

# MUSCLE RESPONSES TO STRETCHING AND DEVELOPING EFFECTIVE INTERVENTIONS IN CHILDREN WITH CEREBRAL PALSY

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# THESIS SUMMARY

Hyper-resistance at the joint is one of the most common symptoms in children with cerebral palsy (CP). There are both neural and non-neural factors contributing to the observed joint hyper-resistance. Non-neural alterations to the musculoskeletal system such as a reduced muscle length, increased tendon length and an increase in joint stiffness are commonly treated by physiotherapy interventions such as stretching exercises. However, the effectiveness of these interventions in terms of improvements in function is of a low magnitude and the response of spastic muscles to stretch is poorly understood.

Therefore, the main aim of the programme of work presented in this thesis was to increase the effectiveness of stretching interventions at the ankle joint. To achieve this, it is important to understand the behaviour of the medial gastrocnemius muscle and Achilles tendon in response to a stretch applied at the joint. For this first sub-goal, three experimental studies were performed. First, the relationship between joint rotation and muscle and tendon contribution during a single stretch is described. We show that a smaller Achilles tendon moment arm leads to a decrease in muscle lengthening during joint rotation. Additionally, we show that the relative stiffness of the muscle to the tendon is increased in children with CP, which further limits lengthening of the muscle during joint rotation. Secondly, we document the acute effect of stretching on medial gastrocnemius fascicle lengthening properties. It was found that medial gastrocnemius muscle stiffness cannot explain the increased ankle joint range of motion acutely following passive stretching. These studies all indicate that the lengthening stimulus to the muscle is reduced in children with CP. Therefore, to increase the effectiveness of stretching exercises, an intervention was designed with the aim to increase this stretching stimulus seen by the muscle. By performing progressive resistance training, we showed an increase in the stiffness of the tendon, and thus a reduction in the relative stiffness of the muscle to the tendon. Therefore, the amount of stretch seen by the muscle was increased. We have shown that due to this combined intervention a remodelling of muscle is possible, as we showed muscle fascicle length to increase. Even though functional benefits were limited, the proof of principle was demonstrated and future work should explore this model of intervention further.

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# PUBLICATIONS AND CONFERENCE PRESENTATIONS

## Full papers

- Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Bass A, Holmes G, Barton GJ, Desloovere K, O'Brien TD (2017) Medial gastrocnemius muscle stiffness cannot explain the increased ankle joint range of motion following passive stretching in children with cerebral palsy. *J exp physiol*. DOI: 10.1113/EP086738
- Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Holmes G, Bass A, Barton GJ, Desloovere K, O'Brien TD (2017) Achilles tendon moment arm length is smaller in children with cerebral palsy than in typically developing children. *J Biomech*. **56**, 48-54

## Abstracts in scientific conferences and meetings

- *September 2017: 26<sup>th</sup> annual meeting of the European Society for Movement analysis in Adults and Children (ESMAC), Trondheim, Norway:* Kalkman BK, Holmes G, Maganaris CN, Barton GJ, Bar-On L, Bass A, Wright D, Walton R, O'Brien TD. Increasing tendon stiffness enhances the effectiveness of stretching interventions in children with cerebral palsy.
- *July 2017: The 26<sup>th</sup> Congress of the International Society of Biomechanics (ISB), Brisbane, Australia:* Kalkman BK, Bar-On L, Cenni F, Holmes G, Bass A, Maganaris CN, Barton GJ, Desloovere K, O'Brien TD. Muscle-tendon contribution to an increased range of motion following passive stretching in children with cerebral palsy.
- *May 2017: The 29<sup>th</sup> Annual Meeting of the European Academy of Childhood Disability (EACD), Amsterdam, The Netherlands:* Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Holmes G, Bass A, Barton GJ, Desloovere K, O'Brien TD. Contribution of muscle and tendon to the increased range of movement following passive stretching in children with cerebral palsy.
- *March 2017: The BASES Biomechanics Interest Group Meeting, Porthsmouth, UK:* Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Holmes G, Bass A, Barton GJ, Desloovere K, O'Brien

TD. The relationship between medial gastrocnemius lengthening properties and spasticity in cerebral palsy.

- *September 2016: 25<sup>th</sup> annual meeting of the European Society for Movement analysis in Adults and Children (ESMAC), Seville, Spain:* Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Holmes G, Bass A, Barton GJ, Desloovere K, O'Brien TD. The effect of cerebral palsy on Achilles tendon moment arm length. *ESMAC, Seville, Spain*
- *September 2016: 25<sup>th</sup> annual meeting of the European Society for Movement analysis in Adults and Children (ESMAC), Seville, Spain:* Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Holmes G, Bass A, Barton GJ, Desloovere K, O'Brien TD. Passive muscle and tendon properties during ankle joint rotation in children with cerebral palsy.
- *March 2016: The BASES Biomechanics Interest Group Meeting, Liverpool, UK:* Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Holmes G, Bass A, Barton GJ, Desloovere K, O'Brien TD. The effect of cerebral palsy on Achilles tendon moment arm length. *BASES Biomechanics Interest Group Annual meeting, Liverpool UK*

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## FREQUENTLY USED ABBREVIATIONS

CP	Cerebral Palsy
DF	Dorsiflexion
EMG	Electromyography
GMFCS	Gross motor functional classification scale
GRF	Ground reaction force
LL	Leg length
MA <sub>AT</sub>	Achilles tendon moment arm
MTJ	Muscle-tendon junction
MTU	Muscle-tendon unit
PCSA	Physiological cross-sectional area
ROM	Range of motion
SEM	Standard error of measurement
TD	Typically developing
TL	Tibia length

## CHAPTER 1:

### General Introduction

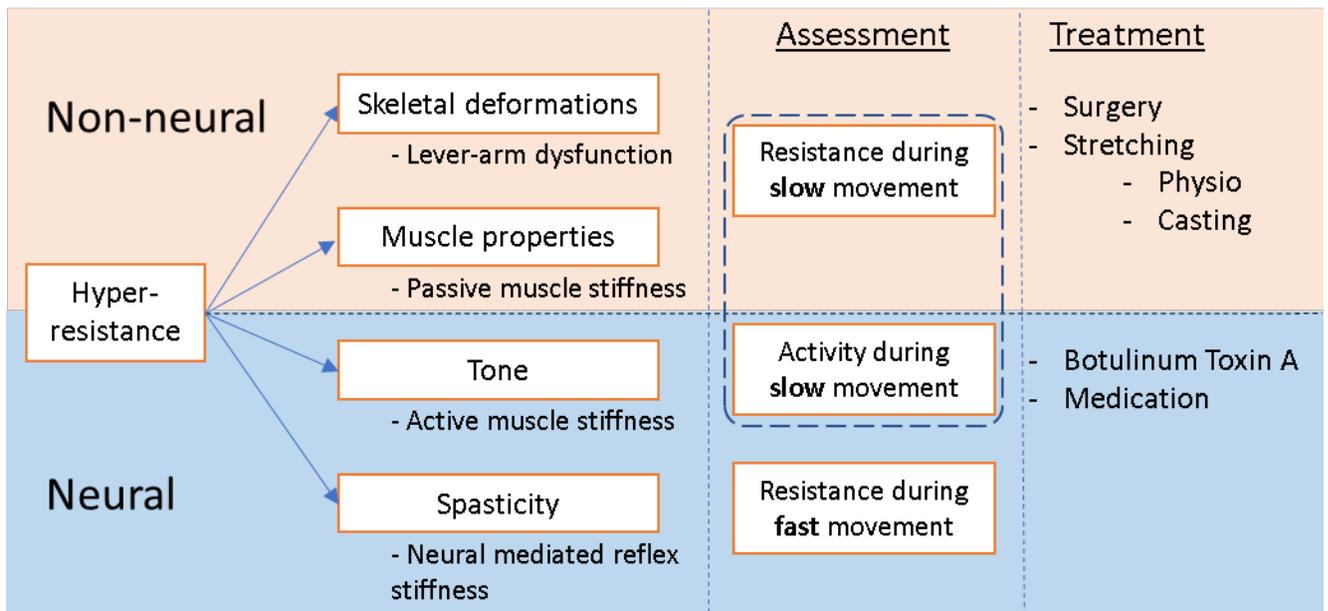
## Spastic cerebral palsy

Cerebral palsy (CP) is the most common cause of childhood disability, with an incidence of 1.5 to 2.5 cases per 1000 live births in the western world (Cans, 2000). Cerebral palsy is caused by a lesion to the immature brain that itself is non-progressive. While the severity of the brain lesion itself does not progress over time, cerebral palsy leads to a disorder of posture and movement that is permanent and can progress with age. Cerebral palsy has a number of different presentations, the most prevalent of which is spastic, affecting around 80% of all patients (Cans, 2000). Primary symptoms associated with CP are muscle weakness, spasticity, a reduced range of motion (ROM) and reduced coordination and an increased joint stiffness. When children grow, these impairments can lead to secondary musculoskeletal adaptations such as muscle contractures and bony-deformities. Although spastic CP is neural in origin, it appears that mechanical muscle and tendon adaptations contribute to the impaired posture and movement control. Furthermore, treatment is often aimed at correcting muscle and tendon lengths or restoring bony misalignments. As a first step in the management of CP, stretching therapies are commonly used with the aim to increase or maintain ROM, based on the assumptions that they increase muscle length and/or reduce its stiffness. However, the effectiveness of these stretching therapies is uncertain (Wiaart *et al.*, 2008; Craig *et al.*, 2016; Eldridge & Lavin, 2016). To provide recommendations as to how to improve the effectiveness of stretching interventions, it is essential to understand the underlying mechanisms of the reduced ROM, or joint hyper-resistance, as well as the mechanisms by which stretching exercises work.

## Joint hyper-resistance

Many of the impairments related to CP mentioned above can be captured under the term joint hyper-resistance, which has been defined as an increased resistance perceived during passive stretch (Noort van den *et al.*, 2017). The different contributions to joint hyper-resistance are captured in figure 1.1. A distinction can be made between neural and non-neural contributions. Since these have a different origin, they are also treated differently. Where neural components are often treated by Botulinum Toxin-A injections or selective dorsal rhizotomy, non-neural components can be treated by orthopaedic surgery or non-invasively with physiotherapy, including stretching exercises. The correct treatment for joint hyper-resistance is dependent on accurate assessment of the underlying mechanisms. However, the validity and reliability of current clinical tests are low (Noort van den *et al.*, 2010). The modified Ashworth scale (Bohannon & Smith, 1987) and the Tardieu scale (Tardieu *et al.*, 1954) are commonly used in clinical practice to assess joint hyper-resistance. During these tests, passive muscle elongations are imposed by

the examiner at fast and slow velocities. Passive resistance perceived at the joint during slow movements is commonly prescribed to any non-neural contributions where high velocities additionally trigger a stretch hyperreflexia response, described by a catch. It is clear from the theoretical framework described in figure 1.1, that by using these tests, it is difficult to accurately distinguish between any neural and non-neural contributions. Furthermore, alterations in mechanical tissue properties itself could change the perceived resistance at the joint, irrespective of what happens at the muscle. This thesis will focus on the non-neural contributions to joint hyper-resistance (top panel of figure 1.1), but both will be discussed briefly.



**Figure 1.1:** Overview of neural and non-neural contributions to joint hyper-resistance in children with CP.

### *Neural contributions to hyper-resistance*

The term spasticity is classically used to describe the neural contribution to joint hyper-resistance and has been described by Lance in 1980 as ‘a motor disorder characterised by a velocity dependent increase in tonic stretch reflexes with exaggerated tendon jerks, resulting from hyper excitability of the stretch reflex’ (Lance, 1980b). This definition highlights the pathophysiological mechanisms of a velocity dependent response. The stretch reflex of a muscle is triggered from the muscle spindles located within the muscle fibres. In CP, a lack of inhibition from the central nervous system leads to an over excitability of the stretch reflex. The increased stretch reflex can be objectively quantified with instrumented assessments by quantifying the EMG bursts and the joint angle at EMG burst onset during fast joint rotations (Salm van

der *et al.*, 2005; Bar-On *et al.*, 2014a). Apart from the classic velocity dependent response, tonic involuntary background activation also contributes to joint hyper-resistance and this activity is non-velocity dependent. This involuntary background activation can be quantified with instrumented assessment by quantifying muscle activation during slow movements. It has recently been shown that the EMG response to stretch is not only dependent on the stretch velocity but also on joint position (muscle length) (Bar-On *et al.*, 2014b) and that these two types of neural contributions vary significantly between different children and muscles. It has been suggested that neural and non-neural contributions to hyper-resistance are not independent of each other (Švehlík *et al.*, 2013; Willerslev-Olsen *et al.*, 2013), therefore, as a supplementary study to the main thesis, we investigated the relationship between medial gastrocnemius lengthening properties and stretch reflex (appendix C).

### *Structural contributions to hyper-resistance*

As mentioned, structural adaptations to muscle tissue have also been reported frequently. Clinically these adaptations are referred to as contracture, which is defined as a fixed shortening of the muscle relative to the length of the bones. This clinical observation can be explained by numerous structural alterations in muscle and tendon tissue. In research settings ultrasound is commonly used to assess these structural alterations of the muscle and tendon in static position or during slow passive movement where neural contributions are minimised (figure 1.1).

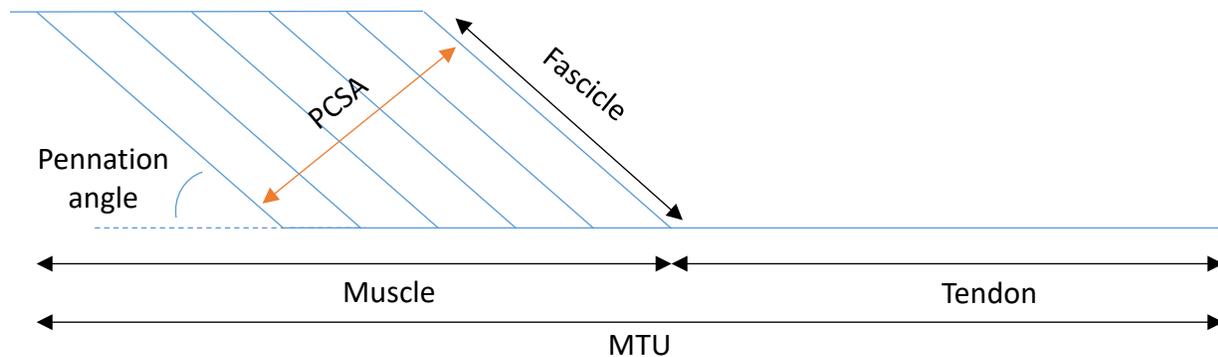
#### *Architecture of spastic muscles*

When studying morphological muscle properties, we consider the anatomical size and shape, or dimension of a muscle and the arrangement of the fascicles. The morphology of a muscle can be studied with medical imaging techniques such as magnetic resonance imaging (MRI) and 2D or 3D ultrasound. The main focus of such studies in CP has been on the medial gastrocnemius muscle. This muscle is functionally important and often affected in children with CP. Furthermore, because the medial gastrocnemius is a superficial muscle, it is easy to image with ultrasound. This section will describe the main morphological alterations seen in spastic muscles and how they may influence its function.

Muscle volume is a global measure that provides us with an overall indication of muscle power (O'Brien *et al.*, 2009a) and growth (Herskind *et al.*, 2016), both longitudinal and cross-sectional. Muscle volume in CP has been assessed both with MRI and 3D ultrasound. It has been shown that muscle volume of several major lower leg muscles is reduced in the paretic limb of children with CP when compared to age-matched TD children (Barber *et al.*, 2011b; Noble *et al.*, 2014; Reid *et al.*, 2015). Furthermore, it has been shown

that when compared to TD children the rate at which muscle volume increases with age is decreased in children with CP (Barber *et al.*, 2016).

The volumetric shape of a muscle is determined by the physiological cross-sectional area (PCSA) and the length of the muscle fascicles (figure 1.2). Decreases in muscle volume as shown in CP can be caused by a reduction in the PCSA, which would lead to muscle weakness, or a reduction in fascicle length, which would reduce the range of movement over which the muscle can exert force. These distinct consequences of a reduced muscle volume indicate that it is important to study muscle properties in more detail. The model used to describe these properties is shown in figure 1.2.



**Figure 1.2:** Schematic representation of a pennate muscle tendon unit (MTU). PCSA: Physiological cross-sectional area.

First of all, the muscle-tendon-unit (MTU) consists of a muscle and a tendon working in series. In the medial gastrocnemius, muscle length is described as the distance between the proximal attachment of the muscle on the medial femoral condyle to the distal muscle-tendon junction (MTJ). Tendon length is described as the distance between the MTJ and the attachment of the Achilles tendon on the calcaneus. There is considerable evidence that the length of the medial gastrocnemius (Malaiya *et al.*, 2007; Fry *et al.*, 2007) and other muscles (Oberhofer *et al.*, 2010) is reduced in the paretic leg of children with CP. Compensatory to that, it has been found that the length of the Achilles tendon in spastic muscles of children with CP (Gao *et al.*, 2011; Barber *et al.*, 2012), but also in spastic stroke patients is increased (Zhao *et al.*, 2009). Secondly, as mentioned above, the length of the muscle belly is dependent on both the PCSA

and the length of the muscle fascicles (figure 1.2). Therefore, the observed alterations in the muscle length are not necessarily accompanied by alterations in fascicle length.

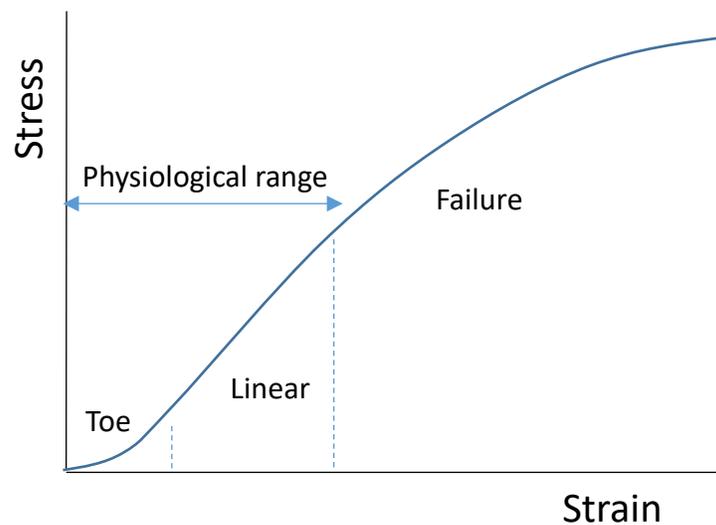
Reports on fascicle length in children with CP are inconsistent. Fascicle length is functionally an important parameter because it has a direct relationship with the excursion range over which the muscle can produce force. This is important for example during the push of phase of the gait cycle, where the calf muscles are lengthened but need to produce a sufficiently large force to achieve an adequate power burst. Shorter fascicles, with fewer sarcomeres in series have a reduced range of motion over which they can produce force. Several studies have reported measures of medial gastrocnemius fascicle lengths, where some studies report no differences (Shortland *et al.*, 2002; Malaiya *et al.*, 2007; Mathewson *et al.*, 2014b) and other studies reported a reduction in fascicle length in children with CP (Mohagheghi *et al.*, 2007, 2008; Gao *et al.*, 2011; Matthiassdottir *et al.*, 2014). Difference in findings might be attributed to methodological issues such as the heterogeneity of the patient group and normalization technique. A proper conclusion is lacking.

The reported deficits in muscle volume are much larger (18%-58%) than the reported decreases in fascicle length (0-29%) in the paretic leg of children with CP (Barrett & Lichtwark, 2010). Pennation angle in CP has not been found to differ from TD children (Shortland *et al.*, 2002; Malaiya *et al.*, 2007), therefore it is argued that the small and short muscles in CP are, among others, caused by smaller PCSA and a lack of cross-sectional growth (Malaiya *et al.*, 2007).

#### *Mechanical properties of spastic muscles*

The mechanical properties (stiffness) of the muscle determine the change in length (or joint angle) in response to an applied force (or torque). This will depend partly on the architectural structure of the muscle, as well as on the intrinsic tissue properties in the muscle. The passive mechanical properties of any tissue can be assessed by applying a known force to the tissue and measuring the amount of elongation/deformation that will occur (Maganaris, 2002). This way a force-elongation curve can be constructed and tissue stiffness is determined as the slope of this relationship in the linear portion (figure 1.3). *In vivo* this becomes a bit more complicated as there are anatomical constraints acting on the structure to be studied. This makes it difficult to assess *in vivo* muscle (fascicle) and tendon stiffness and some assumptions need to be made. By combining 2D ultrasound, EMG and dynamometry we can approximate some of the passive mechanical properties of the medial gastrocnemius muscle. When applying a passive moment around the ankle joint to dorsiflex the foot, the lengthening of the medial gastrocnemius muscle-tendon unit is among others dependent on the muscle's moment arm, any passive

structures within the joint (i.e. ligaments) and the properties of all muscles acting around the joint (agonists and antagonists). These factors might change as a function of ankle angle, making it difficult to quantify the force acting through the muscle and tendon during passive joint rotation. Alternatively, more invasive methods have been used to assess muscle mechanical properties in children with CP. From muscle biopsies, the stiffness of single fibres and fibres bundles can be studied and sarcomere length can be determined *in vivo* with intraoperative laser diffraction.



**Figure 1.3:** Relationship between stress and strain in physiological tissue

It is well known that in children with CP, passive joint stiffness, calculated from the joint moment-angle relationship during passive joint rotation is increased when compared to TD children (Alhusaini *et al.*, 2010). To assess the cause of this increased joint stiffness, previous studies have looked at muscle and fascicle lengthening when passively rotating the ankle joint in children with CP. It has been shown that muscle lengthening is reduced (Matthiasdottir *et al.*, 2014; Kruse *et al.*, 2017) in children with CP when compared to TD children. While some authors show that fascicle lengthening is unaltered in children with CP (Matthiasdottir *et al.*, 2014), others show a reduction in fascicle lengthening (Barber *et al.*, 2011a). Methodological discrepancies between studies could explain the different findings. Some authors compare CP and TD groups over their full ROM, whereas others compare groups over a common ROM. It is well known that ROM is reduced in children with CP, therefore assessing muscle lengthening properties over each individual's full ROM would automatically lead to a smaller muscle lengthening in CP children.

Additionally, increases in joint stiffness also need to be considered when comparisons in lengthening properties are made between groups. An increased joint stiffness in children with CP will cause less MTU lengthening when the same torque is applied at the joint. So far, a consensus on how muscle fascicles and the connective tissue, both within and in series with the muscle, interact to achieve ROM is lacking.

Several studies have assessed the contribution of altered tissue properties to the increased muscle stiffness (table 1.1). *Ex vivo* stiffness of individual muscle fibres in children with CP and TD children has been assessed from muscle biopsies and was shown to be increased in spastic muscles (Friden & Lieber, 2003; Lieber *et al.*, 2003; Foran *et al.*, 2006). An increase that could be attributed to passive connective tissue within the muscle cell. Titin, a large molecule spanning from the Z-disc to the M-band in a sarcomere has been mentioned to play a role in mediating muscle stiffness (Herzog *et al.*, 2012). Also, passive collagen structures surrounding individual muscle cells which we will refer to as the extracellular matrix (ECM) have been suggested to play a role in mediating muscle stiffness (Smith *et al.*, 2011). Alternatively, it has been suggested that the increased muscle stiffness is caused by an increased sarcomere operating length when compared to healthy muscles. Combining this information with a lack of increase in muscle fascicle length leads to the conclusion that children with CP have fewer sarcomeres in series (Mathewson *et al.*, 2014a).

To conclude, there is some indication that muscle fascicle stiffness is increased in children with CP, which could originate from a decreased sarcomere number or alterations to the connective tissue surrounding individual muscle fibres. However, fundamental knowledge as to how muscle and tendon interact to achieve ROM in children with CP is still lacking. Therefore, it is also still unknown how these individual tissues respond to treatments that aim to alter the structural components of the muscle, but are provided at the level of the joint, such as stretching exercises.

