greater PCSA (see above) and a larger relative volume of GM is activated during a submaximal contraction than of GL (Kinugasa et al., 2005). During an isometric plantarflexion contraction to maximum, Kawakami et al. (1998) observed a greater length change of the GM MTU than the GL MTU. In the same study, curvature of GM fascicles was found to be greater than GL fascicle curvature and increased at higher contraction levels in both gastrocnemius muscles in this study. The authors suggest a direct positive relationship between pennation angle and fascicle curvature. While Namburete and Wakeling (2012) share this view, they also report that the increase in curvature is greater for GL than GM.

Some studies have found differential fascicle behaviour of GM and GL during isometric plantarflexion contractions. Maganaris et al. (1998) report a greater increase in GL pennation angle than GM pennation angle but, did not find differences in fascicle shortening. In contrast, Héroux et al. (2016) and Rana et al. (2013) report a greater pennation angle increase in GM than GL, and Héroux et al. (2016) also report greater fascicle shortening for GM. Furthermore, Higham et al. (2008) and Wakeling (2009) discuss the possibility of functional differences between GM and GL, which may be task-dependent. Specifically, Wakeling (2009) has reported that electromyographic activity of GL is more modulated in response to different cycling tasks than GM. Furthermore, according to Héroux et al. (2014) the contribution of GL to simple balancing tasks is almost absent, and Antonios and Adds (2008) suggest that GL may act as a stabiliser of the talocrural joint, while GM acts as the main contributor to plantarflexion. This makes sense given the different force producing capabilities of both muscles. One study compared mechanical properties of GM and GL (Morrison et al., 2015); while these authors' results confirm differences in MTU geometry between GM and GL, a difference in tendon strain and stiffness was not found.

The number of published studies to date that have investigated the differential anatomy and function of GM and GL are sparse and contradictory. The majority of studies investigating gastrocnemius muscle function and mechanical properties, however, only investigate GM and generalise their findings to both gastrocnemii (Kinugasa et al., 2016; Kato et al., 2010; Arampatzis et al., 2005; Maganaris and Paul, 2002; Muramatsu et al., 2001). This may not be appropriate given the differences in the morphology of GM and GL described above and a possible difference in task-dependent functionality of GM and GL suggested by Wakeling (2009). A thorough investigation into the differences between GM and GL has not yet been undertaken.

1.3 Calcaneal inversion/eversion and the subtalar joint

Calcaneal inversion/eversion is the movement of the calcaneus at the STJ. The STJ is the articulation of the calcaneus and the talus superior to it. The inversion/eversion range of motion of the STJ is limited by medial (e.g. fibulocalcaneal) and lateral (e.g. deltoid) ligaments (Nigg et al., 1990) and has been described as 10° of calcaneal inversion and 28° of calcaneal eversion (Aström and Arvidson, 1995). Due to the orientation of the rotational axis of the STJ, inversion is also accompanied by plantarflexion and adduction (together called supination) and eversion is also accompanied by dorsiflexion and abduction (together called pronation) (Hicks, 1954).

The orientation of the STJ axis can be described by the angle it forms with the sagittal plane (deviation angle) and the angle it forms with the transverse plane (inclination angle). *In vitro* studies (e.g. Isman and Inman, 1969; Elftman and Manter, 1935), invasive *in vivo* studies (Lundberg et al., 1989) and non-invasive *in vivo* studies (Reule et al., 2011; Lewis et al., 2009; van den Bogert et al., 1994) have reported average deviation angles between 18° and 23°, and average inclination angles between 33° and 43°. In general, the exit points of the rotational axis of the STJ can be located on the postero-lateral side of the calcaneus and on the medial side of the head of the talus anteriorly (Goto et al., 2016; Hicks, 1954). According to Kirby (2001), the STJ axis lies on a line between the first intermetatarsal space and the postero-lateral aspect of the calcaneus in feet that function most normally during gait (Fig. 1.5B).

Inversion and eversion of the calcaneus has been observed during gait. Upon heel-strike, the calcaneus is inverted and everts during the first part of the stance-phase. In late stance, the calcaneus inverts again to aid push-off (Perry and Lafortune, 1995). These calcaneal movements are associated with a change in orientation of the STJ axis resulting in a change of the location of the axis with respect to the muscles acting around the STJ (Klein et al., 1996; Hintermann et al., 1994). As the calcaneus inverts, the STJ axis rotates externally, while it is rotated internally as the calcaneus everts (Kirby, 2001).

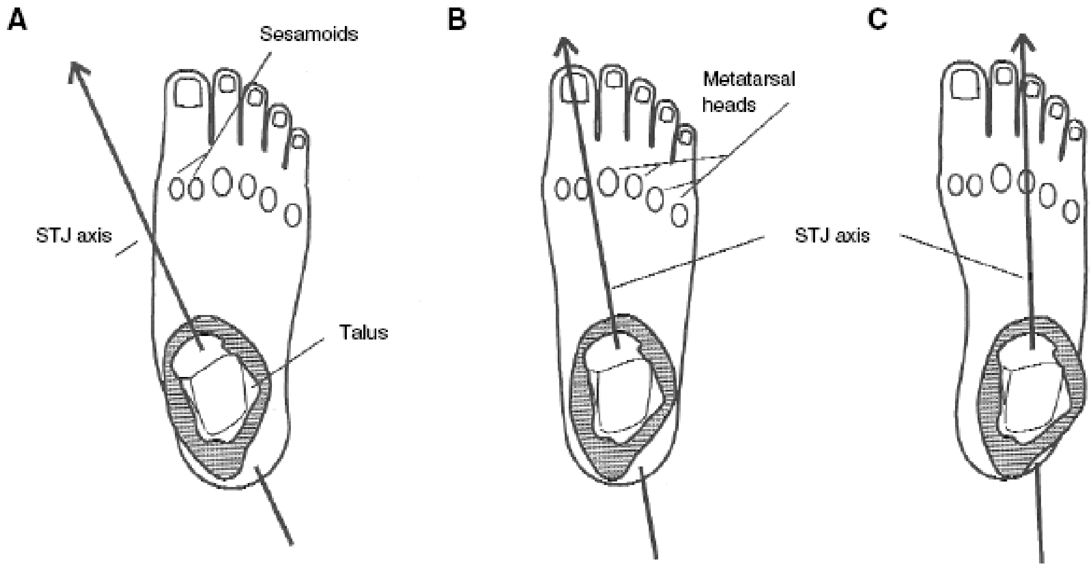


Figure 1.5. The orientation of the STJ in pronated (A), neutral (B) and supinated (C) condition represented as bundle of axes (Kirby, 2001).

Large inter-individual variations in STJ axis orientation have been found (Barbaix et al., 2000; Isman and Inman, 1969; Hicks, 1954). These have been attributed to differences in foot structure since compensation of skeletal misalignment in the foot is thought to take place at the STJ (Root et al., 1977). In relaxed standing, an everted calcaneus has been associated with a medially rotated STJ axis, while the opposite is true for an inverted calcaneus (Kirby, 2001; Tiberio, 1988). Furthermore, Kirby (1987) observed that individuals with a medially rotated STJ axis over-pronate during gait (excessive calcaneal eversion), while individuals with a laterally rotated STJ axis over-supinate (lack of calcaneal eversion) (Fig. 1.5A & C). This seems logical, since inversion is accompanied by forefoot adduction and plantarflexion, and eversion is accompanied by forefoot abduction and dorsiflexion (Hicks, 1954).

1.4 Actions of gastrocnemius medialis and lateralis at the subtalar joint

The triceps surae MTU is the main plantarflexor of the talocrural joint. It also contributes to inversion and eversion at the STJ, although there is little consensus concerning the details of this function. Arndt et al. (1999a), in an *in vitro* study, reported that the triceps surae muscle group exerts an inversion moment when the foot is at a right angle to the lower leg. Examining the contributions of each individual muscle, they found that soleus and GM exert an inversion moment while GL caused an eversion moment at the calcaneus. Others have suggested that the entire triceps surae muscle group acts as an inverter with varying degrees of inversion moment throughout the plantarflexion/dorsiflexion and inversion/eversion ranges of motion (Spoor et al., 1990). Alternatively, a constant inversion moment arm of the triceps surae during pure frontal plane motion has also been suggested (Hintermann et al., 1994). Vieira et al. (2013) showed that isolated activation of GM causes not only plantarflexion of the talocrural joint, but is also accompanied by calcaneal inversion at the STJ. This seems logical since the axis of the STJ has been described as exiting the rearfoot at the postero-lateral side of the calcaneus (Goto et al., 2016; Hicks, 1954). Results from an *in vivo* study, however, yielded slightly different results. When the foot is flat on the ground and at a right angle to the lower leg, GM and GL have been found to produce an inversion moment (Lee and Piazza, 2008). These authors, furthermore, report that GM acts as an inverter and GL as an everter when the foot is everted. With increasing inversion, both muscles become greater inverters. During gait, the entire triceps surae was found to decelerate calcaneal eversion after heel strike and assist in calcaneal inversion at push-off (Segesser and Nigg, 1980).

Despite contradicting results regarding the actions of GM and GL at the STJ, it has become clear that both muscles contribute to inversion and/or eversion of the calcaneus but the exact nature of their actions is still under debate. One reason for the lack of agreement in the studies mentioned above could be the nature of the orientation of the STJ joint. Since its orientation has been found to vary greatly between individuals and also changes its orientation with calcaneal inversion/eversion, the location of the STJ axis is altered with respect to the AT. The possibility has been discussed (Reule et al., 2011) that the STJ axis might sometimes penetrate the AT and sometimes pass laterally or medially to it (Fig. 1.6). This is in agreement with Klein et al. (1996), who reported inversion/eversion moment arms of the triceps surae ranging from approximately 20 mm of eversion moment arm to 28 mm of inversion moment arm throughout the inversion/eversion range of motion thus suggesting that the STJ axis assumes different locations with respect to the AT. It might, therefore, be possible that the gastrocnemii act as antagonists in a situation where, for example, GM acts as inverter of the calcaneus and GL acts as everter.

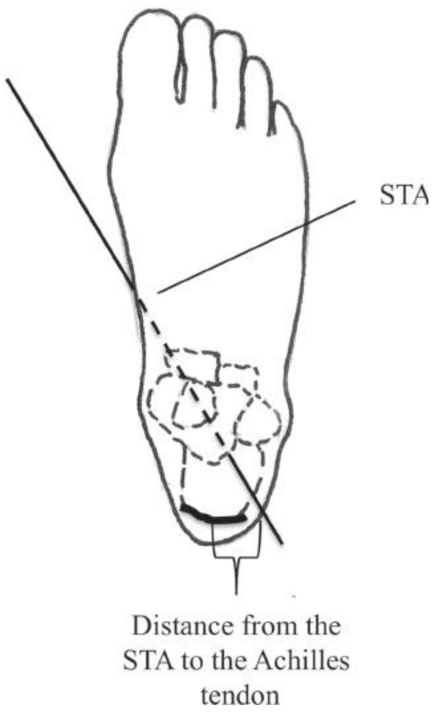


Figure 1.6. A medially rotated STJ axis passing the AT laterally (Reule et al., 2011).

To date, it is not known how the contractile behaviour of GM and GL might be affected by their actions at the STJ. Depending on whether GM and GL act as inverter and/or everter, respectively, the contractile behaviour of the GM and GL MTUs might change. Knee joint angle-dependent changes in passive fascicle behaviour of been reported for GM (Hodson-Tole et al., 2016) but it is not clear whether this holds true for muscle contractions or whether inversion and eversion of the calcaneus might have a similar effect. The contractile behaviour of GM and GL may be affected by calcaneal posture and position.

1.5 Implications of the functional differences of gastrocnemius medialis and lateralis and their actions at the subtalar joint

Functional differences between GM and GL and inversion/eversion movements of the calcaneus at the STJ have been discussed in the literature (see above). Both factors have been identified as possible mechanical causes of non-homogeneous strain distribution in the connective tissue of the gastrocnemius MTUs (Bojsen-Møller and Magnusson, 2015). Non-homogeneous strain distribution within a tissue refers to a situation, in which the strain within this tissue differs between distinct regions. Non-homogeneous strain within the AT may lead to AT injuries. AT injuries constitute over two thirds of injuries in competitive runners such as Achilles tendinopathy (66%), insertional tendinitis (23%), MTJ strains (8%) and complete ruptures (3%) (Kvist, 1994). Multiple factors contribute to AT injuries such as leg length, discrepancy, muscle dysbalances, body mass, joint laxity and foot malalignment (Järvinen et al., 2001). Specifically, the amount of inversion/eversion of the calcaneus has previously been associated with AT injuries. For example, an excessive eversion posture of the calcaneus during relaxed standing has been associated with the occurrence of Achilles tendinitis (Kaufman et al., 1999), while the amount of calcaneal inversion and eversion during running has been linked to AT rupture (Leppilahti and Orava, 1998) and AT overuse injuries (Kvist, 1994). Many of the factors associated with AT injuries are thought to lead to tissue degradation (Kannus and Jozsa, 1991) caused by non-uniform strain distribution and shear in the tissue (Maganaris et al., 2004).

The exact strain distribution within the AT is currently little understood. Reeves and Cooper (2017) hypothesised that larger tissue deformation, and therefore strain, would occur distal to the MTJ of soleus since the CSA of the AT is smallest in this region and most AT ruptures are reported to occur here (Józsa et al., 1989). However, they were unable to confirm this. Complex non-uniform tissue displacement and strain distribution in connective tissue has been identified previously. During submaximal isometric contractions, strain in the triceps surae aponeurosis, proximal AT and free AT have been found to differ, with the free AT experiencing higher longitudinal strain than the aponeurosis and proximal tendon (Farris et al., 2012; Iwanuma et al., 2011; Finni et al., 2003). Similar findings were made using ultrasound imaging (Magnusson et al., 2003). Furthermore, transverse strain within the AT and proximal tendon were shown to differ during contraction, with transverse strain in the free AT being negative (decrease in width) and transverse strain in the aponeurosis being positive (increase in width) (Farris et al., 2012; Iwanuma et al., 2011). Investigations into strain patterns within the free AT showed that strain distribution is non-uniform during passive talocrural joint rotations and walking (Slane and Thelen, 2014; Arndt et al., 2012). Specifically, tensile strain was higher in the anterior, joint-facing side but, lower in the posterior part. One study (Bojsen-Møller et al., 2004) investigated the amount of displacement of the aponeurosis of soleus and the aponeurosis of GM during maximum isometric plantarflexion efforts. These authors found that both aponeuroses displace differently. Specifically, the GM aponeurosis showed a greater displacement than the soleus aponeurosis with the knee fully extended, while the opposite was the case with the knee flexed at 90°. The authors concluded that shear strain occurs between the aponeurosis of GM and soleus.

In their review, Bojsen-Møller and Magnusson (2015) discuss the differential function of GM and GL and calcaneal inversion/eversion as possible causes of non-homogeneous strain distribution. Some evidence was provided by two *in* *vitro* studies. Lersch et al. (2012) showed that inversion/eversion of the calcaneus induces strain differences between the medial and lateral side of the AT. In calcaneal eversion, strain of the medial side of the AT was shown to be positive (tissue lengthening) while strain on the lateral side was negative (tissue shortening). The opposite was true for calcaneal inversion. Arndt et al. (1999b) report that activation of soleus and GM causes higher strain in the medial aspect of the AT and GL predominately causes strain in the lateral aspect of the tendon. It is not clear, however, how well these findings correspond to the compartmentalisation of the AT (Edama et al., 2016; Edama et al., 2015; Szaro et al., 2009). Wallenböck et al. (1995) report that the strain of the lateral part of the AT exceeds the strain of the medial part during a simulated topple-over movement of the talocrural joint. They suggest that the configuration of the STJ may contribute to this strain distribution.

The strain distribution in the AT as result of functional differences of the gastrocnemii and STJ position, however, has not been investigated *in vivo*. Considering the differential force producing capabilities of GM and GL, it is reasonable to assume that strain differences might exist between the tendon portions related to the two gastrocnemius heads. Furthermore, strain differences may be affected by the amount of inversion and eversion of the calcaneus, which would increase the complex nature of GM and GL actions and loading. Strain differences might be affected by calcaneal posture and position.

1.6 Aims of the research

As described above, GM and GL have often been studied as plantarflexors under the assumption that they constitute two heads of one muscle with similar behaviour and properties. However, the above discussions showed that both gastrocnemius heads differ in their muscle architecture, MTU geometry and function, and, furthermore, that both gastrocnemii contribute differently to STJ movement depending on the amount of inversion and eversion of the calcaneus. Therefore, both gastrocnemius heads might behave very differently in different STJ positions. The main aim of this research was to investigate the effect of calcaneal inversion and eversion on the differential contractile behaviour of GM and GL during plantarflexion contractions. The contractile behaviour of the gastrocnemii during plantarflexion contractions might be different between individuals presenting with different amounts of calcaneal inversion and eversion during standing. Furthermore, the contractile behaviour of GM and GL during plantarflexion might be altered in response to externally induced inversion and eversion of the calcaneus. The following five questions needed to be answered:

1. How can the inversion and eversion posture of the calcaneus during standing be assessed reliably?
2. Is the plantarflexion moment arm of the AT affected by calcaneal inversion/eversion, and is it possible to estimate the inversion/eversion moment arm of the AT?
3. What is the effect of calcaneal inversion/eversion on fascicle behaviour and tendon loading of each gastrocnemius head individually during a plantarflexion contraction?
4. Is fascicle behaviour and tendon loading during plantarflexion contractions different between GM and GL?
5. What is the interaction between GM and GL in response to calcaneal inversion/eversion?

In order to answer the five questions above, five studies were conducted with the following objectives:

The first objective of study one is to develop a direct anatomical method to assess the amount of inversion or eversion of the calcaneus during standing using a medical imaging approach and to investigate the reliability of the proposed method. The second objective of study one is to investigate the agreement between the proposed method and the clinical method for the assessment of calcaneal inversion and eversion in order to determine which of the two methods would be most suitable to assess calcaneal posture.

Study two investigates AT moment arms using the centre-of-rotation method. The first objective is to establish whether the plantarflexion AT moment arm differs between individuals with different calcaneal postures and whether the plantarflexion AT moment arm is altered in response to calcaneal positioning in inversion and eversion. The second objective is to explore the applicability of the centre-of-rotation method to determine inversion/eversion AT moment arms.

The objective of study three is to investigate whether fascicle behaviour and tendon loading of each gastrocnemius head individually during an isometric plantarflexion differ between individuals with different calcaneal postures and whether fascicle behaviour and tendon loading of GM and GL is altered in different calcaneal positions.

The objective of study four is to investigate the differences in fascicle behaviour and tendon loading between GM and GL during an isometric plantarflexion contraction.

The objective of study five is to investigate the effect of calcaneal inversion/eversion on the interaction of GM and GL during isometric plantarflexion contractions.

In the context of this thesis, the term “calcaneal posture” refers to the amount of inversion or eversion of the calcaneus assessed during relaxed standing. The term “calcaneal position” refers to an external manipulation of the calcaneus into inversion or eversion. The anatomical method proposed in study one will be used to assess calcaneal posture in the following two studies. The data obtained in the experiment conducted as part of study three forms the basis for study three, four and five. The experimental procedure is described in Section 4.2. Further processing steps and calculations were conducted in study four and five and are described in the corresponding sections of Chapters 5 and 6.

Chapter Two: Assessment of calcaneal posture using an anatomical MRI-based method

2.1 Introduction

In order to be able to investigate whether the contractile behaviour of GM and GL differs between individuals with different calcaneal postures, it is necessary to determine the amount of calcaneal inversion or eversion during standing. The amount of calcaneal inversion or eversion can be identified by determining the bisection line of the posterior calcaneus and measuring the angle it forms with the sagittal plane. This method has been used in podiatric practice to assess rearfoot posture, but its validity has been called into question (Menz, 1995; Wooden, 1990; Elveru et al., 1988). However, the assessment of calcaneal inversion/eversion is still widely practiced as one of the six items of the Foot Posture Index assessment protocol (Redmond et al., 2006).

2.1.1 Determination of calcaneal inversion/eversion in clinical practice

Calcaneal inversion/eversion can be assessed using the calcaneal angle method in the relaxed calcaneal stance position (RCSP), also called the RCSP method (Menz, 1995). In general, the procedure comprises of two parts. First, the calcaneal bisection line is identified visually and through palpation of the posterior calcaneus from the medial and lateral borders (Fig 2.1A). The bisection line is then marked on the participant's skin. In the second part, a goniometer is applied to measure the angle between the bisection line and the vertical (Wooden, 1990) (Fig. 2.1B). The reliability of the RCSP method has previously been reported as good with intra-class correlation (ICC) coefficients for intra-tester reliability of 0.8 (Barton et al., 2010), 0.86 (Levinger and Gilleard, 2004) and 0.87 (Powers et al. 1995), whilst studies investigating inter-tester reliability report ICC coefficients ranging from 0.68 (Sell et al., 1994) to 0.75 (Barton et al., 2010).

A

B

Figure 2.1. (A) Palpation of the calcaneus from the medial and lateral border to identify the calcaneal bisection line. (B) Measurement of the calcaneal angle as the angle between the bisection of the calcaneus and the transverse plane using a protractor goniometer (Levinger & Gilleard, 2004).

2.1.2 Challenges to the RCSP method

Menz (1995) identified two main sources of error with the RCSP method: a) palpation and/or estimation of the location of the calcaneal bisection line, and b) the thickness of the marker pen. Kaye and Sorto (1979) estimate an error of up to 2° when using a 1.4 mm wide marker pen. They, therefore, recommend using thin-line marker pens.

Palpation of anatomical landmarks can introduce error due to relative skin movement over the anatomical site of interest. This problem has also been encountered in motion analysis when using skin-mounted markers (Tranberg and Karlsson, 1998; Holden et al., 1997). Holden et al. (1997) showed considerable differences in marker displacement between markers attached to the skin of the shank and subcutaneous markers. They found displacement movement differences between 3 and 10 mm. A study by Tranberg and Karlsson (1998) investigated the relative movement of skin-mounted markers within individual areas of the foot. They reported that markers in the distal regions of the foot show considerable relative movement. Specifically, a marker on the medial aspect of the calcaneus showed an average movement of ~ 4 mm. The authors suggest that this magnitude of relative movement can be quite substantial in gait analysis studies investigating calcaneal movement. Relative skin movement can also be problematic for the palpation of the calcaneus since it may result in an erroneously marked bisection line. Furthermore, Payne and Richardson (2000) investigated the consistency in the application of the calcaneal angle method with experience. They compared assessments made by first year podiatry students with final year podiatry students and hypothesised that measurement values would be distributed around the true mean value, and that the standard deviation would decrease with greater experience. However, they were unable to confirm this.

One reason for the lack of change in standard deviation in the study by Payne and Richardson (2000) could be a lack of agreement with the actual anatomic structures assessed as they largely depend on palpation and estimation of anatomical reference sites. Hence, the validity of the RCSP method might be questionable. The reliance on palpation to identify the central bisection line may represent a source of error that could lower the validity of this technique (Wooden, 1990) and Elveru et al. (1988), in a pilot study, found relative skin movement over the calcaneus to be of such magnitude that it was impossible for a line on the posterior calcaneus to represent its true orientation.

2.1.3 Validity of the RCSP method

Despite some authors expressing concerns regarding the validity of using the RCSP method (Menz, 1995; Wooden, 1990; Elveru et al., 1988), the validity of this method has not been investigated. A few published studies have investigated the validity of other clinical measurements of foot posture (Wrobel and Armstrong, 2008; Thomson, 1994). Williams and McClay (2000) compared clinical measurements of navicular height with radiographic measurements and found good agreement between the two methods reporting a high intra-class correlation coefficient of 0.918. Similarly, Saltzman et al. (1995) compared clinical measurements of talar height with measurements in radiographic images and reported a Pearson's correlation coefficient of 0.86 with confidence intervals from 0.80 to 0.90, again indicating good agreement between the two methods. The assessment of navicular height and talar height, therefore, can be deemed valid methods for the assessment of foot posture. The skin over these anatomical landmarks is relatively thin measuring approximately 1.2 mm in thickness (Lee and Hwang, 2002) so that these landmarks can be easily palpated and identified. The calcaneus, on the other hand, is surrounded by fibro-fatty tissue. This tissue can be up to 2 cm thick (Campanelli et al., 2011) and may therefore conceal the shape and orientation of the calcaneus. Particularly, the fibro-fatty tissue under the calcaneus may distort its true shape to the examiner. It is described to have flanges on the lateral, medial and posterior sides of the calcaneus with increased thickness of the portion covering the posterior side of the calcaneus up to the AT insertion (Campanelli et al., 2011). Furthermore, the calcaneus has been described as having a relatively irregular shape (Rammelt and Zwipp, 2004; Heger and Wulff, 1985) making it difficult to estimate the true orientation of the bisection line. One study investigated the correlation between radiographic and clinical measurements of the rearfoot and found only a fair correlation between the clinical measurement of calcaneal posture in RCSP and radiographic measurements (Lamm et al., 2005). It appears, to date, that no published studies have investigated the validity of the calcaneal angle in RCSP.

2.1.4 Medical imaging of the rearfoot

Medical imaging of anatomical structures can reveal their true position and orientation. For example, radiographic imaging of the rearfoot has been applied for pre- and post-operative assessment of rearfoot alignment procedures (Hentges et al., 2016). In their study, Hentges et al. (2016) include a procedure to identify the calcaneal bisection on radiographic images but their description of identifying the calcaneal bisection lacks detail. Johnson et al. (1999) also investigated methods to measure calcaneal posture in radiographic images. They describe the posterior calcaneus to have an oval shape, which can be modelled as an ellipse. They propose, therefore, that the bisection of the posterior calcaneus in these images can be identified by fitting an ellipse. The major axis of this ellipse then represents the calcaneal bisection line. Their approach, however, relies on manually overlaying a set of ellipses with pre-defined dimensions over images of the posterior calcaneus so that the true size and orientation of the calcaneus have to be estimated and may not be represented correctly.

MRI of the foot has also been performed previously to generate three-dimensional reconstructions and to study foot joint kinematics (e.g. Udupa et al., 1998). Cahuzac et al. (1999) used MRI to image rearfoot deformities in children with clubfoot. MRI has not, however, been used to investigate the validity of clinical foot posture measures and, in particular, the RCSP method. Therefore, the purpose of this study was to test the validity of RCSP method. Calcaneal angle was measured using the clinical method described by Wooden (1990) and by analysing frontal plane magnetic resonance (MR) images of the posterior calcaneus using a similar approach of fitting an ellipse to the posterior calcaneus as proposed by Johnson et al. (1999).

2.2 Methods

2.2.1 Participants

Eleven participants (5 female, 6 male; age 30.3 ± 8.1 years) took part in the study. Rearfoot posture was assessed in both feet resulting in a total of 22 measurements. Only participants below 18 and 60 years of age and with no musculoskeletal injury to their foot, ankle or lower leg within the previous six months were included. The protocol and procedures were approved by the ethics committee of the Department of Exercise and Sport Science and conformed to the Declaration of Helsinki. Prior to testing, the participants were informed of all the procedures involved and familiarised with the equipment. Written informed signed consent was obtained from each participant prior to taking part in the study.

2.2.2 MRI protocol

Two-dimensional, frontal plane images of the rearfoot were obtained with a 0.2 Tesla MRI scanner (Esaote, Genoa, Italy). Participants were placed in the scanner in an upright standing position with both feet parallel to each other and fully weight-bearing. They were instructed to adopt a relaxed standing posture and to minimise movement.

Frontal plane MR images (TR/TE: 850/26 ms, thickness: 5 mm, gap between slices: 0.5 mm, pixel spacing: 0.625 x 0.625 mm) of the rearfoot were obtained. The field of view was 92.5 mm containing 17 slices. Two scans were conducted, one for each foot with each scan lasting approximately five minutes.

2.2.3 Determination of calcaneal angle in MR images

Each scan resulted in a series of 17 images showing cross-sections of the calcaneus in the frontal plane. The image series were exported from the MRI system and saved on a personal computer. The calcaneal angle was determined in only one image of each series. This image was selected from the posterior part of the calcaneus between the posterior surface and the calcaneal tuberosity (Fig. 2.2A) as this was determined to be the closest representation of the posterior aspect of the calcaneus (Johnson et al., 1999). In this region, the calcaneus has an oval shape in the frontal plane so that the bisection line of this oval shape would represent the general orientation of the posterior calcaneus in the frontal plane (Fig. 2.2B). If two or more slices met this criterion, the slice where the calcaneus exhibited a more evenly oval or elliptical shape was selected.

The selected MR images were analysed using open source image processing software (ImageJ, National Institute of Health, Maryland, USA). In the selected image, a region of interest, i.e. the calcaneus, was defined by manually outlining the boundaries of the bone (Fig. 2.2C). Then, the DrawEllipse macro in ImageJ was used to fit an ellipse to the outlined selection. This algorithm fitted an ellipse with the same area, orientation and centroid as the outlined selection based on a statistical description by (Cramer, 1971) and returned parameters describing the shape and orientation of the ellipse such as length of major and minor axes and the angle of the major axis to the x-axis (horizontal). This angle was used and converted into an angle between the major axis and the y-axis to obtain the calcaneal angle (Fig. 2.2D) as it is specified in the literature (e.g. Barton et al., 2010).