### *Skeletal deformations*

Additionally, to the above mentioned structural alterations, children with CP can develop deformities to skeletal bones, since bone grows and adapts in response to loading. Bony deformities can limit movement and causes lever-arm dysfunction (Theologis, 2013). Lever-arm dysfunction is caused either by a rotation, shortening or change in structure of the muscle's or external moment arm. At the ankle joint (figure 1.4) for example, the external moment arm of the ground reaction force (GRF) relative to the axis of rotation of the ankle can be shortened because of a mid-foot break (flexible moment arm) or tibial torsion (rotated

**Table 1.1:** Overview of literature assessing altered mechanical tissue properties of muscle tissue in children with cerebral palsy. VL: Vastus Lateralis, FCU: Flexor carpi ulnaris, ECRB: Extensor Carpi Radialis Brevis, ECM: Extracellular Matrix, CSA: Cross-sectional area

Author	Methodology	Muscles	Outcome measure	Result
Booth et al. (2001)	Biopsy	VL	Total collagen content	Collagen I accumulation in endomysium
Lieber et al. (2002)	Laser diffraction	FCU	Sarcomere length	Increased
Friden et al. (2003)	Biopsy Single muscle fibre dissection	Variety	Sarcomere length/joint angle curve	Not changed
			Resting sarcomere length	Decreased
			Fibre elastic modulus	Increased
Lieber et al. (2003)	Biopsy	Variety	Fibre CSA	Decreased
			Sarcomere length at fibre failure	Not changed
			Tangent modulus of fibre bundle	ECM modulus decreased in CP
Foran et al. (2006)	Biopsy Single fibre dissection	VL	Tangent modulus of single cells	
			Fibre tangent modulus	
			- With titin	Increased
Ponten et al. (2007)	Laser diffraction	FCU/ECRB	- Without titin	Not changed
			In vivo sarcomere length	Increased sarcomere length
Smith et al. (2011)	Biopsy, single muscle cell dissection & Immunohistochemistry	Hamstring	Titin isoform	Not changed
			Single fibre stiffness	Not changed
			Bundle stiffness	Increased
			Collagen content	Increased
Smith et al. (2013)	Biopsy, mononuclear cell isolation	Hamstring	Satellite cell percentage	Decreased
Mathewson et al. (2014)	Laser diffraction and ultrasound	Soleus	Sarcomere length	Increased
De Bruin et al. (2014)	Biopsy mechanical and histological properties	FCU	Fascicle length	Not changed
			Sarcomere slack length	Not changed
			Sarcomere length-tension relation	Not changed
			Fibre type	Not changed
			Fibre size	Reduced
Dayanidhi et al. (2015)	Immunohistochemistry	Gracilis	Tertiary perimysium	Increased
			Satellite cell number	70% reduction in satellite cells
				Distribution altered

of the ankle can be shortened because of a mid-foot break (flexible moment arm) or tibial torsion (rotated moment arm). A muscle's internal moment arm on the other hand is important because it determines the muscle force needed to generate a moment around the joint according to the following equation:

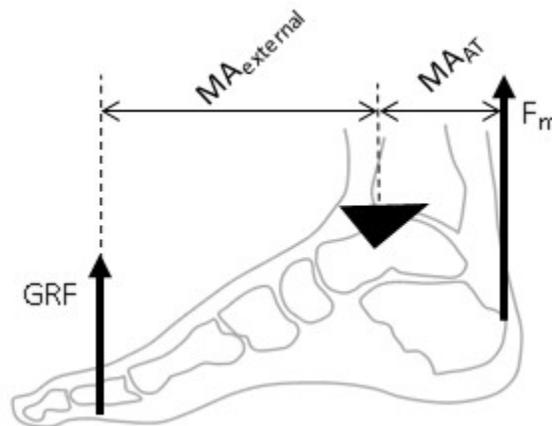
$$M_j = MA * F_m$$

Where  $M_j$  is joint moment, MA is the muscle's moment arm and  $F_m$  is the muscle force. Furthermore, the internal moment arm determines the amount of MTU lengthening during a passive joint rotation:

$$\theta_j = MA * \Delta l_{MTU}$$

Where  $\theta_j$  is the joint angle, MA is the muscle's moment arm and  $\Delta l_{MTU}$  is the change in MTU length.

In combination, the external moment arm and the internal moment arm determine the mechanical advantage of a joint. Knowledge about the mechanical advantage is important, because it determines the amount of muscle force that is needed to overcome an external resistance and is related to energy consumption (Biewener *et al.*, 2004). Furthermore, when studying the relation between muscle properties and joint movement it is vital to take into account the length of the internal moment arm of a muscle.



**Figure 1.4:** Schematic drawing of the internal and external moment arm around the ankle

## Management of spastic CP

As outlined above, a large part of the treatment of spastic CP is typically aimed at the musculoskeletal system (non-neural contributions), specifically, muscle stiffness, contracture and bony deformities (Gage, 2010). Stretching exercises, in the form of physiotherapy, orthotics, casting or any combination are important in the early management of joint hyper-resistance in children with spastic CP. These treatments

aim to maintain or improve ROM for functional movement, increase muscle extensibility and prevent or delay the need for orthopaedic surgery later in life (Wiar *et al.*, 2008). Although commonly applied in clinical settings, recent scientific studies have shown that evidence for the effectiveness of stretching interventions is limited (Pin *et al.*, 2006; Wiar *et al.*, 2008). Also, the outcome measures reported, such as ROM and joint stiffness, are relatively global and do not give us any insight in the contribution of muscle, tendon and connective tissue. This makes it even more difficult to explain the limited effectiveness of stretching interventions. Clearly there is a significant gap between the clinical rationale for stretching and the supporting evidence. Although considered a non-invasive treatment, stretching exercises cause pain and discomfort to the child, they are time consuming and demanding for children and their families (Hadden & Von Baeyer, 2002). Therefore, stronger scientific evidence is needed to support these interventions.

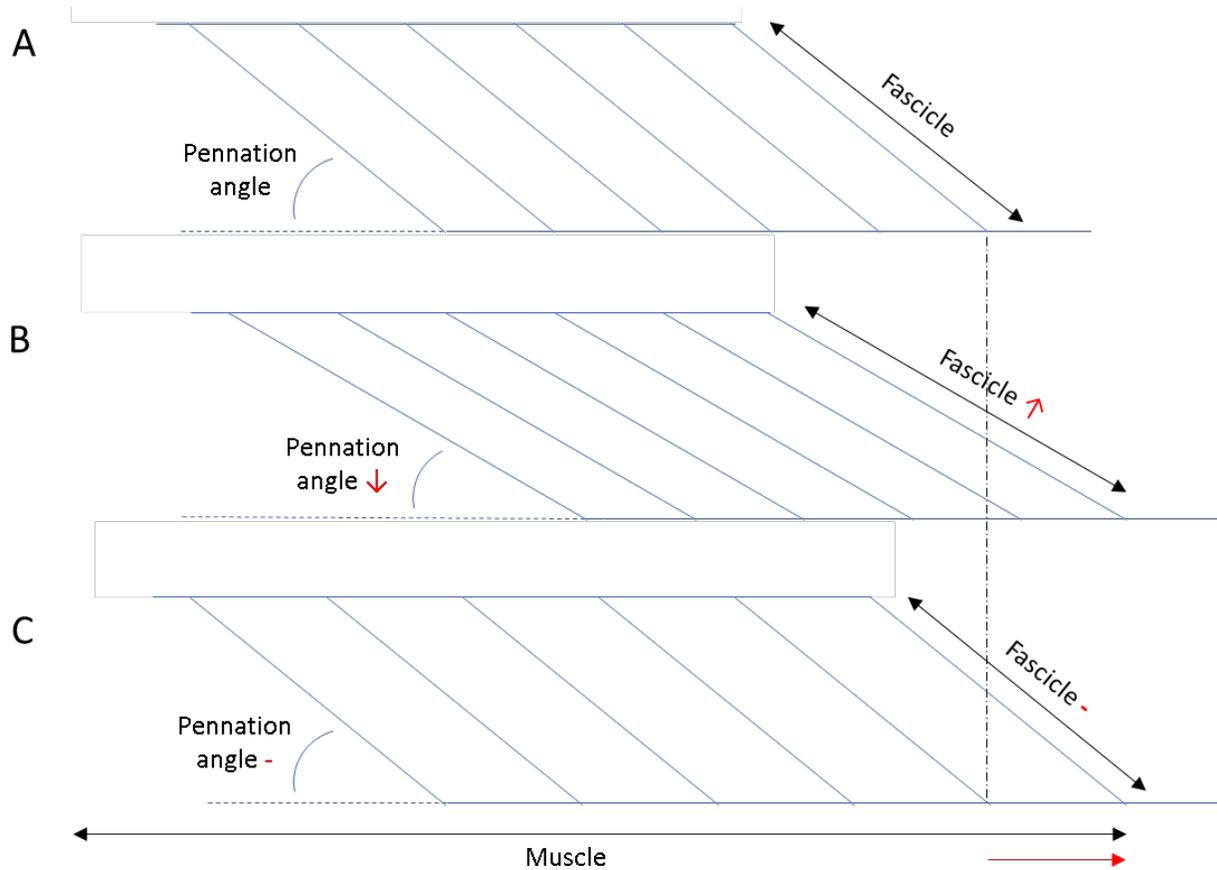
### *Biomechanical response during passive stretching.*

For any physiological changes to occur in the muscle, it is necessary that the structures we expect to change do receive an adequate stretching stimulus, in other words, the muscle needs to lengthen. In pennate muscles, such as the medial gastrocnemius, lengthening of the muscle-tendon-unit can be achieved in different ways. Firstly, when stretching the MTU, lengthening can come from either the muscle or the tendon, see figure 1.2. Here, the relative stiffness between the muscular and tendinous tissue is important because it will be the more compliant structure that will take up most of the stretch. Changes in muscle and tendon stiffness in children with CP could therefore potentially alter the response to stretching interventions. Secondly, lengthening of the muscle itself can be achieved in two different ways. Either, the muscle can lengthen by a rotation and lengthening of the fascicles, as is shown in figure 1.5B. Alternatively, the muscle belly can lengthen while the fascicles stay the same length and maintain the same pennation angle by stretching the intramuscular connective tissue. This in its turn depends on the relative stiffness properties of the fascicles compared to the connective tissue.

### *Physiological adaptations to passive stretching*

In theory, passive stretching may stimulate an increase in the number of sarcomeres in series, this has been shown in multiple animal models, where prolonged positioning of muscles at increased length over several weeks resulted in increased fibre length and in series sarcomere number (Williams & Goldspink, 1973). Because of the changes in muscle architecture and passive properties described above, it is not known if the same mechanism applies to spastic human muscles. The reduction of in-series sarcomere

number seen in spastic muscles (Mathewson *et al.*, 2014b) in combination with a reduction in satellite cell number (Dayanidhi *et al.*, 2015), questions whether spastic muscles are able to achieve any remodelling. Alternatively stretching may alter the intermuscular connective tissue and thereby reduce the stiffness of the muscle. This has been shown to contribute to the increase in ROM seen acutely after a single bout of stretching exercises in typically developing adults (Morse *et al.*, 2008).



**Figure 1.5:** A) Schematic model of a pennate muscle. B) Lengthening of the muscle belly occurs due to a lengthening and rotation of the muscle fascicles. C) Lengthening of the muscle belly occurs without any length changes in the muscle fascicles.

## Summary

Hyper-resistance at the joint is one of the most common symptoms in children with CP. There are both neural and non-neural factors contributing to the observed joint hyper-resistance. Non-neural alterations to the musculoskeletal system such as a reduced muscle length, increased tendon length and an increase

in passive joint stiffness are commonly treated by physiotherapy interventions such as stretching exercises. However, the effectiveness of these interventions in terms of improvements in function is of a low magnitude and the response of spastic muscles to stretch is poorly understood.

## Purpose and outline of this thesis

The overall purpose of this research was to increase the effectiveness of stretching interventions in children with spastic CP. For this purpose, four main experiments were performed. In chapter 2 and 3 we assess the relationship between joint rotation and muscle and tendon contribution *during* a single stretch. More specifically, in chapter 2 we describe alterations in the Achilles tendon moment arm in children with CP and how this affects muscle excursion relative to joint rotation. In chapter 3 we describe the material and mechanical properties of the medial gastrocnemius muscle relative to the Achilles tendon in children with CP compared to TD children. As a next step in chapter 4 we assess the acute effect of stretching on medial gastrocnemius fascicle lengthening properties and we describe how the increase in ROM acutely after stretching can be explained. Following on from the findings of these 3 experiments an intervention was designed with the aim to increase the stretching stimulus seen by the muscle. Consequently, in chapter 5 we perform a combined strengthening and stretching intervention. Strengthening exercises are performed with the aim to increase the stiffness of the tendon so that the relative stiffness of the muscle to the tendon is decreased and the muscle will see more of the stretch during stretching exercises. Chapter 5 shows the effect of such an intervention on muscle properties and gait. The thesis is then closed with a general discussion of the findings and future recommendations.



## CHAPTER 2

Achilles tendon moment arm length is smaller in children with cerebral palsy than in typically developing children

*The information presented in this chapter has been reported in the paper:*

Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Holmes G, Bass A, Barton GJ, Desloovere K, O'Brien TD (2017) Achilles tendon moment arm length is smaller in children with cerebral palsy than in typically developing children. *J Biomech.* **56**, 48-54

## Abstract

When studying muscle and whole-body function in children with cerebral palsy (CP), knowledge about both internal and external moment arms is essential since they determine the mechanical advantage of a muscle over an external force. Here we asked if Achilles tendon moment arm ( $MA_{AT}$ ) length is different in children with CP and age-matched typically developing (TD) children, and if  $MA_{AT}$  can be predicted from anthropometric measurements. Sixteen children with CP (age: 10y 7m  $\pm$ 3y, 7 hemiplegia, 12 diplegia, GMFCS level: I (11) and II (8)) and twenty TD children (age: 10y 6mo  $\pm$ 3y) participated in this case-control study.  $MA_{AT}$  was calculated at 20° plantarflexion by differentiating calcaneus displacement with respect to ankle angle. Seven anthropometric variables were measured and related to  $MA_{AT}$ . We found normalised  $MA_{AT}$  to be 15% (~7mm) smaller in children with CP compared to TD children ( $p=0.003$ ).  $MA_{AT}$  could be predicted by all anthropometric measurements with tibia length explaining 79% and 72% of variance in children with CP and TD children, respectively. Our findings have important implications for clinical decision making since  $MA_{AT}$  influences the mechanical advantage about the ankle, which contributes to movement function and is manipulated surgically.

## Introduction

Cerebral palsy (CP) commonly presents with bony deformities due to increased muscle forces acting on the bones (Morrell *et al.*, 2002), which can cause ‘lever-arm dysfunction’ (Novacheck & Gage, 2007). At the ankle joint, the external moment arm is defined as the perpendicular distance from the ground reaction force (GRF) to the joint centre of rotation. An altered external moment arm may be related to equinus (toe-walking), a midfoot break or tibial torsion, all of which shorten the external moment arm. Surgical interventions are recommended to increase external moment arm length. In the case of equinus, surgical lengthening of the Achilles tendon can improve kinematics, but weakness of the plantar flexors can occur as an adverse outcome of surgery (Gage *et al.*, 2009). To understand the effects of surgical interventions that alter external moment arm, we need to consider how they will change the mechanical advantage. Mechanical advantage is defined as the ratio of internal to external moment arm length. Where the internal moment arm is defined as the perpendicular distance from the muscle-tendon line of action to the joint centre of rotation. However, the length of the Achilles tendon moment arm ( $MA_{AT}$ ), its contribution to mechanical advantage, and the influence it has on surgical outcomes is often not considered in clinical decision making.

The mechanical advantage of a joint determines the internal muscle force needed to overcome an external resistance, e.g. GRF, and has been shown to influence joint and whole body function (Lee & Piazza, 2009). Differences in either moment arm can alter the outcome of muscle contraction, even if muscle function remains unaltered. In situations where movement function may be impaired and the study of muscle function is important, such as CP (Barber *et al.*, 2011a), knowledge of moment arm length and mechanical advantage is vital if we are to understand the nature of impairment and how best to intervene.

The internal moment arm about which a muscle operates also determines more fundamental measures of joint function (Lieber & Friden, 2000). The internal moment arm is proportional to maximum joint torque and the excursion range over which a muscle acts. Thus, differences in moment arm will affect the force-length properties of the muscle. Moreover, an altered muscle excursion range due to differences in the internal moment arm would also necessitate a different muscle shortening velocity, which influences the force-velocity characteristics (Lieber, 2002).

Furthermore, moment arm length is an important feature that determines the outcomes of musculoskeletal modelling. When using these models in children, typically developing (TD) or with CP, moment arm lengths are typically scaled down from adults using a 1:1 proportionality across all structures and dimensions (Sloot *et al.*, 2015), but it is not clear if this is a valid way to scale (O'Brien *et al.*, 2009b; Waugh *et al.*, 2011). Inappropriate scaling can lead to erroneous conclusions concerning muscle length and produced forces (Scheys *et al.*, 2008).

In children with CP, the plantarflexor muscles are often affected by spasticity and contracture, and the foot and ankle joint by deformities. Currently, the focus lies on how these deformities affect the external MA, but we do not know how  $MA_{AT}$  may be altered. A previous study reported smaller muscle excursion over a common ROM in CP vs. TD children, which the authors hypothesised may be related to shorter  $MA_{AT}$  in the CP group (Matthiasdottir *et al.*, 2014). However, since excursion was measured at the myotendinous junction (MTJ) and possible alterations in Achilles tendon lengthening during ankle joint rotation are unknown, the smaller muscle excursion may be caused by either a shorter  $MA_{AT}$  or greater Achilles tendon deformation. So far, no studies have directly measured  $MA_{AT}$  lengths in children with CP. Therefore, the purpose of this study was to quantify  $MA_{AT}$  length in children with CP and TD children using the tendon excursion (TE) method with tendon displacement measured at its distal attachment to the calcaneus. Also, we wanted to establish if  $MA_{AT}$  in both groups can be predicted from anthropometric measurements. We hypothesised that  $MA_{AT}$  is smaller in children with CP compared with TD children, and that in both groups  $MA_{AT}$  is predictable from anthropometry.

# Methods

## *Participants*

Thirty-six children aged 6-16 years were recruited for participation (table 2.1). Sixteen children were diagnosed with spastic CP and twenty age-matched TD children served as a control group. Exclusion criteria were having botulinum toxin-A injection to the lower limb muscles within six months prior to testing, a baclofen pump or any lower limb neuro- or orthopaedic surgery. All TD children were free from neuromuscular or skeletal disorders. Children were recruited through the gait lab of Alder Hey Children's Hospital in Liverpool and the University Hospital in Leuven. The study was approved by the Institutional as well as the NHS research ethics committee in the UK and the University Hospital's ethics committee in Leuven. The study was conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained from the parents, and written assent was given by children in accordance with local regulations.

**Table 2.1:** Participant characteristics

	<b>CP (n=15)</b>	<b>TD (n=20)</b>
Age (years)	11.1 (3.05)	10.4 (3.4)
Male/female (n)	10/5	11/9
Height (cm)	140.5 (20.6)	141.5 (16.6)
Mass (kg)	34.6 (18)	37 (14)
Tibia length (mm)	333 (58)	334 (43)
GMFCS (I-IV) (n)	10 I, 5 II	n/a
Diagnosis (n)	9 Diplegia, 6 Hemiplegia	n/a
Modified Ashworth Score (n=7) and Average Modified Tardieu (n=7) *	MAS: 1 (n=1), 1.5 (n=5), 3 (n=1) Tardieu: 2 (n=5), 3 (n=2)	n/a

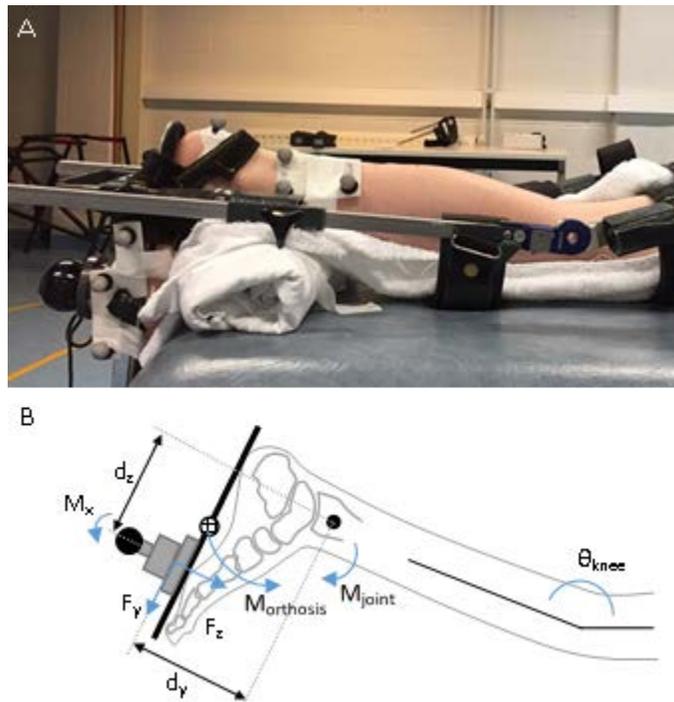
Data are mean (SD) unless otherwise stated. CP: cerebral palsy; TD: typically developing; GMFCS: gross motor functional classification scale; n/a: not applicable.

\*Tardieu scores from children recruited at Alder Hey Children's Hospital in Liverpool. MAS from children recruited at University Hospital in Leuven. One participant unknown.

## *Procedure*

Participants lay prone on a bed with their leg in a custom-made orthosis, to lock knee angle at 20° flexion and control ankle movement to occur only in the sagittal plane (figure 2.1A). The axis of rotation of the orthosis was aligned with the lateral malleolus. The leg tested was the most affected, defined by spasticity scores, in the CP group and the left in the TD group. During each trial, the foot was passively rotated by an experimenter from maximum plantarflexion to maximum dorsiflexion (DF), taking five seconds to

complete the ROM while ankle angle, calcaneus displacement, muscle activity and joint torque were measured.



**Figure 2.1:** **A)** Experimental setup of the ankle in the orthosis. Two clusters of markers were used to calculate ankle angle and a marker on the calcaneus was used to calculate calcaneus displacement. The load cell was used to calculate joint torque. During the experiment, the ankle was moved from plantar to dorsiflexion. **B)** Free body diagram of the foot and foot plate.  $d_y$  and  $d_z$  correspond to the moment arm distances from the point of force application, respectively  $F_y$  and  $F_z$ , of the load-cell to the lateral malleolus.  $M_x$  is the torque exerted on the load cell in the x direction.  $M_{orthosis}$  is the calculated torque caused only by the weight of the orthosis. The joint torque is given by:  $M_{joint} = -F_z d_z - F_y d_y - M_x - M_{orthosis}$

This procedure was repeated six times with a minimum of 10 s of rest between each repetition. Participants were instructed to relax their muscles during the movements, which was checked for post-processing by inspection of surface electromyography (sEMG) of the Triceps Surae muscles at 1600 Hz (Zerowire, Cometa, Milan, IT). Forces and torques applied at the ankle were measured using a six degrees of freedom force sensor load-cell (ATI mini45: Industrial Automation) attached to the orthosis under the ball of the foot. Ankle angle and calcaneus displacement along the length of the shank were calculated from the 3D position of two clusters of markers placed on the foot-plate of the orthosis and the shank and

a single marker on the most superficial part of the posterior calcaneal tuberosity (Optitrack, US). A separate validation was performed to assure the marker on the calcaneus moves synchronous with the attachment of the Achilles tendon (Appendix B). Kinematic data was sampled at 120 HZ and kinetic data at 200 Hz.

### Data analysis

Data analysis was carried out using custom made software (Matlab R2015a, Python 2.7.11). Kinematic and kinetic data were filtered using a 2<sup>nd</sup> order low pass Butterworth filter with a cut-off frequency of 6 Hz. Net ankle joint torque was calculated according to equation 1:

$$M_{joint} = -F_z d_z - F_y d_y - M_x - M_{orth}$$

Where  $F_z$ ,  $F_y$  and  $M_x$  are the forces and torques exerted on the load cell in the z, y and x direction respectively,  $d_z$  and  $d_y$  are the moment arm distances from the point of force application of the load-cell to the lateral malleolus (see free body diagram, figure 2.1B) and  $M_{orth}$  is the predicted torque caused by gravity on the orthotic (Bar-On *et al.*, 2013b; Schless *et al.*, 2015).

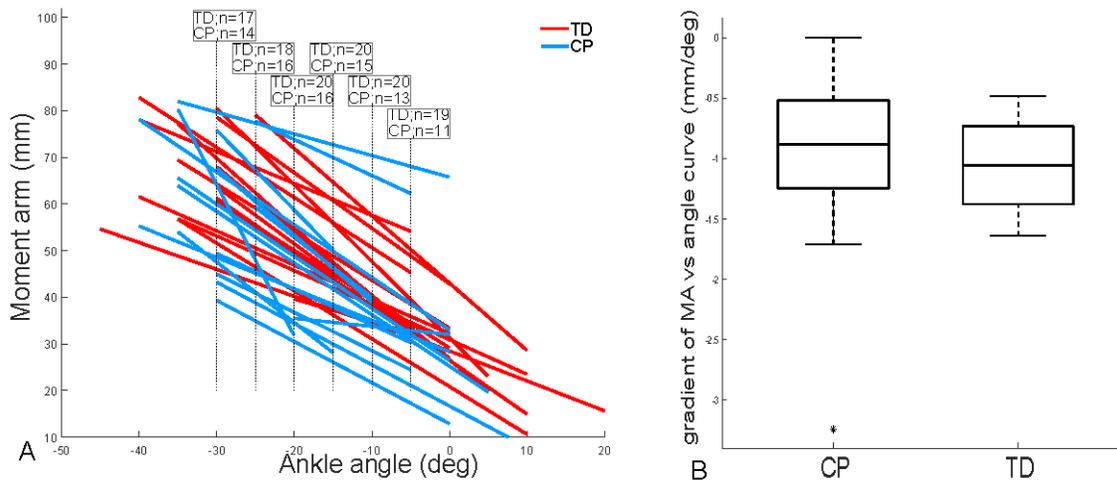
#### Calculation of $MA_{AT}$

$MA_{AT}$  was determined using the tendon excursion method (An *et al.*, 1984; Ito *et al.*, 2000), which defines moment arm as the ratio between linear displacement of the tendon and the change in joint angle ( $MA_{AT} = \frac{\delta_{calc}}{\theta}$ ). Here, linear displacement of the Achilles tendon was defined at its insertion on the calcaneus, and measured from the displacement of a marker on the calcaneus along the direction of the tibia ( $\delta_{calc}$ ), corrected for marker size and skin thickness. A separate pilot experiment has shown that the marker on the skin was a valid representation of the distal insertion of the Achilles tendon (Supplementary material).

For each participant, calcaneus displacement vs. ankle angle curves were constructed for six passive dorsiflexion movements. In some children, a mid-foot break or heel lift out of the orthosis was apparent

**Table 2.2:** minimal, maximal and average joint torques measured at each ankle angle in the common ROM in typically developing (TD) and cerebral palsy (CP) participants.

	-30°		-25°		-20°		-15°		-10°		-5°	
	TD	CP										
Min (Nm)	-1.3	-0.9	-1.4	-0.9	-1.2	-0.8	-0.9	-0.6	-0.7	-0.4	-0.5	-0.2
Max (Nm)	-0.7	-0.1	-0.6	-0.1	-0.3	0.2	0.2	1.0	1.0	2.4	2.3	4.9
Mean (Nm)	-0.9	-0.7	-0.9	-0.6	-0.7	-0.4	-0.3	0.1	0.1	0.8	0.7	2.3



**Figure 2.2:** A: Absolute Achilles tendon moment arm ( $MA_{AT}$ ) length vs ankle angle for typically developing children (TD, red) and children with cerebral palsy (CP, blue). Six angles common to most participants at 5° intervals between -30° and -5° were identified where we could record calcaneus excursion accurately. The boxes above each joint angle indicate the number of children in each group for which  $MA_{AT}$  could be calculated at that specific angle. -20° was the angle achieved by the most children. B: Box and whisker plot of gradient of the  $MA_{AT}$  vs angle curves in TD children and children with CP. \* represents one outlier that was removed from further analysis. The edges of the box correspond to the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The whiskers correspond to  $2.7\sigma$  and cover 99.3 percent of all data

towards maximum dorsiflexion, identifiable by plateauing of the displacement-angle curve. In these cases, curves were trimmed to a smaller ROM. The calculated joint torques were checked over this trimmed ROM. At angles common to most participants, passive joint torques were typically ~1 Nm (table 2.2) and were considered to be sufficiently low to not violate the assumptions of the tendon excursion method and allow us to continue the calculation of  $MA_{AT}$  at all angles (Olszewski *et al.*, 2015). Next, a second order polynomial was fitted through the six trimmed displacement-angle relationships ( $0.97 < r^2 < 0.99$ ). A second order polynomial allowed for the possibility for  $MA_{AT}$  to change with ankle angle. These polynomials were differentiated to construct a  $MA_{AT}$ -ankle angle relationship for each participant. These individual  $MA_{AT}$ -angle graphs within the trimmed ROM are plotted in figure 2.2A. As the ROM over which  $MA_{AT}$  could be calculated was different for each participant, we selected  $MA_{AT}$  at one common ankle angle that most participants could achieve (-20°, with negative angles expressing plantarflexed position) for further calculations. Valid comparison of  $MA_{AT}$  at a single angle relies on equal slopes of the  $MA_{AT}$ -angle

relationship between subjects. To test this assumption, the average gradients of the MA<sub>AT</sub>-angle relations were compared between groups. In the CP group, one participant was excluded because the gradient of the MA<sub>AT</sub>-angle relationship was determined as an outlier, i.e. exceeding 'Q3 ± 1.5 \* (Q3 - Q1)', with Q1 and Q3 being the 25<sup>th</sup> and 75<sup>th</sup> percentiles. Furthermore, this profile was judged to be physiologically implausible (4cm change over a couple degrees). After removing the outlier, within groups, all gradients were within 2.7 SD from the mean and considered similar within and between groups (p=0.27, 95% CI [-0.1, 0.37]) (figure 2.2B). These lengths were then corrected for marker size (11 mm diameter) by subtracting 5.5 mm. Finally, the individual distance measured from the surface of the skin to the attachment of the Achilles tendon on the calcaneus was measured with B-mode ultrasound and subtracted from the MA<sub>AT</sub>.

### *Anthropometric measurements*

Seven anthropometric variables (table 2.3) were measured by a trained physiotherapist: height, body mass, leg length (LL), tibia length (TL), foot length, distance between medial and lateral malleolus and age. These parameters were assessed for predictive power and normalization of MA<sub>AT</sub>. Tibial torsion was measured while the participant was lying prone with the knee in 90° flexion as the angle between the line of the longitudinal axis of the thigh and a line perpendicular to the axis connecting the most prominent points of the medial and lateral malleolus.

**Table 2.3:** definitions of anthropometric variables

<b>Measure</b>	<b>Method</b>
Tibia length	Distance from the tibiofemoral joint space to lateral malleolus
Leg length	Distance from the anterior superior iliac spine to the medial malleolus
Foot length	Distance from lateral malleolus to the head of metatarsal two
Inter-malleolar distance	Distance from the medial to lateral malleolus measured with 3d motion capture.

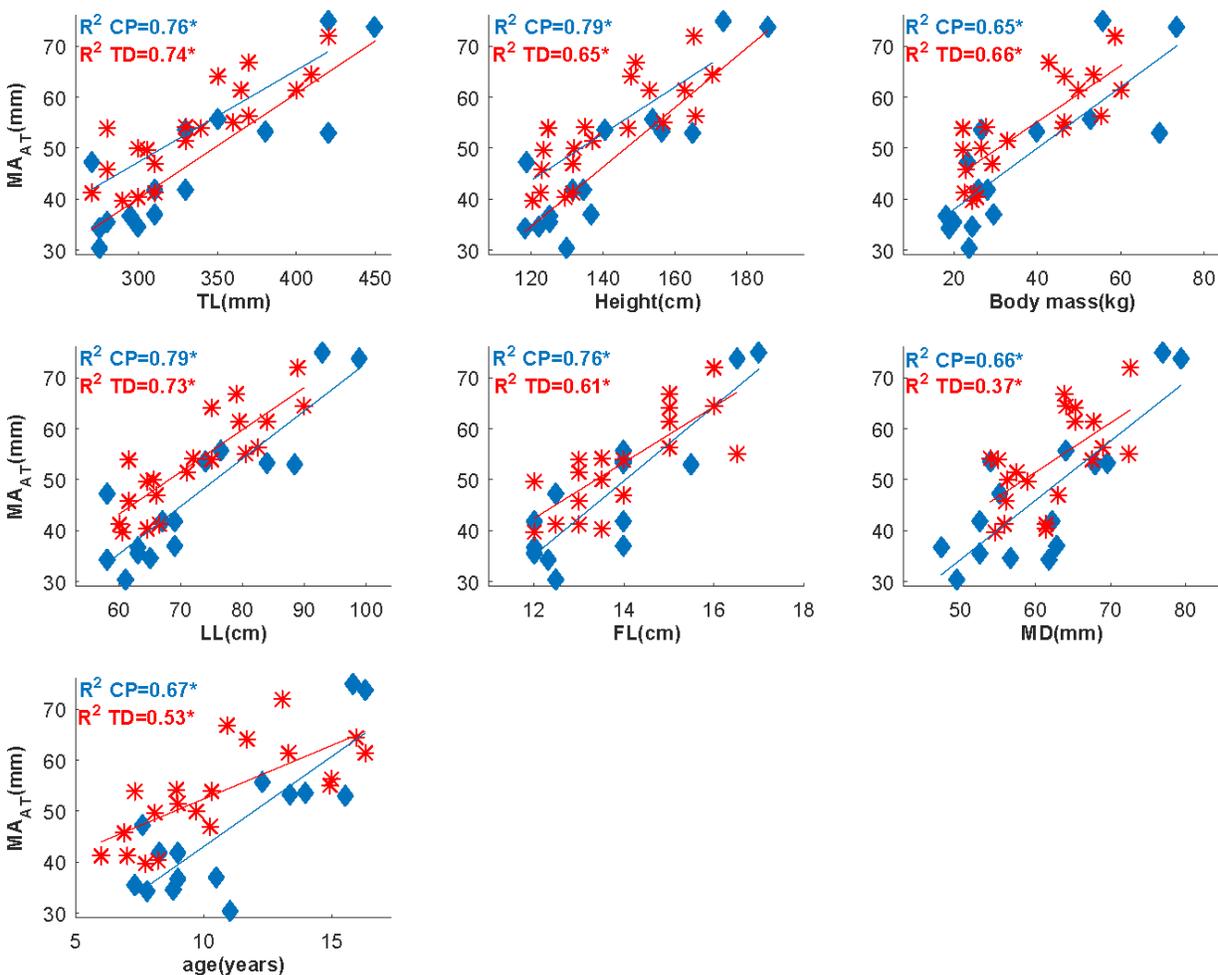
### *Statistics*

All parameters were checked to be normally distributed using the Shapiro-Wilk test and by inspection of the q-q plots. To determine if MA<sub>AT</sub> could be predicted from anthropometric measurements, a backwards stepwise multiple regression analysis was performed, starting with all 7 anthropometric variables described above predictors are then removed based on p-value (p>0.05). Secondly, linear regressions between MA<sub>AT</sub> and each anthropometric measurement separately were performed, of which Pearson r<sup>2</sup>-values were calculated. Based on the r<sup>2</sup>-values of significant relationships it was decided whether, and to which, anthropometric dimension MA<sub>AT</sub> could be normalised. Absolute and normalised MA<sub>AT</sub> were then

compared between groups with a 2-sample independent t-test. The level of statistical significance was set at  $p \leq 0.05$ .

## Results

Data of fifteen children with CP and twenty TD children was used for the final  $MA_{AT}$  calculations. No differences were found between the groups for any of the anthropometric measurements. In the current sample of children with CP, Tibial torsion was between  $0^\circ < \alpha < 20^\circ$  (Median:  $15^\circ$ , IQR:  $10-20^\circ$ ), which is similar to the tibial torsion range considered as “typical” (Mudge *et al.*, 2014).



**Figure 2.3:** Correlations between anthropometric variables and Achilles tendon moment arms ( $MA_{AT}$ ) in children with cerebral palsy (CP) and typically developing children (TD). TL: tibia length, LL: leg length, FL: foot length and MD: inter malleolar distance.

Correlations between MA<sub>AT</sub> at -20° and each anthropometric measure showed mostly strong positive relationships ( $p < 0.01$ ,  $r^2 = 0.36-0.81$ , figure 2.3). It was decided most appropriate to scale MA<sub>AT</sub> to TL, because of the high combined  $R^2$  values across CP and TD groups (figure 2.3).

When MA<sub>AT</sub> was normalised to TL, children with CP had significantly smaller MA<sub>AT</sub> than TD children ( $p = 0.001$ , 95% CI [1.05, 3.96]). This difference was consistent over the ROM studied (-30° to -5°) and its magnitude ranged from 1.4 to 2.5 %TL across the ROM. Absolute MA<sub>AT</sub> were smaller in CP but did not reach significant difference between groups ( $p = 0.0544$ , 95% CI [-0.15, 14.91]).

Linear regressions were performed with all anthropometric variables as separate predictors (table 2.4). MA<sub>AT</sub> could be significantly predicted by all variables independently in both children with CP and TD children. Inclusion of more variables in the regression did not improve predictive power (single regressions'  $r^2 \sim 0.7$ , vs. multiple regression  $r^2 \sim 0.65$ ).

**Table 2.4:** predictive equations to calculate MA<sub>AT</sub> at -20°,  $r^2$  and  $p$  values for all anthropometric variables are shown. TL: tibia length, LL: leg length, FL: foot length, MD: distance between medial and lateral malleoli.

	Formula	$r^2$	$p$
<b>CP</b>			
TL (mm)	MA <sub>CP</sub> = -26.1 + 0.20 * TL	0.79	<0.01
Height (cm)	MA <sub>CP</sub> = -38.7 + 0.57 * Height	0.80	<0.01
Body mass (kg)	MA <sub>CP</sub> = 20.4 + 0.60 * Body mass	0.66	<0.01
LL (cm)	MA <sub>CP</sub> = -26.2 + 0.93 * LL	0.81	<0.01
FL (cm)	MA <sub>CP</sub> = -57.3 + 7.23 * FL	0.79	<0.01
MD (mm)	MA <sub>CP</sub> = -27.9 + 1.13 * MD	0.67	<0.01
Age (y)	MA <sub>CP</sub> = 2.45 + 3.52 * age	0.70	<0.01
<b>TD</b>			
TL (mm)	MA <sub>TD</sub> = -8.2 + 0.17 * TL	0.72	<0.01
Height (cm)	MA <sub>TD</sub> = -13.1 + 0.44 * Height	0.65	<0.01
Body mass (kg)	MA <sub>TD</sub> = 28.9 + 0.53 * Body mass	0.68	<0.01
LL (cm)	MA <sub>TD</sub> = -7.8 + 0.78 * LL	0.71	<0.01
FL (cm)	MA <sub>TD</sub> = -22.1 + 5.00 * FL	0.57	<0.01
MD (mm)	MA <sub>TD</sub> = -7.2 + 0.90 * MD	0.36	<0.01
Age (y)	MA <sub>TD</sub> = 27.1 + 2.04 * age	0.55	<0.01

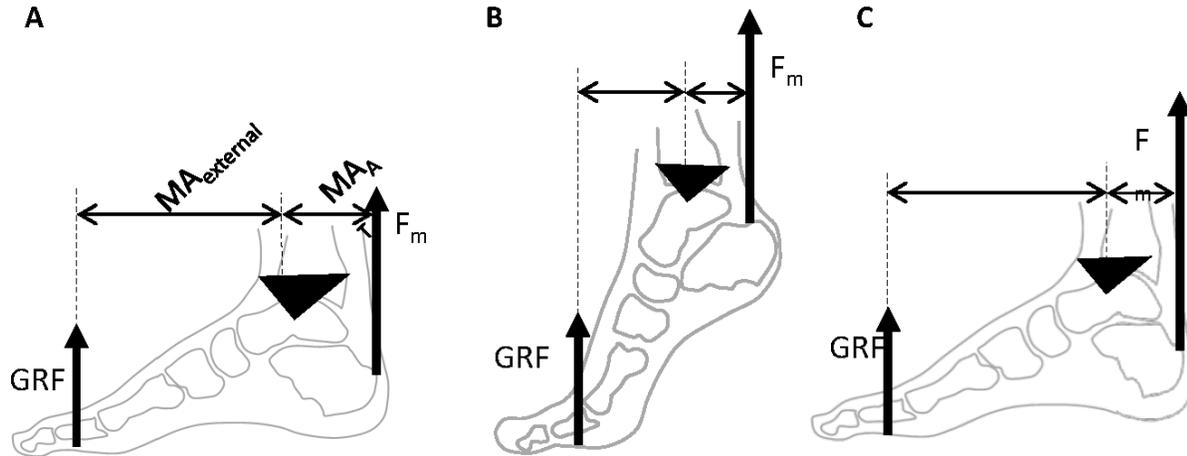
## Discussion

The aim of this study was to quantify MA<sub>AT</sub> in children with CP and TD children, and to determine whether this can be predicted from anthropometrics. We found that MA<sub>AT</sub> in children with CP is 15% (~7mm) smaller than in TD children throughout an ankle ROM of -30° to -5°. Also, it was shown that in both groups MA<sub>AT</sub> scales with, and can be predicted using a range of anthropometric measurements.

The  $MA_{AT}$  found in TD children in this study was slightly large compared to literature (Waugh *et al.*, 2011), which might have been caused by the inclusion of older children in this study. The profile of  $MA_{AT}$  vs ankle angle is slightly different from what is reported in the literature. We report about 1mm decrease in  $MA_{AT}$  per degree for both TD and CP children. Waugh *et al.*, (2011) report a change of 4mm over 10 degrees in children, where both Fath *et al.*, (2010) and Rugg *et al.*, (1990) report a change of 10mm over 15 degrees in adults. These differences might be caused by the different methodologies used. The use of the tendon excursion method over a large ROM in this study might lead to a larger change in moment arm with ankle angle when compared to an instantaneous method such as the centre of rotation method (Rugg *et al.*, 1990);

The relationship between internal and external moment arm around the joint reflects the mechanical advantage. This is an important feature when trying to explain ankle joint function in pathological gait. Considering a simplified scenario when a child is standing stationary on the forefoot, the smaller  $MA_{AT}$  in children with CP, would be associated with a smaller mechanical advantage at the ankle if it was not for the external moment arm also being smaller, for example due to equinus or other external moment arm deformities. The combination may actually mean that mechanical advantage does not turn out to be very different to typical (figure 2.4A&B). Orthopaedic treatment, typically aiming to correct external moment arm length by optimizing foot shape and foot progression angle, will increase the external moment arm and thereby reduce the mechanical advantage. This would happen regardless of  $MA_{AT}$  length, but our results indicate that the mechanical advantage would not be restored to typical as intended, but in fact be reduced to less than typical (figure 2.4C). This could be one of the contributing factors to the observed weakness of the plantar flexor muscles after equinus correcting surgery (Orendurff *et al.*, 2002; Gage *et al.*, 2009). Further research should focus on the specific influence of orthopaedic surgeries correcting lever arm dysfunction on the mechanical advantage of the ankle. Also, studies should seek to determine  $MA_{AT}$  length during gait, and under loading conditions (Rasske *et al.*, 2016).

A smaller  $MA_{AT}$  also brings about changes in the torque-angle relationship. Shorter  $MA_{AT}$  would generate smaller maximal joint torques even when the same maximal muscle force is generated. Also, with a shorter  $MA_{AT}$ , the same muscle excursion will cause a larger joint rotation. Consequently, torque can be produced over a broader ROM (figure 2.5A). As less muscle excursion during joint rotation corresponds to lower angular velocities, joint torque would decline less as joint velocity increases (figure 2.5B). Consequently, the shorter  $MA_{AT}$  of children with CP has the effect of creating joint function similar to that associated with a muscle with long fascicles and small physiological cross-sectional area (Lieber & Friden, 2000).

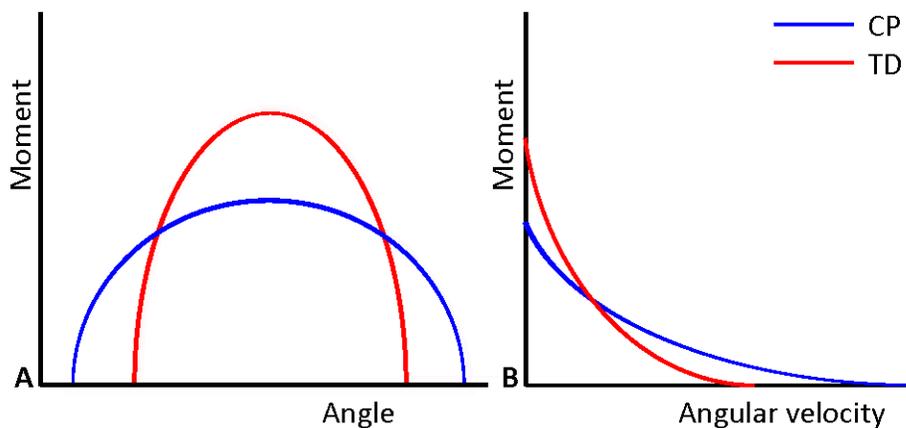


**Figure 2.4:** Schematic drawing of the foot with the ground reaction force (GRF) and muscle force ( $F_m$ ) acting over the external moment arm ( $MA_{ext}$ ) and Achilles tendon moment arm ( $MA_{AT}$ ) respectively. a: TD child, b: a child with CP in equinus and c: a child with CP where surgery has lengthened the  $MA_{ext}$  but not altered the  $MA_{AT}$ . For simplicity, GRF and  $F_m$  are shown to act at right angles to the horizontal. In a and b, the ratio between  $MA_{AT}$  and  $MA_{ext}$  reflects the mechanical advantage and will be close to typical in the child with CP. c: When the  $MA_{ext}$  is lengthened the mechanical advantage will be decreased.

Spasticity is a major problem in children with CP, generally defined as a ‘velocity-dependent increase in muscle activity’ (Lance, 1980b). Assessments of spasticity rotate the joint at high velocities to elicit a response (Bar-On *et al.*, 2013b). Since a shorter moment arm leads to a smaller muscle lengthening velocity when rotating the joint, relating angular velocities to muscle stretch will likely underestimate the true sensitivity of the muscle (Bar-On *et al.*, 2014c).

The smaller  $MA_{AT}$  in children with CP might be due to alterations in bony architecture or the development of bony deformities. Future studies should investigate possible bony deformities at the ankle that can cause the smaller  $MA_{AT}$  with imaging techniques such as MRI or X-ray. Muscle thickness could also play a role (Maganaris *et al.*, 1998), since this would influence the trajectory of the tendon’s action line and so its distance from the centre of joint rotation. Children with CP have a smaller muscle cross sectional area than TD children (Barber *et al.*, 2011a), so this could contribute to the smaller  $MA_{AT}$ . Future studies should aim to confirm the exact cause of the decreased  $MA_{AT}$  in children with CP.