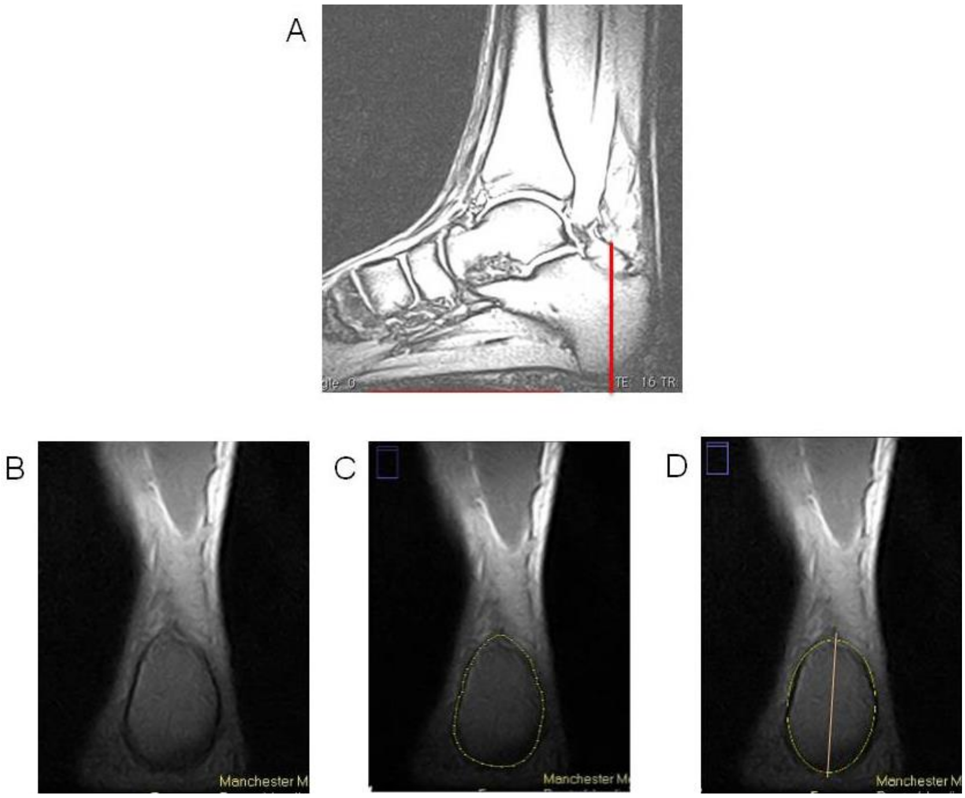


Figure 2.2. MR images of one participant. (A) Sagittal plane image of the rearfoot. The red line indicates the position of the slice chosen for analysis. (B) The slice selected for the analysis showing the oval shape of the posterior part of the calcaneus. (C) MR image with free-hand selection of the calcaneus. (D) The ellipse fitted based on the free-hand selection. The major axis of the ellipse (yellow line) was added manually to illustrate the location of the calcaneal bisection line.

The principle investigator (Tester 1) repeated the selection of the image from the image series, the manual outlining of the area of interest (calcaneus) and the ellipse fitting on two different days a minimum of one week apart (Day 1 and Day 2) to obtain intra-tester reliability. For inter-tester reliability, an independent investigator (Tester 2) also analysed the images including image selection, outlining of the area of interest and ellipse fitting.

Figure 2.3. The participant in the figure-four position.

2.2.4 Determination of the calcaneal angle in the relaxed calcaneal stance position

Clinical measurements were obtained using the method described by Wooden (1990). The participant lay prone on an examination bench in the figure-four position (Fig. 2.3). The posterior aspect of the calcaneus of the extended leg was palpated from the medial and lateral edges in order to identify the calcaneal bisection line, which was then marked with a pen. This procedure was repeated for the second foot. After marking the reference line, the participant was asked to stand on a 30 cm high rigid, wooden box and to adopt a normal relaxed stance position with both feet parallel to each other (Fig. 2.4). The angle between the calcaneal midline and the transverse plane was measured using an analogue goniometer (Prestige Medical, Coventry, England). This angle was then converted into an angle between the midline and the sagittal plane. The calcaneal angle was measured once for each foot. For the assessment of intra-tester reliability, participants were asked to report back to the laboratory at least one week after the initial measurement where the assessment was repeated. All assessments were performed by the principle investigator.

Figure 2.4. The participant on the wooden box with the calcaneal bisection identified on both feet.

2.2.5 Statistical analyses

Statistical analyses were performed using SPSS (version 16.0, Chicago, Illinois). To test for intra-tester reliability of the proposed MRI method a one-way random effects intra-class correlation coefficient (ICC) model with 95% confidence intervals (CI) was used comparing the calcaneal angle obtained by the principal investigator (Tester 1) on Day 1 and Day 2, respectively. Inter-tester reliability of the proposed MRI method was analysed with a two-way mixed effects ICC model with 95% CIs comparing the calcaneal angles obtained by Tester 1 on Day 1 and Tester 2. To test for intra-tester reliability of the RCSP method, a one-way random effects intra-class correlation coefficient

(ICC) model with 95% CIs was used comparing the calcaneal angle obtained by Tester 1 on Day 1 and Day 2, respectively. The agreement between the clinical and the proposed MRI method was determined with a two-way mixed effects ICC model with 95% CIs comparing the calcaneal angle obtained by Tester 1 on Day 1 using the RCSP method and the proposed MRI method. Confidence intervals between 0.8 and 1.0 were considered to indicate good reliability. Systematic measurement error was analysed with a two-tailed dependent t-test. The level of significance was set at $P < 0.05$. The level of agreement between all measurements (MRI method Tester 1 on Day 1 vs. Day 2, MRI method Tester 1 vs. Tester 2, RCSP method Tester 1 on Day 1 vs. Day 2, RCSP method vs. MRI method) was determined with Bland-Altman plots with 95% limits of agreement. All data are presented as mean ± S.D.

2.3 Results

The images of one participant were removed from the study because the foot positioning during the MRI scan was not parallel. In addition, images of the left foot of another participant could not be analysed due to insufficient image quality. Hence, the calcaneal angle from 19 feet is reported (Table 2.1).

Table 2.1. Calcaneal angles obtained in the RCSP and in MR images.

	RCSP	MRI	MRI	
			Tester 1	Tester 2
Day 1	3.6° ± 5.1°	1.1° ± 6.0°	1.1° ± 6.0°	2.5° ± 5.5°
Day 2	4.5° ± 5.0°	1.8° ± 5.2°		
Average	3.9° ± 5.0°	1.4° ± 5.6°		

The test for intra-tester reliability of the MRI method gave an ICC coefficient of 0.91 ($P < 0.01$) and ICC CIs from 0.79 to 0.96. The measurements of Tester 1 differed by 0.7 ± 0.8° between Day 1 and Day 2 resulting in an absolute systematic error of 0.75°, which was not significant (P = 0.169). Upper and lower limits of agreement were 4.3° and -5.8°, respectively. Systematic error and limits of agreement are depicted in Figure 2.5A.

The ICC coefficient for inter-tester reliability of the MRI method was 0.88 (P < 0.01) with CIs between 0.73 and 0.95. The measurements of Tester 2 were 1.1° ± 0.1° larger than those of Tester 1. A systematic error of -1.5° was determined, which was not significant (P = 0.052). The upper limit of agreement was 4.25° and the lower limit of agreement was -7.42°. The Bland-Altman plot in Fig. 2.5B shows the systematic error and levels of agreement.

The assessment of intra-tester reliability of the RCSP angle method gave an ICC coefficient of 0.85 (P < 0.01) and CI values ranging from 0.6 to 0.95. The values of Day 2 were 0.9° ± 0.1° larger than values of Day 1. This systematic error was 0.87° and was not significant (P = 0.169). Upper and lower limits of agreement were -5.15° and 6.88°, respectively. Figure 2.5C shows the Bland-Altman plot indicating the systematic error and levels of agreement between the two measurement days.

The comparison of the RCSP and MRI method produced an ICC coefficient of 0.075 (P = 368) with CIs between -0.34 and 0.48. RCSP values were 2.48° ± 0.9° larger than values obtained with the MRI method. The systematic measurement error was 2.82° and was not significant (P = 0.229). Limits of agreement were -11.62° and 17.27° and are shown in the Bland-Altman plot in Figure 2.5D.

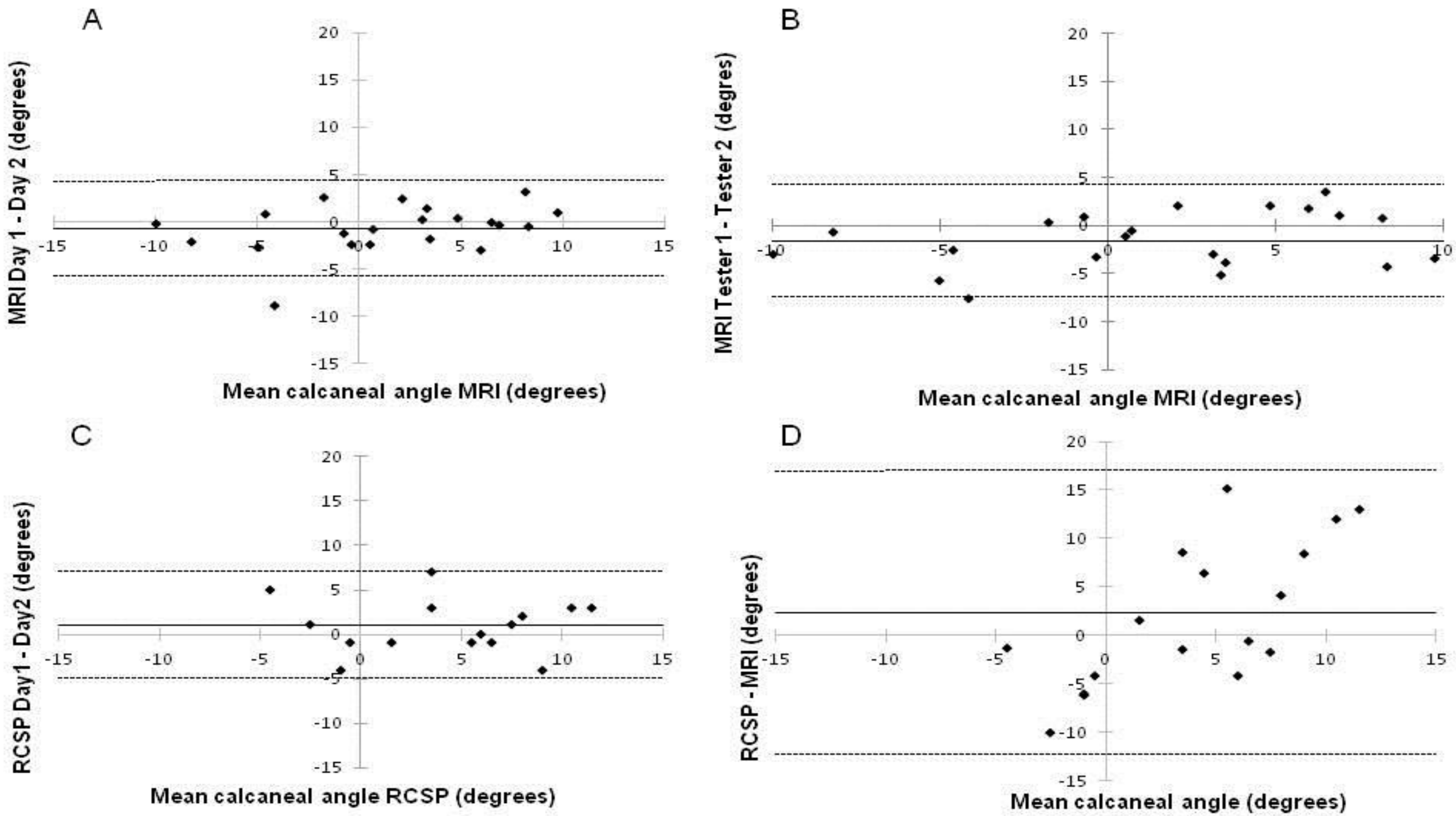


Figure 2.5. Bland-Altman plots showing measurement error (solid lines) and upper and lower levels of agreement (dashed lines) for intra-tester assessment using the RCSP method (A) and the MRI method (B), inter-tester assessment using the MRI method (C) and agreement between RCSP method and MRI method (D).

2.4 Discussion

This study describes a novel MRI-based approach for the assessment of the calcaneal angle. Intra-tester and inter-tester reliability of this method were tested and the validity of the RCSP method was determined. This method is the first to assess the calcaneal angle with a direct MRI-based anatomical approach in a weight-bearing position. In clinical practice, calcaneal angle is measured by visually identifying the calcaneal bisection and measuring the angle it makes with the horizontal surface, which may be prone to considerable measurement error (Menz, 1995).

The MRI-based approach proposes that the bisection line of the calcaneus can be identified by fitting an ellipse to the posterior aspect of the bone. The major axis of the fitted ellipse represents the calcaneal bisection line. A similar approach was described by Johnson et al. (1999), who fitted ellipses of pre-defined dimensions to radiographic images of the posterior calcaneus. Despite providing a true view of the posterior calcaneus, however, the method proposed by Johnson et al. (1999) to identify the calcaneal bisection line may be erroneous due to the manual overlaying of ellipses with pre-defined dimensions. This procedure involves a certain amount of estimation of the ellipse orientation. The method proposed in the study of the current chapter overcomes these challenges by automatically fitting an ellipse to a frontal plane MR image of the posterior calcaneus after manually outlining the boundaries of the bone. Therefore, the fitted ellipse is a true representation of the orientation of the posterior calcaneus since it is fitted based on the orientation and size of the manual outline.

The reliability of the clinical method has previously been reported as good with ICC coefficients between 0.8 and 0.87 (Barton et al., 2010; Levinger et al., 2004; Powers et al., 1995). In the study presented in this chapter, higher ICC coefficients were found for the proposed MRI method, with values of 0.91 for intra-tester measurements and 0.86 for inter-tester measurements. These suggest high and good reliability, respectively (Atkinson and Nevill, 1998), but

CIs were outside the acceptable range of 0.8-1.0. Furthermore, levels of agreement were wide ranging from -5.8° to 4.8° and from -7.42° to 4.25° for intra-tester and inter-tester reliability, respectively. Therefore, intra-tester and inter-tester reliability cannot be assumed for the proposed MRI method.

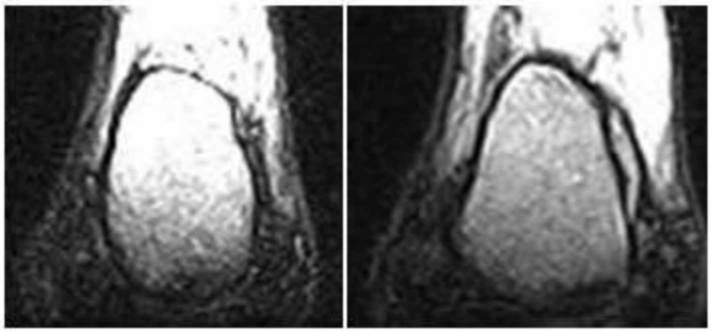


Figure 2.6. Two adjacent slices of the same foot of one participant showing considerable differences in the shape of the calcaneus.

Further analysis of the data set revealed that the main source of error lies in the selection of the image to be analysed. The calcaneus is described as a very irregularly shaped bone (Rammelt and Zwipp, 2003; Heger and Wulff, 1995) and the posterior aspect, as investigated here, shows considerable variations between two adjacent images (Fig. 2.6). The image selected, therefore, can considerably influence the calcaneal angle obtained. Out of 19 images analysed, the same image was selected 16 times by Tester 1 on Day 1 and Day 2, respectively, and Tester 2 selected the same image as Tester 1 eight times. A comparison of Day 1 vs. Day 2 measurements and Tester 1 vs. Tester 2 measurements of only these images greatly improves the reliability of the proposed method. The statistical analysis is summarised in Table 3.2 and highlights how image selection is an aspect of the approach which requires careful control and training.

Table 2.2. Statistics of the reliability analysis when adjusted for image selection. [1]Analysis of 16 images selected by Tester 1 on Day and Day 2. [2]Analysis of 8 images selected by Tester 1 and Tester 2.

	Day 1 vs. Day 2[1]	Tester 1 vs. Tester 2[2]
ICC	0.96	0.96
95% CI	0.89 - 0.99	0.82 - 0.99
Systematic measurement error	0.17°	0.17°
Limits of agreement	-4.84° - 5.18°	-3.79° - 3.45°

The measurements of the calcaneal angle using the RCSP method showed a similar degree of reliability as reported previously in the literature (Barton et al., 2010; Levinger et al., 2004; Powers et al. 1995) with an ICC of 0.85. When testing the agreement between the MRI method and the RCSP method, the systematic error was not significant. An ICC coefficient of 0.075 with wide CI from -0.34 to 0.48 as well as wide levels of agreement between -11.62 and 17.27, however, suggest poor agreement between both methods. The comparison of the RCSP method and the proposed MRI method, therefore, appears to show low validity of measurements using the RCSP method. When using the RCSP method to assess rearfoot posture, a calcaneal angle between 2° of inversion and 2° of eversion has been classified as neutral (Razeghi and Batt, 2002) and an estimated calcaneal angle of approximately 0° (bisection line vertical) is classed as neutral in the FPI (Redmond et al., 2006). However, a large systematic error of 2.82° found in the study presented in the current chapter suggests that the estimation of rearfoot posture should be done cautiously.

Previous investigations into the validity of clinical assessment methods for foot posture indicated good agreement with measurements taken on radiographic images (e.g. Williams and McClay, 2000, Saltzman et al., 1995). These studies took measurement from the midfoot region (navicular height, talar height) where the skin is relatively thin measuring approximately 1.2 mm in thickness (Lee and Hwang, 2002). The RCSP method, on the other hand, takes measurements from the posterior aspect of the calcaneus, which is surrounded by fibro-fatty tissue. This tissue can be up to 2 cm thick (Campanelli et al., 2011) and may therefore conceal the shape and orientation of the calcaneus considerably leading to an erroneous identification of the calcaneal bisection.

Relative skin movement over the calcaneus, the irregular anatomy of the bone and the thickness of the fibro-fatty tissue surrounding it have been identified as potential sources of error. By using MR imaging and applying a direct anatomical approach, it was possible to eliminate these sources of error. When eliminating the error introduced by image selection, higher reliability can be obtained with the proposed MRI method making it a reliable tool to assess the calcaneal angle in weight-bearing conditions. The method can be applied to both MR and radiographic images. It is, therefore, proposed as a tool for orthopaedic surgeons for pre- and post-operative assessment of rearfoot alignment procedures and for researchers studying the rearfoot. Furthermore, the assessment of rearfoot posture based on calcaneal inversion/eversion in clinical practice (e.g. as part of the Foot Posture Index) should be exercised with caution since a large measurement error may result in incorrectly assessing an individual's rearfoot alignment.

The study presented in the current chapter showed that the RCSP method for the assessment of calcaneal posture is not a valid measure. Therefore, in the following chapters the proposed MRI method will be applied to assess individuals’ calcaneal posture during relaxed standing. The next chapter will investigate the correlation of plantarflexion AT moment arm with calcaneal posture and changes in AT moment arm with respect to a change in calcaneal position. The subsequent chapter will address changes in the contractile behaviour of the gastrocnemius muscles with respect to calcaneal posture and position.

Chapter Three: Determination of plantarflexion Achilles tendon moment arms and inversion/eversion Achilles tendon moment arms in different calcaneal postures and position

3.1 Introduction

The AT is the common tendon of soleus, GM and GL. It inserts at the posterior aspect of the calcaneus and through force production of the triceps surae generates a plantarflexion moment at the talocrural joint. The magnitude of triceps surae muscle force that is converted into joint moment is determined by the plantarflexion moment arm of the AT. The moment arm is the perpendicular distance from the tendon to the joint centre of rotation (COR) (Fig. 3.1) and the COR represents one point along the rotational axis of the respective joint. The plantarflexion AT moment arm is frequently determined in order to estimate tendon forces in musculoskeletal models, for example during running (Sano et al., 2015; Rasske et al., 2016).

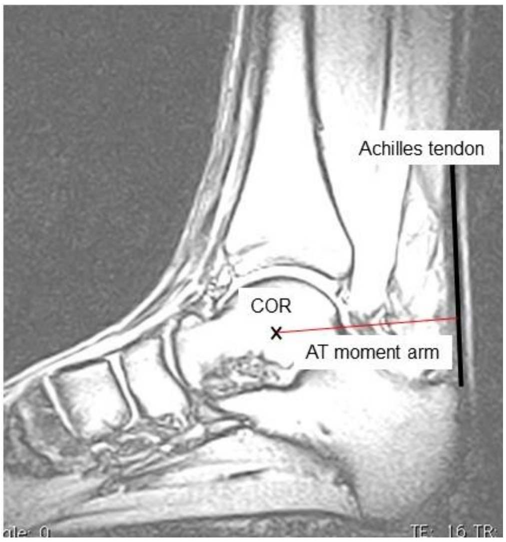


Figure 3.1. Sagittal plane image of left rearfoot depicting the moment arm of the AT as the perpendicular distance from the AT to the COR of the talocrural joint.

The length of plantarflexion AT moment arms is not constant, but rather is dependent on talocrural joint angle, muscle activation level and calculation method (Olszewski et al., 2015; Fath et al., 2010). For example, in neutral talocrural joint position (foot perpendicular to the lower leg), Fath et al. (2013) reported moment arms of 34.3 ± 4.5 mm calculated with the tendon excursion method, while the moment arm in the same position was longer with 51.7 ± 4.3 mm using the centre-of-rotation (COR) method. The authors reported that both methods correlate strongly with each other and show similar changes in AT moment arm length depending on muscle activation and talocrural joint position. With an increase in plantarflexion effort, the triceps surae bulges and pushes the AT away from the joint COR. Hence, the plantarflexion AT moment arm increases (Olszewski et al., 2015; Fath et al., 2013; Maganaris et al, 1998). Furthermore, the moment arm of the AT decreases the more dorsiflexed the talocrural joint is and it is larger in a more plantarflexed position (Fath et al., 2013; Maganaris et al., 1998). This is due to a rotation of the talocrural joint axis in anterior direction away from the AT in plantarflexion (Leardini, et al., 1999).

The effect of plantarflexion and dorsiflexion on the plantarflexion AT moment arm is well established, but the effect of calcaneal inversion and eversion has not been studied extensively. Inversion and eversion movements of the calcaneus have an effect on the rotational axis of the talocrural joint causing a rotation of the talocrural joint axis in the sagittal and frontal plane, but not in the transverse plane (away or towards the AT) (Lundberg et al., 1989). This suggests that plantarflexion AT moment arm is unaffected by calcaneal inversion/eversion but it is not known whether the inversion/eversion position of the calcaneus and the associated variation in talocrural joint axis position has an effect on the plantarflexion moment arm of the AT.

Plantarflexion AT moment arms also differ largely between individuals. For example, Baxter and Piazza (2014) report an AT moment arm of 53.4 ± 5.6 mm with a range from approximately 45 mm to 65 mm obtained with the COR method, and Lee and Piazza (2009) found a difference of 10 mm between the AT moment arms of sprinters and non-sprinters. These differences may be a result of calcaneal posture and may be associated with individual differences in the orientation of the talocrural joint axis. Studies investigating the orientation of the talocrural joint axis have also repeatedly reported large inter-individual variations (Leitch et al., 2010; van den Bogert et al., 1994; Isman and Inman, 1969), but it is not known whether a relationship exists between the orientation of the talocrural joint axis and the posture of the calcaneus during relaxed standing, and the relationship between plantarflexion AT moment arm and calcaneal posture has not been investigated. Only Parr et al. (2012) investigated the relationship between the talocrural joint axis and the STJ axis but, they did not find variations in the relationship between both joint axes between different ethnical populations and males and females.

It is necessary to know whether plantarflexion AT moment arm changes occur when the inversion/eversion position of the calcaneus is altered or whether plantarflexion AT moment arm is different between individuals with different calcaneal postures to study mechanical properties of the AT (e.g. stiffness) in relation to calcaneal inversion and eversion. Calcaneal inversion/eversion has been suggested to be a contributing factor to regionality of loading patterns within the triceps surae muscle and the AT (Bojsen-Møller and Magnusson, 2015) and a recent *in vitro* study found that strain in the AT increased when the calcaneus is in an inverted position while strain is increased on the medial side of the AT when the calcaneus is in an everted position (Lersch et al., 2012). To determine AT loading during plantarflexions, the plantarflexion moment arm of the AT must be determined as precisely as possible for calculation of tendon forces and stiffness. It is therefore important to understand whether the plantarflexion AT moment arm differs in individuals with different calcaneal postures since differences in plantarflexion AT moment arms would indicate differences in the force transmission capability of the AT during plantarflexions. It is also important to understand whether the plantarflexion AT moment arm is altered in different calcaneal positions when studying AT mechanical properties.

Plantarflexion AT moment arms have previously been determined. The triceps surae muscle group and the associated AT, however, have also been found to contribute to inversion and eversion of the calcaneus (Arndt et al., 1999a). To date, little is known about the length of inversion/eversion AT moment arms and differences in AT moment arms in different calcaneal inversion/eversion positions. Calcaneal inversion and eversion is the movement of the STJ and the orientation of its axis of rotation varies considerably throughout its range of motion (Sheehan, 2010; Lewis et al., 2009). The axis of rotation of the STJ is not located perpendicular to anatomical planes, but rather at an angle to the transverse plane (inclination angle) and the sagittal plane (deviation angle). A recent *in vivo* study on the orientation of the STJ axis reports an average inclination angle of 42 ± 16° and a deviation angle of 11 ± 23° (Reule et al., 2011). With the foot in a neutral position (neither inverted nor everted), the axis is described as piercing the rearfoot at the postero-lateral side of the posterior calcaneus (Hicks, 1954) so that the AT would be located medially to it. Hence, the AT would exert an inversion moment at the calcaneus, and, therefore have an inversion moment arm (Lee and Piazza, 2008; Arndt et al., 1999a) (Fig. 3.2). In fact, the *in* vitro study by Arndt et al. (1999a) showed that the entire triceps surae exerts an inversion moment at the calcaneus when the foot is flat on the ground, while the individual heads exert different moments. Specifically, GM and soleus invert the calcaneus and GL acts as everter. Using the tendon excursion method for the estimation of frontal plane AT moment arm, Lee & Piazza (2008) found that GM and GL have an inversion moment arm in an *in vivo* study when the foot is neither inverted nor everted. In eversion, GM had a smaller inversion moment arm while GL had an eversion moment arm. With increased inversion, the inversion moment arm of both muscles increased.

Considering the orientation of the STJ axis and the compartmentalisation of the AT, it is possible for the individual heads of the triceps surae to exert inversion or eversion moments at the calcaneus, which may cause non-homogenous strain distributions within the AT. The orientation of the STJ axis varies widely between individuals (Barbaix et al., 2000; Isman and Inman, 1969) and also changes its orientation during STJ rotation; therefore, moment arms of the AT and its individual compartments may differ between individuals and in different positions of the STJ.

The inversion/eversion moment arms of the AT have not been investigated thoroughly *in vivo* and the study by Lee & Piazza (2008) is the only published *in vivo* study to date. Lee & Piazza (2008) used the tendon excursion method to estimate moment arms. Upon application of the tendon excursion method, however, the assumptions are made that no internal forces are present in the joint of interest and that any tissue excursion observed is due to joint rotation, thereby neglecting the effect of slack within the tendon (Maganaris et al., 2001). Furthermore, tendon excursion during inversion and eversion of the calcaneus is small, and the movement observed by Lee & Piazza (2008) may well be within the measurement resolution of commonly used ultrasound devices. Therefore, the accuracy of the tendon excursion method to estimate inversion/eversion AT moment arms is doubtful. The COR method, on the other hand, overcomes the assumptions made for the tendon excursion method, and the AT moment arm is measured directly based on the underlying anatomy. Its applicability for the measurement of inversion/eversion moment arms of the AT has not previously been explored.

With the COR method, a COR rotation of the joint in question is identified, which lies on a point along the rotational axis of this joint. If the rotational axis is perpendicular to the anatomical plane in which the COR is identified (e.g. sagittal plane for identification of the COR of the talocrural joint), the distance from the tendon to this COR will be the same regardless of where along the joint axis of rotation the COR was identified. However, the axis of rotation of most joints is not perpendicular to an anatomical plane. For example, the axis of rotation of the talocrural joint is located at an angle from the sagittal plane (Hashizume et al., 2012; Leitch et al., 2010; van den Bogert et al., 1994; Isman & Inman, 1969) resulting in a small error in the estimation of the plantarflexion moment arm of the AT (Hashizume et al., 2012; Maganaris et al., 2000). The inversion/eversion moment arm of the AT is identified in the frontal plane. Inversion and eversion are frontal plane movements of the STJ and its axis of rotation is located at considerable angles from all anatomical planes (Lewis et al., 2007). Therefore, the identification of suitable reference points is crucial when applying the COR method to determine inversion/eversion moment arms of the AT. These reference points must be located on the calcaneus as rotating segment, must be reliably identifiable on all calcanei and must be selected so that the identified COR lies in, or close to, the same plane of action of the AT. As described in the study presented in the previous chapter (Chapter 2), the calcaneus exhibits an oval shape posterior to the calcaneal tuberosity but becomes more irregular in the region anterior to the calcaneal tuberosity so that it may not be possible to identify reference points near the AT.

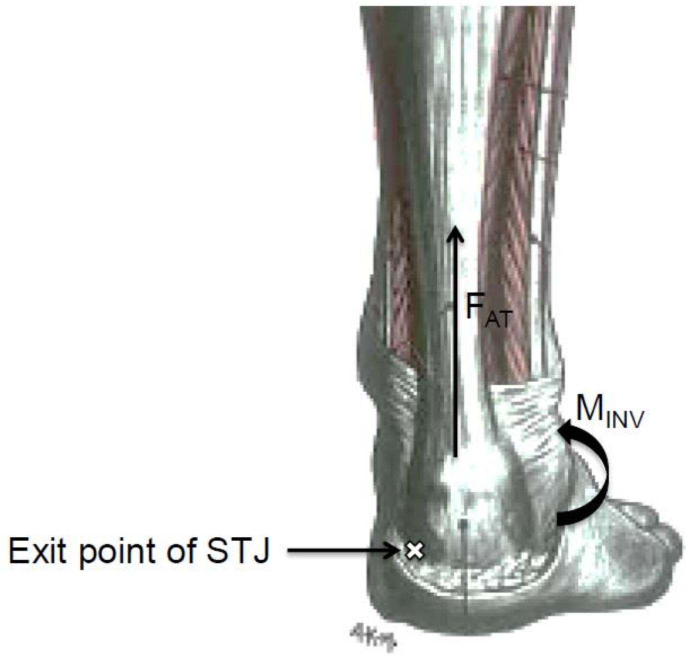


Figure 3.2. Schematic depiction of the left rearfoot showing the subtalar joint axis piercing the rearfoot on the lateral side of the AT. Upon triceps surae activation, the AT would exert a force F_{AT} resulting in an inversion moment M_{INV} at the calcaneus. Adapted from Gray's Anatomy.

The purpose of the study in the present chapter was threefold: a) to examine the effect calcaneal position on plantarflexion AT moment arm, b) to examine the relationship of the plantarflexion AT moment arm with calcaneal posture, and c) to explore the possibility of applying the COR method to identify a suitable COR lying on the axis of rotation of the STJ for later calculation of inversion/eversion AT moment arms.

3.2 Methods

3.2.1 Participants

A total of 24 participants (12 male, 12 female, age 37.8 ± 10.7 years, height 175 ± 9.2 cm, body mass 75.0 ± 14.7 kg) between the age of 18 and 60 years and with no musculoskeletal injury to their foot, ankle or lower leg within the previous six months took part in this study. The protocol and procedures were approved by the ethics committee of the Department of Exercise and Sport Science and conformed to the Declaration of Helsinki. Prior to testing, the participants were informed of all the procedures involved and familiarised with the equipment. Written informed signed consent was obtained from each participant prior to taking part in the study.

3.2.2 MR image acquisition

For calcaneal posture assessment, frontal plane MRI scans of the participants' left rearfoot were obtained in an unshod, weight-bearing position to assess the amount of calcaneal inversion or eversion during relaxed standing. The method was described in Section 2.2.

Following the scans for calcaneal posture assessment, sagittal plane scans of the rearfoot were obtained for plantarflexion AT moment arm analysis. For this part of the study, all participants were asked to lie down in a supine position on the bed of a 0.2 Tesla MRI scanner (Esaote, Italy). TR/TE/flip angle was set at 580/16/75 degrees. 12 slices with a thickness of 4 mm were obtained during each scan with a gap between slices of 0.4 mm resulting in a field of view of 52.8 mm. The duration of each scan was 1:55 minutes. Styrofoam wedges were used to alter the participants' foot position in the frontal and sagittal plane. Three scans of the participants' left ankle were taken with the left foot neither everted nor inverted in 15° plantarflexion, 0° neutral and 15° dorsiflexion, respectively. During the scans, the participants were asked to maintain good contact with the wedge throughout the scan and care was taken to align the foot vertically each time. To investigate the effect of manipulating the foot into an inverted or everted position, 12 of the participants (6 male, 6 female, age 28.1 ± 4.8 years, height 174.2 ± 9.3 cm, body mass 70.1 ± 11.2 kg) were randomly selected and underwent an additional six scans, three with the foot in 10° inversion and 10° eversion.

Frontal plane scans of the left rearfoot of a further subset of 12 randomly selected participants (6 male, 6 female, age 30.8 ± 5.7 years, height 173.6 ± 10.2 cm, body mass 67.8 ± 10.9 kg) were made to investigate the possible application of the COR method for calculations of the inversion/eversion AT moment arm. With the foot at a right angle to the lower leg, scans were obtained with the rearfoot in 20° eversion, 10° eversion, 0° neutral, 10° inversion and 10° inversion.

3.2.3 Data analyses

The resulting image series were analysed using the COR method as described previously (Fath et al., 2010; Maganaris et al., 1998) to identify the position of the COR of the talocrural joint, the location of the AT (representing the location of the force vector) and thus the plantarflexion moment arm of the AT in the talocrural joint neutral position. Based on the description by Reuleaux (1875), the tibia was assumed to be the non-moving part of the joint, with the talus rotating around it. The posterior and the lateral process of the talus were chosen as anatomical reference points. In the image of the talocrural joint in 15° dorsiflexion, the two reference points were connected with a straight line. A 10 cm long line was then drawn upwards and at a right angle from the first line, starting from the posterior process of the talus. The end of this line represents point A. Starting from this point A, another 10 cm long line was drawn at a right angle to the previous line in an anterior direction. The end of this line represents point B. The same procedure was repeated for the image of the talocrural joint in 15° plantarflexion giving points A’ and B’. The images of the ankle in 15° dorsiflexion and 15° plantarflexion were then superimposed on the image of the talocrural joint in neutral so that the tibia was exactly aligned. The COR could then be found at the intersection of the two lines drawn at a right angle from the straight lines connecting point A with point A', and point B with point B'. Subsequently, the moment arm of the AT is the perpendicular distance between this intersection and the bisection of the AT in the sagittal plane. The procedure is illustrated in Figure 3.3. The COR and, therefore, the moment arm of the AT was determined for the calcaneus in neutral (neither inverted nor everted) for all participants and with the foot in 10° eversion and 10° inversion for a group of 12 participants.

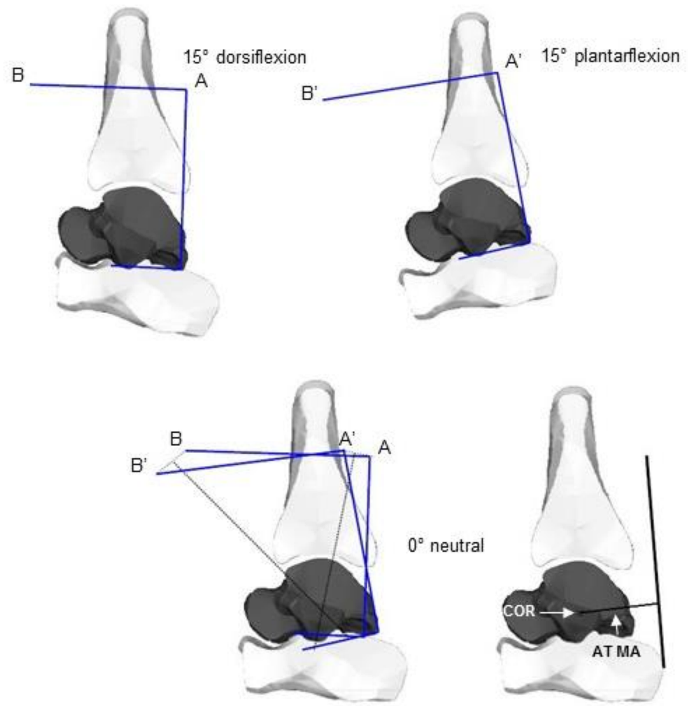


Figure 3.3. Schematic depiction of the COR method to determine the AT moment arm. Two anatomical landmarks are marked on the talus in dorsiflexed and plantarflexed position. From the straight line connecting them, two more straight lines are drawn at a perpendicular angle. The resulting figures are overlaid on the neutral position image. The intersection of the straight lines drawn from A-A' and B-B' represents the COR. The AT moment arm is the shortest distance between the tendon and the COR.

The COR method was applied in the same way to the frontal plane scans. As described in Section 3.1 above, the identification of anatomical reference points is crucial. These points have to be clearly identifiable in all participants in all conditions. The calcaneus was defined as the rotating element of the STJ rotating around the talus as the stationary element. Hence, on the calcaneus, the lateral and the medial edge of the articulate surface between calcaneus and talus were chosen as reference points as they satisfy the criterion of easy identification (Fig. 3.4). However, these points are not located in, or close to, the plane of action of the AT but approximately 5 cm anterior to it. The COR method was then applied in the same manner as described above (Fig. 3.5). The perpendicular distance of the identified COR to the bisection of the AT was measured in the frontal plane to represent the COR location in relation to the AT. It is important to note here that this is not a true frontal plane AT moment arm as the COR is identified 5 cm anterior to the AT. The identified COR represents a point on the STJ axis. Since the STJ axis is not perpendicular to the frontal plane (Reule et al., 2009), the true inversion/eversion AT moment arm would be the distance of the AT to a COR point in the plane of action of the AT (see Fig. 4.8). COR-to-AT distances were obtained for the foot in 10° eversion, 0° neutral and 10° inversion. A positive value indicates that the COR is located medial to the AT, and a negative value indicates that the COR is located lateral to the AT. The AT angle was measured as the angle between the AT bisection and the vertical to account for possible changes in moment arm.

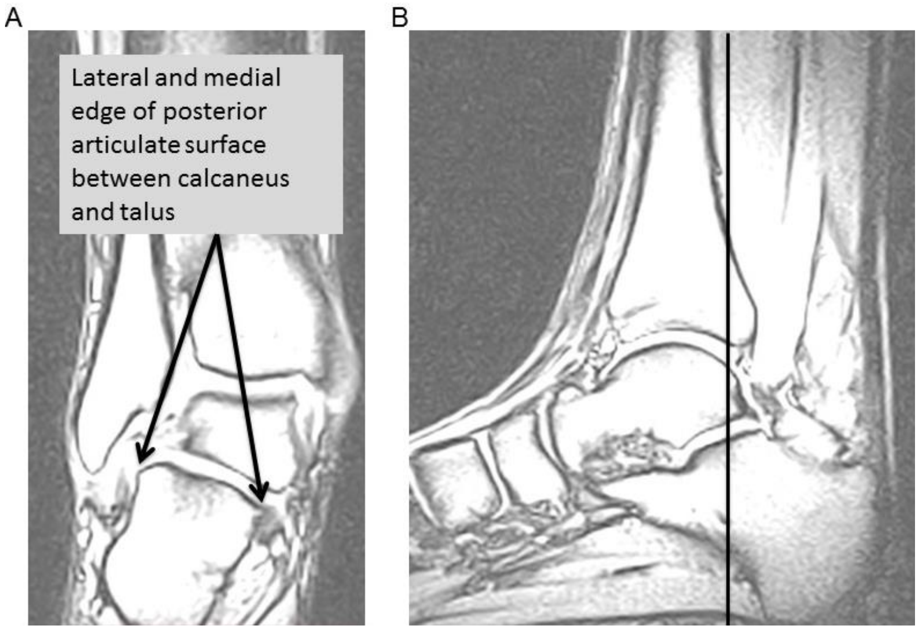


Figure 3.4. Example of a frontal plane and sagittal plane MRI scan of the rearfoot of one participant in neutral calcaneal position. (A) Frontal plane image showing the calcaneus, talus, tibia and fibula. The two black arrow indicate the selected anatomical landmarks that formed the starting points for the application of the COR method to inversion/eversion moment arm determination. (B) Sagittal plane image showing the calcaneus, talus, tibia, navicular and cuneiform. The black vertical line indicates the location of the reference points shown in (A).

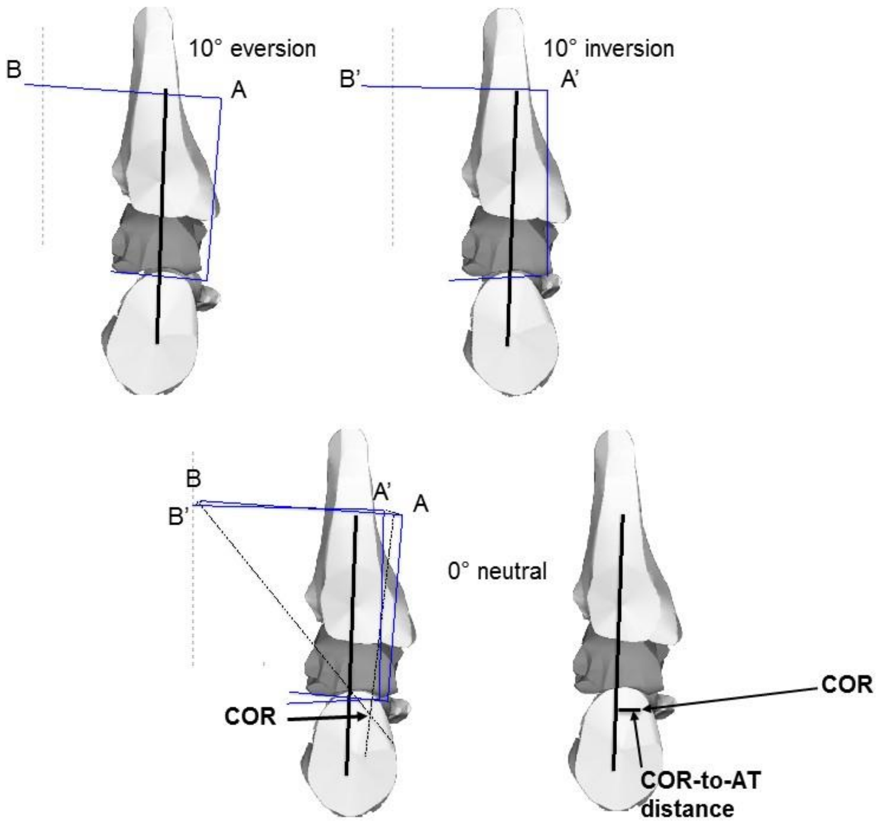


Figure 3.5. Application of the COR method to identify the instantaneous COR of the STJ and to determine the COR-to-AT distance.

3.2.4 Statistical analyses

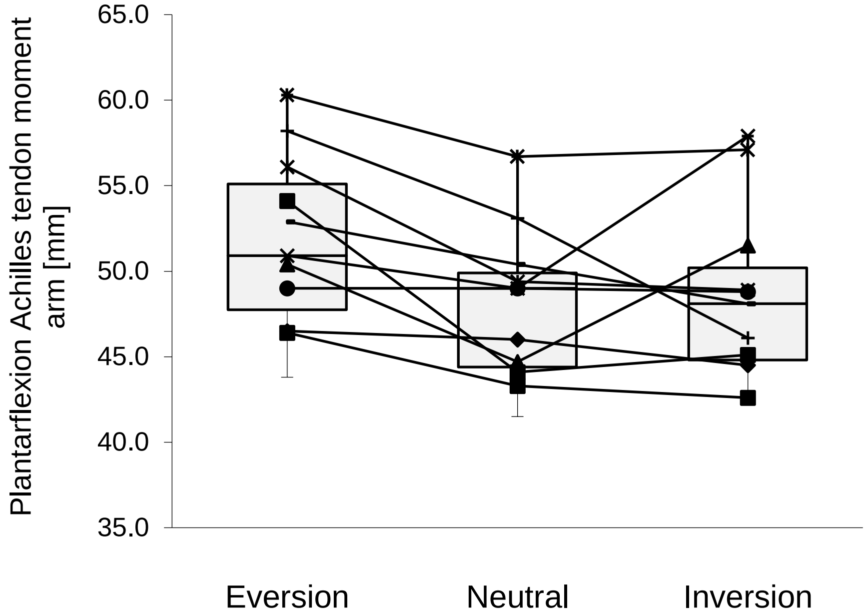
Statistical analyses of the obtained data were performed in SPSS (version 22.0, Chicago, Illinois). Values for calcaneal angles in relaxed standing, AT moment arms and AT angles with the vertical are represented as mean ± S.D. As the data met parametric assumptions, a One-way ANOVA was used to test for differences in sagittal and frontal plane AT moment arms between the three calcaneal positions. A Bonferroni *post hoc* test was performed when differences between groups were indicated. Pearson's r was used to investigate a possible correlation between calcaneal angle in relaxed stance and AT moment arm. The level of significance was $\alpha = 0.05$.

A *post hoc* power analysis was conducted in G*Power (v. 3.1.9.2, Universität Kiel, Germany) to assess the power achieved for comparisons of sagittal plane AT moment arms in different calcaneal positions, frontal plane AT moment arms in different calcaneal positions and the correlation between sagittal plane AT moment arm and calcaneal posture.

3.3 Results

Standing calcaneal posture for all participants was 0.08 ± 3.93° ranging from -6.4° of inversion to 8.9° of eversion. The plantarflexion AT moment arm for all participants in neutral calcaneal position was 47.93 ± 4.54 mm. Plantarflexion AT moment arm length did not increase in everted or inverted calcaneal position ($p=0.823$, Fig. 3.6A). There was weak negative relationship between plantarflexion AT moment arm and calcaneal posture, which was not significant ($r = -0.306$, $p = 0.145$) (Fig. 3.6B). For the comparison of AT moment arms in different calcaneal positions, the *post hoc* power analysis yielded a statistical power of 69% for an effect size of 0.45 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) for the given effect size, a sample size of $n = 51$ would be required. The power analysis for the correlation between AT moment arm and calcaneal posture gave a statistical power of 83% for an effect size of 0.56 at the 0.05 α-level.

A



B

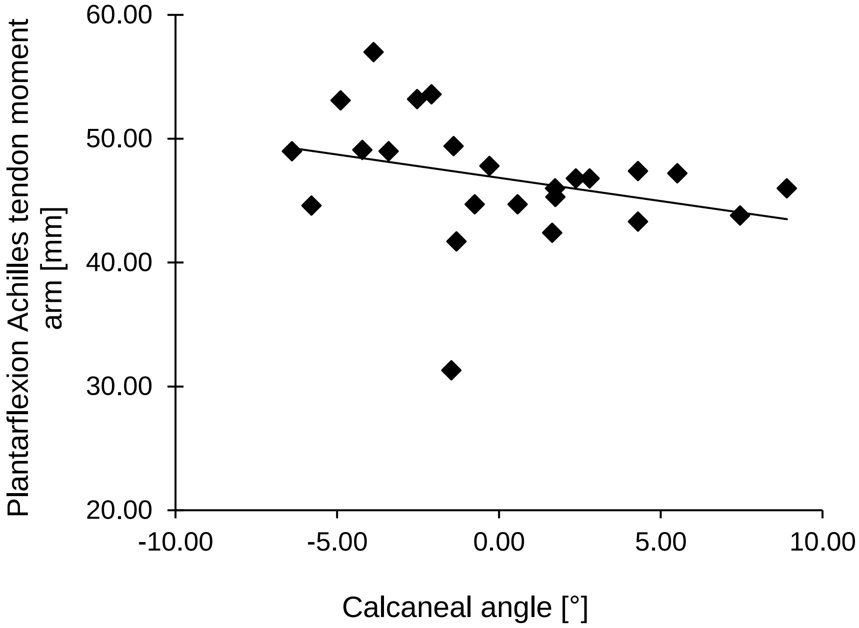


Figure 3.6. (A) Box-and-Whisker plot of the entire data set. One box represents the data distribution for one foot position. Line graphs represent individual participants. The differences in plantarflexion AT moment arm in everted, neutral and inverted position were not statistically significant (n = 12). (B) Scatter plot of calcaneal posture over plantarflexion AT moment arm (n = 12).

The instantaneous centres of rotation identified in the frontal plane were all located medial to the AT. The COR-to-AT distance measured 3.6 ± 3.9 mm, 11.4 ± 4.1 mm and 10.2 ± 7.0 mm in neutral, everted and inverted calcaneal position, respectively (Fig. 3.7). The difference in COR-to-AT distance in everted and inverted position compared to neutral position was significant ($p < 0.05$). AT angle did not differ between the three positions. For the comparison of COR-to-AT distances in different calcaneal positions, the *post hoc* power analysis yielded a statistical power of 95% for an effect size of 0.69 at the 0.05 α-level.

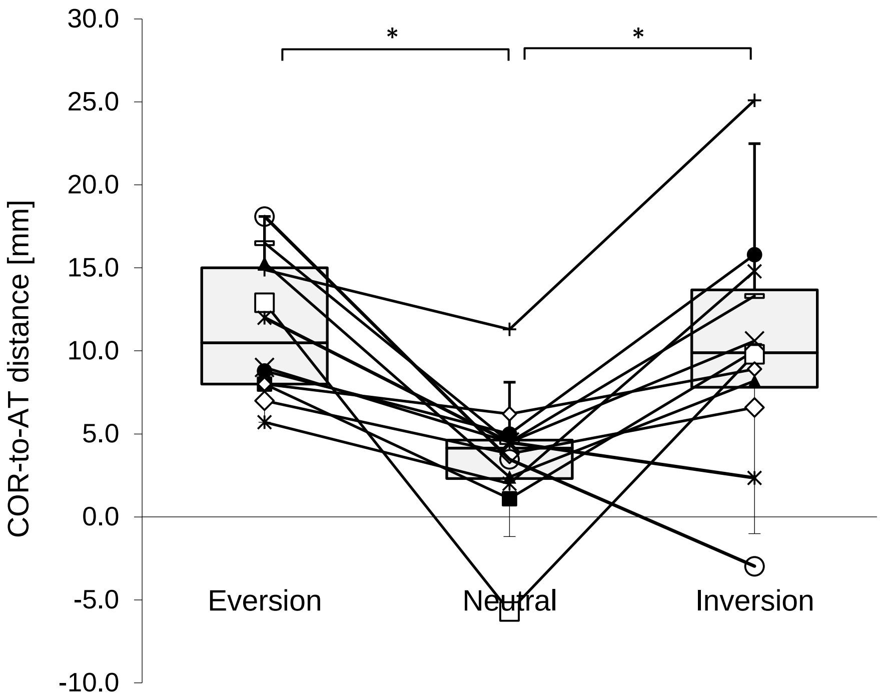


Figure 3.7. Box-and-whisker plots for the data set (n = 12) with each box plot representing the data distribution for one foot position (eversion, neutral, inversion). The line graphs show COR-to-AT distances for each individual participant in each of the three conditions with one line corresponding to one participant. Positive values indicate that the COR was medial to the AT.

3.4 Discussion

This study investigated the relationship between the plantarflexion AT moment arm and calcaneal posture and position. Previous studies have identified a change in AT moment arm with dorsiflexion and plantarflexion of the talocrural joint (Fath et al., 2013; Maganaris et al, 1998), but the relationship of AT moment to calcaneal inversion and eversion has not been previously investigated. The results of this study show a weak, non-significant relationship between AT moment arms and calcaneal posture and position indicating that the location of talocrural joint axis with respect to the AT is not dependent on calcaneal inversion and eversion.

Calcaneal inversion and eversion occur at the STJ and a change in orientation of the talocrural joint axis with STJ movement has been reported only as rotation in the frontal and sagittal plane, but not in the transverse plane (Leardini et al., 1999; Lundberg et al., 1989). The axes of both subtalar and talocrural joint have been studied extensively and large variations in joint axis locations have been reported (Leitch et al., 2010; Lundberg and Svensson, 1993; Lundberg et al., 1989; Isman and Inman, 1969). The relationship between subtalar and talocrural joint axis, however, has not been studied in great detail. Parr et al. (2012) used a 3D vector angle between both joint axes to describe their relationship, but they did not find differences between different ethnical groups and between males and females. The results presented here provide further support for this. A difference in plantarflexion AT moment arm between individuals with different calcaneal inversion/eversion postures was not found suggesting that the location of the talocrural joint axis of rotation with respect to the AT is not related to the location of the STJ axis of rotation.

In the present study, MRI scans were taken in non-weight bearing, but with the foot in good contact with the wedge so that a closed kinematic chain situation can be assumed. In this situation, inversion and eversion of the calcaneus can be achieved by external and internal rotation of the tibia, respectively. This motion is directly transferred to the calcaneus as the talus is firmly located in the ankle mortise (Huson, 1991) and movement of the talus is further limited by ligaments (Leardini et al., 2001). Therefore, movement of the STJ may not have an effect on the orientation of the talocrural joint angle.

A limitation of the COR method is its two-dimensional approach. The assumption is made that the talocrural joint axis is located perpendicular to the sagittal plane. Previous studies, however, show a deviation from the sagittal plane of varying magnitude (Hashizume et al., 2012; Leitch et al., 2010; van den Bogert et al., 1994; Isman and Inman, 1969) which can introduce a calculation error. Maganaris et al. (2000) make the suggestion to multiply the moment arm determined with the COR method with the deviation angle of the talocrural joint axis to arrive at the actual moment arm. While this calculation indicated that the COR method overestimates the AT moment arm, it does not seem suitable given the large range of deviation angles reported for the talocrural joint axis. Recently, methods to determine AT moment arm in three dimensions (3D) based on MR or computed tomography imaging have been developed and these studies show that AT moment arms are smaller when calculated with this (3D) approach compared to a two-dimensional (2D) approach (Hashizume et al., 2012; Sheehan, 2012). In an talocrural joint neutral position, however, the magnitudes of the 3D AT moment arm reported cluster around the same value of about 50 mm as for previously reported 2D values (Clarke et al., 2015; Hashizume et al., 2012; Sheehan, 2012). Therefore, the COR method can be considered as a fast and valid imaging-based method for the determination of AT moment arms.

The use of the COR method to identify one instantaneous COR of the STJ was investigated in the present study. The correct identification of suitable reference points is crucial for the COR method. In this instance, reference points were selected based on the condition that they were reliably identifiable for every participant in each condition. The lateral and medial edges of the posterior calcaneal articulate surface with the talus met these criteria. Using these reference points it was possible to identify an instantaneous COR located on the STJ axis 5 cm anterior to the AT. The distance of this COR to the AT was measured to demonstrate its location in relation to the AT. With the calcaneus neither inverted nor everted, the COR was located on the medial side of the AT and the COR-to-AT distance was small. With the calcaneus in an everted position, the COR was also located on the medial side but the COR-to-AT distance increased significantly. The increase in the COR-to-AT distance measured was not due to a shift of the AT, since the AT angle did not change as a result of inversion and eversion of the calcaneus. Instead, this indicates a change in STJ axis location as reported previously (Kirby, 2001). Kirby (2001) describes that the STJ axis rotates medially with eversion (pronation) of the foot and laterally with inversion (supination). Interestingly, the results of the present study do not support this observation for an inverted calcaneal position. In the present study, the COR was again located medial to the AT and the COR-to-AT distances increased significantly when the calcaneus was in an inverted position. This seems illogical when considering the change of STJ axis location with inversion and eversion described by Kirby (2001). Previous studies, however, have only considered the orientation of the STJ and its change in orientation as rotation in the transverse plane (e.g. van den Bogert et al. 1994). Alternatively, more recent studies have examined the location of the STJ axis in all three dimensions and do not report a simple rotation of the axis during STJ movement, but rather a helical movement (Sheehan, 2010; Lewis et al., 2009). Hence, it is possible for the COR identified in this study to rotate and also translate medially with both eversion and inversion. For example, the identified COR was located medial to the AT in inversion. This suggests that the STJ axis may have rotated laterally as described by Kirby (2001), but it might also shift medially.

By using the COR method, it was possible to identify a COR located on the STJ axis and to demonstrate the change in STJ axis location. This method could be further developed to identify two centres of rotation which would represent the location of the STJ axis projected onto the transverse plane. Information about the frontal plane AT moment arm could then be obtained. The suggested approach is demonstrated in Figure 3.8.

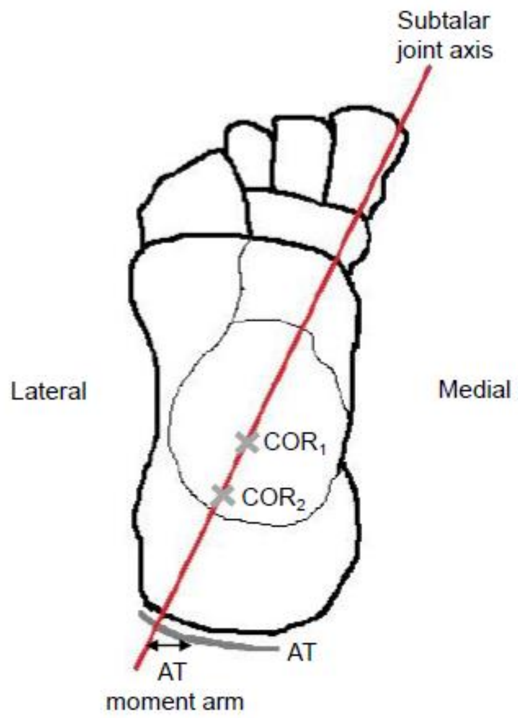


Figure 3.8. Transverse plane image of the left foot viewed from above. The identified centre of rotation (COR1) represents a point on the STJ axis and is located medially to the AT. By applying the COR method using different reference points in a different location, a second COR (COR$_2$) can be identified. The STJ axis can be found by connecting both points. The frontal plane AT moment arm can then be determined as the distance between the STJ axis and the AT bisection.

The study presented in the current chapter showed that there is no difference in plantarflexion moment arm of the AT between individuals with different calcaneal postures. The force transmission capabilities of the AT during plantarflexion are, therefore, not affected by calcaneal posture and plantarflexion moment arms of the AT do not need to be considered in relation to individuals with different calcaneal postures. However, the use of generic plantarflexion AT moment arms is discouraged given the large inter-individual differences. Furthermore, plantarflexion AT moment arms did not change in response to changes of calcaneal position. When investigating mechanical properties of the AT, it may, therefore, not be necessary to take into account a change in plantarflexion AT moment arm.

In the following Chapter 4, contractile behaviour of GM and GL during a submaximal ramped isometric plantarflexion contraction will be investigated. Contractile behaviour will be investigated in individuals with different calcaneal postures and when performing plantarflexion contractions in different calcaneal positions. Stiffness of the GM tendon and the GL tendon will be calculated to study mechanical properties of the AT in individuals with different calcaneal postures and during plantarflexion contractions in different calcaneal positions. Stiffness values will be calculated using each individual's plantarflexion AT moment arm. Based on the results of the study presented in the current chapter, the plantarflexion AT moment arm determined in calcaneal neutral position will be used to calculate stiffness in all three calcaneal positions (neutral, inversion and eversion) since a difference in plantarflexion AT moment arm was not found between the three calcaneal positions.

Chapter Four: Morphology of gastrocnemius medialis and lateralis during submaximal plantarflexion contractions in different calcaneal postures and positions

4.1 Introduction

GM and GL are bi-articular muscles acting as flexors of the knee and plantarflexors of the talocrural joint. The muscle architecture, MTU geometry and function of both gastrocnemii have been described as differing considerably (e.g. Héroux et al., 2016; Wakeling, 2009a; Antonios and Adds, 2008). The study presented in this chapter investigated the morphology of GM and GL and the contractile behaviour of both gastrocnemii in response to calcaneal inversion and eversion. In Chapter 2, a new MRI-based method was proposed for the assessment of calcaneal posture during relaxed standing. This method will be applied in this study in order to assess calcaneal posture and to group participants on the basis of the amount of calcaneal inversion or eversion measured.

Despite being the primary plantarflexors of the talocrural joint, GM and GL also contribute to calcaneal inversion and eversion at the STJ. Depending on the amount of muscle activation in each gastrocnemius head (Arndt et al., 1999b) and the inversion/eversion position of the calcaneus (Lee and Piazza, 2008) each head can contribute differently to inversion or eversion of the calcaneus. Specifically, an increased activation of GM causes an inversion moment at the calcaneus, while increased activation of GL causes an eversion moment in an *in vitro* experiment when the foot is flat on the ground (Arndt et al., 1999a). In an *in vivo* experiment, however, Lee and Piazza (2008) found that this is only true with the calcaneus in an everted position, and both gastrocnemius heads become stronger inverters the more the calcaneus is in an inverted position.