When using musculoskeletal models to study movement function, MAs are central in calculating muscle forces and lengths. Here we confirm that conventional scaling using a 1:1 proportionality across all structures and dimensions is not valid for scaling between adults and children. In addition, we show that applying any scaling algorithm similarly in children with CP and TD children is invalid. Therefore, we provide predictive equations to calculate  $MA_{AT}$  at  $-20^\circ$  in children aged 6-16 years. We found the profile of the MA-angle relationship to be not significantly different within and between groups, this means that a) comparing  $MA_{AT}$  at a single joint angle is valid for the ROM described, and b) we can combine the predicted  $MA_{AT}$  with the known gradient ( $-0.9$  vs  $-1.03$  mm/deg, for CP and TD respectively) to calculate  $MA_{AT}$  at angles between  $-30^\circ$  to  $-5^\circ$ . More research is needed to extend these relationships over a full ROM.



**Figure 2.5:** Schematic torque-angle (a) and torque-angular velocity (b) relationships of two identical muscles with different moment arm lengths. Muscles of children with CP have smaller moment arms than TD children, all other muscle properties are considered to be identical. TD muscle will produce a larger peak torque for a given muscle force, however the active range of force production is larger in CP joint. Similarly, peak joint torque is larger in TD muscles, however, with increasing velocity the decline in joint torque is less in CP muscles.

A multiple regression analysis was performed in this study. However, because of the large degree of collinearity between the anthropometric variables, it was decided to perform an independent linear regression for each predictor. Similar results concerning the predictability of patellar tendon moment arm have been shown in TD children (O'Brien *et al.*, 2009b), where tibia length was shown to be one of the main predictors. On the other hand, Waugh *et al.* (2011) concluded that due to low  $r^2$ -values,  $MA_{AT}$  could

not be predicted from anthropometrics in TD children aged 5-12 years. The contrast with the present findings may be explained by the larger age range used in this study and thus an increased power to detect correlations.

This study has a few limitations. The sample size is relatively small for a heterogeneous population like children with CP. However, the statistical power of our comparison of MA<sub>AT</sub> length between groups was 0.88, which suggests the sample was adequate for our purpose (Chow *et al.*, 2003). Normalisation of MA<sub>AT</sub> was performed by dividing MA<sub>AT</sub> with tibia length, to reduce the variability within the data due to the large range in length of the children and thus obtain a dimensionless number for MA<sub>AT</sub> (Hof, 1996). Additionally, from the predictive equations we can observe that the slope of MA<sub>AT</sub> vs TL is similar in TD and CP, the difference in intercept however indicates that there is indeed a difference in the absolute size of MA<sub>AT</sub> between groups.

Different methods can be used to calculate MA<sub>AT</sub> length, and each has some limitations. In this study we used the tendon excursion method to define MA<sub>AT</sub>, which has been shown to correlate well with MRI based methods (Fath *et al.*, 2010). To exclude deformation of the tendon as a possible source of error we measured tendon displacement at the distal end of the tendon instead of proximally at the MTJ (Maganaris *et al.*, 2000). Even with this step, the tendon excursion method used relies on several assumptions. First, it is assumed that the tensile load applied to the muscle-tendon was constantly low and thus it does not cause any deformations (Olszewski *et al.*, 2015). We confirmed passive net joint torques to be low (~0-1 Nm) across the ROM studied, therefore we believe that no confounding tissue deformations have been introduced. Second, the tendon excursion method assumes ankle rotation to occur within the sagittal plane (Maganaris, 2004). However, the tibio-talar joint axis is typically externally rotated by an angle of 15±5°, which leads to an overestimation of MA<sub>AT</sub>. In children with CP, tibial torsion can cause this angle to deviate from typical values. In the current study, tibial torsion, as measured by the bimalleolar angle, was within typical range, reducing the likelihood that it would have confounded our comparisons (Mudge *et al.*, 2014). Unfortunately, this method of assessing tibial torsion has low correlation to more accurate radiological measures (Lee *et al.*, 2009). To individually correct MA<sub>AT</sub> for tibial torsion, better imaging techniques are necessary. Furthermore, other foot deformities such as varus/valgus or an increased mobility in the foot could have affected our results because of the assumptions inherent to the tendon excursion method as discussed above. In further work it should be aimed to assess the exact relationship between foot deformities and moment arm measures.

To conclude, this study has shown for the first time that Achilles tendon moment arm ( $MA_{AT}$ ) is smaller in children with CP compared to TD children. This has important implications in clinical decision making since  $MA_{AT}$  influences the mechanical advantage about the ankle, which contributes to movement function and is manipulated surgically. We also found that both in TD children and in children with CP,  $MA_{AT}$  can be predicted from anthropometric measurements, which allows realistic quantification for relevant modelling applications without access to medical imaging facilities.



## CHAPTER 3

### Muscle and tendon lengthening behaviour during ankle joint rotation in children with cerebral palsy

*The information presented in this chapter has been reported in the paper:*

Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Bass A, Holmes G, Barton GJ, Desloovere K, O'Brien TD (2017) Muscle and tendon lengthening behavior during ankle joint rotation in children with cerebral palsy. *J experimental Physiology*. Submitted

## Abstract

Children with cerebral palsy (CP) commonly present with reduced ankle range of motion (ROM) partly due to non-neural alterations of the muscle-tendon-unit (MTU). Detailed information about how muscle and tendon interact to contribute to joint ROM is currently lacking, but may provide essential information to guide treatment. The purpose of this study was to quantify which structures contribute to MTU lengthening and thus receive the stretch during passive ankle joint rotation. Fifteen children with CP (age:11.4±3y) and 16 typically developing (TD) children (age:10.2±3y) participated. Ultrasound was combined with motion tracking, joint torque and electromyography to record fascicle, muscle and tendon lengthening of the medial gastrocnemius during passive ankle joint rotations over the full and a common ROM. In children with CP, relative to MTU lengthening, muscle and fascicles lengthened less (CP: 50.4%, TD: 63% of MTU lengthening;  $p<0.04$ ) and tendon lengthened more (CP: 49.6%, TD: 37% of MTU lengthening,  $p<0.01$ ) regardless the ROM studied. Differences between groups in the amount of lengthening of the underlying structures during similar amount of joint and MTU displacement indicate possible differences in structural properties due to CP which are not evident by assessment on a joint level. These factors should be considered when assessing and treating muscle function in children with CP.

## Introduction

Children with spastic CP usually show an increased ankle joint stiffness and reduced range of motion (ROM) compared to typically developing (TD) children. It has been reported that muscles of children with CP undergo significant morphological changes, which contribute to the reduced ROM (Mathewson & Lieber, 2015). Previous studies of medial gastrocnemius muscle structure in CP using ultrasound consistently report shorter muscle bellies compared to TD subjects (Fry *et al.*, 2004; Barrett & Lichtwark, 2010). In addition, longer Achilles tendon has been reported in children with CP (Wren *et al.*, 2010; Barber *et al.*, 2012), which could be an adaptation to compensate for the shorter muscle belly. Furthermore, some studies have reported smaller resting muscle fascicle lengths in children with CP than TD children (Mohagheghi *et al.*, 2008; Matthiasdottir *et al.*, 2014), but others have not detected differences (Shortland *et al.*, 2002).

When trying to assess and treat impairments in joint function it is important to understand the mechanical properties of the underlying structures. The stiffness of a muscle relative to the tendon will determine how

these two structures interact when the joint is rotated and the whole MTU lengthened. In TD adults it has been shown that when stretched, muscle fascicles undergo much smaller changes in length than the whole muscle-tendon unit (MTU) (Herbert & Moseley, 2002; Morse *et al.*, 2008). This can be explained by a classic muscle model where the fascicles (contractile element) are arranged in series with the tendon (series elastic element). However, this interaction of muscle and tendon, and how they contribute to achieve ROM has not yet been studied in CP. It has been shown that, when passively rotating the joint in children with CP, the medial gastrocnemius muscle belly lengthens less when compared to TD children (Matthiasdottir *et al.*, 2014). Additionally, this lengthening of the muscle belly itself will depend on both the properties of the fascicles and the connective tissue that ties them together. Compared to TD peers, medial gastrocnemius fascicle lengthening has been shown to be smaller in young adults with CP (Barber *et al.*, 2011a), but similar in children with CP (Matthiasdottir *et al.*, 2014). These conflicting findings could possibly be explained by different ways of comparing groups. A decreased ROM in children with CP could confound findings when comparisons are made over the full ROM. In fact, any comparison between CP and controls in terms of absolute joint angles is inherently limited, because differences in the muscle's moment arm (Kalkman *et al.*, 2017) and passive joint torque (Alhusaini *et al.*, 2010) will influence the relationship between angular rotation and passive tissue lengthening. Thus, care must be taken when interpreting data acquired from ultrasound imaging. Unquestionably, ultrasound has proved a valuable tool to improve understanding of *in vivo* behaviour of muscle and tendon during contraction and joint rotation. However, a calculation of the tissues' mechanical properties during passive joint rotation is more difficult as several assumptions are inferred. The passive torque measured around the ankle is a combination of different muscles and passive structures, and the contribution of each force-bearing structure to the net joint torque neither can be quantified *in vivo* nor can it be assumed to remain constant throughout the range of motion. Nevertheless, measuring, with ultrasound, the resulting passive elongations of the muscle and the tendon in response to stretch will allow drawing conclusions about the relative contribution of the muscular and tendinous structures to ROM.

The purpose of this study was to quantify which structures contribute to MTU lengthening and thus receive the stretch during passive ankle joint rotation. We hypothesised that the muscle and the fascicles would lengthen less in CP compared to TD children and that the relatively less stiff tendon would lengthen more.

# Methods

## *Participants*

Thirty-one children aged 6-16 years were recruited for participation. Patient characteristics can be found in table 3.1. Five of the TD children were assessed with the same protocol for a second time after a two-hour break to determine reliability of the full measurement protocol. Exclusion criteria were having botulinum toxin-A injection to the lower limb muscles within six months prior to testing, a baclofen pump, or any lower limb neuro- or orthopaedic surgery. All TD children were free from neuromuscular or skeletal disorders. Children were recruited through the gait lab of Alder Hey Children's Hospital in Liverpool and the University Hospital in Leuven. The study was approved by the Institutional as well as the NHS research ethics committee in the UK and the University Hospital's ethics committee in Leuven. The study was conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained from the parents, and written assent was given by children in accordance with local regulations.

**Table 3.1. Participant characteristics**

	<b>CP (n=15)</b>	<b>TD (n=16)</b>
Age (years, months)	11.4 (2.9)	10.4 (3.4)
Male/female (n)	10/5	7/9
Height (cm)	142 (20.3)	138.1 (19.1)
Mass (kg)	36 (18)	35 (15)
Tibia length (mm)	339.7 (54.3)	329.4 (52.7)
GMFCS (I-IV) (n)	9 I, 6 II	n/a
Diagnosis (n)	8 Diplegia, 7 Hemiplegia	n/a
Modified Ashworth Score (n=7) and Average Modified Tardieu (n=8)*	MAS: 1.5 (n=6); 3 (n=1) Tardieu: 2 (n=5); 3 (n=3)	n/a

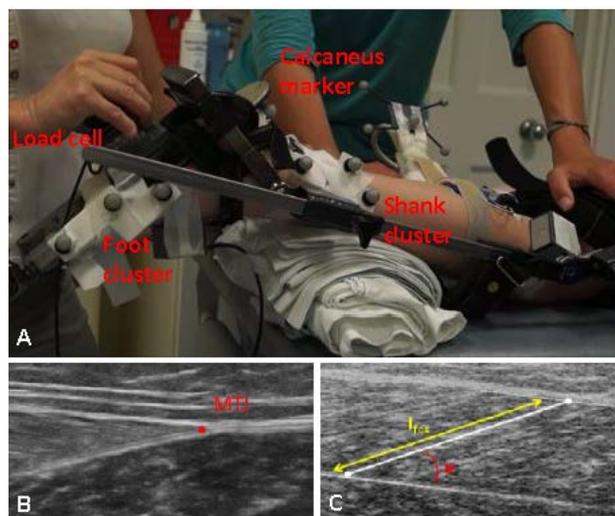
Data are mean (SD) unless otherwise stated. CP: cerebral palsy; TD: typically developing; GMFCS: gross motor functional classification scale; n/a: not applicable.

\*Tardieu scores from children recruited at Alder Hey Children's Hospital in Liverpool. MAS from children recruited at University Hospital in Leuven.

## *Experimental protocol*

Participants lay prone on a bed with the lower leg supported on an inclined cushion such that the knee was ~20° flexed, the leg was positioned in a custom-made orthosis, to control ankle movement in the sagittal plane (figure 3.1A). The axis of rotation of the orthosis was aligned with the lateral malleolus. The leg tested was the most affected, defined by clinical spasticity scores (Tardieu *et al.*, 1954; Bohannon & Smith, 1987) in CP, and the left in TD. Each participant underwent two trials involving three cycles of ankle rotation by manually rotating the foot from maximal plantarflexion to maximal dorsiflexion taking 5

seconds to complete with at least 10 seconds rest in between individual repetitions. This speed of movement is considered sufficiently slow not to elicit a stretch reflex in children with CP (Bar-On *et al.*, 2013a). Forces and torques around the ankle were measured at 200Hz using a six degrees-of-freedom force sensor load-cell (ATI mini45: Industrial Automation) attached to the orthosis under the ball of the foot. The point of attachment of the load-cell to the orthosis could be adjusted according to foot length. 3D kinematics were collected with 3 cameras at 120Hz from 2 clusters of 3 markers placed on the foot-plate of the orthosis and on the shank and a single marker placed on the most superficial part of the posterior calcaneal tuberosity (Optitrack, US). Surface electromyography (sEMG), placement defined with ultrasound, collected signals at 1600Hz from the lateral gastrocnemius and soleus muscles during all trials and from the medial gastrocnemius during the trials measuring muscle lengthening (Zerowire, Cometa, Milan, IT). When, during joint rotation the sEMG signal exceeded 10% of the maximum voluntary contraction value (collected prior to the stretch trials), the corresponding trial was discarded.



**Figure 3.1.** **A.** Experimental design showing leg placement in a custom-made orthosis. A hand-held force sensor load-cell was used to measure net joint torque at the foot plate during passive stretch. Two clusters of reflective markers on the shank and foot were tracked with motion analysis and used to calculate the foot-plate angle in 3D. A single marker was placed on the most distal part of the calcaneus and additionally tracked in 3D using motion analysis. The ultrasound probe was placed above the medial gastrocnemius muscle-tendon junction (MTJ), or on the muscle belly, and the position and orientation of the image was tracked using motion analysis by means of a cluster of markers attached to the probe. **B.** Visualisation of muscle-tendon-junction (MTJ). **C.** Visualisation of fascicle length and Pennation angle.

### *Ultrasound*

A B-mode ultrasound scanner (Telemed Echoblaster, Lithuania) with a 59mm linear transducer rigidly fitted with a cluster of 4 markers was used to identify the location of the medial femoral condyle in a local reference frame defined by the shank cluster.

To define myotendinous junction (MTJ) displacement, the probe with cluster was securely fixed over the MTJ using a custom-made holder. The long axis of the probe was aligned with the line of action of the muscle to minimise out of plane movement. The MTJ was tracked at 30Hz in the local reference frame on the shank during the first 3 ankle rotations.

Then, because MTJ and fascicles could not be visualised simultaneously, the US probe was fixed over the muscle belly to measure fascicle lengthening at 60Hz during the second three passive movements. Guidance regarding probe alignment was adhered to for minimising measurement errors (Bénard *et al.*, 2009).

### *Data analysis*

Data analysis was carried out using custom made software (Matlab R2015a, Python 2.7.11). Anatomical calibration of the shank and foot reference frames was applied to obtain ankle angle (Leardini *et al.*, 2007). During movement, displacement of the MTJ was manually tracked (figure 3.1B) and muscle and tendon lengths were defined as the linear distances between the medial femoral condyle and the MTJ; and between the MTJ and the marker on the calcaneus, respectively. The MTU length was defined as the summation of muscle and tendon length. A modified semi-automated tracking software (Cronin *et al.*, 2011; Gillett *et al.*, 2013) was used to track the fascicle and the aponeuroses. Fascicle length ( $l_{fas}$ ) was calculated by extrapolating the fascicle to the intersection point with the aponeuroses. Pennation angle ( $\alpha$ ) was measured as the angle between the fascicle and the deep aponeurosis. Next, fascicle length resolved along the axis of the MTU was calculated trigonometrically:  $l_{fas\_resolved} = l_{fas} / \cos \alpha$ . For a justification on the (automated) tracking mechanisms used in this study, see appendix A. The net ankle joint torque was calculated from the exerted torques and forces on the load-cell, measured external moment arms, and the predicted torque caused by gravity on the foot and orthotic (Bar-On *et al.*, 2013b). All kinematic and kinetic variables were filtered using a 2<sup>nd</sup> order Butterworth filter with a cut-off frequency of 6Hz and averaged over 3 stretches. Starting length was subtracted from absolute muscle, tendon and fascicle length to compare lengthening of these structures over the full ROM and over a common ROM that could be achieved by all participants (-5° to -25°). Furthermore, all lengthening

parameters were assessed over a common joint torque from 0Nm (defined as slack length) to 3Nm and over a common amount of MTU lengthening (20mm). For the different comparisons, muscle, fascicle and tendon lengthening was additionally expressed as a percentage of MTU lengthening. The curve-wise parameters described above were calculated for the individual data curves. For visualization purposes, average curves were calculated by first applying a spatial normalization to all variables relative to the full stretch cycle and subsequently averaging the variables. These average curves are shown in figure 3.2.

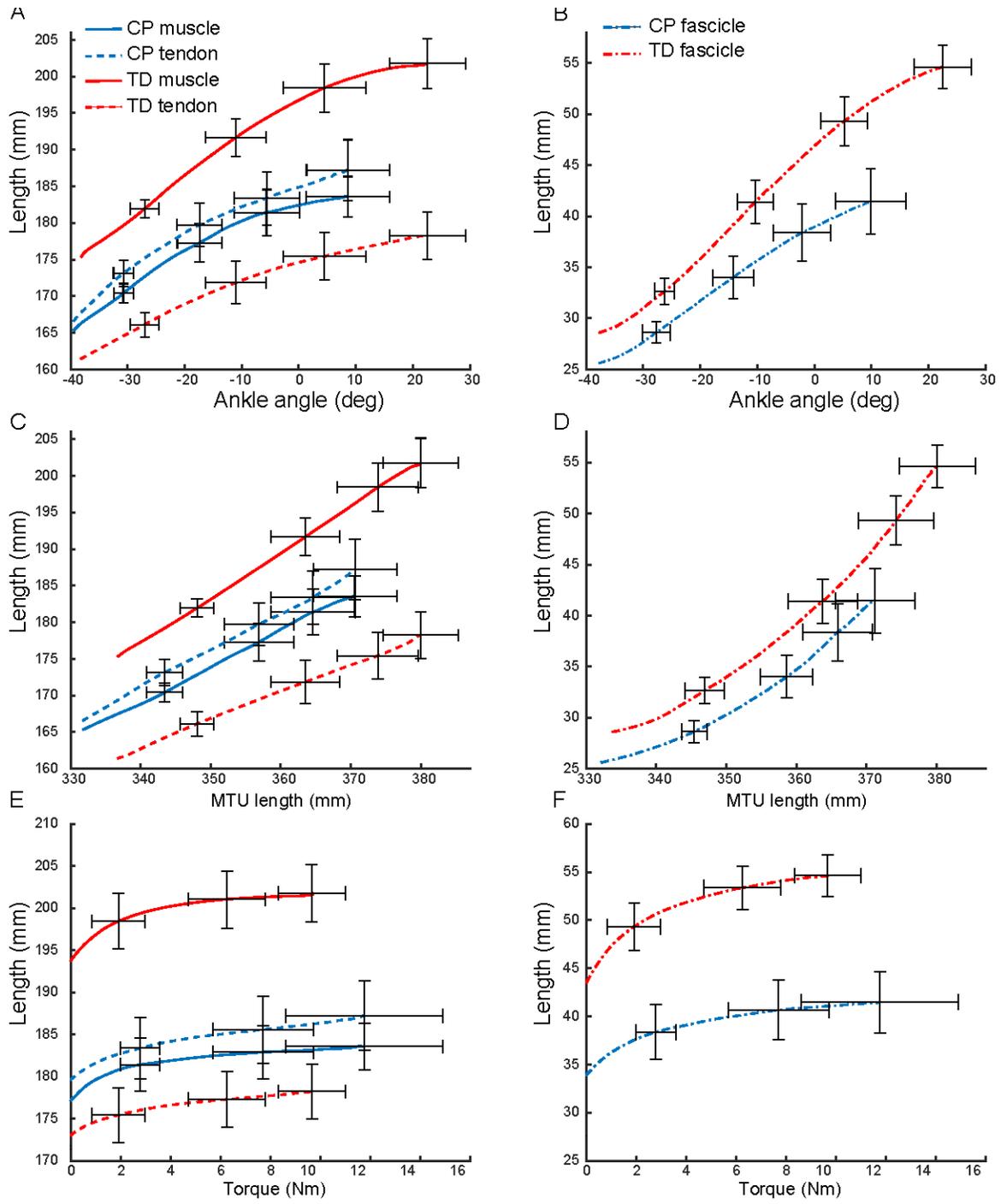
### *Statistics*

All parameters were checked to be normally distributed using the Shapiro-Wilk test and by inspection of the q-q plots. The between session reliability of lengthening was analysed using intra-correlation coefficients (ICC, 3,k) and the standard error of measurement (SEM), calculated from one-way ANOVA. A 2-sample independent t-test was used to compare lengthening between CP and TD groups. Relations between muscle/tendon lengthening, ROM and age were made using Pearsons  $r^2$ -values. The threshold of significance was set at  $p=0.05$ .

## Results

No significant differences in anthropometric measurements were found between groups (table 3.1). ICC values of the inter-session reliability ranged from 0.50-0.70. The SEM values are shown in table 3.2.

At the starting position (maximal plantarflexion), joint angle was not different between TD and CP groups (mean (SD); CP:  $-38.3^\circ$  (7.2), TD:  $-36.6^\circ$  (9.4),  $p=0.59$ , CI [-7.85 4.53]). At this angle, torque (CP: -1.5Nm (0.9), TD: -1.8Nm (0.5),  $p=0.25$ , CI [-0.22 0.81]), absolute muscle (CP: 164.1mm (28.8), TD: 174.7mm (30.9),  $p=0.4$ , CI [-32.47 13.4]), tendon (CP: 166.9mm (29.6), TD: 159.6mm (24.7),  $p=0.54$ , CI [-13.74 25.85]) and fascicle lengths (CP: 25.0mm (6.6), TD: 28.4mm (4.1),  $p=0.08$ , CI [-7.34 0.42]) were not significantly different between children with CP and TD children.



**Figure 3.2** (A) Muscle/tendon length versus ankle angle, (B) fascicle length versus ankle angle, (C) muscle/tendon length versus muscle-tendon-unit (MTU) length, (D) fascicle length versus MTU length, (E) muscle/tendon length versus ankle torque and (F) fascicle length versus ankle torque. Data are average curves calculated by first applying a spatial normalization to all variables relative to the full stretch cycle and subsequently averaging the variables. 95% Confidence Intervals are shown at 4 representative time points.

**Table 3.2** Mean (SD) lengthening values in children with cerebral palsy (CP) and typically developing (TD) children during passive ankle rotation.