The amount of inversion or eversion of the calcaneus may result in an alteration in the MTU behaviour of GM and GL. Joint dependent changes in muscle belly architecture of GM have previously been demonstrated during passive plantarflexion movements in relation to knee joint position but not in relation to STJ position (Hodson-Tole et al., 2016). Lersch et al. (2012) demonstrated that inversion and eversion of the calcaneus leads to differential displacement of tendinous tissue within the proximal and free AT and Vieira et al. (2013) showed a dependency of plantarflexion and inversion movement during contraction of GM.

To date, published studies have not investigated whether inversion/eversion of the calcaneus at the STJ leads to an alteration in MTU behaviour of GM and GL during muscle contraction. During an isometric plantarflexion contraction, fascicles shorten accompanied by an increase in pennation angle (Maganaris et al., 1998) and tendon elongation (Ito et al., 1998). Fascicle shortening, pennation angle increase and tendon elongation may be altered in relation to inversion/eversion of the calcaneus.

The amount of inversion/eversion of the calcaneus during relaxed standing (calcaneal posture) is expressed as the angle between the bisection of the calcaneus with the vertical (see Chapter 3). It varies widely between individuals with calcaneal angle values ranging from 2° of inversion to 15° of eversion (Sobel et al., 1999). Furthermore, the calcaneus adopts inversion and eversion positions (calcaneal position) during gait. Inversion and eversion of the calcaneus has been linked to AT injuries. For example, excessive eversion of the calcaneus during relaxed standing has been associated with the occurrence of Achilles tendinitis (Kaufman et al., 1999), while the amount of calcaneal inversion and eversion during running has been linked to AT rupture (Leppilahti and Orava, 1998) and AT overuse injuries (Kvist, 1994); see section 2.5.

Inversion/eversion of the calcaneus has been shown to cause non-homogenous strain distribution in the AT (Lersch et al., 2012) which may contribute to AT injury (Bojsen-Møller and Magnusson, 2015; Maganaris et al., 2004), but the mechanisms behind this relationship still remain unclear. An excessive inverted/everted posture of the calcaneus during relaxed standing or excessive inversion/eversion positions of the calcaneus during gait may alter the contractile behaviour and loading of the MTUs of GM and GL. It is currently not known whether fascicle behaviour of GM and GL during a plantarflexion contraction differs between individuals with different calcaneal postures or whether fascicle behaviour is altered when performing plantarflexion contractions in different calcaneal positions. Similarly, it is not known whether tendon loading and tendon mechanical properties are different in individuals with different calcaneal postures during a plantarflexion contraction or whether tendon loading and mechanical properties are altered when performing plantarflexion contractions in different calcaneal positions.

The purpose of the study presented in this chapter was to investigate (a) whether fascicle behaviour and tendon loading of GM and GL during a plantarflexion contraction are different in individuals with different calcaneal postures, and (b) whether fascicle behaviour and tendon loading of GM and GL are altered during plantarflexion contractions in different calcaneal positions.

4.2 Methods

4.2.1 Participants

33 participants (age 37.8 ± 10.4 years, height 175 ± 9.7 cm, body mass 75.2 ± 15.6 kg) between the age of 18 and 60 years and with no musculoskeletal injury to their foot, ankle or lower leg within the previous six months gave their informed consent to participate in the experiment. The protocol and procedures were approved by the ethics committee of the Department of Exercise and Sport Science and conformed to the Declaration of Helsinki. Prior to testing, the participants were informed of all the procedures involved and familiarised with

the equipment. Written informed signed consent was obtained from each participant prior to taking part in the study.

4.2.2 Experimental setup

Participants were asked to lie prone on the bench of an isokinetic dynamometer (HUMAC®, NORM™ 770, Computer Sports Medicine Inc., Stoughton, MA, USA) with their hips and knees fully extended. The dynamometer was controlled by HUMAC NORM™ software (HUMAC2015, v. 15, Computer Sports Medicine Inc., Stoughton, USA) running on a dedicated personal computer. A custom-built footplate that allowed rotation of the foot in the frontal plane (inversion/eversion) was mounted onto the foot adapter of the dynamometer (Fig. 4.1). This footplate allowed positioning of the foot in neutral, inverted or everted position at any selected angle. The participants' left foot was placed onto the footplate at a talocrural joint angle of 90° between foot and lower leg. The talocrural joint COR (here identified as the lateral malleolus) was carefully aligned with the dynamometer axis of rotation. Calcaneal position was altered by rotating the footplate to the desired angle, where 0° represents a neutral calcaneal position, -10° an inverted calcaneal position and 10° an everted calcaneal position. To limit relative movement of the talocrural joint during plantarflexion efforts, the ankle was firmly strapped into place.

Fascicle information of GM and GL was obtained with B-mode ultrasound (LogicScan 128 EXT-1Z, TLEMED, Vilnius, Lithuania). A 50 mm ultrasound transducer (LV.7.5/60/128Z-2) was placed over the mid-portion of the muscle belly. The MTJ of GM and GL was also imaged using B-mode ultrasound (Echo Blaster, TELEMED, Vilnius, Lithuania) with a 50 mm ultrasound transducer (LV.7.5/60/128Z-2). Hyperechoic gel was applied to both transducers to improve image quality. Each transducer was inserted into a custom-made holder and strapped firmly to the lower leg using elastic bandage (3M Coban™, Neuss, Germany).

Isometric plantarflexion torque from the dynamometer was sampled as voltage output at 2000 Hz using a data acquisition box (USB 6210, National Instruments Corp., Austin, TX, USA) and custom-written software (LabView 8.6, National Instruments Corp.) running on a laptop computer. The data acquisition box was connected to this laptop via USB. The LogicScan ultrasound device was connected to a laptop computer and videos were recorded using Telemed software (Echowave II 3.5.2, TELEMED, Vilnius, Lithuania) at 40 Hz. Videos were saved to the hard drive of the laptop in *.tvd format. The Echo Blaster ultrasound device was connected to another laptop computer and videos were recorded using Telemed software (Echowave 3.5.9, TELEMED, Vilnius, Lithuania) at 40 Hz. Videos were saved to the hard drive of the laptop in *.avi format. Both ultrasound systems were connected to the data acquisition box. All systems were synchronised with a single trigger control. This trigger was connected to both ultrasound laptops via USB. Pressing of the trigger started acquisition of the ultrasound videos. Upon start of the acquisition, both ultrasound devices output a square-wave voltage signal, which was fed into the data acquisition box. To start a trial, torque data acquisition was started in LabView followed by pressing the synchronisation switch to start ultrasound acquisition. A delay of approx. 200 ms was measured between the start of data acquisition of the LogicScan ultrasound and the remaining systems. This delay was calculated as the temporal difference in the trigger signal between the Echo Blaster ultrasound and the LogicScan ultrasound and taken into account for the analysis of the data.

4.2.3 Experimental procedure

Calcaneal posture was assessed in frontal plane MRI scans of the participants' left rearfoot as described in Section 2.2. Prior to testing, resting length of the GM and GL tendon was determined in neutral calcaneal position under ultrasound guidance with the participants' foot secured to the foot adapter of the dynamometer at a right angle to the lower leg as had been done previously (Waugh et al., 2012). A small strip of tape was placed over the ultrasound transducer to provide a visible marker in the ultrasound image that was used to identify the MTJ of GM and GL, respectively, as well as the insertion of the AT on the calcaneus. All three reference points were marked on the participants' skin with a marker pen. The length of GM and GL tendon was measured as the distance between the AT insertion and the GM MTJ and the GL MTJ, respectively, using a tape measure.

To determine the behaviour of the GM and GL MTU, participants performed ramped isometric plantarflexion contractions on the isokinetic dynamometer. As pre-conditioning, the participants performed five isometric submaximal plantarflexions (Maganaris, 2003) with the foot in a neutral position (neither inverted nor everted and foot at a right angle to the lower leg). Subsequently, the individual maximum voluntary contraction (MVC) level of each participant was determined in two maximum effort isometric plantarflexion contractions in neutral calcaneal position, which were averaged to obtain the individual plantarflexion MVC level. If the torque of two MVC trials differed by more than 5%, the trial was repeated and the torque of the two most similar trials was averaged. Achieved torque values were displayed and noted for each plantarflexion MVC trial. The participants then performed a set of six ramped isometric plantarflexion trials to 70% of their individual MVC level, during which fascicle behaviour and MTJ displacement of GM were imaged using ultrasound. The first two plantarflexion trials were performed with the foot in neutral position, followed by two trials with the foot in an inversion position and two trials with the foot in an eversion position. During each trial, participants gradually increased the effort over four seconds. Visual feedback was provided in real-time to the participants, who were instructed to keep the target torque line between two dots placed ± 5% of the target torque. The participants were given a resting period of at least two minutes between each trial to avoid fatigue. After the first set of plantarflexion trials, participants were asked to perform another plantarflexion MVC to confirm the absence of fatigue. This was followed by a second set of six plantarflexion trials, during which fascicle behaviour and MTJ displacement of GL were imaged using ultrasound. The order of trials in neutral, inversion and eversion position was the same as described above.

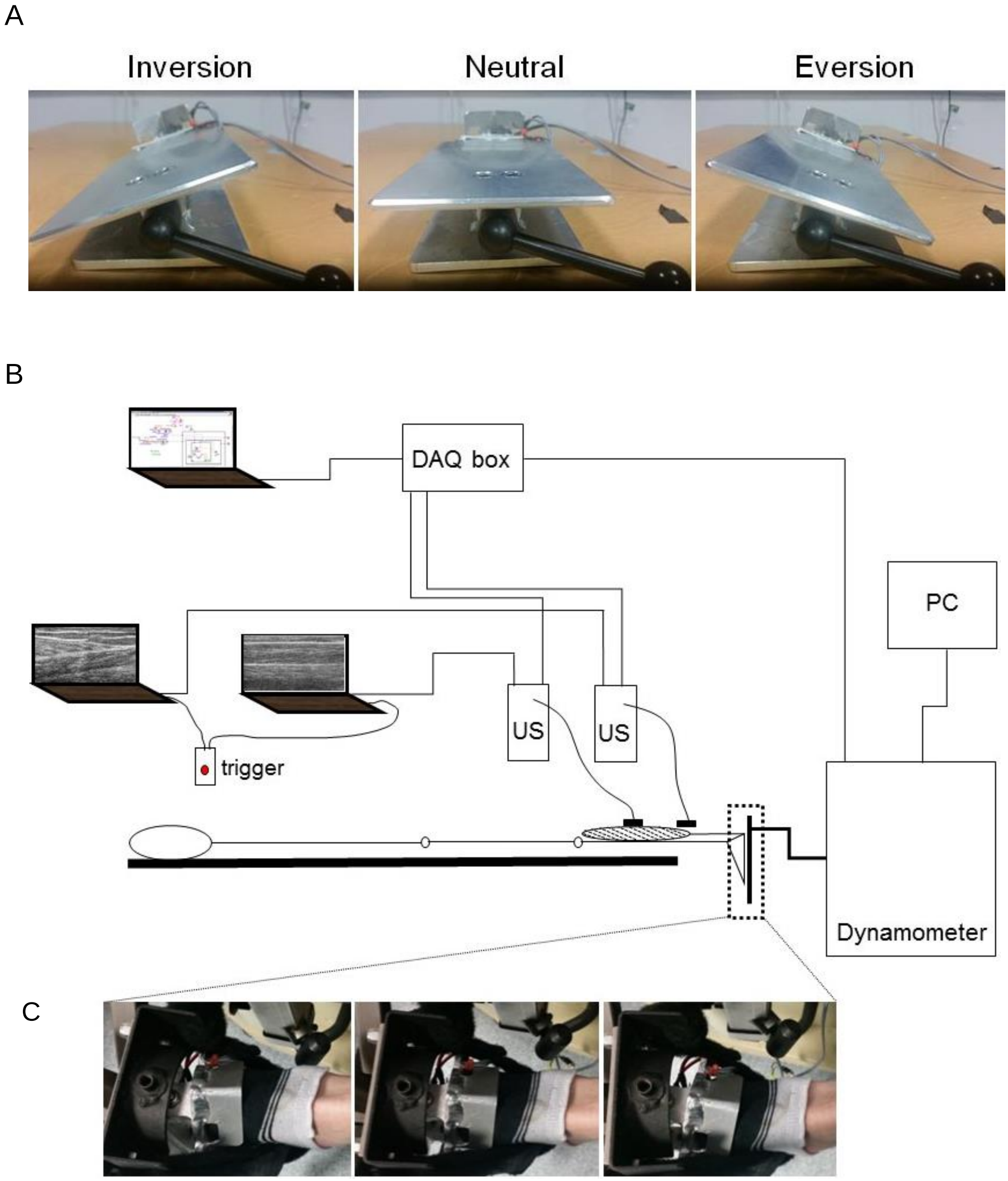


Figure 4.1. (A) The custom-made footplate dismounted from the dynamometer foot adapter. (B) Experimental setup showing the DAQ box, dynamometer and both ultrasound (US) systems. (C) Enlargement of the foot-plate mounted to the dynamometer foot adapter and a participant's foot in eversion, neutral and inversion position (left to right).

4.2.4 Data processing and analysis

Torque data from the dynamometer were sampled as voltage and processed with custom-written Mathematica code (v. 10.2, Wolfram, Champaign, IL, USA). The voltage-to-torque calibration was determined by plotting the MVC torque recorded with the dynamometer for each trial of every participant against the associated voltage values. A linear least-squares fit was applied to determine the conversion between voltage and torque (Fig. 4.2).

$$torque = -111.219 \times voltage - 2.4 \qquad \text{(Equation. 4.1)}$$

Raw voltage was converted into raw torque data using Equation 4.1. The converted torque data was then low-pass filtered for further processing using the built-in LowpassFilter function in Mathematica with a cut-off frequency of 5 Hz.

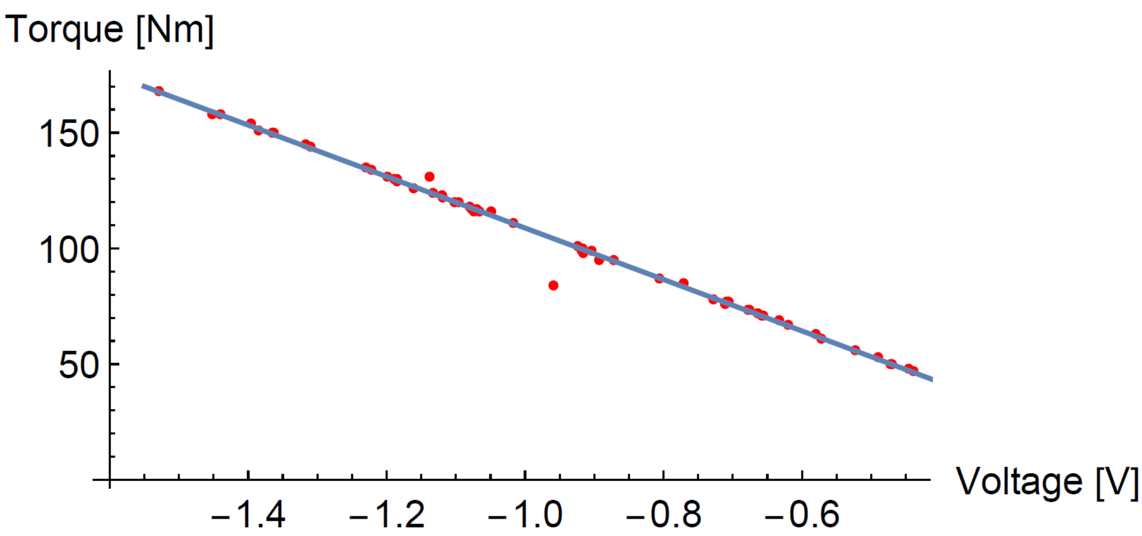
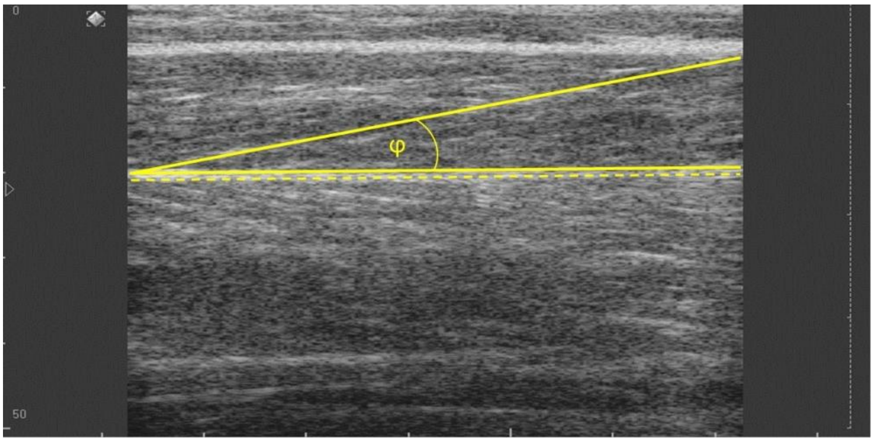


Figure 4.2. Torque-voltage scatter plot and linear fit graph.

Ultrasound videos of the muscle belly were analysed using automatic tracking software (UltraTrack, v.4.1, Farris and Lichtwark (2016)) to obtain fascicle length and pennation angle information for each frame over the entire trial. The validity and reliability of this approach were reported to be high (Cronin et al., 2011).

A



B

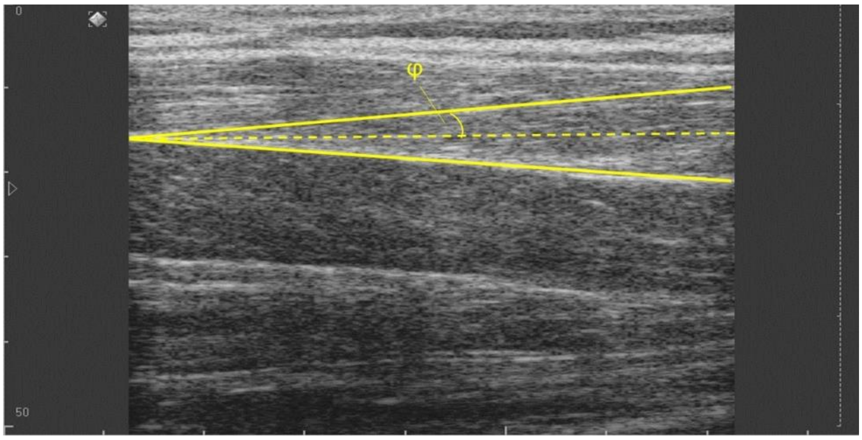


Figure 4.3. UltraTrack determines pennation angle φ as the angle between the fascicle and the horizontal image axis. If the aponeurosis is aligned with the horizontal image axis (A), φ is the same as the angle between the fascicle and the aponeurosis. If the aponeurosis is at an angle to the horizontal image axis (B), φ is underestimated.

With the software, a region of interest (the muscle of interest) is defined with the boundaries being the left and right edge of the video image as well as the deep and superficial aponeuroses of the muscle of interest. A fascicle is then defined within this region of interest. The software tracks the end points of the defined fascicle in the series of frames of the video using an affine extension to a Lucas-Kanade optical flow algorithm. Tracking data output contains fascicle length and pennation angle. The pennation angle provided is the angle between the defined fascicle and the horizontal image axis and not the anatomical pennation angle (Fig. 4.3). Therefore, changes in the orientation of the aponeuroses during contraction did not affect the change of pennation angle in contrast to studies that define pennation angle as the angle formed between the fascicle and the aponeurosis (Héroux et al., 2016; Maganaris et al., 1998). If fascicles extended outside the image, total fascicle length was estimated as a function of pennation angle and muscle thickness:

$$L_F = \frac{d}{\sin\varphi} \qquad \text{(Equation. 4.2)}$$

Where L_F is estimated fascicle length, d is muscle thickness and φ is pennation angle. Muscle thickness was obtained manually in the Telemed software (Echowave II 3.5.2, TELEMED, Vilnius, Lithuania) and pennation angle provided by the UltraTrack software was used.

After extracting information about fascicle length and pennation angle from ultrasound videos of the muscle belly, a frame-time correction was applied. Ultrasound devices generally provide the sampling frame rate as one single value. However, frames are not sampled at constant intervals and manufacturers of ultrasound devices have begun to provide information about inter-frame intervals in video header files. Seynnes et al. (2015) discuss problems arising from insufficiently timed ultrasound data acquisition and Finni et al. (2012) report that calculations of AT hysteresis can be significantly affected due to an offset between the acquisition of torque data and ultrasound data. The offset of ultrasound data with and without frame-time correction was recently shown to be as high as four times the duration between two consecutive frames for an acquisition duration of 5 s (Miguez et al., 2017).

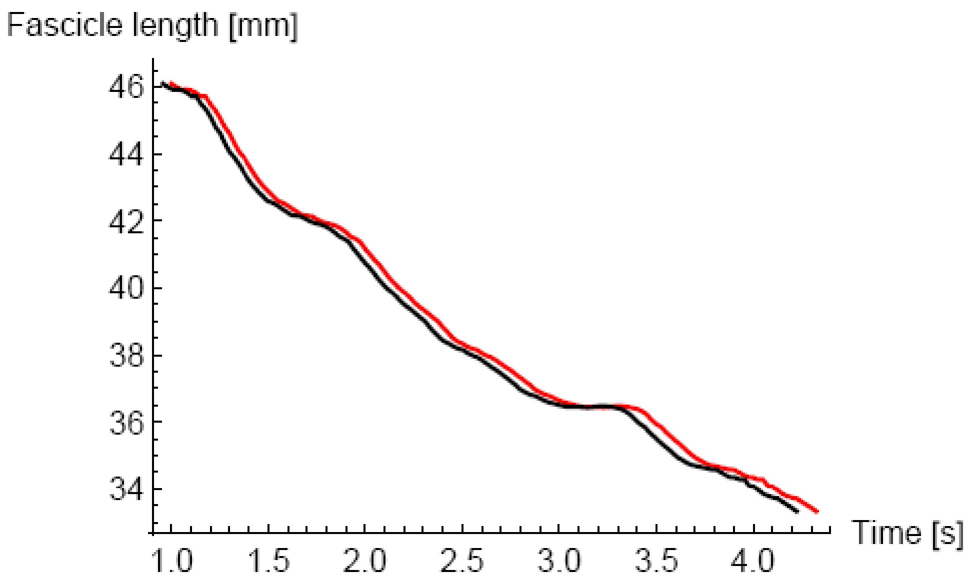


Figure 4.4. Fascicle length for one participant plotted over time with (black) and without (red) frame-time correction.

The LogicScan ultrasound device and Telemed software (Echowave II 3.5.2, TELEMED, Vilnius, Lithuania) were used to acquire ultrasound videos of the muscle belly in *.tvd format. This file format contains information about inter-frame intervals, which can be accessed. Custom-written Mathematica code (v. 10.2, Wolfram, Champaign, IL, USA) based on a publically available Matlab code (available at http://www.telemedultrasound.com/science-research/) was used to correct frame time information. The offset due to variable inter-frame intervals is depicted in Figure. 4.4.

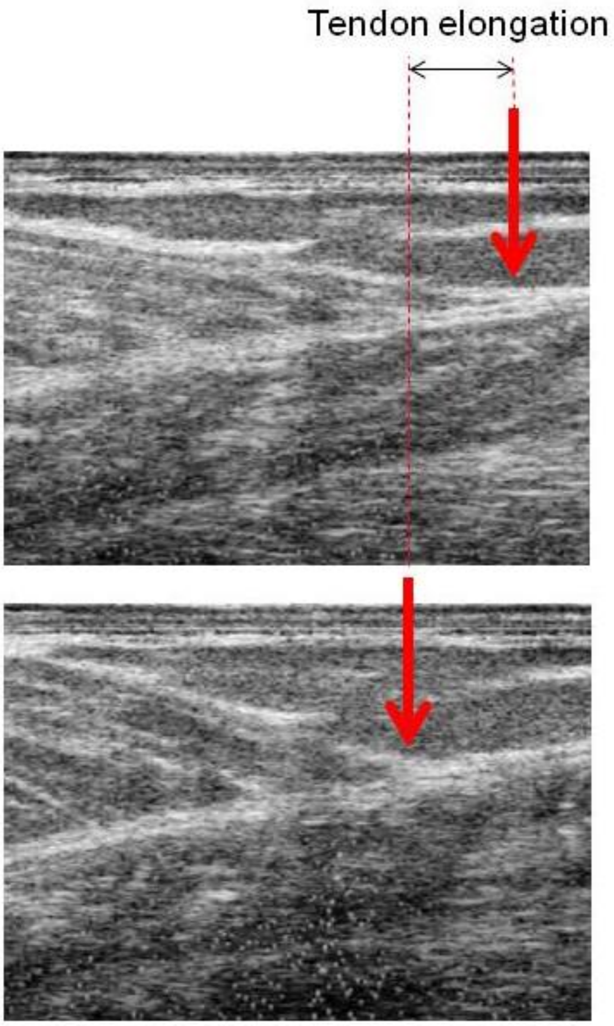


Figure 4.5. Ultrasound images of the MTJ at rest (top) and at a contraction level of 70% MVC (bottom).

Tendon elongation was measured as proximal displacement of the MTJ recorded by the second ultrasound imaging device (Fig. 4.5). MTJ displacement was determined for each frame over the entire trial with custom-written Matlab code (Cunningham, personal communication) using the point tracker function, which uses pyramidal implementation of a Kanade-Lucas-Tomasi feature tracking algorithm. Automated tracking of the displacement of the MTJ has been performed previously with a similar algorithm (Peltonen et al., 2012; Magnusson et al., 2003) and the repeatability of this method was reported to be 98% (Magnusson et al., 2003). To track the MTJ displacement, a point was placed near the muscle tendon junction inside the muscle belly. A square of 21 x 21 pixels was then created around this point, inside which features were tracked. The point coordinates in vertical (y) and horizontal (x) direction were recorded in each frame as pixel positions and then converted into millimetres. The image size of the ultrasound videos was 512 x 512 pixels corresponding to 50 mm x 50 mm giving a pixel size of 0.098 mm x 0.098 mm. Displacement was calculated as

$$d = \sqrt{(x_{P_1} - x_{P_2})^2 + (y_{P_1} - y_{P_2})^2} \qquad \text{(Equation. 4.3)}$$

Where: d … point (MTJ) displacement

x_{P1} … x-coordinate of the point in the first frame

x_{P2} … x-coordinate of the point in the following frame

y_{P1} … y-coordinate of the point in the first frame

y_{P2} … y-coordinate of the point in the following frame

Ultrasound videos of the MTJ were captured in *.avi format. This video file format does not contain information about inter-frame intervals. It was, therefore, not possible to perform a frame time correction for ultrasound videos of the MTJ

Data for converted torque, fascicle length, pennation angle and tendon elongation were saved in separate text files and further analysed using custom-written Mathematica code (v. 10.2, Wolfram, Champaign, USA). Torque, fascicle length, pennation angle and tendon elongation data were extracted from torque onset to 70% MVC and then time normalised. Torque data from onset to 70% MVC consisted of approximately 8,500 to 9,500 data points so that all data were time-normalised by interpolating to 10,000 data points.

To identify possible probe movement during the trials, tendon elongation data were plotted over torque (Fig. 4.6). Trials were discarded if probe movement occurred. This procedure was not necessary for fascicle data since measurements of fascicle length and pennation angle are related to absolute reference points within one single frame and, therefore, are affected the same by possible transducer movement.

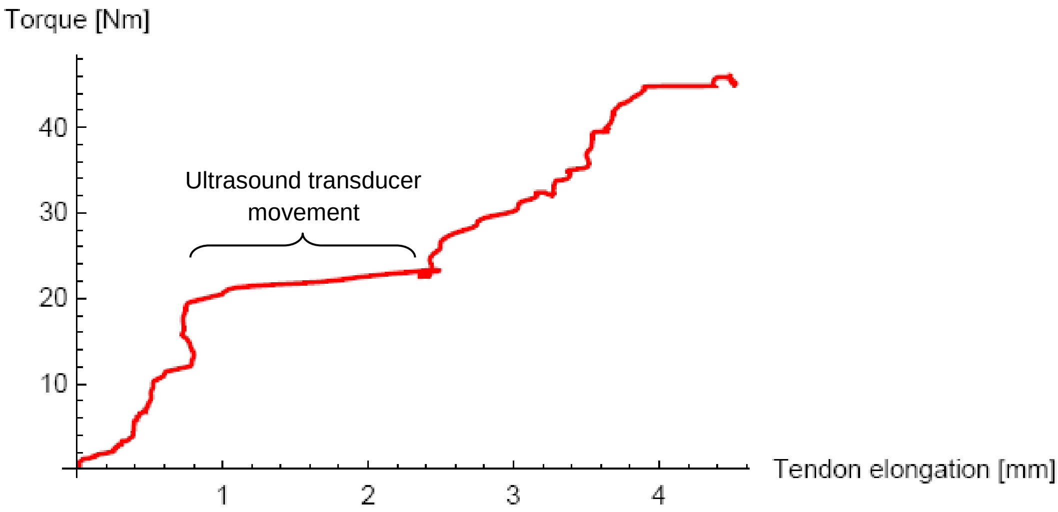


Figure 4.6. Example of a discarded trial due to ultrasound transducer movement. Movement of the probe is indicated by elongation of the tendon with no increase in torque.

Fascicle length, pennation angle and tendon elongation were determined from rest to 70% MVC in torque intervals of 10%. The data points corresponding to each torque interval were identified and then averaged with the preceding 100 data points and 100 subsequent data points to average possible local fluctuations.

Tendon stiffness of GM and GL was calculated as stiffness of the global tendon including proximal and free AT. First, torque was converted into tendon force using the AT moment arm values of each individual obtained from MRI scans (Section 4.3) as

$$F_{AT} = \frac{M_{PF}}{L_{AT}} \qquad \text{(Equation 4.4)}$$

Where F_{AT} is AT force, M_{PF} is plantarflexion torque measured by the dynamometer, and L_{AT} is length of the AT moment arm. Tendon force specific to

GM and GL was calculated based on the values provided by Albracht et al. (2008) as

$$F_{GM} = 0.26 \times F_{AT} \qquad \text{(Equation 4.5)}$$

$$F_{GL} = 0.12 \times F_{AT} \qquad \text{(Equation 4.6)}$$

where F_{GM} is tendon force specific to GM, and F_{GL} is tendon force specific to GL. Tendon force specific to GM and GL was then plotted over tendon elongation of GM and GL, respectively. Tendon stiffness was calculated as the slope of the least-squares linear fit from 10% MVC to 70% MVC (Fig. 4.7) (Peltonen et al., 2012).

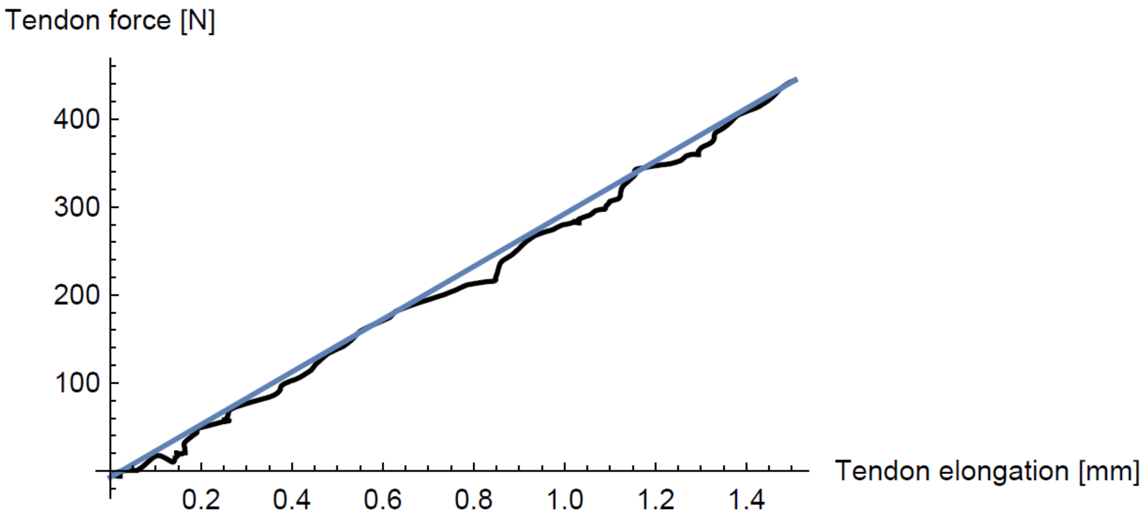


Figure 4.7. Force-elongation curve for GM of one participant (black) and line of linear fit (blue).

Dataset for calcaneal position

To determine the effect of calcaneal position on fascicle behaviour and tendon loading of GM and GL, data sets from all participants were considered. Complete datasets from nine participants were rejected due to probe movement, incomplete trials or considerable failure to follow the visual feedback provided. Out of the remaining 24 datasets, the best trial of each condition was selected resulting in six trials being analysed for each participant. The best trial out of two was accepted to be the trial where the participant followed the provided feedback most closely. Fascicle length, pennation angle and tendon elongation of GM and GL were compared in three different calcaneal positions (0° neutral, -10° inversion, 10° eversion).

Dataset for calcaneal posture

To determine the effect of calcaneal posture on fascicle behaviour and tendon loading of GM and GL, the datasets of the 24 participants remaining after rejecting unsuccessful trials were considered. Participants were grouped into three calcaneal posture groups (neutral, inverter, everter) based on the calcaneal angle measurements obtained using the MRI-based method proposed in Chapter 2. The intra-tester reliability of this method was shown to be high with an intra-class correlation (ICC) coefficient of 0.91. In clinical practice a person's rearfoot posture is classified based on the calcaneal angle. The calcaneal angle is assessed with the participant standing in RCSP and a calcaneal angle between 2° of inversion and 2° of eversion has been classified as neutral (Razeghi and Batt, 2002). However, the findings discussed in Chapter 3 showed that the agreement between the RCSP method and the MRI-based method proposed in the same chapter is poor (ICC = 0.075). Therefore, it was not possible to group the participants of this study following this classification scheme. Instead, five participants with the highest degree of calcaneal inversion were included in an inverter group, five participants with the highest degree of calcaneal eversion were included in an everter group and five participants with a calcaneal posture closest to zero were included in a neutral group resulting in a datasets of 15 participants to be included. This was done to investigate fascicle length, pennation angle and tendon elongation of GM and GL in individuals with the largest and smallest calcaneal angles. Fascicle length, pennation angle and tendon elongation of GM and GL were determined in neutral calcaneal position and were compared between three different calcaneal posture groups (neutral, inverter, everter).

4.2.5 Statistical analyses

Statistical analysis of the data was performed using SPSS (v. 22.0.0.1, IBM Corporation, USA). Data for GM and GL fascicle length, pennation angle, tendon elongation and tendon stiffness are presented. All variables were tested for normality using the Kolmogorov-Smirnov test of normality and Levene's test of equal variances. The data met parametric assumptions and are shown as means ± S.D. P

Differences in plantarflexion MVC torque before the first set of six submaximal isometric plantarflexion contractions and before the second set of submaximal isometric plantarflexion contractions were determined with a paired-samples two-tailed t-test.

To determine the effect of calcaneal posture, differences in fascicle length, pennation angle, tendon elongation and tendon stiffness of GM and GL were compared at all contraction levels between the three calcaneal posture groups (neutral, inverter, everter) with the foot in a neutral position on the foot-plate. To determine the effect of calcaneal position, differences in fascicle length, pennation angle, tendon elongation and tendon stiffness of GM and GL were compared at all contraction levels between the three calcaneal positions (0° neutral, -10° inversion, 10° eversion).

Differences in fascicle length, pennation angle and tendon elongation between contraction levels were determined with a Repeated-Measures ANOVA. Between-position and between-posture differences were determined with a One-Way ANOVA with Bonferroni *post hoc* adjustment, if statistical significances were found. The significance level for all tests was set at $\alpha=0.05$.

A *post hoc* power analysis was conducted in G*Power (v. 3.1.9.2, Universität Kiel, Germany). Fascicle length and pennation angle at rest, tendon elongation at 10% MVC as well as tendon stiffnesses were considered for the analysis.

Statistical power was determined for comparisons of these variables between the calcaneal postures and positions.

4.3 Results

4.3.1 Participant characteristics and measurements at rest

Calcaneal posture for all 24 participants included in the analysis was 0.85 ± 4.62°. Calcaneal posture for the neutral, inverter and everter groups was -0.04 ± 0.51°, -4.96 ± 0.94° and 6.49 ± 3.05°, respectively. Calcaneal posture differed significantly between the three groups ($p < 0.05$). Average plantarflexion MVC torque of all 24 participants was 101.03 ± 35.15 Nm before the first set of six ramped plantarflexion trials and 102.22 ± 34.03 Nm before the second set of six ramped plantarflexion trials. There was no difference in plantarflexion MVC torque from the first to second set of six MVCs ($p > 0.05$). Measurements of fascicle length, pennation angle and tendon length at rest differed significantly between GM and GL (all $p < 0.001$) and are presented in Table 4.1. A statistical power of 100% was achieved for all comparisons.

Table 4.1. Morphological measures of MTJ architecture of GM and GL at rest. (*) denotes a significant difference to GM ($p < 0.001$).

Variable	GM	GL
Fascicle length [mm]	50.9 ± 7.39	109.8 ± 46.81*
Pennation angle [°]	16.1 ± 5.0	10.0 ± 4.3*
Tendon length [mm]	213.6 ± 28.0	250.5 ± 29.0*

4.3.2 Effects of calcaneal position on muscle-tendon unit behaviour of medial and lateral gastrocnemius

There was no difference in GM and GL fascicle length between the neutral, inversion and eversion position at all contraction levels (Fig. 4.8A). In all calcaneal positions, GM fascicle length decreased from rest to 30% of plantarflexion MVC by between 8 mm and 9 mm ($p < 0.05$), after which there was no further decrease in fascicle length. For comparison of GM fascicle length at rest between the three calcaneal positions, a statistical power of 10% for an effect size of 0.09 was achieved at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 1161 would be required. GL fascicle length decreased from rest to 40% of MVC by between 30 mm and 37 mm ($p < 0.05$), after which there was no further change in GL fascicle length ($p > 0.05$). For comparison of GL fascicle length at rest between the three calcaneal positions, a statistical power of 15% for an effect size of 0.13 was achieved at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 537 would be required.

GM and GL pennation angle did not differ between the neutral, inversion and eversion positions at all contraction levels (Fig. 4.8B). In all calcaneal positions, GM pennation angle increased from rest to 40% MVC of plantarflexion MVC by 6.3° and 7.0° ($p < 0.05$), after which there was no further change in GM pennation angle. For comparison of GM pennation angle at rest between the three calcaneal positions, a statistical power of 8% for an effect size of 0.08 was achieved at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 1488 would be required. GL pennation angle increased from rest to 40% MVC by between 3.1° and 4.0° ($P > 0.05$), after which there was no further change in GM pennation angle ($P > 0.05$). For comparison of GL pennation angle at rest between the three calcaneal positions, a statistical power of 11% was achieved for an effect size of 0.12 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 831 would be required.

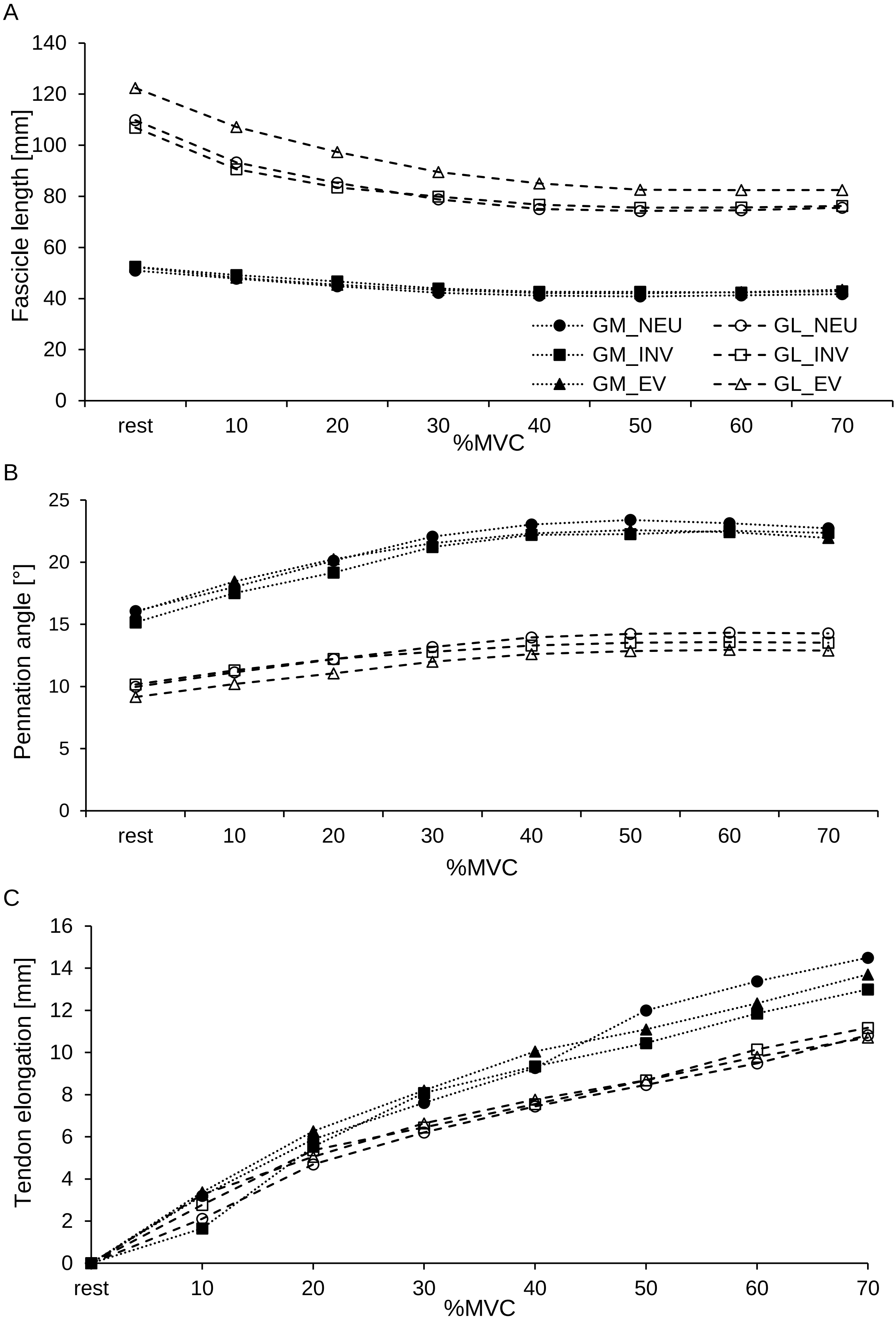


Figure 4.8. Change in fascicle length (A), pennation angle (B) and tendon elongation (C) of GM and GL from rest to 70% MVC. Error bars were omitted for clarity.

There was no difference in GM tendon elongation between the neutral, inversion and eversion positions at all contraction levels and in GL tendon elongation between the neutral, inversion and eversion positions at all contraction levels (Fig. 4.8C). In all groups, the GM and GL tendons lengthened from rest to 70% MVC by between 13 mm and 14 mm ($p < 0.05$), and GL tendon lengthened from rest to 70% MVC by between 10 mm and 11 mm ($p < 0.05$). For comparison of GM tendon elongation at 10% MVC between the three calcaneal positions, a statistical power of 68% was achieved for an effect size of 0.33 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 93 per group would be required. For comparison of GL tendon elongation at 10% MVC between the three calcaneal positions, a statistical power of 31% was achieved for an effect size of 0.21 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 234 per group would be required.

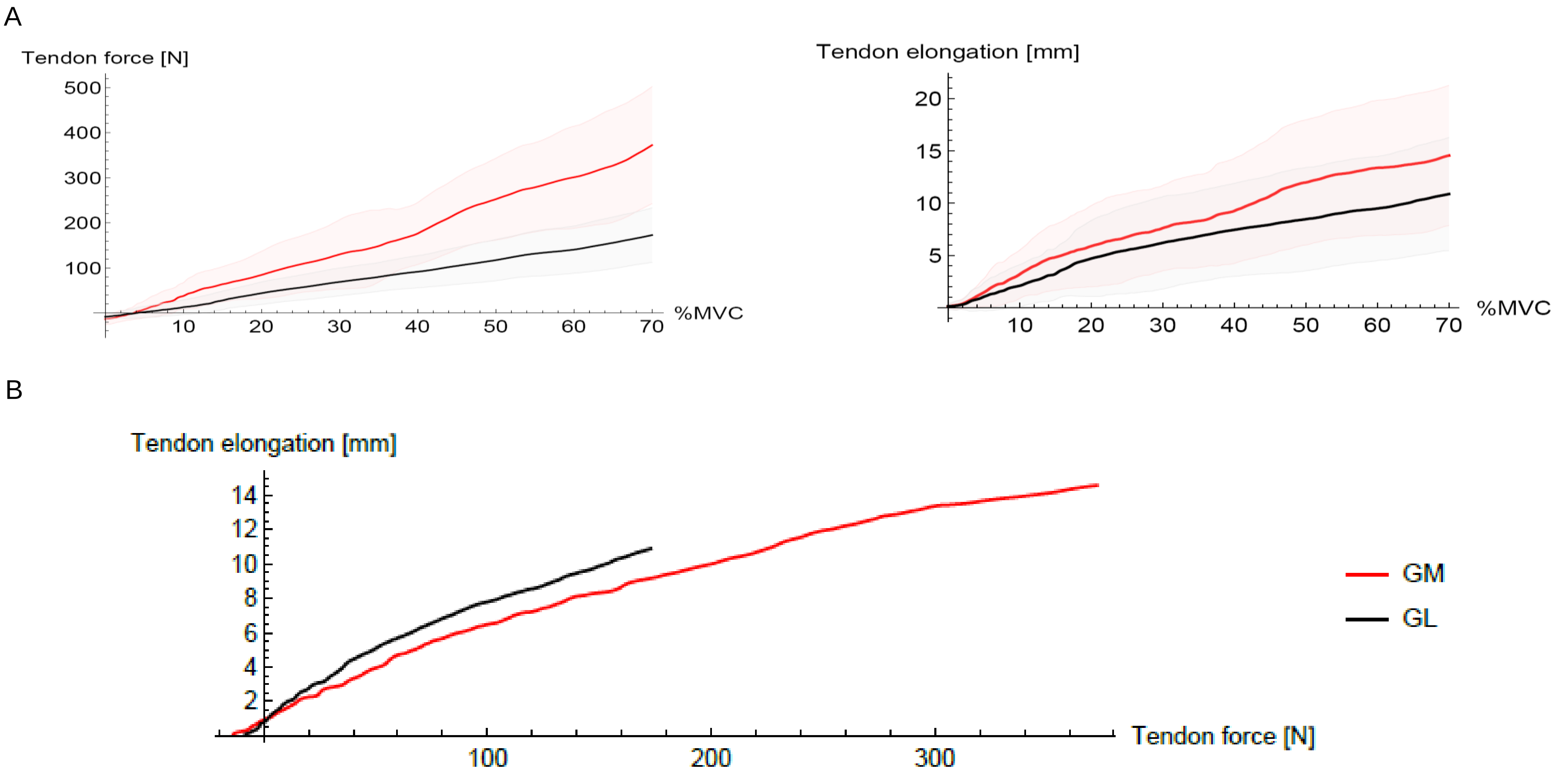


Figure 4.9. (A) Mean tendon force and elongation of GM and GL from rest to 70% MVC with S.D clouds for all participants in neutral calcaneal position. (B) Mean tendon elongation of GM and GL plotted over mean tendon force of GM and GL.

Tendon force of GM at 70% MVC was 372.38 ± 129.69 N in calcaneal neutral position. GM tendon elongation at the same contraction level was 14.5 ± 6.69 mm (Fig. 4.9). GM tendon stiffness was 35.94 ± 27.22 N/mm, 42.14 ± 31.08 N/mm and 39.4 ± 17.97 N/mm for the calcaneal neutral, inversion and eversion positions, respectively. The difference in GM tendon stiffness was not statistically different between the calcaneal positions ($p = 0.646$). For comparison of GM tendon stiffness between the three calcaneal positions, a statistical power of 11% was achieved for an effect size of 0.10 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 867 would be required. Tendon force of GL at 70% MVC was 172.87 ± 60.37 N for all participants. GL tendon elongation at the same contraction level was 11.17 ± 5.93 mm (Fig. 4.9). GL tendon stiffness was 24.18 ± 15.85 N/mm, 23.06 ± 9.97 N/mm and 25.16 ± 12.27 N/mm for the calcaneal neutral, inverter and everter groups, respectively. The difference in GL tendon stiffness was not statistically different between the groups ($p = 0.853$). For comparison of GL tendon stiffness between the three calcaneal positions, a statistical power of 8% was achieved for an effect size of 0.07 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 1878 would be required.

4.3.3 Effects of calcaneal posture on muscle-tendon unit architecture of medial and lateral gastrocnemius

There was no difference in GM and GL fascicle length between the neutral, inverter and everter groups at all contraction levels (Fig. 4.10A). In all groups, GM fascicle length decreased from rest to 40% of plantarflexion MVC by between 9 mm and 13 mm ($p < 0.05$), after which there was no further decrease in fascicle length. For comparison of GM fascicle length at rest between the three calcaneal postures, a statistical power of 5% for an effect size of 0.04 was achieved at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 5166 would be required. GL fascicle length decreased from rest to 50% of MVC by between 19 mm and 20 mm ($p < 0.05$), after which there was no further change in GL fascicle length ($p > 0.05$). For comparison of GL fascicle length at rest between the three calcaneal postures, a statistical power of 6% for an effect size of 0.08 was achieved at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 1458 would be required.

GM and GL pennation angle did not differ between the neutral, inverter and everter groups at all contraction levels (Fig. 4.10B). In all groups, GM pennation angle increased from rest to 40% MVC of plantarflexion MVC by between 5.8° and 8.4° ($p < 0.05$), after which there was no further change in GM pennation angle. For comparison of GM pennation angle at rest between the three calcaneal postures, a statistical power of 19% for an effect size of 0.38 was achieved at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 72 would be required. GL pennation angle increased from rest to 20% MVC by 1.8° and 2.7° ($p > 0.05$), after which there was no further change in GM pennation angle ($p > 0.05$). For comparison of GL pennation angle at rest between the three calcaneal postures, a statistical power of 15% for an effect size of 0.31 was achieved at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 102 would be required.

There was no difference in GM tendon elongation between the neutral, inverter and everter groups at all contraction levels and in GL tendon elongation between the neutral, inverter and everter groups at all contraction levels (Fig. 4.10C). In all groups, the GM tendon lengthened from rest to 70% MVC by between 11 mm and 17 mm ($p < 0.05$), and the GL tendon lengthened from rest to 70% MVC by between 7 mm and 12 mm ($p < 0.05$). A statistical power of 42% was achieved for comparisons of tendon elongation between the three calcaneal postures. For comparison of GM tendon elongation at 10% MVC between the three calcaneal postures, a statistical power of 73% was achieved for an effect size of 0.84 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 18 per group would be required. For comparison of GL tendon elongation at 10% MVC between the three calcaneal postures, a statistical power of 64% was achieved for an effect size of 0.77 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 21 per group would be required.

Tendon force of GM at 70% MVC was 394.41 ± 131.49 N for all participants. GM tendon elongation at the same contraction level was 13.29 ± 7.96 mm. GM tendon stiffness was 44.94 ± 20.78 N/mm, 37.38 ± 21.85 N/mm and 23.65 ± 7.34 N/mm for the calcaneal neutral, inverter and everter groups, respectively. The difference in GM tendon stiffness was not statistically different between the groups ($p = 0.201$). For comparison of GM tendon stiffness between the three calcaneal postures, a statistical power of 11% was achieved for an effect size of 0.12 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 831 per group would be required. Tendon force of GL at 70% MVC was 182.29 ± 60.46 N for all participants. GL tendon elongation at the same contraction level was 9.62 ± 5.67 mm. GL tendon stiffness was 28.14 ± 4.97 N/mm, 33.22 ± 32.13 N/mm and 15.59 ± 5.65 N/mm for the calcaneal neutral, inverter and everter groups, respectively. The difference in GL tendon stiffness was not statistically different between the groups ($p = 0.357$). For comparison of GL tendon stiffness between the three calcaneal postures, a statistical power of 60% was achieved for an effect size of 0.73 at the 0.05 α-level. To achieve a high power of 80% (Cohen, 1988) at the given effect size, a sample size of n = 24 per group would be required.

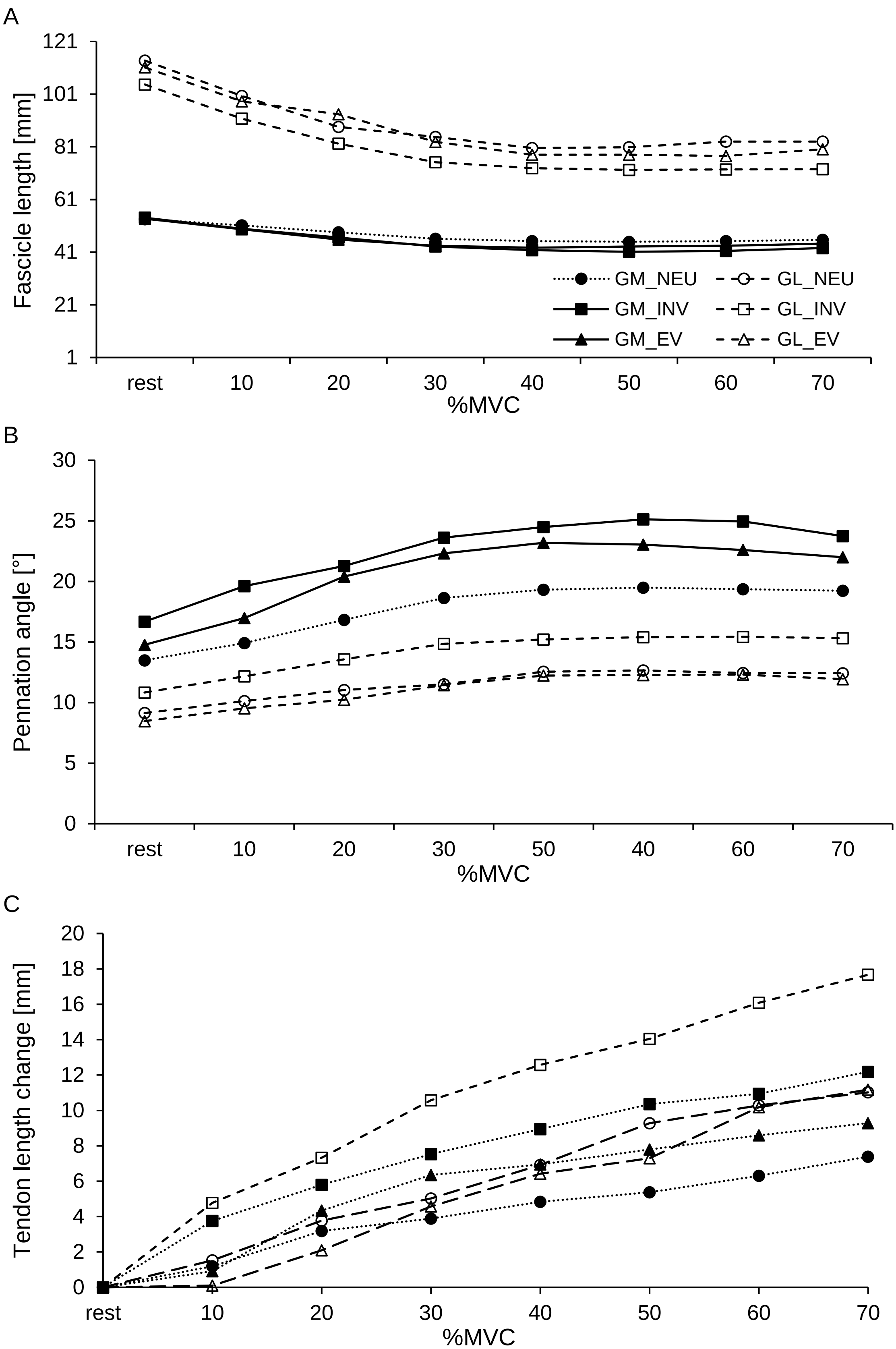


Figure 4.10. Change in fascicle length (A), pennation angle (B) and tendon elongation (C) of GM and GL from rest to 70% MVC. Error bars were omitted for clarity.

4.4 Discussion

4.4.1 Differences in the morphology of the medial and lateral gastrocnemius

The study presented in this chapter compared morphological measures of the GM and GL MTUs in relation to calcaneal inversion and eversion. Descriptive measures of MTU geometry and architecture were also made and showed that GM and GL differ considerably in their anatomical composition. Previous studies have reported that fascicles of GM are shorter than fascicles of GL, while GM pennation angle is larger than GL pennation angle (Héroux et al., 2016; Morse et al., 2005; Maganaris et al., 1998). The results of the present study are in agreement with previously reported values of GM fascicle length and pennation angle of GM and GL. The average fascicle length of GL in this study, however, was 110 mm, which is considerably longer than GL fascicle lengths of previous reports ranging from 63 mm to 70 mm (Héroux et al., 2016; Morse et al., 2005; Maganaris et al., 1998). There is evidence of compartmentalisation within GL (Segal and Song, 2005; Wolf et al., 1993; Segal et al., 1991) and fascicles within this muscle exhibit different lengths and pennation angles (Wakeling, 2009b). In the study presented here, the ultrasound transducer was placed over the “A” head of GL, which exhibits greater fascicle lengths than other regions within GL (Wolf et al., 1993). Previous ultrasound studies reporting fascicle lengths measured in different regions of GL, however, did not find differences (Chow et al., 2000; Maganaris et al., 1998). Furthermore, there was great inter-individual variation in the GL fascicle lengths measured in the present study ranging from 34 mm to 217 mm; such an inter-individual range of GL fascicle length has previously not been reported. The sample size of the study presented in this chapter was sufficiently large and a statistical power of 100% was achieved for comparisons of fascicle lengths between GM and GL. The likelihood of a realistic representation of the population is, therefore, given.

The lengths of the tendons of GM and GL were found to differ considerably in this study with GM tendon being shorter than GL tendon. Little attention has been paid to this point in the literature, and only two studies report length measurements for the muscle bellies of GM and GL (Albracht et al., 2008; Antonios and Adds, 2008). In both of these studies, the GM muscle belly is longer than the GL muscle belly; therefore, the tendon of GM must be shorter than the tendon of GM as is the case in this study. One other study reports that the GM tendon is significantly shorter than the GL tendon (Morrison et al., 2016). It is important to consider the length of a tendon since the amount of strain experienced by a tendon is dependent on its length since tendon strain is an expression of the length change of the tendon with respect to its resting length. Furthermore, the length of a tendon also influences the shortening capability of the entire MTU (Wakeling et al., 2011).

4.4.2 Effects of calcaneal position on muscle-tendon unit behaviour of medial and lateral gastrocnemius

Measures of fascicle length, pennation angle, tendon elongation and tendon stiffness did not differ between the three calcaneal positions (neutral, inversion, eversion). Inversion/eversion of the calcaneus resulted in a change in the resting length of the MTU but it was not possible to accurately measure this change in resting length with the selected methods. Theoretically, inversion of the calcaneus would result in shorting of the GM MTU and lengthening of the GL MTU. The reverse would be true for eversion of the calcaneus.

If the amount of inversion/eversion applied in this study was sufficient to considerably change the resting length of the MTUs, a change in muscle architecture would have been observed. Passive lengthening of the gastrocnemius MTU leads to lengthening of fascicles, a decrease in pennation angle and elongation of the tendon (Oliveira et al., 2015; Abellaneda et al., 2009). Fascicle length and pennation angle at rest, however, were not found to be different in the three calcaneal positions in this study. On the other hand, the change in MTU resting length might have occurred at the tendon level. At shorter lengths, the tendon contribution to MTU lengthening is greater than the contribution of the muscle belly (Herbert et al., 2011). However, the possible length change was probably too small to have an effect on tendon stiffness (Hug et al., 2013).