	Absolute lengthening (mm)				% of MTU lengthening	
	CP	TD	ES Hedge's g	SEM	CP	TD
<u>Over the full ROM</u>					<u>Over the full ROM</u>	
ROM (°)	48.0 (12.8)	60.6 (11.0) *	-1.03	9.5		
Fascicle	15.9 (6.2)	26.0 (4.3) **	-1.27	2.2	40.7 (10.7)	58.1 (14.3) **
Muscle	18.2 (5.4)	26.5 (7.0) **	-1.87	3.9	48.1 (9.2)	62.4 (9.2) **
Tendon	20.7 (8.1)	16.8 (6.7)	0.51	3.3	52.5 (8.8)	37.6 (9.2) **
<u>Over common ROM (-25° to -5°)</u>					<u>Over common ROM (-25° to -5°)</u>	
Fascicle	7.9 (3.2)	11.1 (2.1) **	-1.32	2.0	50.6 (20.4)	59.3 (14.6)
Muscle	8.5 (2.3)	11.4 (2.8) **	-1.20	1.8	53.9 (9.0)	64.9 (9.9) **
Tendon	7.6 (3.1)	6.45 (2.3)	0.32	2.0	46.1 (9.0)	35.1 (9.9) **
<u>From maximum 0 to 3Nm</u>					<u>From maximum 0 to 3Nm</u>	
ROM (°)	14.2(3.2)	17.4(5.6)	-0.81	9.0		
Fascicle	4.1(1.6)	7.6(3.2)**	-1.29	1.7	37.5 (9.6)	56.3 (14.9) **
Muscle	3.5(1.9)	5.7(2.5) *	-0.91	1.4	50.4 (9.3)	63.4 (8.5) **
Tendon	3.8(2.9)	2.8(1.3)	0.38	1.7	49.6 (9.3)	36.6 (8.5) **
<u>Over common MTU range (0-20mm)</u>					<u>Over common MTU range (0-20mm)</u>	
ROM (°)	17.7 (5.5)	18.7 (5.7)	-0.33	3.5		
Fascicle	5.7 (2.1)	7.6 (3.9) *	-0.52	1.6	25.4 (12.3)	39.1 (19.5) *
Muscle	9.9 (2.3)	12.1 (2.5) **	-1.08	1.0	49.3 (11.5)	61.4 (12.9) **
Tendon	10.1 (2.2)	7.9 (2.5) **	1.08	1.0	50.5 (11.2)	38.6 (12.8) **

ROM: range of motion; PF: plantar flexion; MTU: muscle-tendon unit; CI: confidence interval; SEM: inter-session standard error of measurement; ES: effect size (Hentschke & Stüttgen, 2011)

\* Significant difference between CP and TD at  $p < 0.05$  (\*\*  $p < 0.01$ )

The full ROM was 13° smaller towards dorsiflexion in the CP group. Absolute muscle and fascicle lengthening over full ROM were on average 9mm smaller in CP. Absolute tendon lengthening was similar between groups. Over the common ROM that could be achieved by all participants (-25° to -5°) absolute muscle and fascicle lengthening was on average 3mm smaller in CP and absolute tendon lengthening did not differ between groups (figure 3.2A & 3.2B). At -5°, being the most dorsiflexed position all participants could achieve, joint torques were significantly larger in children with CP (2.34±1.77Nm) than TD children (0.49±0.94Nm). To account for this difference, comparisons made over a common joint torque (0Nm to 3Nm) (figure 3.2E& 3.2F), revealed that absolute muscle and fascicle lengthening was on average 3.2mm smaller in CP, and absolute tendon lengthening did not differ between groups. When analysed over a common range of MTU lengthening (20mm), absolute muscle and fascicle lengthening were on average

2.5mm smaller in CP and absolute tendon lengthening was on average 2.6mm larger in CP (figure 3.2C & 3.2D). Finally, when expressed as a percentage of MTU lengthening, relative muscle lengthening was smaller and relative tendon lengthening larger in children with CP over all the studied ROMs (table 3.2).

Fascicle lengthening resolved along the axis of the MTU was  $8.2\pm 3.2$ mm and  $11.5\pm 2.0$ mm respectively for CP and TD children over the common ROM ( $p<0.01$ ). Over the full ROM this was  $16.3\pm 6.3$ mm and  $26.5\pm 7.0$ mm for CP and TD children ( $p<0.01$ ).

Muscle lengthening increased significantly with age in TD children while in CP children, tendon lengthening increased with age (Figure 3.3). Significant correlations were found between muscle and tendon lengthening with ROM in children with CP (Figure 3.4).

## Discussion

Regardless of whether groups were compared according to common joint angle, joint torque, or relative to MTU lengthening, muscle and fascicle lengthening were always smaller in children with CP than TD children (table 3.2). This confirms previous findings of smaller muscle and fascicle lengthening during passive ankle dorsiflexion in children with CP when compared to TD children (Barber *et al.*, 2011a). By simultaneously studying the relative contributions of the muscle and tendon to MTU lengthening, we also found that in TD children the muscle lengthens more than the tendon (63%-37%) while in children with CP they lengthen equally (50%-50%). These differences between groups in the amount of lengthening of the underlying structures during similar amounts of joint and MTU displacement indicate possible differences in structural properties due to CP which are not evident by assessment on a joint level.

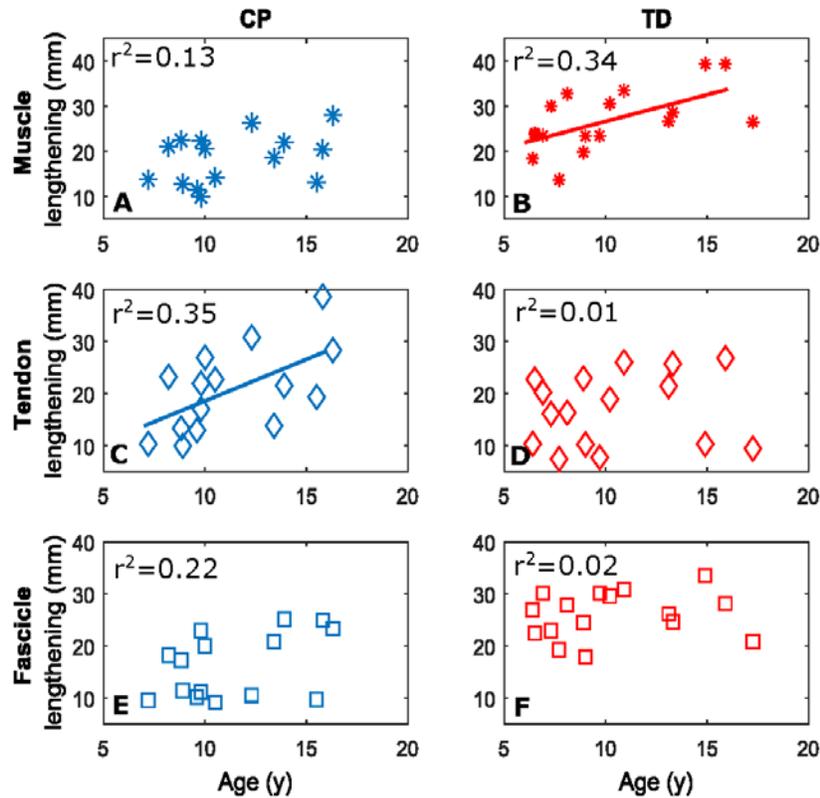
Due to differences in Achilles tendon moment arm (Kalkman *et al.*, 2017) and joint stiffness (Alhusaini *et al.*, 2010) between TD and CP participants, comparison of lengthening parameters between groups only in terms of joint angles should be interpreted with caution. Therefore, while previous studies analysed fascicle lengthening only over the full (Barber *et al.*, 2011a) or a common ROM (Matthiasdottir *et al.*, 2014), we compared our data in two additional ways. First, we used a common joint torque rather than common ankle angles. Children with CP develop torque earlier in their ROM. We found the torque measured at the limit of the common dorsiflexion range ( $-5^\circ$ ) to be higher in children with CP compared to TD children. Therefore, comparing the results at a common torque would circumvent these problems and assure a similar stretching stimulus to the MTU. However, joint torque is also affected by differences in Achilles tendon moment arm and intrinsic joint stiffness. Comparison of lengthening values over a common MTU

lengthening circumvents the influence of a smaller Achilles tendon moment arm in children with CP (Kalkman *et al.*, 2017). Nevertheless, irrespective to the method used, we always found that relative to MTU lengthening, muscle lengthening is smaller and tendon lengthening larger in children with CP. This consistency confirms that these changes in mechanical behaviour of the MTU of children with CP are substantial. In contrast, when assessing the effect of intervention or comparing different subgroups of children with CP, the differences may be less pronounced and the method of analysis will likely be important. This is a vital consideration when decomposing the causes of a reduced ROM in the clinical decision-making process.

This study, and others before us (Morse *et al.*, 2008), observed a discrepancy between the amount of fascicle and muscle lengthening during a passive stretch. This decoupling of the elongation of the fascicles from that of the whole muscle can be explained by the deformation of the aponeurosis and connective tissue between the fascicles (Lieber *et al.*, 2017). Additional analysis of the current data to explore fascicle:muscle lengthening showed that over a common ROM, muscle lengthening could be entirely explained by the resolved fascicle lengthening in both groups. This may imply that the increased resistance to stretch of the muscle in children with CP results from similar changes in the lengthening characteristics of both the fascicles and passive connective tissue. When studied over the full ROM, mean muscle lengthening in the CP group was 1.4mm larger than the resolved fascicle lengthening, while in the TD group muscle lengthening was equal to resolved fascicle lengthening. This could suggest that structures other than the fascicles, such as the perimysium and tissue between the fibres, deform to provide the additional lengthening required to achieve maximal dorsiflexion angles in children with CP, while in TD children this is not the case. Consistent with this interpretation, both intramuscular connective tissue (Malaiya *et al.*, 2007) and the expression of extracellular matrix production-related genes were found to be dramatically increased in spastic muscles and correlated with muscle mechanical properties, such as stiffness (Smith *et al.*, 2012).

It has been shown that muscle contractures already start developing at an early age in children with CP (Willerslev-Olsen *et al.*, 2013) and that growth is an important factor contributing to the development of contractures (Švehlík *et al.*, 2013). Therefore, it is important to capture the critical age at which treatment is most effective and consider the changes that occur in muscle/tendon properties with maturation. Benard *et al.* reported that gastrocnemius muscle length increases with age in TD children (Bénard *et al.*, 2011). However, in the same TD children, muscle lengthening in response to a 4Nm dorsiflexion torque, was not found to increase with age (Bénard *et al.*, 2011). This is not supported by our data, since we found

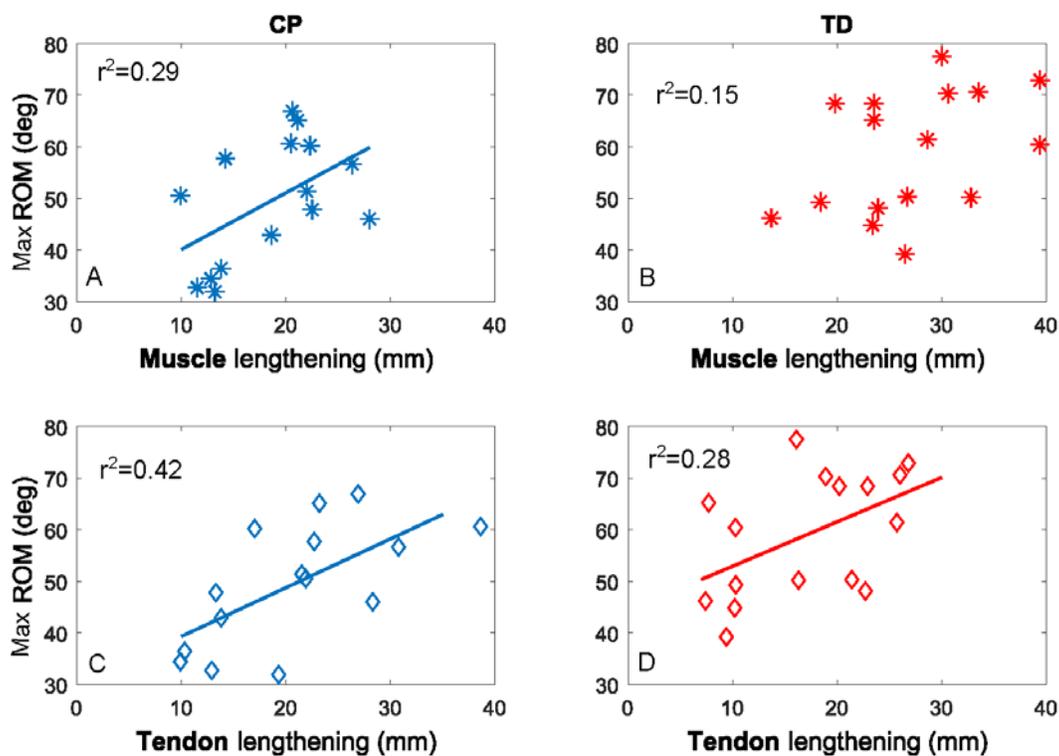
a tendency for muscle lengthening to increase with age in TD children (figure 3.3). Interestingly, this increase in muscle lengthening with age was absent in children with CP. Instead, in children with CP we observed an increase in tendon lengthening with age. This may indicate that muscle stiffness increases with age in children with CP, which is consistent with the progression of disease (Graham *et al.*, 2016). Additionally, it indicates a possibility that the Achilles tendon acts as a compensation mechanism to preserve ankle ROM, despite a shorter and stiffer muscle as children with CP grow.



**Figure 3.3** Correlations between age and muscle (A, B), tendon (C, D) and fascicle (E, F) lengthening across the ROM, for children with CP and TD children. A regression line is shown for significant relationships.

The relative contribution of fascicle, muscle and tendon lengthening to ROM may be important in determining the best treatment. We show that both muscle and tendon lengthening are related to ROM in children with CP (figure 3.4). A lack of this relationship in TD children shows that the medial gastrocnemius does not play an essential role in determining ROM. Stretching is often used to increase ROM in children with CP and is assumed to increase muscle length and reduce muscle stiffness.

However, in this study, we showed that the muscle in children with CP experiences a smaller stretching stimulus during joint rotation than TD children which may explain the lack of effectiveness of stretching therapies (Wiat *et al.*, 2008). For example, passive stretching for a period of six weeks, has been shown effective in increasing ROM, but an increase in muscle or fascicle length was absent (Theis *et al.*, 2015). However, large variability amongst participants in the current study and those reported in literature suggests that patient- and muscle-specific information may be required to facilitate individualised treatment programs.



**Figure 3.4** Correlations between muscle (A, B), and tendon (C, D) lengthening and ROM in children with CP and TD children. A regression line is shown for significant relationships.

This study has some limitations. Currently, it is not possible to measure muscle and tendon stiffness during passive rotation in an intact joint, because there are no *in vivo* techniques to quantify forces in the muscle-tendon unit. Additionally, the force within a single muscle is dependent on the distribution of the joint torque over the different agonist and antagonist muscles. For example, an increase in the stiffness of the

Soleus, a muscle can show intermuscular force transmission to the medial gastrocnemius and attached onto the Achilles tendon could confound our measurements. To partly overcome this problem, measurements of fascicle and muscle excursion of different muscles should be taken at the same time. Nonetheless, the results of our study show less MG MTJ displacement in children with CP compared with TD children. Because in children with CP, the agonist calf muscles are rarely affected in isolation, it is likely that the reduced contribution of the MG muscular component to MTU lengthening can, at least partly, be explained by an increased stiffness in the MG muscle. Although the SEM values of all parameters were lower than the average difference between groups, reliability of the calculated parameters was lower than expected. Future studies could reduce possible sources of error by applying motorised instead of manual movements and automatic tracking algorithms for feature identification. However, we were still able to detect between-group differences consistently. We are therefore confident that this has not confounded the present findings. In the current study, muscle fascicle and tendon length were represented as straight lines, thus neglecting possible effect of curvature. However, the influence of curvature has been reported to be small for passive fascicle length measurements in the medial gastrocnemius (Muramatsu *et al.*, 2002). Neglecting tendon curvature leads to an overestimation of tendon lengthening in both groups especially at more plantarflexed ankle angles where the tendon is below slack length. Since we expect slack length to be at more plantarflexed angles in children with CP, an overestimation of tendon lengthening would be more likely in the TD children. Thus, controlling for tendon curvature would only amplify the between-group difference in tendon lengthening reported here. Ankle angle was measured in the sagittal plane as the angle between the shank and the footplate that supported the foot. To minimise errors, we assured the foot to be rigid to the foot plate during the whole ROM. Finally, the exclusion of movements that showed activation higher than a threshold helped minimise the effects of EMG-activity on the feature displacement. However, small effects of EMG activity below this threshold cannot be fully excluded.

In summary, this study demonstrates that when passively rotating the ankle joint to stretch the calf muscles, the tendon lengthens less than the muscle in TD children, while in children with CP, the muscle lengthens as much as the tendon. This suggests altered material properties of the muscle and tendon in children with CP. This should be considered when assessing and treating muscle function at joint level in children with CP.

## CHAPTER 4

Medial gastrocnemius muscle stiffness cannot explain the increased ankle joint range of motion following passive stretching in children with cerebral palsy

*The information presented in this chapter has been reported in the paper:*

Kalkman BK, Bar-On L, Cenni F, Maganaris CN, Bass A, Holmes G, Barton GJ, Desloovere K, O'Brien TD (2018) Medial gastrocnemius muscle stiffness cannot explain the increased ankle joint range of motion following passive stretching in children with cerebral palsy. *J exp physiol.* **103**(3), 350-357

## Abstract

Stretching is often used to increase/maintain joint range of motion (ROM) in children with cerebral palsy (CP) but the effectiveness of these interventions is limited. Therefore, this study aimed to determine the acute changes in muscle-tendon lengthening properties that contribute to increased ROM after a bout of stretching in children with CP. Eleven children with spastic CP (age:12.1(3)y, 5/6 hemiplegia/diplegia, 7/4 GMFCS level I/II) participated in this study. Each child received 3 sets of 5x20s passive, manual static dorsiflexion stretches separated by 30s rest, and 60s rest between sets. Pre- and immediately post-stretching, ultrasound was used to measure medial gastrocnemius fascicle lengthening continuously over the full ROM and a common ROM that could be achieved per individual pre- to post-stretching. Simultaneously, 3D motion of two marker clusters on the shank and the foot was captured to calculate ankle angle, and ankle joint torque was calculated from manually applied torques and forces on a 6DoF load cell. After stretching, ROM was increased (9.9° (12.0),  $p=0.005$ ). Over a ROM common to both pre and post measurements, there were no changes in fascicle lengthening or torque. The maximal ankle joint torque tolerated by the participants increased (2.9(2.4) Nm,  $p=0.003$ ) and at this highest passive torque maximal fascicle length was 2.8(2.4) mm greater ( $p=0.009$ ) when compared to before stretching. These results indicate that the stiffness of the muscle fascicles in children with CP remain unaltered by an acute bout of stretching and the increased ROM could be due to an increased tolerance to stretch.

## Introduction

Stretching therapies are commonly used as a non-invasive treatment in children with CP. In clinical practice the assumption is that repeated bouts of stretching can increase muscle length, and consequently decrease muscle stiffness and therefore delay the onset of muscle contractures and defer or avoid surgery (Odéen, 1981; Herbert, 2004; Wiart *et al.*, 2008). However, despite an improved ankle ROM (Theis *et al.*, 2015), the scientific evidence does not confirm these assumptions and a significant gap exists between the clinical rationale for stretching and the supporting evidence (for systematic reviews see: Pin *et al.*, 2006; Wiart *et al.*, 2008). Given that stretching interventions cause discomfort to children and are demanding of them and their families (Hadden & Von Baeyer, 2002), stronger evidence is required to support their application and optimise their effectiveness.

We have shown that altered muscle-tendon properties in CP may leads to a reduced ROM (chapter 3), but these alterations may also mediate the response to stretching interventions as seen in typically developing

individuals. For example, previous studies show that in CP, muscles are shorter (Fry *et al.*, 2004; Malaiya *et al.*, 2007; Oberhofer *et al.*, 2010), tendon slack length is longer (Gao *et al.*, 2011; Barber *et al.*, 2012) and relative muscle to tendon stiffness is increased (Kalkman *et al.*, 2016). It is unknown how these altered properties mediate the acute response of a muscle to stretching in individuals with CP. In typically developing adults, acutely after 5 minutes of conditioning stretches, ROM was increased and joint stiffness decreased (Morse *et al.*, 2008). Based on an increase in muscle belly length in the absence of an increase in fascicle length and pennation angle changes, the authors attributed these changes to a reduction in the stiffness of the intramuscular connective tissue. The question then arises, do spastic muscles respond to stretch in a similar way, since changes have been shown in both the quality and amount of intramuscular connective tissue in children with CP (Smith *et al.*, 2011). It has been shown that ankle ROM in children with CP improved immediately after stretching and this was accounted for by an increase in length at maximal joint angles of all three structures that make up the muscle-tendon-unit (MTU) of the medial gastrocnemius, i.e. muscle belly, fascicles and tendon (Theis *et al.*, 2013). However, the gain of MTU lengthening after stretching reported in this study seems extremely large (18.5mm) for an increase in ankle ROM of only 9.8°. Also, no information was reported about any changes in joint torque and thus in the passive properties of the involved structures.

Since muscle and tendon act in series, the lengthening stimulus experienced by the muscle (and thus the fascicles) is dependent on the relative stiffness between muscle and tendon. Higher relative stiffness in the muscle fascicles compared to the tendon in children with CP, would reduce muscle lengthening when rotating the joint compared to typically developing individuals (Kalkman *et al.*, 2016). This reduced strain during ankle stretch might explain why functional improvements, such as gait kinematics, after long term stretching interventions in CP are inconsistent and of low magnitude (Pin *et al.*, 2006; Wiart *et al.*, 2008). Previous studies do not provide information into the relative contribution of muscle fascicles and other structures to the increased ROM observed acutely after stretching. Therefore, the aim of this study was to examine whether ankle ROM could be increased after 20 minutes of stretching and whether the lengthening properties of the structures within the medial gastrocnemius MTU contribute to this increased ROM.

# Methods

## *Participants*

Eleven children aged 6-16 years old, diagnosed with spastic CP were recruited for participation in this study. Patient characteristics can be found in table 4.1. Exclusion criteria were having botulinum toxin-A injection to the lower limb muscles within six months prior to testing, a baclofen pump, or any lower limb neuro- or orthopaedic surgery. Children were recruited through the gait lab of Alder Hey Children's Hospital in Liverpool and the University Hospital in Leuven. The study was approved by the Institutional as well as the NHS research ethics committee in the UK and the University Hospital's ethics committee in Leuven. The study was conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained from the parents, and written assent was given by children in accordance with local regulations.

**Table 4.1.** Participant characteristics

	<b>CP (n=11)</b>
Age (years)	12.1 (3.0)
Male/female (n)	9/2
Height (cm)	147.1 (21.6)
Mass (kg)	40.9 (18.7)
Tibia length (mm)	351.8 (57.6)
GMFCS (I-IV) (n)	7 I, 4 II
Diagnosis (n)	6 Diplegia, 5 Hemiplegia
*Modified Ashworth Score (n=7) and Average Modified	MAS: 1.5 (n=2); 3 (n=1)
Tardieu (n=8)	Tardieu: 2 (n=5); 3 (n=3)

Data are mean (SD) unless otherwise stated. CP: cerebral palsy; GMFCS: gross motor function classification system;

\* MAS from children recruited at the University hospital in Leuven, Tardieu scores from children recruited at Alder Hey Children's Hospital in Liverpool

## *Experimental design*

Participants attended the hospital on one occasion. During this visit, participants underwent an acute bout of passive ankle dorsiflexion stretches applied by a physiotherapist. Stretches were applied to the leg that was most affected as defined by spasticity scores. Before and within 10 minutes after the stretching session, measurements of ankle angle, passive joint torque and medial gastrocnemius muscle fascicle lengthening during a passive stretch were taken. During these measurements, participants lay prone on a

bed with the lower leg supported on an inclined cushion such that the knee was  $\sim 20^\circ$  flexed. The leg was positioned in a custom-made orthosis, to control ankle movement in the sagittal plane (figure 2.1). The axis of rotation of the orthosis was aligned with the lateral malleolus. Each participant underwent 3 trials of passive ankle dorsiflexion movements taking 5 seconds to complete one movement, which resulted in an average velocity of 10deg/s. At least 10 seconds rest was taken between individual repetitions. The maximal ROM was defined as the point where either the participant indicated the threshold or the examiner felt the joint reach the end of the passive movement. Forces and torques at the ankle were measured at 200 Hz using a six degrees-of-freedom force sensor load-cell (ATI mini45: Industrial Automation) attached to the orthosis under the ball of the foot. 3D kinematics were collected with 3 cameras at 120 Hz from 2 clusters of 3 markers placed on the foot-plate of the orthosis and on the shank (Optitrack, US). Surface electromyography (sEMG), placement defined with ultrasound, was used to collect signals at 1600 Hz from the lateral gastrocnemius and soleus muscles (Zerowire, Cometa, Milan, IT). When, during joint rotation, the sEMG signal exceeded 10% of the maximum voluntary contraction value (collected with a hand-held dynamometer prior to the stretch trials), the corresponding trial was discarded. To measure muscle fascicle lengthening, a B-mode ultrasound probe (Telemed Echoblaster, Lithuania, 60 Hz) was securely fixed over the mid belly of the medial gastrocnemius muscle. Guidance regarding probe alignment was adhered to for minimising measurement errors (Bénard *et al.*, 2009; Bolsterlee *et al.*, 2016). Resting fascicle length was measured with the knee flexed at  $\sim 20^\circ$  and the foot hanging off the edge of the bed.