It is also possible that the amount of inversion/eversion applied in this study was not sufficient to introduce a length change of the GM and GL MTUs. The foot-plate was tilted by 10° to invert and evert the foot, respectively. An inversion/eversion tilt of the foot-plate of 10° was selected as it seems to be within the range of motion of every person (Aström and Arvidson, 1995; Nigg et al., 1990). It is not known, however, whether the amount of inversion/eversion at the calcaneus was indeed 10°. It is reasonable to assume that this was not the case due to the deformation of the calcaneal fat pad. Hence, the amount of inversion/eversion at the calcaneus was unlikely to introduce a considerable resting length change of the GM and GL MTUs.

In addition to the exact amount of inversion/eversion being unknown, one must also consider that the footplate rotated the entire foot in the frontal plane along its long axis. The STJ axis, however, is oriented at an angle to the frontal and transverse plane (see Section 2.3). The response of GM and GL to positioning of the foot along the STJ axis might have been different since deeper muscles in the lower leg as well as GM and GL might have contributed differently to the isometric plantarflexion effort. Support in the literature is sparse but one study (De Ridder et al., 2014) showed that electromyographic activity of lower leg muscles including GM during a balancing task on a uniaxial balance board is altered when the axis of the balance board is changed. Furthermore, the effect of calcaneal position on contractile behaviour of GM and GL during a ramped submaximal isometric plantarflexion contraction was tested on a sample containing individuals with various calcaneal postures. The contractile behaviour of GM and GL during an isometric plantarflexion was not found to differ between individuals with different postures (see Section 5.4.2 above) but it is possible that their response to altering calcaneal position might differ between these groups. Altering calcaneal position does not affect the plantarflexion moment arm of the AT nor was the plantarflexion moment arm of the AT found to be different in individuals with different calcaneal postures (Chapter 2). This suggests that the plantarflexion actions of GM and GL are not affected. The plantarflexion moment arm of deep plantarflexor muscles such as tibialis posterior or flexor hallucis longus might have changed since their plantarflexion and inversion/inversion moment arms are highly variable (Klein et al. 1996, Spoor et al. 1990). Thus, their contribution to plantarflexion efforts might be altered, which might affect the function of GM and GL during plantarflexion contractions.

4.4.3 Effects of calcaneal posture on muscle-tendon unit architecture of medial and lateral gastrocnemius

Measures of fascicle length, pennation angle, tendon elongation and tendon strain did not differ between the three calcaneal posture groups (neutral, inverters, everters). A relationship between rearfoot anatomy and the contractile behaviour of the GM and GL MTUs during isometric plantarflexions cannot, therefore, be established.

Muscle architecture changes with age (Morse et al., 2005) and body mass (Tomlinson et al., 2014), and the behaviour of the gastrocnemius MTU during hopping and running tasks is altered with age (Sano et al., 2015; Hoffrén et al., 2012). The present study, however, showed that the anatomical configuration of the rearfoot (calcaneal posture) is not related to morphological measures of GM and GL MTUs. Excessive eversion of the calcaneus during relaxed standing has been linked to the occurrence of Achilles tendinitis (Kaufman et al., 1999) but this may not be due to a change in MTU behaviour of GM and GL.

An interesting observation was that fascicle length and pennation angle only changed significantly up to approximately 40% MVC. Since continuous tendon elongation was observed at all contraction levels, it is unlikely that fascicles would act isometrically at higher contraction levels. The ultrasound transducers and their holders were firmly strapped around the lower leg to limit probe movement, but it is possible that the pressure exerted in this way may have caused erroneous measurements of fascicle length and pennation angle. Muscle compression due to elastic strapping has been shown to decrease muscle thickness and reduce pennation angle of GM during submaximal plantarflexion contractions (Wakeling et al., 2013) and muscle bulging at higher contraction levels may have increased this effect. Bolsterlee et al. (2015) showed recently that pressure between the ultrasound transducer and the skin can lead to underestimation of pennation angle measurements at rest by approximately 25%. Furthermore, it is also possible that lateral displacement of the muscle belly during contraction (Farris et al., 2012) altered the orientation of the fascicles with respect to the imaging plane.

The study presented in this chapter showed that fascicle behaviour and tendon loading during a submaximal isometric plantarflexion is not different between individuals with different calcaneal postures. Furthermore, fascicle behaviour and tendon loading was not found to change when performing submaximal isometric plantarflexion contractions in different calcaneal positions. These results suggest that non-homogeneous loading of the AT cannot be explained by differences in contractile behaviour of GM and GL due to calcaneal inversion/eversion as has been suggested (Bojsen-Møller and Magnusson, 2015). (Bojsen-Møller and Magnusson, 2015) furthermore suggest that the differential force producing capabilities of GM and GL may contribute to non-homogeneous loading of the AT. The study presented in this chapter showed that GM and GL differ in their architecture and geometry, which may suggest differential behaviour of both gastrocnemii. The following study will address these differences in fascicle behaviour and tendon loading between GM and GL in order to investigate whether these differences could contribute to non-homogenous AT loading.

Chapter Five: Differential behaviour of the gastrocnemius muscle-tendon unit during a submaximal plantarflexion contraction

5.1 Introduction

The study in the previous chapter suggested that the individual behaviour of GM and GL is unaffected by calcaneal inversion and eversion. Differences in the anatomical composition, however, were found suggesting that GM and GL may have different functions. The differential actions of GM and GL have been discussed as a possible cause of non-homogeneous strain in the AT (Bojsen-Møller and Magnusson, 2015) leading to tissue degeneration and subsequently to injury (Kvist, 1994; Kannus and Jozsa, 1991); for a detailed explanation see Section 2.5. GM and GL have often been studied in isolation (e. g. Lichtwark et al. 2007, Ito et al. 1998) and have also been mathematically modelled as one muscle with identical characteristics (Kinugasa et al., 2016). Studies have shown, however, that GM and GL differ considerably in their volume (Kinugasa et al., 2005), muscle belly length (Antonios and Adds, 2008), physiological cross-sectional area (Albracht et al., 2008; Morse et al., 2005), architecture (Héroux et al. 2016, Wolf & Kim 1997), force producing capabilities (Arndt et al., 1998; Albracht and Arampatzis, 2013) and function (Héroux et al., 2014).

GM with its larger physiological cross-sectional area contributes approximately 26% to maximum plantarflexion torque while GL contributes approximately 12% (Albracht et al., 2008). During submaximal plantarflexion contractions, GM shows a higher electromyographic activation than GL (Masood et al., 2014) with a larger relative volume of GM being activated compared to GL (Kinugasa et al., 2005) and Héroux et al. (2014) demonstrated an absence of electromyographic activity of GL during standing suggesting that the actions of GM and GL differ and are task specific. Furthermore, GM and GL exhibit differential inversion/eversion actions at the calcaneus (e. g. Lee and Piazza, 2008; Arndt et al., 1999a; Elftman, 1969). Inversion and eversion are the main actions of deep muscles in the lower leg such as peroneus longus (everter) and flexor hallucis longus (inverter). Despite being the primary plantarflexors of the talocrural joint, however, GM and GL also contribute to inversion/eversion at the STJ (e. g. Lee and Piazza, 2008; Arndt et al., 1999a; Elftman, 1969).

The differential actions of GM and GL and the inversion/eversion position of the calcaneus have been discussed as contributing factors to non-homogeneous strain distribution in the AT (Magnusson and Bojsen-Møller, 2015). The study in the previous chapter (Chapter 4) showed that calcaneal inversion/eversion may not provide a good explanation for non-homogeneous strain distribution in the AT during an isometric plantarflexion contraction since the contractile behaviour of GM and GL was not found to be different in individuals with different calcaneal postures nor was it found to be altered when performing this type of contraction in different calcaneal positions. Alternatively, Arndt et al. (1999b) in an *in vitro* study showed that an increased activation of GM leads to higher strain on the medial side of the AT, while increased activation of GL leads to higher strain on the lateral side.

Evidence for non-homogeneous loading of the triceps surae has also been demonstrated *in vivo*. For example, strain in the free AT (between the soleus MTJ and the AT insertion at the calcaneus, see Fig. 1.2) was shown to be higher in the deep and middle portion of the AT than in more posterior portions during passive talocrural joint rotations and during walking (Slane and Thelen, 2014; Arndt et al., 2012). During isometric plantarflexion, the free AT and proximal AT (between the MTJs of gastrocnemius to the soleus MTJ, see Fig. 2.2) lengthen resulting in longitudinal strain, which was found to be higher in the free AT than in the proximal AT (Farris et al., 2012; Iwanuma et al., 2011).

Non-homogeneous strain distribution within the AT may indicate more independent actions of GM and GL. Both heads insert into the proximal AT and the collagenous fibres corresponding to each head are clearly distinguishable well into the free AT (Edama et al., 2015; Szaro et al., 2009). It is, therefore, conceivable that GM and GL could act as relatively separate entities undergoing very different loading during plantarflexion contractions. A difference in fascicle behaviour and tendon loading of GM and GL during plantarflexion contractions has not previously been investigated. To date, only one study has demonstrated differential displacement of the GM and soleus aponeuroses during isometric plantarflexions at different knee angles potentially leading to non-homogeneous strain distribution within the aponeuroses (Bojsen-Møller, et al. 2004). Therefore, the purpose of this chapter was to investigate whether fascicle behaviour and tendon loading is different in GM and GL during a submaximal isometric plantarflexion contraction.

5.2 Methods

Data for fascicle length, pennation angle, tendon length and tendon elongation collected and processed in the previous chapter (Chapter 5.2) were used. To be able to compare fascicle lengths and pennation angles between GM and GL at all contraction levels, fascicle length and pennation angles were normalised to their respective values at rest for both muscles. Tendon strain was calculated for the tendons of both muscles. For comparison of MTU behaviour between GM and GL, data from all 24 participants in calcaneal neutral position (see Section 5.2.4) were used. To determine the effect of calcaneal position, data from all 24 participants in calcaneal neutral, inversion and eversion position were used.

5.2.1 Normalisation of fascicle length and pennation angle

For comparison of GM and GL fascicle behaviour during a ramped isometric plantarflexion contraction, fascicle strain and rate of pennation angle change were calculated. Fascicle strain was calculated with respect to fascicle length at rest as

$$\varepsilon_F = \frac{\Delta L_F}{L_{Frest}} \times 100\% \qquad \text{(Equation 5.1)}$$

where ε_F is fascicle strain, ΔL_F is fascicle length change calculated as difference between fascicle resting length and fascicle length at the corresponding contraction level, and L_{Frest} is fascicle length at rest. As fascicles shorten during an isometric plantarflexion effort, fascicle strain values are negative.

The normalised pennation angle from rest to 70% MVC was calculated with respect to pennation angle at rest for all contraction levels as

$$\varphi_{norm} = \frac{\varphi}{\varphi_{rest}} \qquad \text{(Equation 5.2)}$$

where φ_{norm} is the normalised pennation angle, φ is the pennation angle at the corresponding contraction level, and φ_{rest} the pennation angle at rest. A φ_{norm} of one indicates no change, while a value larger than one indicates an increase in pennation angle and a value smaller than one indicates a decrease in pennation angle.

5.2.2 Calculation of tendon strain

Tendon strain was calculated for the global tendons of GM and GL (proximal tendon and free AT combined) as done previously (Oliveira et al., 2015; Albracht and Arampatzis, 2013; Lichtwark and Wilson, 2005). Values for GM and GL tendon length and tendon elongation as presented in Section 4.3.2 were used. Tendon strain was calculated as

$$\varepsilon_T = \frac{\Delta L_T}{L_{Trest}} \times 100\% \qquad \text{(Equation 5.3)}$$

where ε_T is tendon strain, ΔL_T is tendon elongation, and L_{Trest} is tendon resting length. Strain was calculated for the tendon elongation values at the corresponding contraction levels.

5.2.3 Calculation of tendon stiffness

Tendon stiffness was calculated for GM and GL in the previous chapter (Section 4.2). The stiffness values calculated for GM and GL during submaximal isometric plantarflexions in calcaneal neutral position will be included in the analysis in this chapter.

5.2.4 Statistical analyses

Statistical analyses of the data were performed using SPSS (v. 22.0.0.1, IBM Corporation, USA). Fascicle strain, normalised pennation angle and tendon strain are presented for GM and GL. All variables were tested for normal distribution using the Kolmogorov-Smirnov test of normality and for homogeneity using Levene's test of equal variances. Data are shown as means ± S.D.

Differences in fascicle strain, normalised pennation angle, tendon strain and tendon stiffness between GM and GL were determined for data from all 24 participants in neutral calcaneal position using a paired-samples one-tailed t-test. Statistical comparisons of fascicle strain, normalised pennation and tendon strain were conducted at each contraction level from 10% to 70% MVC. The level of significance was set at $\alpha = 0.05$.

A *post hoc* power analysis was conducted in G*Power (v. 3.1.9.2, Universität Kiel, Germany). Fascicle strain, normalised pennation angle and tendon elongation at 10% MVC as well as tendon stiffnesses were considered for the analysis. Statistical power was determined for comparisons of these variables between GM and GL.

5.3 Results

Fascicles of GM shortened by 9.2 mm from rest to 70% MVC resulting in a fascicle strain of -22.5 ± 9.0% at 70% MVC. Fascicles of GL shortened by 34.3 mm from rest to 70% MVC resulting in a fascicle strain of -37.8 ± 39.6% at 70% MVC. Fascicle strain differed significantly between both muscles at all contraction levels ($p < 0.05$, Fig. 5.2A). The difference in fascicle strain between GM and GL increased from 6.7% at 10% MVC to 17.0% at 70% MVC. For the comparison of fascicle strain at 10% MVC, the *post hoc* power analysis yielded a statistical power of 76% for an effect size of 0.5 at the 0.05 α-level was achieved. To achieve a high power of 0.8 (Cohen, 1988) for the given effect size, a sample size of n = 27 would be required.

Pennation angle of GM increased by 6.7° from rest to 70% MVC and normalised pennation angle was 1.45 ± 0.25 at 70% MVC. Pennation angle of GL increased by 4.3° from rest to 70% MVC and normalised pennation angle was 1.47 ± 0.35 at 70% MVC. Pennation angle did not differ significantly between muscles at any contraction level ($p > 0.05$, Fig. 5.2B). For the comparison of normalised pennation angle at 10% MVC, the *post hoc* power analysis yielded a statistical power of 9% for an effect size of 0.06 at the 0.05 α-level was achieved. To achieve a high power of 0.8 (Cohen, 1988) for the given effect size, a sample size of n = 1719 would be required.

The tendon of GL lengthened by 8.7 mm from rest to 70% MVC resulting in a tendon strain of 4.4 ± 2.1% at 70% MVC. Strain differed significantly between both muscles from 20% MVC to 70% MVC ($p < 0.05$, Fig. 5.2C). The difference in tendon strain between GM and GL increased from 0.64% at 10% MVC to 2.49% at 70% MVC. For the comparison of fascicle strain at 10% MVC, the *post hoc* power analysis yielded a high statistical power of 98% for an effect size of 0.76 at the 0.05 α-level was achieved.

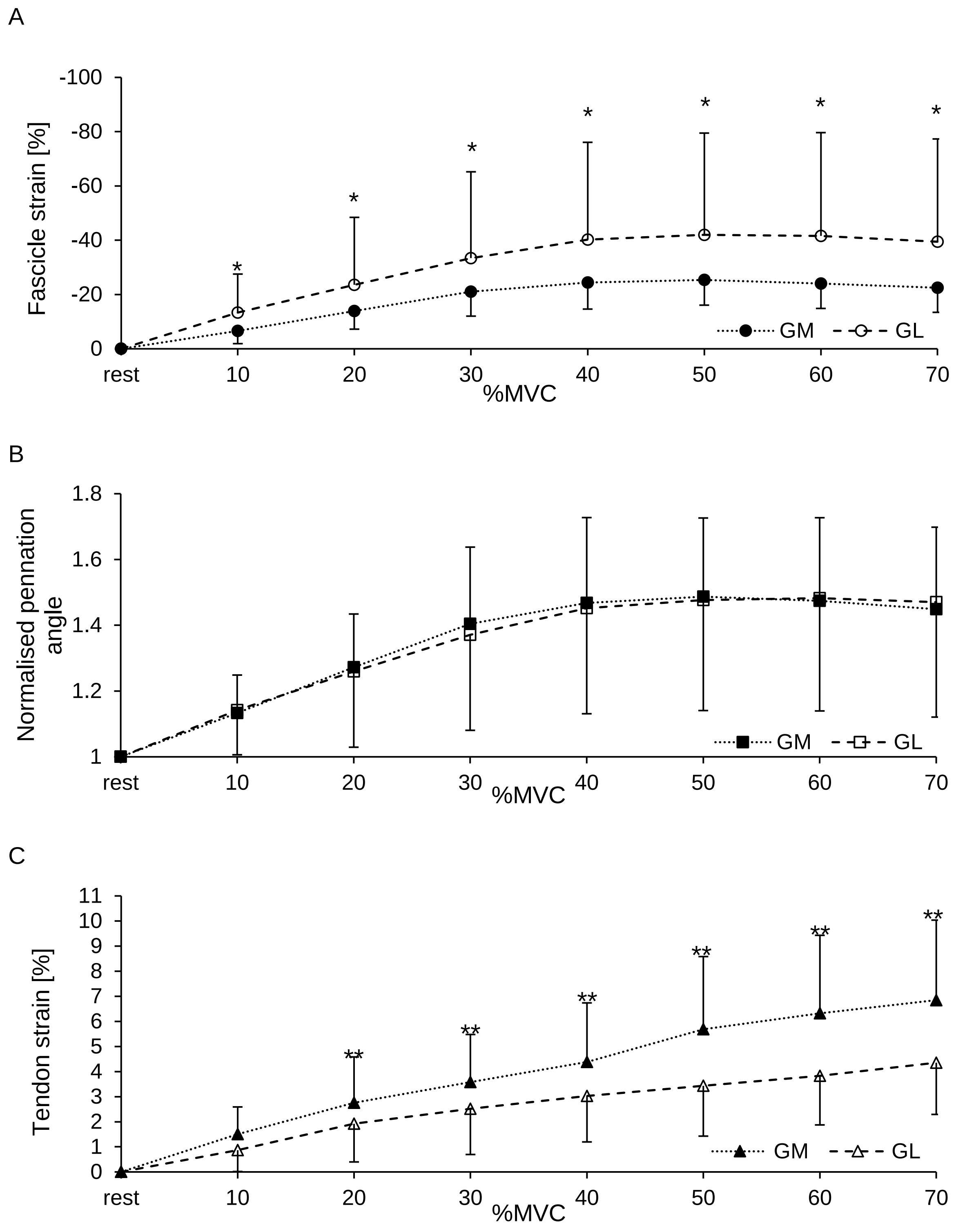


Figure 5.1. Change in fascicle length (A), pennation angle (B) and tendon elongation (C) of GM and GL from rest to 70% MVC. Error bars were omitted for clarity. The y-axis was inverted for better readability. (*) denotes a statistically significant difference between GM and GL at the 0.05 significance level. (**) denotes a statistically significant difference between GM and GL at the 0.001 significance level.

Maximum tendon force of GM and GL at 70% MVC was 372.38 ± 129.69 N and 172.87 ± 60.36 N, respectively, and tendon elongation at the same contraction level was 14.5 ± 6.7 mm for GM and 10.8 ± 5.4 mm for GL. Stiffness of the GM tendon exceeded stiffness of the GL tendon by 11.7 N/mm, which was significant ($p < 0.001$, Fig. 6.3). For the comparison of tendon stiffness between GM and GL, a statistical power of 99% was achieved for an effect size of 0.8 at the 0.05 α-level.

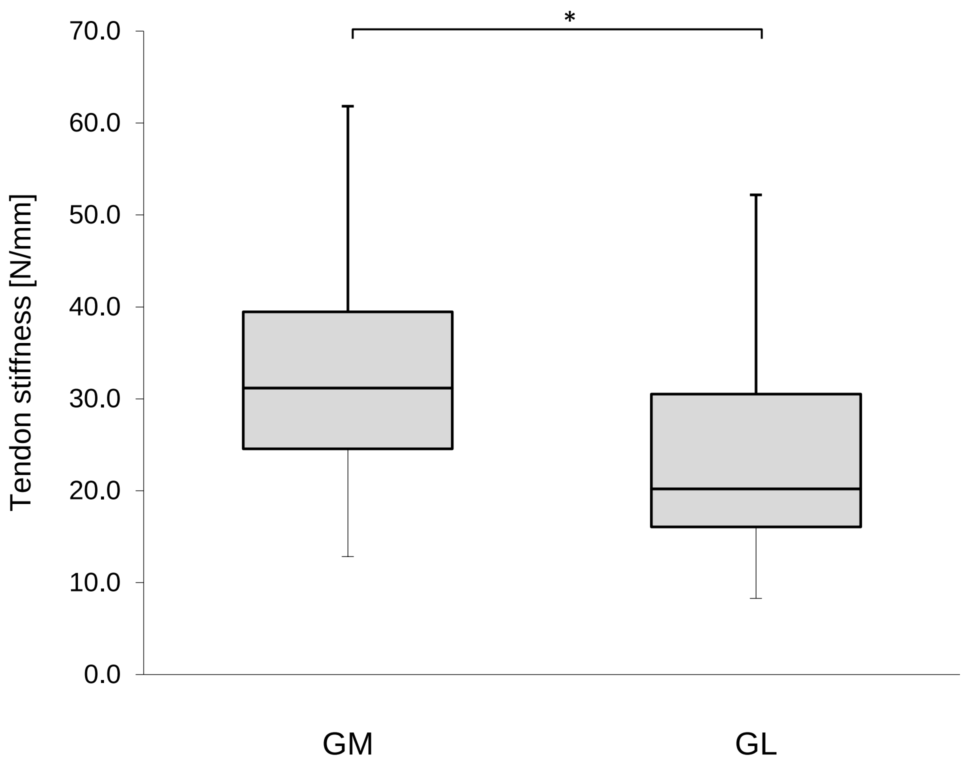


Figure 5.2. Box-and-Whisker plot showing the distribution of tendon stiffness data of GM and GL, respectively. (*) denotes a statistically significant difference of tendon strain between GM and GL ($p < 0.001$).

5.4 Discussion

This study investigated the differences of the GM and GL MTU behaviour during ramped isometric submaximal plantarflexion contraction. Differences in fascicle strain, tendon strain and tendon stiffness between GM and GL were observed.

5.4.1 Comparison of fascicle behaviour of gastrocnemius medialis and lateralis

The fascicles of GM shortened significantly less than the fascicles of GL at all contraction levels with the difference between GM and GL fascicle shortening increasing the higher the contraction level. A similar observation was previously made by Héroux et al. (2016) at lower contraction levels (25% MVC). Maganaris et al. (1998) also reported differences in absolute fascicle length between GM and GL but did not find differential fascicle shortening behaviour. Fascicles of GM are often reported to be significantly shorter than fascicles of GL (Héroux et al., 2016; Morse et al., 2005; Maganaris et al., 1998), and this observation was also made in Chapter 6. Shorter fascicles contain fibres with a smaller number of sarcomeres in series compared to long fascicles. The number of sarcomeres in series is directly related to the shortening capacity of a muscle fibre with longer fibres having greater shortening capacity (Wickiewicz et al., 1984). Therefore, consistent with the observations presented in the present chapter, GL fascicles would be expected to shorten more under contraction.

Héroux et al. (2016) reported that the pennation angle of GM increases less than the pennation angle of GL during a low-level plantarflexion contraction to 25% MVC. In contrast, this study did not find a difference in pennation angle change between GM and GL. During an isometric contraction, as the muscle fascicles shorten, they rotate resulting in an increase in pennation angle (e.g. Henriksson-Larsen et al., 1992). In study presented in this chapter, GM and GL pennation angles were found to increase to the same degree for both muscles. Pennation angle here was defined as the angle formed between the fascicle and the horizontal image axis, which results in an underestimation of pennation angle (see Chapter 5.2). For a comparison of the change in pennation angle between GM and GL, normalised pennation angle was reported in this study, which normalised absolute pennation angle values to pennation angle at rest. Therefore, the effect of absolute pennation angle was eliminated and results from studies with different definitions of pennation angle could be compared. This is only true, however, if the relative displacement of fascicles and aponeurosis with respect to each other during contraction remains the same for all definitions of pennation angle but this is not the case. During muscle contraction, the orientation of the aponeurosis (especially the deep aponeurosis) changes with respect to the image axes due to muscle bulging resulting in a larger relative pennation angle increase with respect to pennation angle at rest. On the other hand, the horizontal image axis is a fixed reference, which is independent of muscle bulging so that pennation angles according to the definition used here represent the absolute rotation of the fascicle during contraction. Therefore, relative pennation angle increase with respect to pennation angle at rest is smaller. Specifically, Maganaris et al. (1998) reported an increase of pennation angle of 90-110% and 175-256% for GM and GL, respectively. In this study, normalised pennation angle for GL increased from 1.0 at rest to 1.44 at 70% MVC, while normalised pennation angle for GL increased from 1.0 at rest to 1.47 at 70% MVC. The increase in pennation angle for both gastrocnemii corresponds to an increase of 70%, which is considerably lower than the reported values. Therefore, the absolute rotation of the fascicles of both muscles is identical, but the rotation of the fascicles with respect to the aponeurosis may differ.

5.4.2 Comparison of tendon properties of gastrocnemius medialis and lateralis

There was no difference in tendon strain of GM and GL at very low contraction levels, but tendon strain of GM significantly exceeded tendon strain of GL from 20% MVC onwards. With an increase in contraction intensity, the difference in tendon strain between GM and GL increased. Strain for the GM tendon in this study is slightly higher than strain values reported in the literature (e.g. Arampatzis et al., 2005; Muramatsu et al., 2001). Only one study reported GL tendon strain in children of 2.5% (Neugebauer and Hawkins, 2012), which is lower than the GL strain reported here.

GM and GL have been shown to differ substantially in their geometry and force producing capabilities, which could result in the tendon strain differences observed between GM and GL. The tendon of GM is considerably shorter than the tendon of GL (Chapter 5.3) resulting in larger strain in the GM tendon, since strain is a measure of length change with respect to resting length. Furthermore, GM contributes a greater amount of force to the overall plantarflexion torque during an isometric contraction (Albracht and Arampatzis, 2013) due to its larger physiological cross-sectional area (Albracht and Arampatzis, 2013), muscle volume (Morse et al., 2005) and larger relative volume of electromyographic activation (Masood et al., 2014; Kinugasa et al., 2005). One other study compared strain of the tendons of GM and GL in a similar experiment to the one presented here (Morrison et al., 2015). While a significant difference in resting length of the GM tendon and the GL tendon was observed, a difference in maximum tendon strain at MVC level was not reported. In the present study, tendon strain values were calculated up to 70% MVC in increments of 10%. It is possible that both tendons experience similar strain at maximum contraction levels, but not at submaximal contraction levels. Therefore, strain distribution within the AT may be more non-homogeneous during sub-maximal plantarflexion efforts than at maximal plantarflexion efforts.

Both tendons also differ in their mechanical properties. Tendon stiffness was determined as the slope of a linear fit to the tendon force-elongation relationship between 10% MVC and 70% MVC. Tendon stiffness of GM and GL was 36 N/mm and 24 N/mm, respectively. Similar stiffness values have been reported for GM by Morrissey et al. (2011) who used a similar calculation method. Only one study reported stiffness for the GL tendon of on average 129 N/mm in children (Neugebauer & Hawkins, 2012). This is a much greater stiffness than found here and is surprising, since stiffness is increased in adults compared to children (Waugh et al., 2012). Neugebauer & Hawkins, however, did not take force contributions of individual muscles to total AT force into account and, furthermore, they reported that data from the GM tendon were also included in this sample. In contrast to the findings here, Morrison et al. (2015) did not report a difference in tendon stiffness between GM and GL. The different methods applied to determine tendon stiffness could be a reason for this. In the present study, individual muscle contribution to overall tendon force were considered and tendon elongation was automatically tracked for the entire trial, while Morrison et al. used total AT plantarflexion force and tracked tendon elongation manually. Moreover, stiffness in this study was determined as the slope of the least-squares linear fit line from 10% MVC to 70% MVC, while Morrison et al. used a least-squares linear fit from rest to MVC.

Another surprising finding of the study presented in this chapter is the relationship of strain and stiffness in GM and GL. Strain of the GM tendon was greater than strain of the GL tendon, but stiffness of the GM tendon was higher than GL stiffness. This is counterintuitive since stiffer tendons exhibit less elongation during an isometric contraction, which results in less strain (Onambele et al., 2006; Reeves et al., 2003). Stiffness was defined as the slope of the linear tendon force-elongation curve. In general, stiffness is the ratio of force change over length change. Therefore, differences in stiffness between the tendons of the two muscles can be explained by their differential length change and the different force contributions to plantarflexion torque, which were taken into account. Tendon elongation of GM considerably exceeded tendon elongation of GL, which would have resulted in smaller GM tendon stiffness. On the other hand, force contribution of GM to total AT force exceeds the contribution of GL more than two-fold, which resulted in the greater GM tendon stiffness found here.

The stiffness calculations performed in the study presented here did not take into account contributions of deep muscles of the lower leg to overall plantarflexion torque. Based on PCSA data of lower leg muscles (GM, GL, soleus, tibialis posterior, flexor halluces longus and flexor digitorum longus) from Fukunaga et al. (1992), GM and GL contribute 17% and 7%, respectively. Calculating stiffness from these values would have resulted in smaller specific force values for GM and GL and, therefore, smaller stiffness values. However, the ratio of contribution of GM and GL to plantarflexion torque remains approximately 2:1 so that stiffness of the GM tendon would still be greater than stiffness of the GL tendon.

The stiffness calculations performed here make the assumption that contributions of GM and GL to overall plantarflexion torque are the same at every contraction level. The PCSA is directly related to the maximum amount of force a muscle can produce (Albracht et al. 2008). This measure, therefore, is suitable for estimating the individual contributions of plantarflexor muscles to overall plantarflexion torque during contractions to maximum level. However, at submaximal contraction levels, the individual contributions of the plantarflexor muscles might be altered. Using high-resolution positron emission tomography (PET), Masood et al. (2014) showed that the contribution of GM and GL to submaximal plantarflexion torque of 30% of MVC is 29% and 25%, respectively, which indicates that both gastrocnemii may contribute more evenly to plantarflexion torque at submaximal contraction levels. The authors also estimated individual contribution of plantarflexor muscles to submaximal plantarflexion contractions from surface EMG and report a contribution of 29% for GM and 19% for GL. These results indicate that the contributions of GM and GL to overall plantarflexion torque at submaximal contraction levels are dissimilar to plantarflexion torque maximum contraction levels. An accurate method to determine contributions to overall plantarflexion torque is currently not available so that tendon stiffnesses calculated for submaximal contraction levels should be interpreted with caution.

5.4.3 Comparison of whole muscle-tendon unit behaviour of gastrocnemius medialis and lateralis

The study presented in this chapter showed that the contractile behaviour of GM and GL differs during a submaximal plantarflexion contraction. The tendon of GM lengthened more than the tendon of GL suggesting that the muscle belly of GM shortened more than the muscle belly of GL. On the other hand, greater shortening was observed for fascicles of GL, which would suggest greater shortening of the GL belly.

Greater shortening of the muscle belly of GM as observed here would be possible if pennation angle increase in GM was greater than in GL but this was not observed. Pennation angles increased to the same degree in both muscles. The definition of pennation angle used in the studies presented in this thesis could be an explanation for this (see Section 5.2.4 for a detailed explanation of the definition of pennation angle and Section 6.4.2 above for a discussion of the implications). Another explanation for the discrepancy between fascicle shortening and muscle belly shortening of GM and GL could lie in the mechanical properties of the tendinous portion and the aponeurosis. The aponeurosis of GL might be more compliant than the tissue of the GL tendon. The displacement of aponeurotic tissue can be calculated using the fascicle and tendon elongation data presented in the previous chapter (see Section 5.3). According to the equation presented by Kawakami et al. (1998), muscle length change ΔL_m can be calculated as:

$$\Delta l_m = L_{f1} \times \cos \varphi_1 - L_{f2} \times \cos \varphi_2 \qquad \text{(Equation 5.7)}$$

Where L_{f1} is fascicle length at rest, L_{f1} is fascicle length at 70% MVC, φ_1 is pennation angle at rest and φ_2 is pennation angle at 70% MVC. In using this Equation 5.7, Kawakami et al. (1998) make the assumption that length change along the muscle-tendon unit during an isometric contraction is uniform. However, this is not the case and the length change calculated with Equation 5.7 refers to the proximal displacement of the fascicle’s attachment point on the deep aponeurosis and can, therefore, provide an estimate for tissue displacement of the aponeurosis. By using this equation, aponeurosis tissue displacements of 10 mm and 35 mm can be calculated for GM and GL, respectively. In comparison, tendon elongation measured as proximal displacement of the MTJ was 12 mm for GM and 9 mm for GL.

This calculation shows that the tissue displacement of the GM aponeurosis and the GM tendon is similar and both tissues may have similar mechanical properties, which allows a direct transfer of force from fascicles to the connective tissues and the calcaneus. The muscle architecture of GM (short and highly pennate fascicle arrangement, see Section 5.3.1) allows a high force producing capability and an aponeurosis and tendon of similar stiffness would be required for a direct force transfer. Therefore, GM seems to be equipped to act as an important force contributor during plantarflexion tasks as has been previously suggested (Antonios & Adds, 2008).

For GL, the tissue displacement difference between aponeurosis and tendon is 24 mm, which suggests that the aponeurosis has a smaller stiffness than the tendon in the region where fascicle lengths and pennation angles were measured. It is possible that the stiffness of the aponeurosis is different in other areas. Complex tissue displacement has been shown previously within the soleus aponeurosis (Finni et al., 2003) and within the aponeuroses of the quadriceps femoris (Finni et al., 2008). A more compliant aponeurosis paired with a stiffer tendon and fascicles with high shortening capabilities would allow GL to finely tune the transfer of force from muscle fascicles to the tendon and calcaneus. Therefore, its task may be to modulate its force output depending on the requirements of a given task. In fact, Wakeling (2009) showed that during cycling, electromyographic activity of GL is modulated in response to varying velocity and power demands.

5.4.4 Conclusions

Fascicle strain and tendon strain were found to differ between GM and GL, which suggests differential contractile behaviour of GM and GL during a submaximal isometric plantarflexion effort. Tissue displacement of the GM aponeurosis and the GM tendon was similar, while a large difference in aponeurosis displacement and tendon elongation was found for GL. This suggests that GM is better suited to act as important force contributor while GL is better suited to finely tune its force contribution during plantarflexion contractions. Both gastrocnemii are important plantarflexors of the talocrural joint and their differential contributions to overall plantarflexion torque are well known. In addition to their role as plantarflexors, both gastrocnemii also may have different roles in modulating the force output depending on the task requirements.

Differential contractile behaviour of GM and GL was found particularly at higher contraction levels. By determining fascicle strain and tendon strain at increments of 10% of MVC, it is possible to state that GL fascicles shorten more than GM fascicles from 10% MVC onwards, and tendon strain of GM exceeds tendon strain of GL from 20% MVC onwards. Due to the selection of discrete torque increments, however, it is not possible to determine the exact contraction level at which differences between GM and GL start to occur. This information is important since it provides a better insight into the loading patterns of the gastrocnemii, during which injuries are most likely to occur. Furthermore, it is important to investigate differences in fascicle strain and tendon strain between GM and GL further to understand their interaction in different calcaneal positions. The contraction levels at which strain differences occur between the gastrocnemii may be different in different calcaneal positions. Since data for fascicle length and tendon elongation were analysed continuously for each frame, it is possible to apply statistical parametric mapping (SPM) for a statistical analysis of this data continuously from rest to 70% MVC. In the next chapter, SPM will be applied to exactly determine, where differences in fascicle strain and tendon strain between GM and GL occur. Fascicle strain and tendon strain will be compared between GM and GL for all three calcaneal positions (neutral, inversion, eversion) to determine possible differences in the contraction levels, at which differences occur and to understand the interaction between GM and GL at different calcaneal inversion/eversion positions.

Chapter Six: Interaction of medial and lateral gastrocnemius in different calcaneal positions

6.1 Introduction

GM and GL have often been considered as having identical functions as plantarflexors of the talocrural joint and have been mathematically modelled as one muscle (Kinugasa et al., 2016). The results of Chapter 5 and other studies, however, showed that their architecture (Morse et al., 2005; Maganaris et al., 1998) and geometry differs considerably (Morrison et al., 2015, Antonios and Adds, 2008). Other studies have indicated that they can have differential actions at the STJ, with GL contributing to eversion and GM contributing to inversion (Lee & Piazza 2008; Arndt et al. 1999a).

The results of the previous chapter also show that fascicle strain and tendon strain of the GM and GL MTUs differ during ramped isometric contractions in neutral calcaneal position, especially at higher contraction levels (>10% MVC, see Fig. 6.2). During a ramped isometric submaximal plantarflexion contraction with the foot neither inverted nor everted, GM fascicle strain was shown to be greater than in GL fascicles indicating that GL fascicles shorten more than GM fascicles. At the tendon level, GM tendon exhibited greater strain than GL tendon. Differences in fascicle and tendon strain between GM and GL were not present at all contraction levels. Fascicle strain differed from 10% MVC and tendon strain differed from 20% MVC onwards. However, it is not clear whether the same observation would be made in calcaneal inversion or eversion position.

Inversion and eversion occur at the STJ and its rotational axis has been shown to rotate into a different position during calcaneal inversion/eversion (Kirby, 2001), which could alter the function of GM and GL at the STJ. For example, Lee and Piazza (2008) showed that GL acts as everter in a calcaneal eversion position but becomes an inverter in calcaneal inversion position, which may be a result of the positional change of the STJ axis. A calcaneal position-dependent change in the function of GM and GL could alter the interaction between both gastrocnemii. The interaction between GM and soleus during isometric plantarflexion contractions has previously been shown to be dependent on knee joint position (Bojsen-Møller et al., 2004) but a STJ-joint dependent interaction between GM and GL has not be investigated.

If the interaction between GM and GL is altered depending on the inversion/eversion position of the calcaneus, the differences in fascicle strain and tendon strain observed for a calcaneal neutral position in the study presented in Chapter 5 may be different in calcaneal inversion or eversion position. Comparisons of fascicle strain and tendon strain between the two muscles were made at selected data points, here percentage of MVC, and a statistical test (t-test) was conducted repeatedly for each selected data point. This selection was done on an *ad hoc* basis and has routinely been applied to data related to MTU behaviour during ramped isometric contractions (e.g. Arampatzis et al., 2005). However, an *ad hoc* selection of data points creates an abstract representation of the data with the potential to bias results and may even introduce an error since the behaviour of the data immediately before and after the selected data point is not considered. Furthermore, to be able to exactly determine the STJ-dependent interaction of GM and GL, it is necessary to identify the exact contraction level(s) at which fascicle shortening of GL exceeds fascicle shortening of GM and at which tendon strain of GM exceeds tendon strain of GL. Data for fascicle and tendon strain are available as continuous curves so that statistical comparisons should be made on this curve and not for discrete pre-selected data points.

Statistical parametric mapping (SPM) allows a statistical comparison of continuous curves therefore eliminating potential selection bias that may exist due to the pre-selection of discrete data points. SPM was first developed for the analysis of cerebral blood flow in functional MRI data (Friston, 1995). The technique is based on random field theory and conducts statistical tests for one-dimensional data (curves, e.g. ground reaction forces) or two-dimensional data (spatial distributions, e.g. plantar pressure). Two fundamental assumptions must be met to be able to apply this technique: (1) the data are spatiotemporally smooth (the value of one observation is dependent on the values of the previous and following observations), and (2) the data are bounded (datasets can be registered with each other based on events, e.g. heel-strike or toe-off). Biomechanical data often meets these criteria because measurements are made above the Nyquist frequency and because of the viscoelastic nature of biological tissue (Fung, 1981).

SPM has previously been applied for the comparison of biomechanical variables. For example, Vanrenterghem et al. (2012) compared knee flexion angle and knee valgus loading during a side-cutting manoeuvre at different running speeds and, more recently, Deschamps et al. (2017) investigated the relationship of foot segment kinematics with age in young boys using a linear regression model.

To date, SPM has not been applied to measures of MTU behaviour. In the previous study (Chapter 5), MTU behaviour was determined for discrete points selected in the data (10% MVC and multiples thereof) and statistical tests were conducted individually for each of these points. Conducting multiple statistical tests on the same data, however, can result in falsely rejecting the null hypothesis (Pataky et al., 2013) leading to misinterpretation of the results. Furthermore, using SPM it is possible to identify exactly at what contraction level(s) statistically significant differences between GM fascicle/tendon strain and GL fascicle/tendon strain occur. This is of particular interest since this information could provide insight into the loading levels of the gastrocnemius MTUs, at which injuries are more likely to occur in a given calcaneal position. The purpose of this study, therefore, was to investigate differences in fascicle strain and tendon strain between GM and GL in different calcaneal positions (neutral, inversion, eversion) using SPM.

6.2 Methods

6.2.1 Datasets

Datasets of all 24 participants of study 3 were used. As described in Chapter 5, data for fascicle length and tendon elongation were interpolated to 10,000 nodes, from which fascicle strain and tendon strain were calculated (Chapter 6) to provide spatiotemporally smooth changes in strain resulting from ramped isometric contractions, suitable for SPM analysis.

6.2.2 Data analyses

To compare fascicle strains and tendon strains, one-dimensional SPM was employed (Pataky, 2012; Friston, 1995). This made it possible to compare fascicle strain and tendon strain of GM and GL over the entire trial from torque onset to 70% MVC. A one-tailed paired t-test was conducted to compare GM fascicle strain vs. GL fascicle strain as well as GM tendon strain vs. GL tendon strain in all three calcaneal positions (neutral, inversion, eversion). The scalar output statistic (here t-value) was calculated separately for each node resulting in six SPM{t} curves (2 muscles x 3 calcaneal positions). An SPM{t} curve is the trajectory of the scalar statistic variable over the contraction level from rest to 70% MVC. Each SPM{t} curve only represents the magnitude of the difference between GM fascicle/tendon strain and GL fascicle/tendon strain, but cannot be used alone to accept a significant difference between them. To test for significance, a critical threshold was calculated at the significance level $\alpha = 0.05$ based on estimates of trajectory smoothness (Friston et al. 1995) and random field theory. If the SPM{t} curve crosses the critical threshold, a significant difference between the variables is assumed. Due to the smooth nature of the data, several points often cross this threshold. These areas are referred to as “supra-threshold clusters” (Pataky et al., 2013). Cluster-specific p-values are calculated, which indicate the probability that a randomly generated data set could have produced the same result. If supra-threshold clusters were found, the first and the last node of the cluster were determined to specify the contraction levels at which fascicle/tendon strain differed significantly between GM and GL. The node number was converted into %MVC as

$$\%MVC = \frac{n_{total} \times n_{current}}{70} \times 100\% \qquad \text{(Equation. 6.1)}$$

where n_{total} is the total number of all nodes (here 10,000) and $n_{current}$ is the number of the node where the critical threshold is exceeded. All SPM analyses were conducted with custom-written code in Python (Enthought Canopy v. 1.7.4, Enthought Inc., Austin, USA) using the open-source spm1d software package (v. 0.4, available at http://www.spm1d.org/Downloads.html).

6.3 Results

Statistical tests using SPM revealed no differences in fascicle strain for all calcaneal positions. The critical thresholds of t = 3.83, t = 3.833 and t = 3.844 for calcaneal neutral, inversion and eversion position, respectively, were not exceeded (Fig. 6.1). Tendon strain in calcaneal neutral position was similar between GM and GL at very low contraction levels (< 6%MVC) (Fig. 6.2A), but GM tendon strain was significantly higher than GL tendon strain from 6% MVC to 70% MVC, where the critical threshold of t = 3.07 was exceeded by a supra-threshold cluster (Fig. 6.2C). Tendon strain did not differ between GM and GL in the calcaneal inversion position; the critical threshold of t = 2.945 was not exceeded (Fig. 6.2B). In calcaneal eversion position, tendon strain was similar between GM and GL at low contraction levels (< 22%MVC) but GM tendon strain was significantly higher than GL tendon strain from 21.6% MVC to 70% MVC, where a supra-threshold cluster exceeded the critical threshold of t = 3.03.

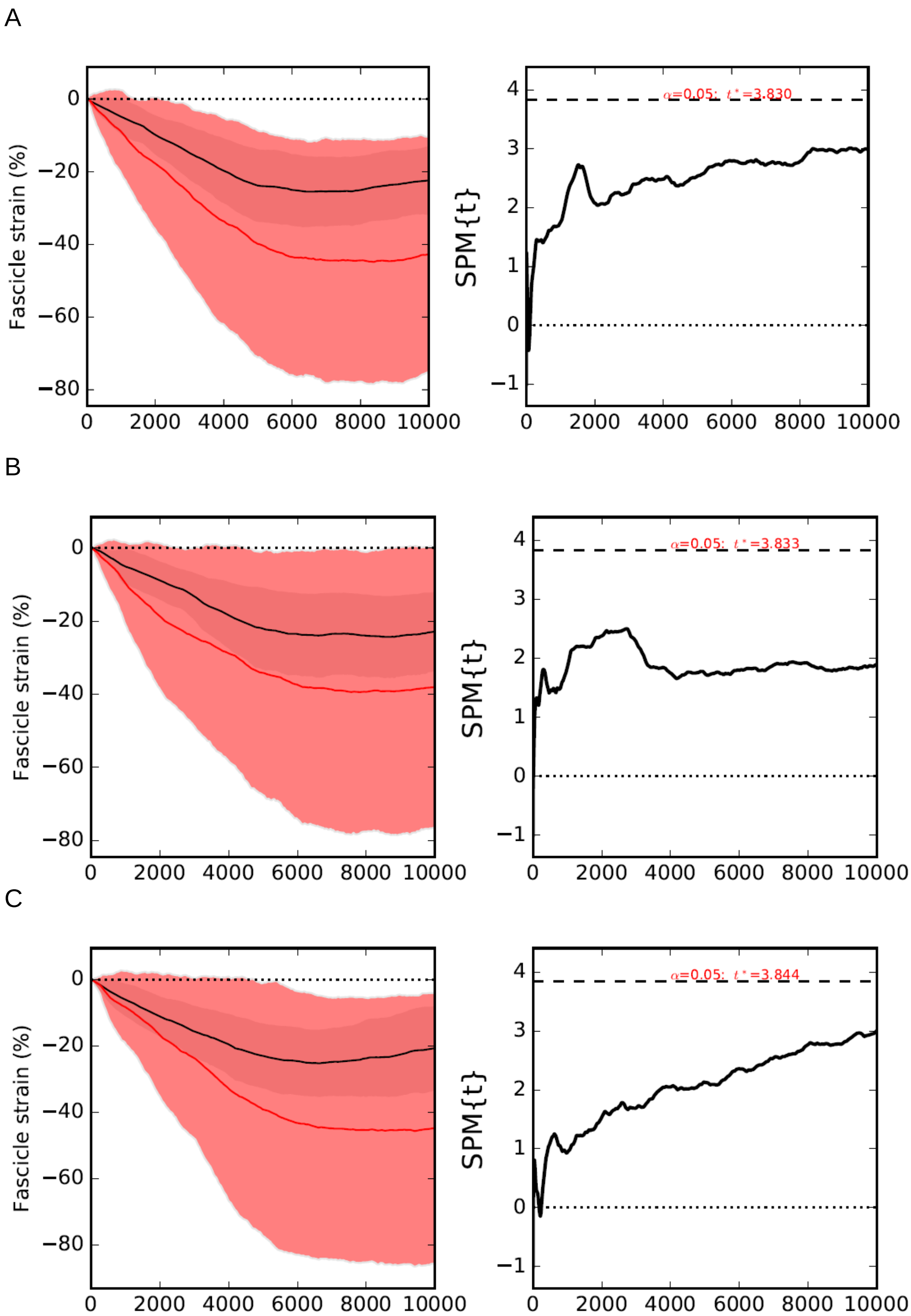


Figure 6.1. SPM output for fascicle strain in calcaneal neutral (A), inversion (B) and eversion (C) position. Left: Mean fascicle strain calcaneal position for GM (black) and GL (red) and S.D. (red and grey shaded area) for every node. Right: SPM{t} curve showing t-values for every node and the critical threshold at the significance level (dashed vertical line).

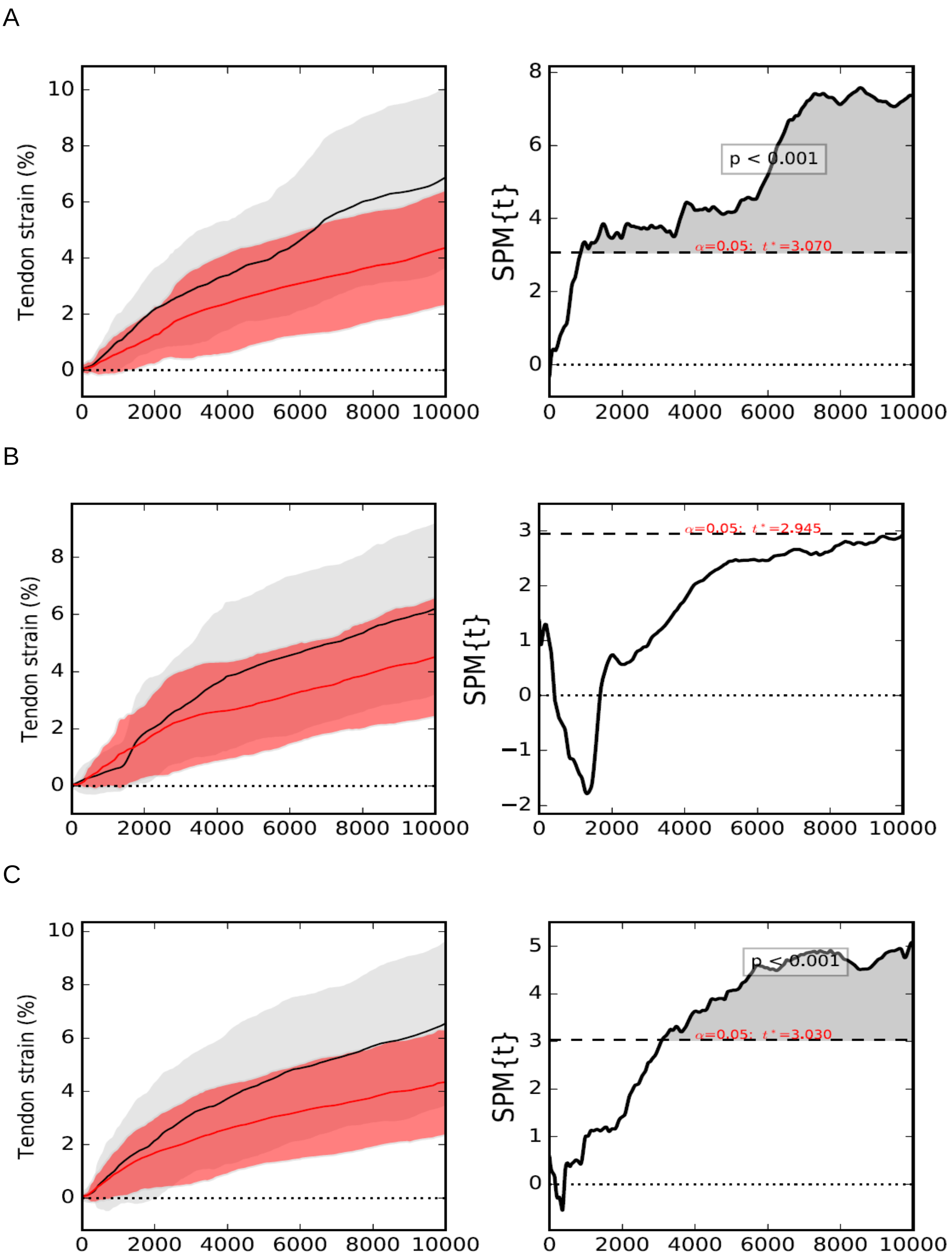


Figure 6.2. Left: Tendon strain curves for GM (black) and GL (red) and S.D. (red and grey shaded areas) for every node in calcaneal neutral (A), inversion (B) and eversion (B). Right: Corresponding SPM{t} curves showing t-values for every node and the critical threshold at the significance level (dashed vertical line). The grey shaded area indicates the supra-threshold cluster, where tendon strain differs significantly between GM and GL.

6.4 Discussion

This study applied SPM to investigate the differences in fascicle and tendon strain between GM and GL in different calcaneal inversion/eversion positions. Fascicle strain did not differ between GM and GL in all three calcaneal positions. A difference in tendon strain between GM and GL was observed in the calcaneal neutral and eversion positions, but not in the inversion position.

In the previous study (Chapter 5), fascicle strain was found to differ significantly between GM and GL in neutral calcaneal position. In contrast to these findings, fascicle strain did not differ between GM and GL in the neutral calcaneal position as well as in the calcaneal inversion and eversion positions. In the previous study, fascicle strain was compared between GM and GL by conducting a t-test at discrete points of contraction level (multiples of 10% MVC). That means that one threshold was calculated for each of these pre-selected scalar data points. In SPM, only the maximum threshold value is calculated for the entire curve. The threshold calculated for a curve is higher than the threshold calculated for a scalar value since the smoothness of the curve is taken into account (Pataky et al., 2013). Using SPM for the comparison of fascicle strains between GM and GL showed that fascicle strain is not different between the gastrocnemius heads. This is in contrast to the findings of the previous study (Chapter 5) and the question must be asked; which of the statistical methods used is more suitable. Fascicle strain was calculated from fascicle lengths of muscles during a ramped isometric plantarflexion contraction, resulting in a smooth continuous curve of fascicle lengths and subsequently fascicle strains, where one data point is correlated to the previous and the subsequent data point. SPM is a more suitable statistical method for this type of data, since it takes this interrelation of data points within the curve into account. Therefore, it can be concluded that a significant difference between GM fascicle strain and GL fascicle strain does not exist during a ramped isometric plantarflexion contraction.

Applying SPM to the comparison of fascicle strain between GM and GL, it was shown that fascicles of GM and GL shorten to the same degree during a ramped isometric plantarflexion contraction to 70% MVC. This is in agreement with the findings of Héroux et al. (2016) who report similar fascicle shortening for GM and GL during isometric plantarflexion efforts at low contraction levels. The authors of this study did not use SPM, but compared the slopes of the fascicle length-torque curves for GM and GL and found no difference. The present study shows that fascicle shortening of GM and GL is also similar at higher contraction levels irrespective of the inversion/eversion position of the calcaneus. It appears that the response of the MTUs of GM and GL to alterations in calcaneal position does not occur at the fascicle level. Alterations of calcaneal position due to inversion/eversion at the STJ may lead to small alterations of the resting lengths of the GM and GL MTUs (see Chapter 4.4). Passive joint-dependent length changes of the triceps surae MTU are a result of tendon elongation and not fascicle elongation when passive joint movement is small (Herbert et al., 2011). Small length changes in the GM and GL MTUs due to calcaneal inversion/eversion may also be reflected in the behaviour of the tendon rather than the fascicle behaviour.

GM tendon strain was shown to exceed GL tendon strain in calcaneal neutral and eversion position, but not in calcaneal inversion position. Using SPM, it was possible to specify the exact contraction level at which differences in tendon strain between GM and GL occur. These results suggest that the contraction levels at which tendon strain differences occur between GM and GL during an isometric plantarflexion differ depending on the position of the STJ, which may indicate that the interaction between GM and GL may be dependent on the STJ position.

A knee joint-dependent change in tissue displacement during an isometric plantarflexion contraction was previously reported for the aponeuroses of GM and soleus (Bojsen-Møller et al., 2004). Specifically, the aponeurosis of GM showed greater displacement than the aponeurosis of soleus during an isometric plantarflexion with the knee extended. The opposite was the case with the knee flexed. Knee flexion puts the bi-articular GM into a disadvantageous position to exert a plantarflexion moment at the foot and the authors discuss the possibility that the contribution of GM to plantarflexion torque is reduced in this position. Contributions of the individual heads of the gastrocnemius to the total force produced by the triceps surae during isometric plantarflexion contractions to MVC were determined to be 26% for GM and 12% for GL (Albracht et al., 2013). The relative contribution of each gastrocnemius head, however, might change. GL, in particular, has been reported to show different levels of electromyographic activation patterns during different tasks such as balancing (Héroux et al., 2014), gait (Higham et al., 2008) and cycling (Wakeling, 2009). Therefore, the relative contributions of GL and GM to plantarflexion force might be dependent on task and joint configurations of knee, talocrural joint and STJ. This, in turn, would affect differential tissue displacement and strain between the tendons of both gastrocnemius heads during different tasks.

The relative contributions of GM and GL to plantarflexion torque might also be altered in different inversion/eversion positions of the calcaneus. Inversion/eversion of the calcaneus is related to the orientation of the STJ axis with a calcaneal inversion position being associated with a more laterally rotated STJ axis and a calcaneal eversion position being associated with a more medially rotated STJ axis (Kirby, 2001). Therefore, the location of the STJ axis with respect to the AT differs in different calcaneal positions and may in some calcaneal positions penetrate the AT or it might pass the AT on its medial or lateral side in other calcaneal positions. This would result in different inversion/eversion moment arms of the AT and in particular the AT portions corresponding to GM and GL. Collagenous fibres from GM and GL are clearly distinguishable well into the free AT (Edama et al., 2015, Szaro et al., 2009). It is, therefore, possible for both muscles to act relatively independent of each other and it was previously shown that GM and GL can have antagonistic roles at the STJ (Lee and Piazza 2008, Arndt et al., 1999a). For example, in the *in vitro* study by Arndt et al. (1999a) it was reported that GM acts as inverter of the calcaneus while GL acts as everter of the calcaneus when the foot is flat on the ground. On the other hand, in the *in vivo* study by Lee and Piazza (2008), this was only the case in an eversion position of the foot. Furthermore, Vieira et al. (2013) showed that plantarflexion induced by electrically stimulating GM in isolation is accompanied by inversion. This suggests that GM simultaneously acts at the talocrural joint and at the STJ.

Despite contradicting evidence, it appears that GM and GL can act on the calcaneus as synergists (e.g. GM and GL as inverters) or antagonists (e.g. GM as inverter and GL as everter) depending on the inversion/eversion position of the calcaneus and, therefore, the location of the STJ with respect to the AT portions associated with GM and GL. The greatest differences in tendon strain between GM and GL were found in calcaneal neutral position. In this position, the location of the STJ axis might be such that GM and GL both act as synergists and GM might contribute relatively more to the plantarflexion torque than GL as suggested by Albracht et al. (2013). On the other hand, no differences in tendon strain between GM and GL were found in calcaneal inversion position. This may suggest that the STJ axis passes through the AT in such a way that GM and GL may act as antagonist on the calcaneus. In an effort to stabilise the ankle complex and to counteract GM, activation of GL might be higher than in other situations contributing relatively more to plantarflexion torque. This would result in greater GL tendon elongation and, hence, GL tendon strain so that GL tendon strain would be more similar to GM tendon strain as observed in the present study. In calcaneal eversion position, differences in tendon strain between GM and GL are smaller and occur at a higher contraction level. The actions of GM and GL at the calcaneus might be inversion or eversion depending on the location of the STJ axis with respect to the GM and GL tendon portions of the AT and may be specific to each individual.

This study showed that strain in the GM tendon exceeds strain in the GL tendon at submaximal plantarflexion contraction levels, which may indicate that the occurrence of GM strain injuries is higher than that of GL strain injuries. In fact, strain injuries are more common in GM than GL (Dixon, 2009). Evidence of incidence rates is sparse, but Millar (1975) reported that MTJ ruptures of the

GM make up 53% of all gastrocnemius strain injuries compared to only 3% of GL MTJ ruptures. Furthermore, strain differences are also likely to occur during locomotion and may lead to shear strain and subsequently tissue degeneration and overuse injury (Kannus and Jozsa, 1991). AT overuse injuries were reported to occur predominately in relation to excessive eversion during the stance phase of walking and running (Kvist, 1994). The greatest strain difference between GM and GL, however, was found in the calcaneal neutral position, which is in contrast to the above reports on AT injury incidence.

In conclusion, this study showed that tendon strain differs between GM and GL during higher contraction levels of a ramped isometric plantarflexion effort. The strain differences between GM and GL were shown to occur at different contraction levels in different calcaneal positions suggesting that the position of the STJ might influence the function of both gastrocnemii.