### *Stretching intervention*

Participants lay supine on a bed with the physiotherapist positioned on the side of the bed. Initial stretch position was achieved by lifting the leg with the knee flexed to  $90^\circ$ . To initiate the stretch, the physiotherapist dorsiflexed the foot by applying force manually at the sole of the foot. While maintaining dorsiflexion, the knee was slowly guided into extension. Pressure at the ankle continued to be applied by the physiotherapist until the participant indicated the point of discomfort. This maximum dorsiflexed position was held for 20 s in total and participants received 3 sets of 5x20 s passive, static dorsiflexion stretches separated by 30 s rest, and 1 min rest between sets.

### *Data analysis*

Data analysis was carried out using custom-made software (Matlab R2015 and Python 2.7.11). Kinematic and kinetic data were filtered using a 2<sup>nd</sup> order low pass Butterworth filter with a cut-off frequency of 6 Hz

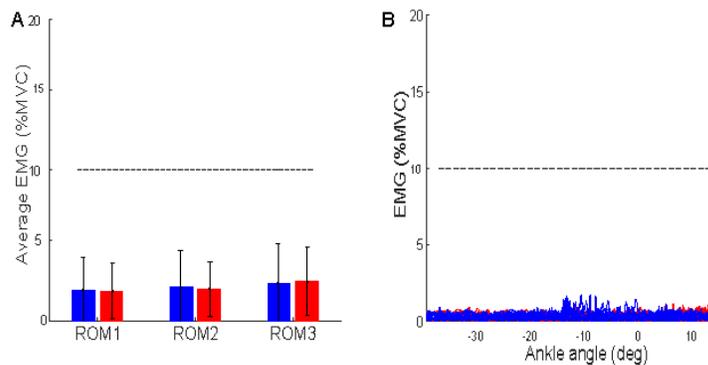
and averaged over the 3 stretches for each individual. Anatomical calibration of the shank and foot reference frames were applied to obtain the ankle angle (Leardini *et al.*, 2007). The calculation of net ankle joint moment is described in figure 2.1 (Bar-On *et al.*, 2013b; Schless *et al.*, 2015). A modified semi-automated tracking software (Cronin *et al.*, 2011; Gillett *et al.*, 2013) was used to track the fascicles and aponeuroses. Fascicle length was calculated by extrapolating the fascicle as a straight line to the intersection point with the aponeuroses. Pennation angle ( $\alpha$ ) was measured as the angle between the fascicle and the deep aponeurosis. Next, to determine separate contribution of fascicles and the tendon-aponeurosis to total MTU lengthening, fascicle length resolved along the axis of the MTU was calculated ( $l_{fas\_resolved} = l_{fas} * \cos \alpha$ ). For a justification on the (automated) tracking mechanisms used in this study, see appendix A. Changes in fascicle lengthening were analysed over the full ROM (to maximal dorsiflexion angles) and over a ROM common to all subjects from  $-25^\circ$  to  $-5^\circ$  (with negative angles reflecting plantarflexion). To define ankle stiffness, a second-order polynomial was fitted for each individual through the 3 repetitions of the passive torque-angle curve, the slope of this polynomial was defined at 5 equally distributed torque values between 0 and 12 Nm that could be achieved by all participants. Raw EMG signals were filtered with a 6<sup>th</sup> order zero-phase Butterworth bandpass filter from 20 to 500 Hz. The root mean square envelope of the sEMG (RMS-EMG) was extracted by applying a low-pass 30 Hz 6<sup>th</sup> order zero-phase Butterworth filter on the squared signal. To assess any change in RMS-EMG post stretching the RMS-EMG signal was quantified over three equal zones of the ROM. The zones were defined as the time windows corresponding to 10-36.6% ROM, 36.6-63.3% ROM and 63.3-90% ROM. Average RMS-EMG per position zone was defined as the area underneath the RMS-EMG curve divided by the duration of the corresponding time zone. All RMS-EMG values are expressed relative to the maximum voluntary contraction value (collected prior to the stretch trials).

### *Statistics*

All parameters were checked for normal distribution using the Shapiro-Wilk test and by inspection of the q-q plots. All data except for the maximally applied torque were found to be normally distributed. Separate paired t-tests or Wilcoxon signed rank tests were used to compare lengthening, ROM, maximal torque and EMG parameters before and after the stretching intervention. A MANOVA was used to compare joint stiffness at different torque values before and after intervention. All statistical analyses were performed in Matlab (Mathworks, R2015). Alpha-level was set at 0.05 and effect sizes were expressed as Cohen's *d*. Threshold values were 0.2, 0.5 and 0.8 for small, medium and large effects. (Cohen, 1977).

## Results

Eleven trials in 9 participants were excluded based on excessive RMS-EMG activity. This equates to 20% of the total number of trials. There were at least 2 trials per participant available for analysis. No differences were found pre- to post-stretching in the average RMS-EMG in any of the movement zones analysed for the lateral gastrocnemius ( $p=0.25$ ) or the soleus ( $p=0.96$ , figure 4.1).



**Figure 4.1:** (A) Average RMS-EMG response of the lateral gastrocnemius and soleus combined pre (blue) and post (red) intervention. (B) Individual RMS-EMG signals versus ankle angle of the lateral gastrocnemius and soleus pre (blue) and post (red) intervention.

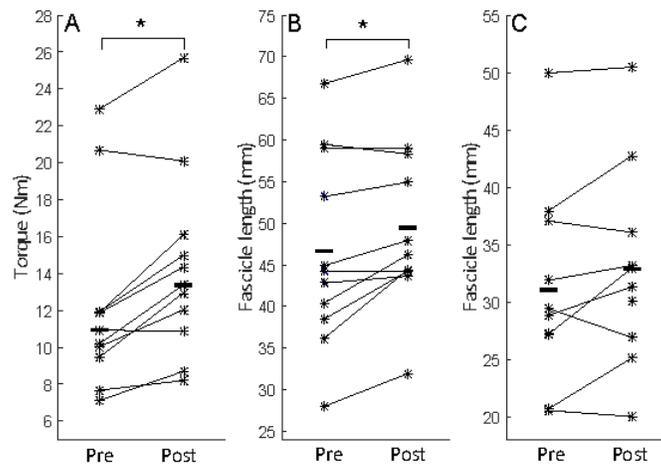
Resting fascicle length (figure 4.2C) or resting ankle angle did not appear to change post-stretching (table 4.2). ROM increased significantly by  $9.9^\circ$  ( $12^\circ$ ) ( $p=0.01$ ). This was accompanied by a  $3.9(3.7)$  mm increase in MTU lengthening ( $p=0.01$ ) and a  $3.0(2.4)$  mm increase in fascicle lengthening ( $p=0.01$ ) over the full ROM (table 4.2). There was an increase of  $2.4(2.1)$  Nm in the maximal torque that was applied to the ankle after stretching (figure 4.2A). The change in pennation angle during muscle lengthening was not altered post-stretching ( $p=0.230$ ), thus fascicle length resolved along the axis of the MTU increased by  $3.1(2.6)$  mm after stretching ( $p=0.007$ ).

**Table 4.2.** Mean (SD) lengthening values during passive ankle rotation pre- and post-stretching.

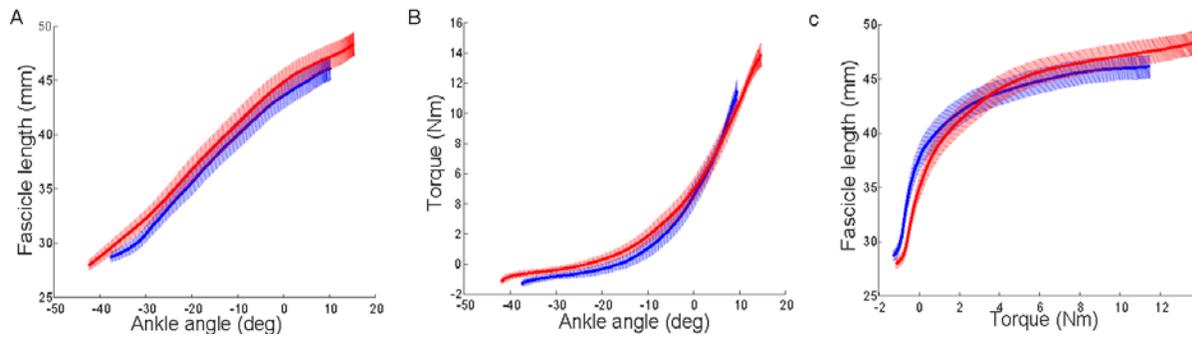
Variable	Pre-stretching	Post-stretching	Absolute change	ES	p	CI
<b>Ankle joint level</b>						
Resting ankle angle (°)	-31.1 (12.6)	-26.9 (16.6)	4.2	0.23	0.263	[-12.71 3.9]
ROM (°)	47.8 (14.1)	57.8 (14.2)	<b>10 *</b>	<b>0.67</b>	<b>0.036</b>	[0.78 19.07]
MTU lengthening (mm)	39.5 (12.1)	43.4 (13.0)	<b>3.9 *</b>	<b>0.30</b>	<b>0.009</b>	[1.21 6.55]
Torque at max. DF (Nm)	12.6 (6.1)	14.9 (5.0)	<b>2.3 *</b>	<b>0.46</b>	<b>0.007</b>	[0.87 3.86]
<b>Fascicle level</b>						
Resting fascicle length (mm)	31.1 (8.8)	32.9 (8.7)	1.8	0.21	0.113	[-3.91 0.51]
Fascicle length at max. DF (mm)	46.6 (11.6)	49.5 (10.2)	<b>2.9 *</b>	<b>0.26</b>	<b>0.009</b>	[0.84 4.77]
Fascicle lengthening full ROM (mm)	17.4 (6.7)	20.4 (7.2)	<b>3.0 *</b>	<b>0.39</b>	<b>0.006</b>	[0.95 4.97]
Fascicle lengthening common ROM (mm)	8.2(3.6)	8.3(3.5)	0.1	0.22	0.301	[-0.98 2.69]
Change in pennation angle (°)	-6.5(3.1)	-7.6(2.5)	1.1	0.29	0.230	[-0.72 2.55]
Resolved Fascicle lengthening (mm)	17.6 (7.1)	20.7 (7.5)	<b>3.1 *</b>	<b>0.38</b>	<b>0.007</b>	[0.82 5.23]

ES: effect size; CI: 95% Confidence interval (non-parametric test: Hodges-Lehmann estimator); ROM: range of motion; MTU: muscle-tendon-unit; DF: dorsiflexion; Negative ankle angles refer to a plantarflexed position

No changes were found in the amount of fascicle lengthening over a common ROM ( $p=0.301$ ) pre- to post-stretching. Ankle stiffness calculated at 5 common torque values between 0 and 12 Nm were not different pre- to post-stretching ( $p=0.63$ ). Fascicle lengthening vs change in ankle angle and torque are visualised in figure 4.3.



**Figure 4.2:** Individual data for (A) maximal torque applied around the ankle during a stretch, (B) fascicle length at end-range dorsiflexion angle and (C) resting fascicle length. Data are shown pre and post intervention. Individual data points (\*) and group mean values (-).



**Figure 4.3:** Data shown are mean ( $\pm 95\%$  CI calculated on normalised data (Cousineau, 2005)) values pre- (blue) and post- (red) stretching of **(A)** fascicle length versus ankle angle, **(B)** joint torque versus ankle angle, **(C)** fascicle length versus joint torque.

## Discussion

The present study has shown that after an acute bout of stretching, children with CP achieve an increase in the ROM. However, no changes were found to occur in either joint stiffness or the lengthening characteristics of muscle fascicles. This indicates that the mechanical properties of the muscle and joint did not change after an acute bout of stretching. The increased ROM can be attributed to a higher maximal torque that was applied manually by the experimenter. This increase in dorsiflexion ROM resulted in an increase in maximal fascicle length.

In healthy adults, the mechanical properties of the muscle could be altered after repeated stretches. Morse et al. (2008) concluded that elastic properties of the connective tissue elements within the muscle change acutely after stretching in typically developed young adults. We did not find evidence to support this hypothesis in children with CP, since fascicle properties over a common ROM and joint stiffness did not change due to repeated stretches. A lack of change in passive torque over a common ROM further indicates that muscle-tendon structures were not altered post-stretching. Nonetheless, ROM did increase acutely after stretching, and in the absence of any changes in muscle-tendon properties, this can be attributed to the greater maximal torque applied by the examiner.

This study was designed to assess any changes in muscle-tendon properties in response to the clinical practice of a therapist manually stretching the ankle to its end ROM. As such, we did not control, or set out to identify, what determines the maximum joint torque that can be applied or tolerated. However, there are a few possible explanations for this change after stretching that may be considered. The maximal ROM, when determined by the examiner is clinically defined as the “end-feel” of movement due to tissue

stretch (Magee, 2014). The position at which this end-feel occurs will depend, among others, on pain tolerance, warm-up, or acquaintance between clinician and patient. These factors would all change after a bout of stretching and could contribute to the greater joint torque applied after stretch, as observed in this study. Due to practicalities in the current study design, the assessor was not blinded to the condition of the patient. A blinding of the assessor could tease out some of the subjective factors that influence the amount of torque applied to reach the end ROM, as mentioned above. Additionally, we may hypothesise that the children experienced an increased stretch tolerance. It has been shown repeatedly in healthy adults that an increased tolerance to an uncomfortable stretch sensation is related to an increased ROM after stretching (Magnusson *et al.*, 1996a; Folpp *et al.*, 2006; Konrad & Tilp, 2014). Future work should evaluate whether this has practical implications in the therapy of children with CP.

The greater ROM achieved after the bout of stretching in this study resulted in a 3.9 mm increase in MTU lengthening. Eighty percent of this increase in maximal MTU length was accounted for by resolved fascicle lengthening, which was calculated as the lengthening of the fascicles along the axis of the MTU. The remaining 20% thus should be due to stretching of the in series elastic component, which includes the Achilles tendon distal to the muscle belly and/or the connective tissue within the muscle. These results contradict earlier findings of Theis *et al.* (2013), who showed muscle and tendon to contribute equally to the increase in MTU lengthening seen after an acute bout of stretching in children with CP. However, the gain in MTU lengthening of 18.5mm reported in this study seems extremely large for a change in ankle angle of only 9.8°. Such a displacement of the MTU would imply moment arm values of 11cm which are much larger than those previously reported in children (Waugh *et al.*, 2011; Kalkman *et al.*, 2017) or adults (Maganaris *et al.*, 2000).

Long-term stretching interventions are based on the assumption that they affect muscle fascicle length and stiffness by changing in series sarcomere number or alter the mechanical tissue properties. An advantage of the addition of sarcomeres in series would be to change the active excursion range of the muscle. Such plasticity of muscle fibres to stretch has been shown in several animal studies (Tabary *et al.*, 1972; Williams & Goldspink, 1973) where prolonged positioning of muscles at increased length over several weeks resulted in increased fibre length and in-series sarcomere number (Williams & Goldspink, 1973). However, it is not known whether this finding applies to spastic human muscle, in particular when sarcomeres are already over lengthened (Mathewson *et al.*, 2014b). Nonetheless, for any remodelling of the muscle to take place, the fascicles must experience sufficient stretching stimulus. In a previous study we have shown that when rotating the ankle joint, this stretching stimulus to the muscle fascicles is smaller in children with CP than their typically developing peers (Kalkman *et al.*, 2016). Similarly, it has been

showed that when stretching over the full ROM, muscle and tendon lengthen less than in TD children (Hösl *et al.*, 2015). This may explain the lack of consistent and substantial functional improvements seen after long term stretching interventions in these patients. Here on the other hand, we show that after 20 minutes of stretching, the stretching stimulus to the muscle fascicles can be acutely increased, thereby giving the potential for remodelling of the muscle to occur. Future research should assess whether the increase in ROM seen after long term stretching interventions in children with CP is due to an increase in stretch tolerance, as is shown here acutely, or if indeed any remodelling of the muscle takes place.

A number of assumptions in the present study should be acknowledged. Muscle fascicles were treated as straight lines, thus neglecting possible effect of curvature. However, the influence of curvature has been reported to be small for passive fascicle length changes in the medial gastrocnemius (Muramatsu *et al.*, 2002). Ankle angle was measured in the sagittal plane as the angle between the shank and the footplate that supported the foot. To minimise errors, a custom-made insole assured that the foot was rigid to the footplate during the whole ROM. Not including a control group to check whether any changes are actually due to the intervention, is a limitation in this study. However, we do not expect muscle properties to change over the short time period that was assessed in this study. Therefore, we do not believe the study design has confounded our conclusions. We also chose not to include a control group of TD children in this study, although they might offer some further insight into the altered muscle properties of children with CP, they are not required to answer the primary question of this study, what is the acute effect of stretch in children with CP. Because of the short time-scale studied here, possible changes should be attributed to time-dependent mechanical properties such as creep and we do not expect any muscle remodelling to take place. Therefore, the comparison to a TD control group is of lesser importance. Furthermore, In a separate analysis, four typically developing children were assessed for repeatability by performing the same protocol before and after an hour break (Cenni *et al.*, 2018), no systematic changes were found in these children and the study design was found to be reliable for applications that do not require sub-mm accuracy. It was not possible to collect EMG recordings of the medial gastrocnemius muscle because we could not fit an ultrasound probe and EMG electrodes on the small surface of a child's muscle. As an alternative, we measured EMG of the lateral gastrocnemius and the soleus to assure joint rotations were passive. Also, we need to acknowledge that even though EMG remained below 5% of the MVC values, we cannot ascertain that muscles were fully passive. Also, we measured only the properties of one muscle of the triceps surae group, however because we performed the stretching intervention with relatively more knee extension the influence of the soleus muscle to the increased ROM is considered small. Finally, this study was performed with a relatively small number of participants. Also, we had no information about

stretching interventions children were exposed to in their regular care. Validation of our results is needed in a larger cohort of children with CP.

We conclude that ROM increased acutely after a single bout of passive stretching in children with CP, but the stiffness of the muscle fascicles remains unaltered. Importantly, the increased ROM is accompanied by a longer maximal fascicle length, which means there is a potential for long term adaptations if repeated over multiple weeks.

## CHAPTER 5

The impact of increasing tendon stiffness on the effectiveness of stretching interventions in children with cerebral palsy

## Introduction

In the treatment of cerebral palsy (CP), there are several strategies that aim to increase range of motion (ROM) at the ankle and thereby improve the gait pattern. Invasive treatments such as surgical lengthening of calf muscle contractures have been shown to improve gait characteristics and musculotendinous lengths during gait (Wren *et al.*, 2004). However, surgery is invasive, there is a risk of overcorrection (Segal *et al.*, 1989) and the timing of surgery is essential for its success (Borton *et al.*, 2001). Therefore, at first conservative approaches are preferred. Stretching, as part of a physical therapy intervention, aims to increase ROM and consequently improve function, delay the development of contractures and the need for surgery. It is assumed that stretching leads to an increase in muscle length by promoting an increase in sarcomeres in series. Although ankle ROM can be improved, it is not known whether muscle properties are improved after stretching, and a significant gap exists between the clinical rationale for stretching and the supporting evidence (Pin *et al.*, 2006; Wiart *et al.*, 2008). Given that stretching exercises can be painful, demanding and time consuming for children and their families (Hadden & Von Baeyer, 2002) and they take up a large amount of time in the workload of physiotherapists (Wiart *et al.*, 2008), stronger evidence is needed to support their application and improve their effectiveness.

To increase effectiveness of stretching interventions, we need to understand how a muscle responds to stretch. We know that in children with CP, muscles are shorter (Fry *et al.*, 2004; Barrett & Lichtwark, 2010), tendon slack length is longer (Barber *et al.*, 2012) and the relative stiffness of muscular to tendinous tissue is increased (Chapter 3). Therefore, during a single stretch, muscles of children with CP will lengthen less and the physiological stretching stimulus to the muscle will be decreased. As such, we found that, acutely after a single bout of stretching, there was an increase in ROM, but no changes to the muscle properties in children with CP (Kalkman *et al.*, 2018). A similar mechanism may limit the effect of long term stretching interventions.

To resolve this issue, alternate solutions are needed. One example would be to decrease the relative stiffness of the muscle to the tendon. This can be achieved in different ways. For example, intramuscular botulinum toxin-A injections decrease the neural component of muscle hyper-resistance (Noort van den *et al.*, 2017) thus this would decrease its stiffness relative to the tendon during stretch. When botulinum toxin-A is combined with stretching of the muscle, either by physiotherapy or casting, the stretching stimulus to the muscle will be increased when compared to stretching alone (Booth *et al.*, 2003; Love *et al.*, 2010). However, the effect of botulinum toxin-A is temporary and repeated injections can cause

significant damage to the muscle (Fortuna *et al.*, 2013; Barber *et al.*, 2013). Another way to decrease the relative stiffness of the muscle to the tendon is to increase the stiffness of the tendon. Tendon stiffness is adaptable and shown to increase with resistance training in adults (Couppé *et al.*, 2008), elderly (Reeves *et al.*, 2003) and typically developing prepubertal children (Waugh *et al.*, 2014). Resistance training has been shown to improve strength and muscle volume in children with CP (McNee *et al.*, 2009; Gillett *et al.*, 2016), indicating that spastic muscles can adapt in response to training. Increasing tendon stiffness with resistance training could furthermore have the benefit to increase the stretching stimulus to the muscle when resistance exercises are combined with stretching exercises.

To test this mechanistic theory, we designed a combined intervention where children performed a combination of resistance training and stretching. Stretching exercises of the calf muscle were performed for six weeks. While resistance training (heel-raises) started four weeks prior, to increase Achilles tendon stiffness before starting the stretching exercises. A control group performed only stretching exercises, but to assure the same physiological load and contact hours with the research team, this group performed resistance training exercises of the upper limbs. Hence, the main aim of this study was to increase the effectiveness of passive stretching in terms of improvements in fascicle length and functional outcomes. Secondly, we assessed the mechanisms by which the combined intervention was effective. We hypothesised that children in the intervention group, in contrast to the control group, would show an increase in fascicle slack length and functional improvements as assessed by gait analysis.

## Methods

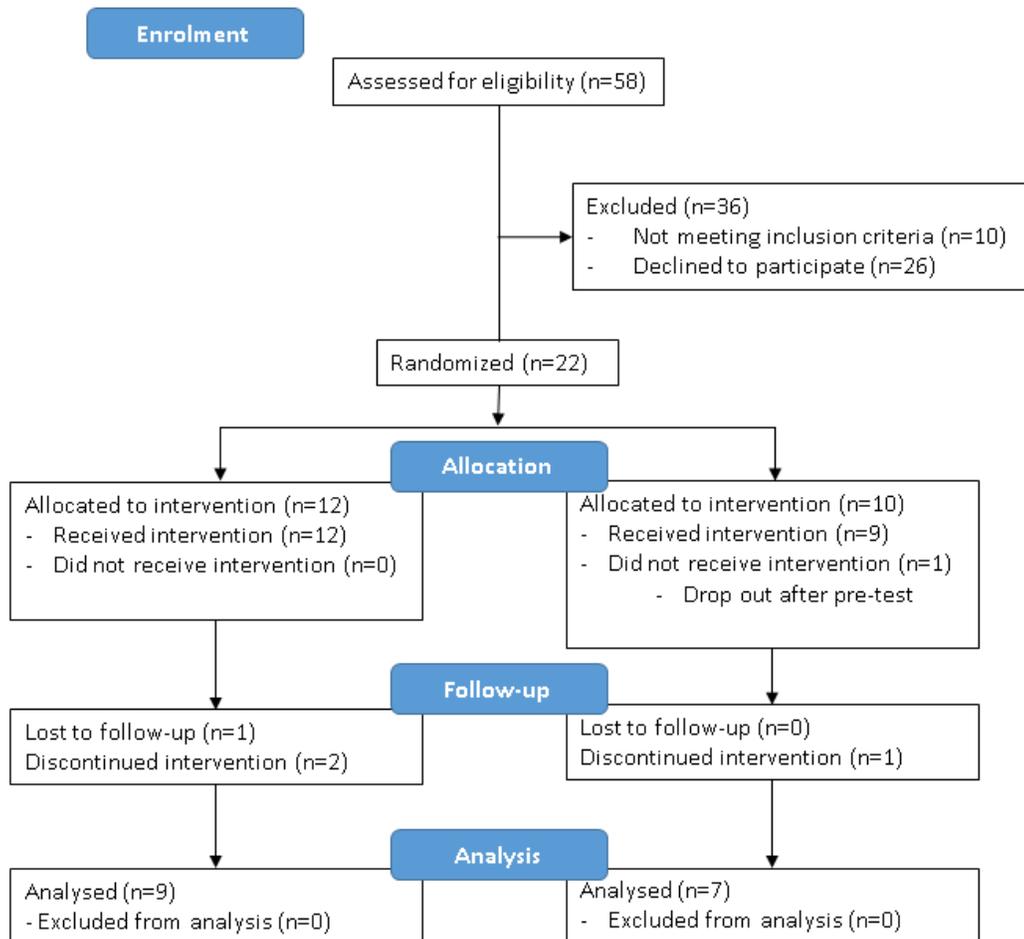
### *Participants*

Twenty-two children with CP aged between 7 and 14 years old were recruited for participation in this study (mean age 9.9(1.9) years, 11 Diplegia and 11 Hemiplegia). Children were excluded from participation if they had received botulinum toxin-A injections to the lower limb muscles 6 months prior to testing, a baclofen pump, or any lower limb neuro- or orthopaedic surgery. Children were recruited through the gait

**Table 5.1.** Participant characteristics

	<b>Intervention (n=12)</b>	<b>Control (n=10)</b>
Age (years)	10.4 (2.0)	9.3 (1.6)
Sex	6 male, 6 female	7 male, 3 female
Diagnosis	6 Diplegia, 6 Hemiplegia	5 Diplegia, 5 Hemiplegia
GMFCS	7 x I, 5 x II	5 x I, 4 x II, 1 x III

lab of Alder Hey Children’s Hospital. The study was approved by the institutional as well as NHS ethics committees. The study was conducted in accordance with the declaration of Helsinki. Written parental consent was obtained and written assent was given by children.



**Figure 5.1:** Flow diagram based on consort guidelines to show the experimental design.

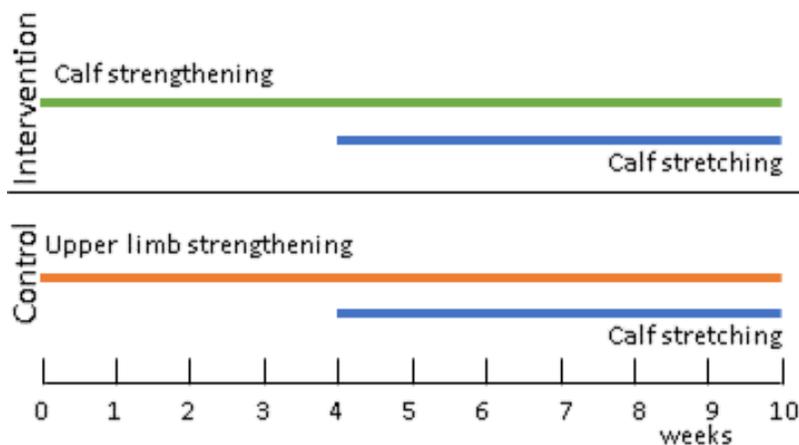
### *Experimental design*

Participants were recruited when scheduled for a routine clinical gait analysis. Children were randomly assigned to either the intervention (n=12) or a control (stretching-only) group (n=10) according to a computerised minimisation algorithm (Saghaei & Saghaei, 2011). When a new patient was ready for randomisation an imbalance score was calculated based on the balance of gender, age and GMFCS score.

The patient was then assigned to the group with the lowest imbalance score. When the scores were equal between groups, the patient was randomly assigned. At the start of the intervention there were no significant differences between groups in terms of age, height and body mass. Sixteen children completed the programme (figure 5.1), patient characteristic of these children can be found in table 5.1.

Children in the intervention group performed strengthening exercises of the calf muscle for 4 weeks, followed by 6 weeks of stretching and strengthening of the calf muscle. Children in the control group performed 6 weeks of stretching exercises of the calf muscles similar to the intervention group. To assure the same amount of contact hours with the research team, the control group performed 4 weeks of strengthening exercises of the upper limb prior to the stretching (figure 5.2). Exercises were performed 4 times a week, of which one session was supervised by the principal investigator and the remaining 3 sessions were performed at home. All participants kept an exercise diary of their progress. In both groups, only the most affected side in terms of ROM was trained.

Measurements of tendon stiffness and muscle morphology were taken at all three time points: Before the start of the intervention (Baseline), after 4 weeks of training (4-weeks) and at 10 weeks after the intervention (10-weeks). A gait analysis was performed before (Baseline) and after (10-weeks) the intervention.



**Figure 5.2:** Time scheme showing the exercises performed by the intervention and the control group

## *Training*

### *Strength training*

Standing heel raises were used to strengthen the ankle plantarflexor muscles following the American College of Sport Medicine guidelines for progressive resistance training (Faigenbaum & Myer, 2010). Exercises were performed in three to four sets of 6-12 repetitions, in which volitional fatigue was reached at the end of each set. When participants could achieve more than 12 repetitions, exercises were progressed by advancing from bilateral to uni-lateral heel raises on the most affected side, and then by adding weight to a rucksack worn on the participants back. The stretching-only group performed a similar protocol with biceps-curls as an exercise, targeting the elbow flexors. This group progressed by increasing the weight of the dumbbells.

### *Stretching*

Stretching of the calf muscle was performed either actively by the participant (self-stretch) or passively applied by the parents. The method of stretching depended on capability (age and physical functioning) of the individual participant to perform the self-stretch. All stretches were held for 1 minute and repeated 10 times with 30 s rest in between stretches (Wiat *et al.*, 2008; Theis *et al.*, 2015). For the self-stretch, to gain the initial stretch position children were instructed to stand facing a wall, with the hands placed against the wall at shoulder height and the leg to be stretched placed behind the body. The contralateral leg was then flexed and to perform the stretch participants were asked to lean forwards and pull their pelvis towards the wall while pressing the heel of the stretching leg into the floor. For the passive stretch children lay on their back on a mat on the floor with the parent positioned at the side of the participant. To gain the initial stretch position the leg was lifted with the knee flexed to 90°. To initiate a stretch the hand was cupped around the heel with the palms of the hand flat against the foot. The ankle was slowly dorsiflexed by applying pressure against the plantar surface of the foot. When in a maximal dorsiflexed position, the knee was slowly guided into maximal extension. This position was then held for 1 min. The stretch was performed 10 times with 30 s rest in between stretches.

### *Measurement protocol*

Participants lay prone on a bed with the lower leg supported on an inclined cushion such that the knee was ~20° flexed. The foot was fixed onto the footplate of a dynamometer (Humac Norm CSMI, MA, USA) and stabilised by a custom-made arch support. The upper leg was fixed to the bed. The axis of rotation of the dynamometer was aligned with the lateral malleolus. Range of motion was determined by manually

rotating the ankle to the point where either the participant indicated the threshold or the examiner felt the joint reached the end of passive movement. Subsequently the stops of the dynamometer were set to this ROM. Surface electromyography (sEMG), electrode placement defined with ultrasound, was collected at 1600Hz from the lateral gastrocnemius and the tibialis anterior muscle during all tests (BioNomadix, Biopac systems, UK). Ankle torque and joint angle were collected at 200Hz by a dynamometer. The net joint torque was corrected for the torque caused by gravity on the weight of the footplate.

### *Passive muscle structure*

#### Measurement of muscle and tendon length

A B-mode ultrasound scanner was used to measure muscle and tendon lengths at 4 different ankle angles. Maximal plantarflexion, maximal dorsiflexion (as defined above), 10° plantarflexion and a resting angle, which was defined as the ankle angle when the foot hung freely off the bed, without the weight of the dynamometer. During all tests, the knee was fixed at 20° flexion. The most superficial point of the medial femoral condyle, the most distal point of the myotendinous junction (MTJ) of the medial gastrocnemius and the most distal point of the attachment of the Achilles tendon on the calcaneus were identified with ultrasound. Subsequently these anatomical landmarks were marked on the skin with help of a thin metal rod placed between the skin and the ultrasound probe, casting a shadow on the ultrasound image (Intziagianni *et al.*, 2015). Muscle and tendon lengths were measured with a segmometer as the linear distances between the medial femoral condyle and the MTJ; and between the MTJ and the attachment on the calcaneus, respectively. Muscle-tendon-unit length was defined as the summation of muscle and tendon length.

#### Measurement of fascicle length

To measure fascicle lengths, the ultrasound probe was placed over the mid belly of the muscle. Guidance regarding probe alignment was adhered to for minimising measurement errors (Bénard *et al.*, 2009). Fascicle length was assessed at resting ankle angle and during a continuous passive movement applied by the dynamometer, from maximal plantarflexion to maximal dorsiflexion performed at 7.5°/s. Ultrasound frequency ranged between 15 and 40 Hz depending on the type of acquisition. Four separate images were taken of resting fascicle length and the passive movement was repeated four times, after which the data was averaged over trials. During the passive movements, a modified semi-automated tracking software (Cronin *et al.*, 2011; Gillett *et al.*, 2013) was used to track the fascicle and both the superficial and the deep aponeurosis. Fascicle length was calculated by extrapolating the fascicle to the intersection point with the

aponeuroses. For each individual, the angle at which the fascicles started to lengthen was determined visually from plotted fascicle vs ankle angle relationship and from the ultrasound video.

### *Achilles tendon stiffness*

#### Achilles tendon force

Force was applied to the Achilles tendon by means of a maximal isometric plantarflexion contraction (MVC). The moment around the ankle joint was measured using an isokinetic dynamometer. During this movement, the maximum ankle joint torque was measured to quantify muscle strength. Children were asked to perform an MVC taking four seconds to reach the maximum force. Despite extensive practice, many of the children found it difficult to perform these slow contractions smoothly. These children were asked to produce their MVCs at their preferred speed. Because the rate of force development was markedly lower than in adults (Moreau *et al.*, 2011), the resolution of our force elongation curves created with an ultrasound sampling frequency of 15-40Hz was adequate for reliable data capture. Two successful repetitions were performed of the MVC with at least one minute rest between them. Achilles tendon force was calculated by:  $F_{tendon} = M_{joint} / MA_{AT}$ , where the Achilles tendon moment arm ( $MA_{AT}$ ) was estimated based on the predictive equation derived from (Kalkman *et al.*, 2017).

#### Tendon elongation

Tendon elongation was measured as the displacement of the medial gastrocnemius MTJ from rest during the MVC trials. An echo-absorptive strip was placed over the skin above the muscle to confirm that the ultrasound probe did not move relative to the skin during the measurement. During the movement, absolute displacement of the MTJ was manually tracked, twice for each movement, using ImageJ imaging software (NIH, USA).

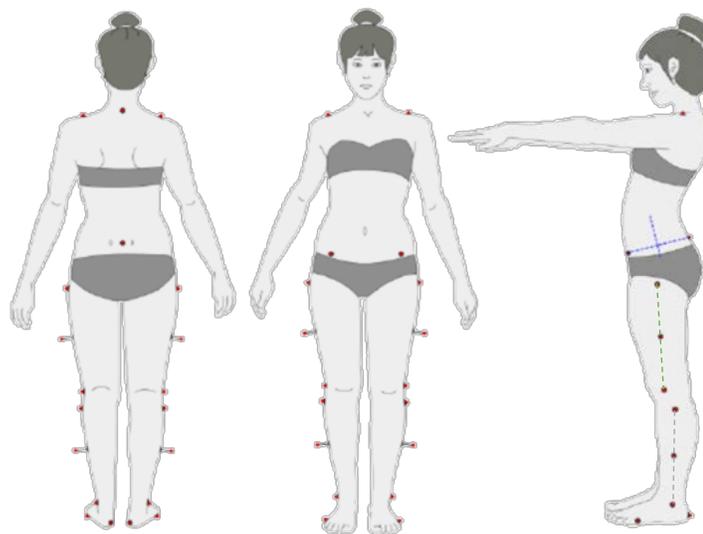
#### Tendon stiffness

Tendon stiffness was calculated as the gradient of each individual force-elongation curve using two different approaches. First, stiffness was calculated as the slope of a linear fit through the individual force-elongation curves between 50-90% of the peak force. This measure may be considered as an average stiffness across the force range for each individual participant. However, because of the non-linear nature of the tendon force-elongation relationship, a tendon stiffness at a common force region for all participants was also calculated. This was done, by fitting a second order polynomial through the force-elongation curve and differentiating at the highest force level that could be achieved by all participants

pre- and post-intervention. The calculated tendon stiffness was then averaged over the four repetitions from two trials.

### *Gait*

Children underwent a 3D gait analysis according to standard clinical practice at the Gait Laboratory at Alder Hey Children's Hospital 0 to 7.5 months before the start of the intervention and immediately after the intervention. Children were asked to walk on an 8.5-metre walkway barefoot at a self-selected walking speed. Twelve infrared cameras (BTS-Gaitlab, BTS Bioengineering, Milan, Italy) were used to capture the 3D motion of reflective markers attached to the skin over anatomical landmarks at a sampling rate of 340 Hz (figure 5.3). Marker trajectories were filtered with a second-order low-pass Butterworth filter at 5 Hz and gap filling was performed with a cubic b-spline function (SMARTtracker 1.10.462.0, BTS Bioengineering, Milan, Italy). Pelvic and hip angles in 3 planes, knee flexion/extension, ankle plantar/dorsiflexion and foot progression angles were reconstructed using the Davis model (Davis *et al.*, 1991). The mean of 3 to 5 trials of the trained leg was analysed. Parameters of interest included maximal knee and ankle angle and ROM during the stance and swing phase in the sagittal plane. Additionally, the movement deviation profile (MDP, Barton *et al.*, 2012) was calculated based on the kinematic results from the hip (3DoF), knee flexion/extension, ankle plantar/dorsiflexion and foot progression angles as well as on a subset of three kinematic angles around the knee and the ankle. The following spatio-temporal parameters were extracted from the gait analysis: self-selected walking velocity, cadence and stride-length.



**Figure 5.3:** Marker placement following the BTS protocol based on the model by Davis *et al.* (1991).

## Statistics

Data analysis was carried out using custom made software (Matlab R2015a). Parameters were checked to be normally distributed using the Shapiro-Wilk test and by inspection of the q-q plots. MVC, tendon stiffness and resting fascicle lengths were compared within group over the three timepoints using a Friedman test. For the variables that showed significant changes, Conover post-hoc tests were applied. Baseline measures were compared between groups using a 2-sample independent t-test or a Mann-Whitney U test. A p-value of  $\leq 0.05$  was considered significant. All statistical analyses were performed in R (R Core Team, 2015).

## Results

A total of 16 children completed the intervention and all the testing sessions. Baseline differences were found in patient characteristics between the intervention and the control group in age, height and mass (table 5.2). However, there were no significant differences in resting fascicle length (intervention:  $36.9 \pm 8.2$  mm, control:  $30.3 \pm 6.6$  mm,  $p=0.11$ ), muscle strength (intervention:  $413.5 \pm 399.3$  N, control:  $251.8 \pm 187.9$  N,  $p=0.33$ ) or tendon stiffness (intervention:  $35.6 \pm 25.7$  N/mm, control:  $24.3 \pm 8.9$  N/mm,  $p=0.32$ ) between groups at baseline.

**Table 5.2:** Characteristics of participants completing the intervention.

	<b>Intervention (n=9)</b>	<b>Control (n=7)</b>
Age (years)	11.1 (2.0)	8.8 (1.0)*
Height (cm)	144.7 (14.1)	126.75 (10.0)*
Mass (kg)	41.1 (14.1)	26.9 (6.9)*
Sex	4 male, 5 female	5 male, 2 female
Diagnosis	5 Diplegia, 4 Hemiplegia	3 Diplegia, 4 Hemiplegia
GMFCS	5 x I, 4 x II	3 x I, 3 x II, 1 x III

\* Significant difference at  $p < 0.05$

## Muscle-tendon properties

### Strength

Of the nine participants in the intervention group who performed calf strengthening exercises, two participants began training with bilateral heel raises and both progressed to uni-lateral heel raises in the second week. Significant increases in the number of heel raises achieved as well as the load added to the backpack were found between baseline and week 10 for the whole group (table 5.3).

Maximal ankle joint torque as measured with a dynamometer increased significantly in the intervention group ( $\chi^2 = 10.457$ ,  $df=2$ ,  $p=0.005$ ). A Conover post hoc test revealed significant differences between baseline and 10-weeks ( $p=0.003$ ). No differences were found between baseline and 4-weeks or 4-weeks and 10-weeks. No increases in MVC were found in the control group ( $\chi^2 = 2.57$ ,  $df=2$ ,  $p=0.277$ , figure 5.4A).

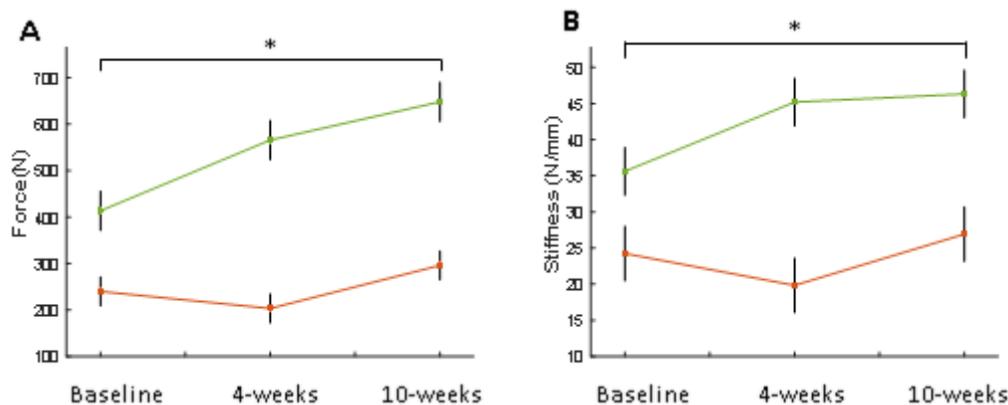
**Table 5.3:** Training related measures

	Baseline	Week-10
Uni-lateral repetitions (#)	6.5 (0-10)	12 (12-12) *
Added load (kg)	0 (0-0)	2.6 (0-6) *
$F_{\text{tendon}}$ during MVC (N)	413.5 (399.3)	648.1 (423.7)

\*Significant difference between week-10 and baseline measurements ( $p<0.001$ ). For repetitions and load, median (range) values are shown. For maximal voluntary contraction (MVC), mean (SD) values are shown.

#### Tendon stiffness

Tendon stiffness at maximal force increased significantly in the intervention group ( $\chi^2 = 11.706$ ,  $df=2$ ,  $p=0.002$ ). A Conover post hoc test revealed significant differences between baseline and 10-weeks ( $p = 0.002$ ). No differences were found between baseline and 4-weeks or 4-weeks and 10-weeks. No changes in stiffness at maximal force were found in the control group ( $\chi^2 = 3$ ,  $df=2$ ,  $p=0.223$ ).

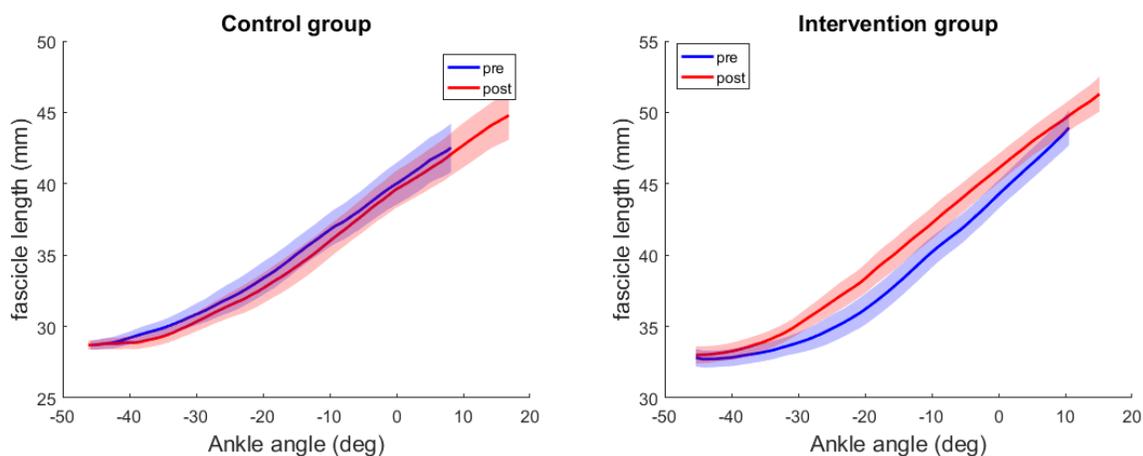


**Figure 5.4:** Tendon force during a maximal voluntary contraction (A) and tendon stiffness at a common force (B) at baseline, after four weeks of training and after ten weeks of training. Orange: control group, Green: intervention group. Significant change was found in the intervention group between baseline and 10-weeks, which was absent in the control group.

Tendon stiffness at a common force increased significantly in the intervention group ( $\chi^2 = 7.47$ ,  $df=2$ ,  $p=0.023$ ). A Conover post hoc test revealed significant differences between baseline and 10-weeks ( $p = 0.040$ ). No differences were found between baseline and 4-weeks or 4-weeks and 10-weeks. No changes in stiffness at a common force were found in the control group ( $\chi^2 = 4$ ,  $df=2$ ,  $p=0.135$ , figure 5.4B).