Chapter Seven: Discussion and conclusions

The purpose of this series of studies was to investigate the contractile behaviour and loading of GM and GL in different calcaneal postures and positions during a ramped submaximal plantarflexion contraction and the interaction of GM and GL with respect to calcaneal inversion and eversion. It was shown in Chapter 5 that GM and GL differ considerably in their muscle architecture and MTU geometry, but the individual behaviour of each gastrocnemius head during a ramped isometric plantarflexion contraction was shown to be unaffected by the amount of calcaneal inversion and eversion. A change in calcaneal inversion/eversion position did not result in a change in contractile behaviour of either gastrocnemius head. Contractile behaviour was also shown to be unaffected by calcaneal posture assessed during relaxed standing. However, the results relating to the effects of calcaneal posture must be interpreted with caution due to the small sample size and, consequently, low statistical power.

Differences in the contractile behaviour between GM and GL were also investigated in Chapters 5 and 6. Despite their different muscle architectures, fascicle behaviour of GM and GL did not differ considerably during a ramped submaximal isometric contraction for the muscle regions investigated. The amount of fascicle shortening and rotation was found to be similar in both. On the other hand, tendon strain of GM was found to exceed tendon strain of GL from a contraction level of 10% MVC. GM tendon strain was 57% greater than GL tendon strain at 10% MVC and 63% greater at 70% MVC. This difference can be attributed to differences in tendon lengths and the differential force contributions of each gastrocnemius head during plantarflexion. The strain difference between GM and GL was also found to be dependent on the position of the calcaneus (Chapter 6). These results indicate that the gastrocnemius heads respond to a change in the position of the STJ and that this response occurs at the tendon level rather than the fascicle level.

7.1 Interaction of gastrocnemius medialis and lateralis

The work presented in this thesis showed that fascicles of GM and GL behave very uniformly during a ramped submaximal isometric plantarflexion contraction regardless of the position of the calcaneus, whilst an interaction between both gastrocnemii can be seen at the tendon level. Rotation of the calcaneus around the STJ is likely to cause a length change of the MTUs of GM and GL. As discussed in Chapter 4, inversion of the calcaneus should induce some lengthening of the GL MTU and shortening of the GM MTU, while the opposite would be true for eversion of the calcaneus. It was not possible to accurately measure resting lengths of the tendons of GM and GL in the three calcaneal positions. It is likely, however, that tendon shortening and lengthening occurred as described above. A change in muscle architecture was not observed, but during passive joint rotation, it is possible that a MTU length change occurs due to a length change of the tendon but not of the muscle belly (Herbert et al. 2011). This indicates a decoupling of fascicle and tendon behaviour. Decoupling of tendon behaviour and fascicle behaviour has been reported previously during passive joint rotation (Herbert et al., 2011) and locomotion (Lichtwark et al., 2007; Kawakami et al., 2002). During passive joint rotations, fascicles were shown to contribute less than 30% to the overall length change of the MTU (Herbert et al., 2011). During locomotion tasks such as level and sloped walking, fascicles were found to behave largely isometrically (Lichtwark and Wilson, 2006) and shorten only slowly during running (Lichtwark et al., 2007). Furthermore, an increase in “task intensity” during hopping was found to result in greater tendon elongation and tendon strain during the stretch phase but the fascicles were found to behave similarly regardless of intensity (Kawakami et al., 2002). This suggests that muscle fascicles operate at mechanically and energetically advantageous lengths and velocities (Roberts and Scales, 2004). The results of this work lend further support to the notion of a decoupling between fascicles and their tendon and they also show that fascicle-tendon decoupling likely occurs in response to STJ rotation.

Differential strain between the tendons of GM and GL indicates an interaction between the gastrocnemii, which changes depending on the position of the STJ. In Chapter 6, the possibility was discussed that relative force contributions of GM and GL during a ramped submaximal isometric plantarflexion contraction may not be the same as reported for maximum effort contractions (Albracht et al., 2013). The relative contributions of GM and GL to plantarflexion torque might also be altered depending on the position of the STJ. It is important to point out that this alteration in relative contribution could be achieved without altering fascicle behaviour but is dependent on the relative position of the AT to the STJ axis. In Chapter 4, the COR method was used to identify a point on the STJ axis and the distance of that point to the AT was measured. It was shown that the distance between this point and the AT differed depending on the inversion/eversion position of the calcaneus. This indicates that the location of the STJ axis may have shifted with respect to the AT. It was shown that, depending on the location of the STJ axis with respect to the AT, GM can be a strong or less strong inverter, while GL can be an inverter or everter of the STJ (Lee and Piazza et al., 2008, Klein et al., 1995). Therefore, GM and GL act not only at the talocrural and knee joint but also at the STJ. This would mean that GM and GL are not bi-articular but, due to their actions at the STJ, should be considered as tri-articular muscles making the gastrocnemii unique compared to other muscles of the lower limbs. A tri-articular action of the gastrocnemii would add to their complex behaviour. During plantarflexion contractions, the gastrocnemii were shown to exhibit complex three-dimensional displacement (Finni et al., 2003, Farris et al., 2013) and fascicle behaviour of GM is dependent on the position of the knee and talocrural joint (Hodson-Tole et al., 2016; Arampatzis et al., 2006; Magnusson et al., 2003). It can be speculated that the behaviour of GM and GL may not only be affected by knee and talocrural joint positions but their interaction may also be dependent on the position of the STJ. The exact nature of this interaction is currently unknown but could provide insights into the aetiology of AT overuse and gastrocnemius strain injuries.

Chapter 5 showed that strain differences exist between the GM tendon and the GL tendon during submaximal ramped isometric plantarflexion contractions. In addition, Chapter 6 further showed that the occurrence of strain differences between the GM tendon and the GL tendon is dependent on the amount of inversion and eversion of the calcaneus. The possibility was discussed that GM and GL may behave very differently (e.g. as antagonists at the STJ) depending on the position of the STJ. Given their differential actions at the STJ, their differential anatomy and force producing capabilities, it might not be appropriate to refer to GM and GL as two heads of the same muscle but as two separate muscles with differential anatomical composition and function. Architectural and functional differences between GM and GL were discussed in Section 1.2.2. Previous studies investigating the behaviour and function of the triceps surae have often studied GM as representative muscle of the entire gastrocnemius and have treated gastrocnemius and soleus as two separate entities (e.g. Kinugasa et al., 2016, Arampatzis et al., 2005). The results of other studies, however, indicate that GM and GL may differ in their function. Specifically, Héroux et al. (2014) report that electromyographic activation of GL during standing is almost absent and Wakeling (2009) reports that the electromyographic activity of GL shows greater modulations in response to load and velocity variations during cycling. Antonios and Adds (2008) observed an earlier onset of GL activation during plantarflexion tasks and suggest that GL acts as a stabiliser of the talocrural joint. The work presented in this thesis lends further support to the notion of functional differences between GM and GL. Based on the evidence provided in previous studies and on the findings of Chapter 6, it can be suggested that future investigations into the contractile behaviour and function of the triceps surae should not generalise findings of GM to behaviour and function of GL but should consider all three heads of the triceps surae as individual muscles.

7.2 Interaction of gastrocnemius medialis, lateralis and soleus

The series of studies presented here investigated the interaction of GM and GL but the contribution of soleus must also be considered. Unlike the gastrocnemii, soleus is a mono-articular muscle acting as a powerful plantarflexor. It makes up the largest volume of the entire triceps surae occupying approximately 55% of the total volume (Kinugasa et al., 2005; Morse et al., 2005; Fukunaga et al., 1996). With a PCSA of 128 cm^2 (Albracht and Arampatzis, 2013; Morse et al., 2005), it is the main contributor to plantarflexion force. Fascicles of soleus are shorter than fascicles of GM and pennation angle is smaller than pennation angle of GM but larger than pennation angle of GL (Maganaris et al. 1998, Kawakami et al., 1998). In contrast to the gastrocnemii, soleus consists predominately of slow-twitch fibres (Edgerton et al., 1975), which makes it an important postural muscle that is mainly involved in tasks requiring greater force but lower velocities (Wakeling et al., 2006).

During ramped, isometric contractions slow-twitch fibres have a lower firing threshold than fast-twitch muscle fibres (Henneman et al., 1974) so that it is likely that soleus contributes relatively more to plantarflexion than the gastrocnemii at low contraction levels resulting in bulging of the muscle belly of soleus. Bulging of the soleus muscle belly might compress the muscle bellies of GM and GL so that shortening and rotation of their fascicles during low plantarflexion contraction levels may partly be a result of interaction between soleus and the gastrocnemii. An interaction between soleus and the gastrocnemii has been reported by Tian et al. (2012). They showed that passive knee flexion with the foot in dorsiflexion results in shortening of GM fascicles, but lengthening of the soleus fascicles. During an isometric contraction, the compression of GM and GL due to bulging of soleus may affect gastrocnemius fascicle behaviour. Therefore, fascicle behaviour observed in GM and GL at low contraction levels may not solely be a result of active muscle contraction. The effect of compression of the gastrocnemii was discussed in Chapter 4. Recently, Bernabei et al. (2017) reported considerably different behaviour of the triceps surae heads during trotting in rats.

Interaction between soleus and the gastrocnemii has also been discussed in terms of myofascial (intermuscular) force transmission. The aponeuroses of soleus and the gastrocnemii exhibit local inter-aponeurotic connections particularly in the area of the distal gastrocnemii (Hodgson et al., 2006; Bojsen-Møller et al., 2004), which would allow force transmission between soleus and the gastrocnemii. To date, myofascial force transmission has previously been shown in animal models (Sandercock and Maas, 2009; Meijer et al., 2008) and in passive knee flexion/extension experiments in humans *in vivo* (Tian et al., 2012; Oda et al., 2007). Only one study to date has reported myofascial force transmission during knee extension with isolated electrical stimulation of GL (Finni et al., 2015). However, the functional importance of myofascial force transmission remains unclear, as only approximately 5 N of force are thought to be transferred between the soleus and GM (Tian et al., 2012). Other studies have suggested a greater level of independence between the three muscles of the triceps surae (Bojsen-Møller et al., 2004, Kawakami et al., 1998). This seems more logical given the evidence to date that GM, GL and soleus might have differential actions at the STJ.

Similar to GM and GL, soleus was also found to act at the STJ and studies report an inversion moment arm when the foot is flat on the ground (Arndt et al., 1999a, Elftman, 1969). Alterations of the soleus moment arm at the STJ in different inversion and eversion positions of the calcaneus has not been investigated. However, it can be assumed that the magnitude and direction of soleus' moment arm at the STJ changes with rotation of the joint in similar fashion to moment arm changes reported for GM and GL (Lee and Piazza, 2008, Klein et al., 1996). The location of the STJ axis differs depending on the amount of inversion and eversion of the calcaneus (Kirby, 2001, Chapter 4) so that its location with respect to the AT portion corresponding to soleus (Pekala et al., 2017; Edama et al., 2015; Szaro et al., 2009) would also be different in different inversion/eversion positions. Hence, the moment arm of soleus at the STJ would be dependent on STJ position. Similar to the suggestion regarding the tri-articular nature of GM and GL mentioned above, it might therefore be more appropriate to refer to the soleus as a bi-articular muscle acting on the talocrural joint and the STJ. At any given STJ position, the calcaneal moments exerted by these three muscles might be different. For example, based on the findings of the studies cited above (Lee and Piazza, 2008, Klein et al., 1996), GM and soleus might exert an inversion moment when the foot is everted but GL might exert an eversion moment resulting in antagonistic actions between GM and soleus on one hand and GL on the other. Chapters 6 and 7 showed that the strain of the GM tendon exceeds strain of the GL tendon during an isometric plantarflexion with the foot flat and in an everted position but not in an inverted position. This indicates that both muscles may act differently depending on the position of the STJ position indicating differential actions of both muscles and the possibility for antagonistic behaviour. Chapter 7 discussed this possibility in light of the compartmentalisation of the AT. It could be speculated that a similar interaction exists between all three muscles of the triceps surae making it a very complex muscle group. To date, the interaction between the gastrocnemius muscles and soleus is not known but, based on the findings of this thesis and evidence in published literature, it is reasonable to assume that differences in tendon elongation and strain also exist between all three muscles. Recently, Bernabei et al. (2017) reported complex interactions within the triceps surae muscles of the rat during locomotion, but details are currently unknown and have not been investigated in human locomotion.

Contraction level-dependent and joint angle-dependent relative force contributions of GM, GL and soleus combined with the possibility of relative independence between them indicate that the triceps surae is a muscle group with complex function, which may be required to meet the various demands of human movement. For example, the movement of the ankle complex (talocrural joint and STJ) during walking and running is multi-planar with plantarflexion/dorsiflexion, inversion/eversion and adduction/abduction often occurring at the same time (Wright et al., 2011; Leardini et al., 2007). To achieve this, the actions of GM, GL and soleus must be concerted and finely tuned. To date, the exact mechanisms of these fine-tuned actions of GM, GL and soleus are still poorly understood and require further investigation. The findings presented in this thesis provide some evidence of interaction between GM and GL during a single-plane isometric plantarflexion, but interactions between GM and GL during multi-planar movements is currently unknown. The interaction with soleus and the three-dimensional actions of the entire triceps surae muscle group at the ankle complex (see Section 1.4 and Chapters 3 and 6) must also be considered. Knowledge of the details of interactions between GM, GL and soleus during complex movements will provide further insight into the aetiology of AT injuries (see Section 1.3) but will also aid in the development of musculoskeletal models of the lower leg and the design of prosthetics.

7.3 Possibility of interaction in other muscle groups

Interaction of muscles within a muscle group, such as those discussed for the triceps surae, is likely to occur also in other muscle groups. Muscle groups that share a common tendon and consist of a combination of mono-articular and bi-articular muscles may exhibit particularly complex interactions since force and velocity requirements of the joint(s) they span are likely to vary greatly. The triceps surae muscle group, for example, acts as knee flexor, plantarflexor of the talocrural joint and also contributes to movement of STJ during a range of movements. Lieber & Fridén (2000) suggest that individual muscles of synergistic muscle groups, such as the triceps surae, should vary in their anatomical composition to meet these various force demands. One other important synergistic muscle group acting at two joints is the quadriceps femoris (QF) muscle group.

QF consists of four muscles; vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VI) and rectus femoris (RF). VL, VM and VI mono-articular knee extensors, and VL and VM also contribute to external and internal rotation of the knee, respectively. RF is bi-articular acting as knee extensor and also as hip flexor. Due to the anatomical structure of QF, the muscles of QF may exhibit an interaction similar to the interaction between GM, GL and soleus discussed above. The findings presented in this thesis indicate a STJ-dependent interaction of GM and GL and previous studies have reported differential actions of GM depending on knee and talocrural joint positions (Arampatzis et al., 2006, Magnusson et al., 2003). A similar mechanism might be present in the QF and the function of individual QF muscles might be dependent on knee and/or hop joint positions.

The individual muscles of QF are unipennate (Noorkoiv et al., 2010; Blazevich et al., 2006; Fukunaga et al., 1997) and contribute differently to knee extension. Based on their PCSA, RF, VM, VL and VI were found to contribute 25%, 12%, 24% and 40%, respectively (Blazevich et al., 2006). Similar to the gastrocnemii, the contribution of the bi-articular RF to knee extension is dependent on the position of the hip joint. With the hip joint flexed, the contribution of RF is greater than when the hip joint is flexed (Maffiuletti and Lepers, 2003; Pavol and Grabiner, 2000). Furthermore, the contributions of all four QF muscles to knee extension is also dependent on the knee joint angle (Watanabe and Akima, 2011), but de Ruiter et al. (2008) suggest that the contribution of VM to knee extension remains the same irrespective of knee joint angle.

All four muscles of the QF conjoin into the QF tendon, which spans the patella and inserts via the patellar tendon (PT) on the anterior side of the tibial tuberosity. Similar to the AT, the QF tendon consists of compartments corresponding to each of the QF muscles, but the compartments are arranged in layers and do not exhibit a twisted structure like the AT (Zeiss et al., 1992).

Non-uniform strain distributions have not been investigated for the QF tendon but have been reported for the PT. Similar to the strain distributions observed in the AT, greater strains have been reported in the joint-facing side of the PT than in the anterior portion (Pearson et al., 2014; Haraldsson et al., 2005), and the strain distribution pattern has been reported to be dependent on knee joint angle (Almekinders et al., 2002) and QF contraction level (Pearson et al., 2014). Strain differences within the PT may be an indication of a layered tendon structure (Pearson et al., 2014; Haraldsson et al., 2004), but the anatomical composition of the PT has not been reported in great detail in published studies to date. One MRI study reported a layered structure of the PT, where the superficial layer corresponds to RF, the deep layer corresponds to VI and the intermediate layer corresponds to VL and VM (Zeiss et al., 1992).

The complexity of the QF muscle group paired with variations in strain distribution within the PT may cause PT injuries as was discussed for the AT in Section 1.5. Like the AT, the PT is a very frequently injured site of the lower leg in the sporting population with a prevalence of 14% among athletes (Østein et al., 2005). The occurrence of patellar tendinopathy is region-dependent, but there is no consensus regarding the relationship between the pathological site within the PT and strain distribution patterns (Haraldsson et al., 2005; Basso et al., 2002). Interestingly, the amount of calcaneal eversion during relaxed standing has been linked to patellofemoral pain syndrome (Barton et al., 2010; Levinger and Gilleard, 2004). Eversion of the calcaneus causes internal rotation of the tibia and has been thought to alter patella tracking (Kaufman et al. 1999). Patella tracking may also be affected by imbalances in the relative contributions of the QF muscles to knee extension torque (Grelsamer and Klein, 1998). Both mechanisms may be a result of the interaction of the muscle of the QF muscle group, but the details have not yet been investigated.

7.4 Methodological considerations

In this series of studies, dynamometry combined with ultrasound was used to investigate the behaviour of GM and GL during a ramped isometric submaximal plantarflexion contraction. Both methods have been used in numerous previous studies (e.g. Farris et al., 2013, Arampatzis et al., 2005, Maganaris et al., 1998). However, limitations exist for both methods, which will be discussed.

7.4.1 Relative talocrural joint rotation

An isometric plantarflexion performed on a dynamometer is never truly isometric since the plantarflexion moment exerted by the triceps surae forces the talocrural joint to rotate resulting in lifting of the heel. Talocrural joint rotations during isometric plantarflexion efforts have been reported to be between 1.2° and 7.6° (Magnusson et al., 2001). This amount of talocrural joint rotation is problematic since it can result in an overestimation of tissue displacement (Magnusson et al., 2001). Correction methods involving passive talocrural joint rotation and the use of a goniometer to assess the amount of talocrural joint rotation have been proposed (Mademli and Arampatzis, 2005; Magnusson et al., 2001). Magnusson et al. (2001) have shown that results of tissue displacement obtained without correction can differ significantly from results obtained with correction. On the other hand, studies have reported to apply tight strapping of the lower leg to the foot adapter of the goniometer to limit talocrural joint rotation (e.g. Farris et al., 2013).

The experimental protocols of the present series of studies did not correct for talocrural joint rotation in order to allow for tight strapping. This was necessary to ensure that the entire foot of the participants remained in contact with the custom-made foot-plate throughout the plantarflexion trial. The footplate allowed rotation of the foot into inversion and eversion and it was important that inversion or eversion of the foot would be maintained throughout the ramped plantarflexion trial. Several straps were applied around the ankle complex to limit talocrural joint rotation to a minimum but it was not possible to apply a goniometer to the participants' ankle in this setup. Hence, the possibility for correction for talocrural joint rotation was sacrificed in order to ensure a minimum of talocrural joint rotation. The absence of joint rotation was visually confirmed; however, the occurrence of talocrural joint rotation cannot be fully excluded. This would have resulted in an overestimation of tendon elongation and strain values reported.

7.4.2 Morphological measurements with ultrasound

Ultrasound was used to determine fascicle length, pennation angle and tendon elongation. This methodology has been shown to be very reliable (Fukunaga et al., 1997, Narici et al., 1996), but some limitations still exist. Ultrasound provides a two-dimensional echoic image of a cross-section of the anatomy scanned. It is important to align the transducer with the anatomical plane of the region of interest, e.g. fascicles, to obtain accurate measurements of fascicle length and pennation angle. The error due to misalignment of the ultrasound transducer has been shown to be small approximately 1.08% (Lichtwark and Wilson, 2005). During a plantarflexion contraction, however, the triceps surae has shown to move laterally (Farris et al., 2012; Finni, 2006; Finni et al., 2003) so that the imaged cross-section moves out of the image plane, potentially introducing an error in the measurements.

Measurements of fascicle lengths as described in Chapter 4, were based on the assumption that fascicles form a straight line between the deep and superficial aponeuroses. However, fascicles of GM and GL were shown to curve and the amount of curvature increases upon fascicle shortening (Namburete and Wakeling, 2012; Muramatsu et al., 2001). Therefore, fascicle lengths may have been underestimated. In fact, Muramatsu et al. (2001) report an error of up to 6% when assuming straight fascicles compared to curved fascicles. Furthermore, all measurements of muscle architecture assume uniform fascicle lengths and pennation angles for the entire muscle belly. Some studies have reported similar fascicle lengths along and across the muscle bellies of GM and GL (Chow et al. 2000, Maganaris et al. 1998) while others described regional variations especially within GL (Segal et al., 2005; English et al., 1993; Wolf et al., 1993). Pennation angles have been found to differ considerably within the muscle belly of GM (Bolsterlee et al., 2015). However, it can be speculated that fascicle behaviour is not non-homogeneous throughout the muscle belly during a ramped submaximal isometric plantarflexion, since fascicle behaviour was not found to differ between GM and GL (Chapter 6).

7.4.3 Tendon resting length

Resting lengths of the GM tendon and the GL tendon were assumed to be constant in all three calcaneal positions, which may not have been the case. It was not possible to distinguish measurements of tendon resting lengths between the three calcaneal positions with the method described in Section 4.2. Inversion and eversion of the calcaneus may introduce a small length change of the tendon compared to tendon length in calcaneal neutral positions. However, it is not known whether a possible length change might have led to different tendon elongation and strain results. The use of motion capture would provide an answer to this question. For example, MTU length could be estimated by tracking the displacement of a marker over the AT insertion on the calcaneus (Farris et al., 2013) or from joint angles (Herbert et al., 2011).

7.4.4 Calculation of tendon force and stiffness

Tendon force was calculated as an individual's plantarflexion torque divided by their AT moment arm length. The AT moment arm was obtained using the COR method. The COR method and its limitations were described in Section 3.2 and 3.4, respectively. The tendon force of the GM tendon and the GL tendon was then calculated based on relative contributions of GM and GL to plantarflexion torque during a maximum plantarflexion effort based on the information provided by Albracht et al. (2013). Alterations in relative contribution of GM and GL at lower contraction levels, however, were not considered. Furthermore, antagonistic co-contraction from tibialis anterior was not taken into account. Co-contraction of tibialis anterior during plantarflexion contractions is well documented and results in a smaller plantarflexion torque measured compared to a plantarflexion effort without the presence of antagonistic co-activation (Magnusson et al., 2001). Several authors (e.g. Mademli et al., 2004; Magnusson et al., 2001) showed that co-contraction can considerably influence the calculation of resulting AT forces, i.e. real AT forces are higher when co-contraction is taken into account. The amount of tibialis anterior co-activation during a plantarflexion contraction can be determined by relating the electromyographic activity of tibialis anterior to the associated force produced during a dorsiflexion effort (Mademli and Arampatzis, 2005). This approach, however, assumes a linear relationship between electromyographic activity of tibialis anterior and the force produced.

In Chapter 5, AT forces were calculated to determine tendon stiffness. Stiffness values were obtained for GM and GL separately based on a linear, least-squares fit to the force-tendon elongation curve (Peltonen et al., 2012). Stiffness values presented in Chapter 5 are consistent with stiffness values reported in previous studies that applied similar calculation methods and did not take co-contraction into account (Morrissey et al., 2011). Stiffness values reported in the present literature vary widely depending on the calculation methods. An exact comparison is, therefore, not possible, but in Chapter 5, tendon stiffness was compared between GM and GL and both muscles would have been affected equally by the amount of tibialis anterior co-contraction. The relative contributions of GM and GL to the total AT force during a submaximal plantarflexion, however, were not known. Knowledge of their contributions would have allowed for a more accurate comparison of tendon stiffness between GM and GL.

7.4.5 Sample size

An important aspect to consider is the sample size. The *post hoc* power analyses yielded statistical powers of less than 80% especially for comparisons of contractile behaviour of GM and GL between different calcaneal postures and positions. To achieve an acceptable level of statistical power of 80% (Cohen, 1988), a considerably larger sample size would have been required Therefore, some results must be interpreted with some caution since falsely accepting the null hypothesis is a possibility.

7.5 Conclusions

The purpose of the series of studies presented in this thesis was to investigate contractile behaviour of GM and GL in different calcaneal postures and position during a ramped submaximal plantarflexion contraction and the effect of calcaneal inversion/eversion on their interaction. It was shown that calcaneal posture and position do not influence the sagittal AT moment arm. Furthermore, the morphology of GM and GL was shown to differ considerably between the two muscles, but the contractile behaviour of GM and GL during an isometric plantarflexion contraction is not affected by calcaneal posture and position. Fascicle behaviour was not found to differ between GM and GL during an isometric plantarflexion, but differences in tendon strain between GM and GL were found. Moreover, the contraction levels, at which these strain differences occur, are affected by calcaneal position. This indicates an interaction between GM and GL, which is STJ-dependent. Based on the findings of these studies and evidence from published literature, it was suggested that GM and GL are not two heads of the same muscle but two individual muscles with relatively independent function. Furthermore, it was suggested that GM and GL may not be bi-articular muscles acting on knee and talocrural joint but tri-articular muscles acting on knee, talocrural joint and STJ.

The results of the studies presented in this thesis indicate that adaptations of GM and GL to STJ position occur during isometric plantarflexion contractions. These adaptions, however, are achieved at the tendon level indicating a decoupling of fascicle behaviour and tendon behaviour. It can, therefore, be concluded that the contractile behaviour of GM and GL is not affected by calcaneal posture and position but the interaction of GM and GL is different depending on calcaneal position. In order to understand the interaction of GM and GL and the effect of calcaneal position better, the following issues need to be addressed:

This series of studies only considered the interaction of GM and GL but not soleus. Possible effects of soleus on the interactions within the entire triceps surae muscle group were discussed in section 6.2 and further work should consider three-way interactions.

The actions of GM, GL and soleus at the subtalar STJ are still poorly understood. An investigation into their inversion/eversion actions would shed more light on their interaction. It was proposed in chapter 6, that GM and GL might act as antagonists at the STJ depending on the location of the STJ axis. This has not been confirmed.

Stiffness calculations in chapter 5 assumed that the relative contributions of GM and GL are similar at every contraction level. This may, however not be the case. A closer investigation into relative contributions of all three muscles of the triceps surae muscle group would allow more accurate calculations of tendon stiffness at sub-maximal contraction levels. Furthermore, the relative contributions of the triceps surae muscles might be altered depending on the position of the STJ. This relationship has also not been investigated. It was speculated in Chapter 6 that relative contributions of GM and GL to plantarflexion torque might be altered in different calcaneal positions.

Finally, the studies presented in chapters 4, 5 and 6 of this thesis investigated the effect of calcaneal posture and position on the contractile behaviour of GM and GL in a standardised experiment using dynamometry. However, the movements of the foot, for example during gait, are complex and three-dimensional. An investigation into the differential behaviour of the three muscles of the triceps surae and their interaction may shed more light on specific loading conditions of the triceps surae MTU and provide greater insight into the aetiology of AT injuries.

7.6 Summary of the findings

- Clinical calcaneal angle assessments in the RCSP were shown to have low validity and a new MRI-based method was developed.

- The plantarflexion action of the triceps surae is not affected by calcaneal posture and position; the AT moment arm does not change.

- The COR method to determine sagittal plane AT moment arms could be adapted to determine frontal plane AT moment arms.

- Fascicle behaviour and tendon elongation are likely to be unaffected by calcaneal posture and position but further investigations with larger sample sizes are required.

- GM and GL differ significantly in their morphology.

- Fascicle behaviour during a ramped submaximal isometric plantarflexion contraction does not differ between GM and GL.

- GM tendon strain exceeds GL tendon strain.

- The contraction levels at which differences in tendon strain between GM and GL occur are dependent on calcaneal position.

7.7 Implication for future research and clinical practice

The findings of the research presented in this thesis provide further evidence for the differential nature of GM and GL. Based on previous literature and the findings of this thesis it is suggested that GM and GL should not be regarded as one muscle with two heads but as two muscles with different characteristics and functions. As such, muscle models of the triceps surae should not generalise findings pertaining to the contractile behaviour and mechanical properties of GM to both gastrocnemius muscles. The architecture, geometry and function of GL and GM differ and both muscles should be considered individually for each gastrocnemius muscle. Furthermore, GM and GL were shown to exhibit interaction, which is dependent not only on the position of the knee and talocrural joint but also on the STJ. Therefore, it was proposed that GM and GL are not bi-articular muscles but tri-articular due to their important contribution to STJ movement. In addition, soleus would not be mono-articular but bi-articular with actions at the talocrural joint and the STJ. The position of all of these joints must be taken into consideration when investigating the triceps surae muscle group. Further research is warranted to understand the nature of the interaction between all three muscles of the triceps surae muscle group, their contributions to talocrural and subtalar joint movement, especially at submaximal contraction levels.

The experimental data presented in this thesis was collected using a very standardised and controlled protocol, which poses limits on the applicability of the findings to clinical practice. However, clinicians should also be aware of the differential function of GM and GL at the ankle complex. AT injuries can result from non-homogeneous strain distributions within the tissue. This thesis provides further support that the differential function and force producing capabilities of GM and GL may be important factors. Furthermore, the function of GM and GL also differs depending on the position of the STJ. Training and rehabilitation programmes for patients with AT disorders should take these factors into account.

Assessments of calcaneal angle in clinical practice have been shown to have very low validity. Calcaneal is no longer assessed in isolation but still forms part of the Foot Posture Index (FPI). The FPI item related to the assessment of calcaneal angle should, therefore, be used with caution. A novel method for the identification of the calcaneal bisection in frontal plane images of the calcaneus was presented, which will be useful for surgeons planning and assessing STJ alignment procedures.

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