### ROM

Range of motion increased significantly in both the control group and the intervention group between baseline and 10-weeks ( $p=0.008$ ). Maximal dorsiflexion angle increased from  $10.6^\circ \pm 8.3$  to  $17.4^\circ \pm 9.8$ . During passive joint rotation, the angle at which muscle fascicles started to lengthen shifted towards a more plantarflexed position in the intervention group (Baseline to 10-weeks:  $-29^\circ$  to  $-35^\circ$ ,  $p=0.03$ , CI [1.0 12.5], figure 5.5)



**Figure 5.5:** Lengthening profile of muscle fascicles vs ankle angle during a passive joint rotation.

### Muscle and fascicle lengths

Muscle and tendon lengths did not change after the intervention at any ankle angles (max. dorsiflexion,  $10^\circ$  plantarflexion, resting, max. plantarflexion) in both the control and the intervention group. Resting fascicle length increased significantly from  $36.9 \pm 8.6$  mm to  $40.1 \pm 8.3$  mm in the intervention group ( $\chi^2 = 14.824$ ,  $df=2$   $p=0.0006$ ). A Conover post-hoc test revealed significant differences between baseline and 10-weeks ( $p=0.010$ ) and between 4-weeks and 10-weeks ( $p=0.0008$ ). The ankle angle at which resting fascicle length was assessed did not change (baseline:  $-30.4^\circ$ , 10-weeks:  $-29.3^\circ$ ). No increases in resting fascicle length were found in the control group ( $\chi^2 = 0.074$ ,  $df=2$ ,  $p=0.964$ ). Lengthening profiles of muscle fascicles are visualised in figure 5.5.

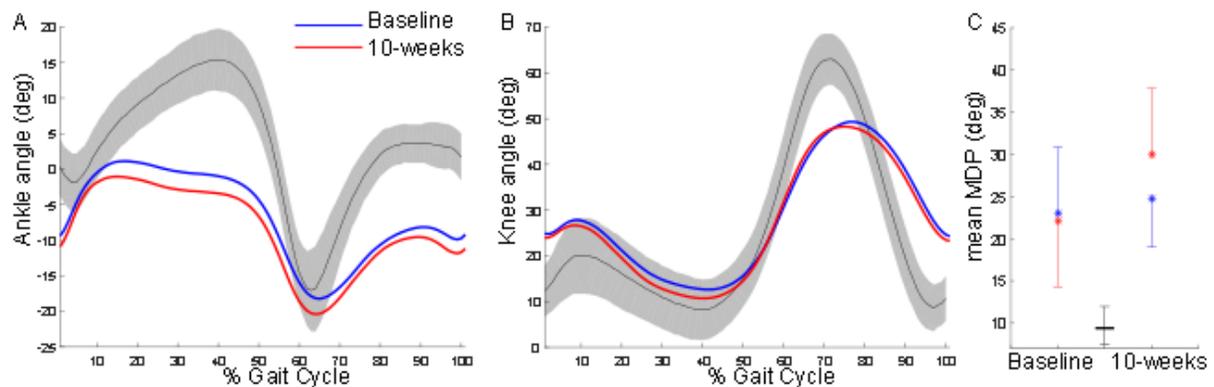
## Gait

No significant changes were found in self-selected walking speed, stride length or cadence after the intervention period in both the intervention and the control group. None of the kinematic parameters selected around the knee and the ankle (figure 5.6 A&B) showed any significant changes (see a selection of the most significant parameters in table 5.4). The mean MDP calculated either with 6 angles or only with the ankle and knee angles did not show any significant changes after the intervention (figure 5.6C).

**Table 5.4:** Mean (SD) gait parameters before and after the intervention.

	Intervention		Control		Typically developing
	Baseline	10-weeks	Baseline	10-weeks	
<b>Spatio-temporal parameters</b>					
Walking velocity (m/s)	1.1(0.4)	1.1(0.2)	1.1(0.2)	1.1(0.2)	1.2(0.1)
Cadence (step/min)	122.1(23.7)	124.1(7.9)	146.0(13.9)	148.3(10.8)	123(10.8)
Stride length (m)	1.1(0.3)	1.0(0.2)	0.9(0.2)	0.9(0.2)	1.18(0.13)
<b>Kinematics</b>					
Maximum ankle DF in stance (°)	7.0(11.1)	6.7(10.1)	0.6(13.9)	-2.1(16.6)	15.4(6.6)
Ankle ROM during the gait cycle (°)	27.9(8.4)	29.1(6.7)	27.4(10.4)	28.4(5.3)	32.4(6.1)
MDP using 6 angles (°)	23.0(7.9)	22.1(7.7)	24.8(5.7)	29.9(7.8)	9.39(2.6)
MDP using 3 angles (°)	8.8(7.6)	8.1(6.8)	10.9(5.3)	15.1(7.8)	3.1(1.4)

Typically developing data are shown for comparison purposes. These data were obtained from the database at the Gait Laboratory of Alder Hey Children's Hospital (N=15).



**Figure 5.6:** Average gait curves for the ankle (A) and knee (B) angle in the sagittal plane. (C) Mean movement deviation profile (MDP) calculated with 6 angles at baseline (blue) and 10-weeks (red) compared to control values of TD children (black)

## Discussion

The present results show that in response to a progressive resistance training programme, tendon stiffness can be increased in children with CP. As a result of this, the stretching stimulus to the muscle was increased. Moreover, when resistance training and stretching were combined, fascicle length increased when measured at a constant resting ankle angle. This indicates that a remodelling of the muscle has occurred.

The designed intervention aimed to increase the effectiveness of traditional stretching exercises. These are typically performed either passively by another person, or actively (the child performs the stretch). The most common reasons for using stretching techniques are to maintain or increase ROM, improve functional tasks and defer or avoid surgery. The use of stretching is based on the assumption that stretching will increase muscle extensibility (Wiar *et al.*, 2008), but there is no scientific evidence to support this statement. Some studies have shown an increase in ROM after stretching (Theis *et al.*, 2015), while others report no changes in flexibility after stretching interventions (Miedaner & Renander, 1988; Darrah *et al.*, 1999). We have shown that after six weeks of performing only stretching exercises (control group), ankle ROM can be increased. However, this was not accompanied by a change in muscle properties. In our study design the examiner manually stretched the ankle to its end ROM, defined as the “end-feel” of movement. The position at which this end-feel of motion occurs, may depend on different factors for example pain tolerance, warm-up of the muscle or acquaintance between the patient and the examiner. Therefore, we may hypothesise that the children experienced an increased stretch tolerance after the intervention, which is the same mechanism that we identified as a factor that could play a role in the increased ROM acutely after a single bout of stretching (Chapter 3). Previous studies have indicated a similar mechanism to explain increases in ROM after stretching interventions in healthy individuals (Magnusson *et al.*, 1996b).

When stretching exercises were preceded by four weeks of resistance training to increase Achilles tendon stiffness, an increase in resting/slack fascicle length was observed. Both an increase in the number or in the length of the individual sarcomeres acting in series would lead to such an effect. During intraoperative measurements of spastic muscles, it has been shown that individual sarcomeres are overstretched in children with CP (Ponten *et al.*, 2007; Smith *et al.*, 2011; Mathewson *et al.*, 2014b). This has lead people to hypothesise that muscles of children with CP are unable to add sarcomeres in series in response to stretch (bone growth). Studies showing a reduction in the number of satellite cells in spastic muscle (Smith

*et al.*, 2013; Dayanidhi *et al.*, 2015) would support this hypothesis because satellite cells are mediators of muscle growth (Dayanidhi & Lieber, 2014). However, Kinney *et al.* showed that, at least in a mouse model, when muscles underwent satellite cell depletion, sarcomeres could still be added in series in response to stretch (Kinney *et al.*, 2017). A clear conclusion is therefore still lacking, and it is conceivable that the increased fascicle length in this study was caused by an increase in the number of sarcomeres in series.

Alternatively, the increased fascicle length can be caused by an increase in the resting length of the sarcomeres. The passive resting length and the passive length/tension relationship of a sarcomere is mainly determined by the large molecule titin (Labeit & Kolmerer, 1995). There is no definitive data to show that titin is altered in spastic muscles, however titin isoforms have been found to be different between muscles (Prado *et al.*, 2005; Granzier & Labeit, 2006) and altered due to disease (Neagoe *et al.*, 2002). Investigations into the alterations that occur in titin in spastic skeletal muscle and after training are warranted. Nonetheless, whichever of these scenarios causes the increased fascicle length seen after a combined strengthening/stretching intervention in this study, both indicate that a remodelling of muscle structure in response to mechanical stimuli is possible in children with CP.

A gait analysis was performed in all children before and after the intervention period. We expected to see improvements in dorsiflexion angle during the stance phase of gait and at initial contact, also we expected ankle ROM over the whole gait cycle to improve. However, no changes were found in gait kinematics after the intervention period. Our results are consistent with previous reports of only non-significant improvements in gait after strengthening or stretching interventions (McNee *et al.*, 2009). At baseline, many of the children showed only mild gait deviations, while for others ankle ROM might not have been the main contribution to their gait deviations. A gait analysis performed in a controlled environment such as the gait laboratory may not be sensitive to any genuine improvements in walking function that these children achieved after training. Other measures aimed at testing higher level function such as fast walking, running, jumping or stair negotiation might better detect improvements after strengthening and stretching. Alternatively, structural alterations to only one muscle group, might not lead to any detectable changes in gait. Functional improvements might become apparent however when the same effect is achieved in multiple muscle groups. Further research is needed to determine the effect of a strengthening and stretching intervention targeting multiple muscle groups.

We did not observe any measurable improvements in gait characteristics after the intervention. However, subjective reports from children and their parents told us that all participants experienced the exercises

as very positive. Most of the time, children enjoyed doing the exercises and were motivated to get better and stronger. Parents valued the one-on-one feedback they received from the lead researcher on the progress of the exercises. Some children reported pain in their muscles after doing the exercises, it was then explained to them that muscle soreness is a consequence of the training and that this is not damaging to the muscle, and followed up to confirm it eased within a few days. After completing the program, some families felt their children could walk further or they endured their regular physiotherapy sessions better. Even though we did not aim to quantify the subjective response of the children and their families to the program, we consider it valuable information that the responses were very positive.

### *Limitations*

There was a significant difference in the baseline characteristics between the control and the intervention group. Participant recruitment was an ongoing process during the data collection of this study. Therefore, it was chosen to randomly assign participants to the intervention or control group based on a minimisation algorithm (Saghaei & Saghaei, 2011). With the aim to recruit thirty participants, it was chosen to control for three parameters: age, gender and GMFCS level. Only twenty-two participants started the intervention, this led to a small, though non-significant, imbalance between the two groups in terms of age. Retrospectively, it might be better for experiments of this size to control randomization only for the one factor that is considered most important. Upon completion of the intervention there was a significant difference in patient characteristics between the groups, which was caused by the drop-outs from the intervention group being the 3 youngest children. To check whether a difference in age would have an effect on the response to the intervention we performed some additional analyses. However, no relationship was found between age and increases in strength ( $r^2=0.23$ ,  $p=0.08$ ) or stiffness ( $r^2=0.13$ ,  $p=0.19$ ). Therefore, we do not expect this to have influenced our results. Secondly, we did not assess the effect of isolated strength training on muscle structure. It has been shown that increases in fascicle length due to resistance training occur mostly in response to eccentric training (Franchi *et al.*, 2014). We cannot exclude that due to the nature of the exercises (children putting their foot flat on the floor) a stretch was induced to the muscle already during the strengthening exercises. The results of an ongoing study on strength training for adolescents with CP (Ryan *et al.*, 2016) will help us to tease out the relative contribution of strength training and stretching to an increased fascicle length. Also, some assumptions were made in the calculation of tendon stiffness. Co-contraction of the antagonist muscle was not accounted for when calculating tendon force because for most of the participants it was too difficult to perform a guided dorsiflexion contraction. If anything, we expect the amount of co-contraction to

decrease after the intervention. Therefore, our calculation of tendon stiffness will be underestimated more after the intervention than at baseline. As a consequence, accounting for co-contraction would only enlarge the increase in tendon stiffness we report. Also, because not all children were able to perform a slow plantarflexion contraction, we could not confirm the strain rate of the tendon to be constant during the MVC. It has been shown that strain rate influences the calculation of tendon stiffness (Theis *et al.*, 2012). However, additional analysis indicated no significant differences in strain rate at 10-weeks vs baseline (10-weeks:  $4.43 \pm 2.3$  mm/s, baseline:  $4.06 \pm 1.6$  mm/s,  $p=0.17$ ). Therefore, we do not expect this to have influenced our results. We did not measure Achilles tendon moment arm for the calculation of tendon force but predicted this based on the results from Chapter 1. The effect of this on tendon stiffness will be negligible since we do not expect the moment arm to change in ten weeks. Finally, the gait analysis of most children did not capture reliable kinetic data. Therefore, it was only possible to interpret gait kinematics, even though moments and powers might have provided additional insight into the effects of the intervention on gait performance.

To conclude, this study provides proof of principle that a combined resistance and stretching intervention can increase muscle fascicle length in children with CP. This is an important finding because it demonstrates that a remodelling of muscle structure after non-invasive interventions is possible in spastic CP. Even though we did not find any immediate functional improvements, the changes in muscle structure might have a long-term positive effect on mobility. Further long-term studies are required to determine this. Since muscle contractures develop over time as children grow, an increase in fascicle length may prevent the development of contractures at later age.



## CHAPTER 6

### General discussion

The overall purpose of this research was to increase the effectiveness of stretching interventions in children with Cerebral Palsy (CP). This information can guide clinical decision making to improve motor function and quality of life in children with CP.

## Summary of experimental findings

While deciphering the relationship between joint rotation and muscle extension, it was found that the Achilles tendon moment arm ( $MA_{AT}$ ) in children with CP is 15% (~7mm) smaller than in typically developing (TD) children (Chapter 2). This has numerous implications, among others for mechanical advantage which is functionally an important parameter (Biewener *et al.*, 2004). But in the context of stretching the implications are that the expected stretch in the muscle-tendon-unit (MTU), and thus the muscle, will be smaller. This is important to realise when applying a stretch at the ankle joint either for treatment or to assess muscle function. This also means that for the same joint rotation velocity the stretch velocity of the muscle during dynamic tasks such as walking is decreased, which might therefore act as a protective mechanism against spasticity.

Next the relationship between the stretch in the MTU and the muscle was further explored (Chapter 3). It was found that the relative stiffness of muscular tissue to tendon was increased in children with CP. During passive ankle joint rotation, in TD children the muscle contributed to ~60% of total MTU lengthening, while in children with CP this was only 50%. These differences, along with the effect of a smaller moment arm, affect the stretching stimulus seen by the muscle during joint rotation in a negative way.

The final two experiments were designed with the goal to understand how these CP-related changes in muscle and tendon properties influence the way the muscle responds to stretching interventions. This question was investigated both acutely (Chapter 4) and after a long term stretching intervention (Chapter 5). Also, the insights from Chapter 3 were used to design an intervention that would optimise the stretching stimulus to the muscle.

In Chapter 4 it was shown that acutely, after 20 minutes of passive stretching, the muscle-tendon properties of the medial gastrocnemius muscle in children with CP were not altered. Importantly, range of motion (ROM) was found to increase significantly by  $10\pm 12^\circ$ . The maximal joint torque that could be applied at the end-feel of movement increased by  $2.9\pm 2.4$  Nm and at this highest joint torque fascicle length increased significantly by  $2.8\pm 2.4$  mm. This indicates that even though the stiffness of the muscle fascicles did not change, at the end of the stretching session children were able to tolerate a higher passive

torque and this increased the stretching stimulus to the muscle fascicles. These results are important to consider when assessing the effectiveness of long term stretching interventions. This raises the question whether the stretch seen by the muscle is large enough to elicit any long-term adaptations.

The new knowledge about muscle stretching (Chapter 2+3) indicates that in order to make stretching interventions more effective, we need to decrease the relative stiffness of muscle to tendon. In Chapter 5, we tested this hypothesis. An intervention was designed with the aim to first increase the stiffness of the tendon, by means of resistance training, so that the relative stiffness of muscle to tendon would be decreased and the muscle would take up more of the stretch. In our intervention group, tendon stiffness was increased by ~30% after 10 weeks of training. Consequently, the muscle fascicles experienced stretch earlier in the ROM and fascicle slack length at a resting ankle angle increased by 3.2mm after the intervention. This indicates that the combined application of strengthening and stretching exercises can remodel muscle structure in children with CP. This, however, did not result in an improved gait pattern, which could be caused by the fact that only one muscle group was trained. Nonetheless, we proved that after this type of intervention muscle remodelling is possible in children with CP.

## Clinical relevance

In addition to the advancement of our fundamental understanding of muscle and tendon function, the findings reported in this thesis have important clinical implications. In this section, we will discuss both the direct consequences of a smaller Achilles tendon moment arm and highlight the importance of our finding that the relative stiffness of the muscle to the tendon can be manipulated by a properly designed physiotherapy intervention.

During gait the bones act as rigid lever arms, allowing muscles and the GRF to apply a torque around the joints. Skeletal deformities, as seen in CP, can affect the function of bones as lever arm and so lever arm dysfunction is a common problem in children with CP (Theologis, 2013). The most usual deformities causing lever arm dysfunction in CP are femoral anteversion and deformities around the foot. Femoral anteversion compromises the lever arm of the hip abductors as the projection of the femoral neck in the frontal plane is shortened. Deformities around the foot, such as tibial torsion, equino-valgus foot deformity and a midfoot break can all compromise the projected length of the foot in the sagittal plane. This leads to a shortening of the external moment arm at the ankle. The internal moment arm however (Achilles tendon moment arm) is not widely considered in clinical practice or in the literature. We found that the

Achilles tendon moment arm is shorter in children with CP (Chapter 2). This has some important implications when considering CP gait and the outcome of orthopaedic surgeries. Surgeries such as gastrocnemius recession or derotation osteotomies often aim to increase the external moment arm around the ankle to typical developing values. It is assumed that because of this the mechanical advantage around the ankle is restored to normal values as well. However, the mechanical advantage of a joint is influenced by both the internal and the external moment arm (mechanical advantage =  $MA_{AT}/MA_{external}$ ). Therefore, a result of the above-mentioned surgeries is that the mechanical advantage is actually reduced to values below typical. So, to counteract the same ground reaction force during gait, the muscle force produced by the plantarflexors needs to be much larger. This could contribute to the observed relative muscle weakness seen after equinus correcting surgery (Orendurff *et al.*, 2002; Gage *et al.*, 2009). While research continues to fully understand the mechanics and develop clinically applicable ways to assess mechanical advantage, clinicians now could at least test muscle strength prior to surgery. Treatments should be planned taking this potential strength loss into account.

Stretching exercises are a very common treatment modality in children with CP. Stretching aims to increase range of motion and consequently improve function, delay the development of contractures and the need for orthopaedic surgery. However, the effectiveness of these interventions is still questionable (Craig *et al.*, 2016; Eldridge & Lavin, 2016). We have shown that, when applied in isolation, stretching exercises can improve range of motion but do not alter muscle properties or gait characteristics (Chapter 5). Therefore, it is unlikely that stretching exercises improve function or delay the development of contractures. The main aim of this thesis was to increase the effectiveness of stretching interventions. To achieve this, an intervention was designed where the stretching stimulus to the muscle would be increased. The amount of stretch in a muscle during joint rotation is dependent on the relative stiffness between the muscle and tendon. We found that in children with CP, this relative stiffness was increased (Chapter 3). Therefore, our intervention aimed to increase the stiffness of the tendon, so that the relative stiffness of muscle to tendon would be decreased and the muscle would receive more of the stretch during a joint rotation. To achieve this, strengthening exercises (to increase tendon stiffness) were performed prior to the application of stretching exercises (to increase fascicle length). This combination of interventions led to a remodelling of muscle structure in terms of increased fascicle lengths (Chapter 5). These are promising results, since it has been questioned whether muscle remodelling is possible in children with CP (Smith *et al.*, 2011). Although these changes were small and did not yield functional gait improvements, the changes in muscle structure may have a long-term positive effect on mobility. Since muscle contractures develop over time

as children grow (Herskind *et al.*, 2016), an increase in fascicle length may prevent the development of contractures at later age and therefore delay or defer the need for orthopaedic surgery.

## Joint hyper resistance and underlying impairments

To identify the appropriate treatment for a child with CP, a first step is to determine the exact underlying impairment. Subjective clinical tools to assess muscle properties exist, but these lack sensitivity (Noort van den *et al.*, 2010). These clinical tools assess muscle properties at the level of the joint, however, we observed a large variability in the amount of muscle lengthening (up to 50% difference between children) relative to ROM (Chapter 3). Furthermore, we have shown that passive ROM can increase in the absence of any changes in muscle properties (Chapter 4). During a passive test, the joint is rotated until the “end-feel” of movement due to tissue stretch is reached. This end-feel is dependent, among others, on pain tolerance, warm-up and acquaintance between clinician and patient. Therefore, the outcome of a clinical test could be more sensitive to this end-feel of movement than on the actual underlying muscle properties.

Additionally, in many neurological conditions, it is a big challenge to distinguish between the neural and mechanical contributions to altered joint motion. Instrumented spasticity assessments, combining kinematic, kinetic and EMG data are being developed to more objectively quantify the amount of spasticity and passive stiffness in a muscle. However, even with these tests it is still difficult to distinguish between neural and structural contributions to joint hyper-resistance. In a further analysis of the data collected for this PhD, ultrasound was combined with an instrumented spasticity assessment (Appendix C). We showed that the medial gastrocnemius exhibits a large variability in the amount of stiffness and spasticity between children with CP. Three behavioural patterns were established. First, muscles with a low mechanical stiffness allowed a high muscle lengthening velocity during fast joint rotation, eliciting a stretch reflex. Secondly, muscles with greater mechanical stiffness, preventing a high muscle lengthening velocity, did not show a velocity dependent stretch reflex but exhibit more length-dependent muscle activation at low velocities. In the third category, muscles had a greater mechanical stiffness, but also showed velocity dependent stretch reflexes. These separate studies highlight that treatments that aim to decrease ankle joint hyper-resistance should ideally be based on quantification of the amount of stiffness and reflex activity at the muscle-tendon tissue level, rather than gross measurements of passive resistance at the whole joint level.

An increased muscle stiffness can be caused by structural changes or an increased muscle tone (figure 1.1, Chapter 1). In neurological conditions, the background neural drive to the muscle is thought to be increased, resulting in an increased muscle tone during slow movements or even at rest (Bar-On *et al.*, 2014b). It is important to distinguish between this altered muscle tone and the above mentioned structural changes, because they require different treatments. However, with routine clinical tests this distinction is difficult to make. In this thesis, all experimental measurements recorded during a slow stretch, were excluded when the EMG exceeded more than 10% of the MVC. In correspondence with others (Bénard *et al.*, 2010; Haberfehlner *et al.*, 2015), we found that 10% of the MVC in children with CP approximately corresponded to 1-2SD above resting EMG levels, given that baseline EMG values for children with CP are typically higher than control. However, a background involuntary muscle tone might still have contributed to the increased muscle stiffness we observed. In clinical practice before the performance of orthopaedic surgery, a final test of joint stiffness is often performed with the child under general anaesthetic, thus minimising the effect of any neural contributions (Zwick *et al.*, 2004). However, this procedure is risky and impractical to perform in most children, meaning the clinical decision-making process is not fully informed and therefore treatment outcomes can be suboptimal. Therefore, further studies should aim to better measure the neural and non-neural contributions to joint hyper-resistance. Alternatively, *in silico* studies can help us to predict the contribution of neural and non-neural factors to joint hyper resistance (Sloot *et al.*, 2015).

## Gait analysis as an outcome measure

In the last few decades there has been an enormous progress in the treatment of gait problems in children with CP. These advancements have been attributed to the development of clinical gait analysis (Sutherland, 1978; Gage, 1994; DeLuca *et al.*, 1997). Quantitative preoperative and postoperative gait analysis inform the clinician with valuable information by which surgical protocols can be judged. However, benefits of clinical gait analysis as a tool to evaluate the effectiveness of interventions has been shown mainly for orthopaedic surgery (Novacheck & Gage, 2007), selective dorsal rhizotomy (Boscarino *et al.*, 1993), intramuscular botulinum toxin injections (Sutherland *et al.*, 1996) and casting (Hösl *et al.*, 2015). The effectiveness of other non-invasive therapies to treat cerebral palsy, such as stretching or strengthening, seem more difficult to assess with gait analysis. Strengthening interventions have been assessed by clinical gait analysis but studies have reported no changes (Maeland *et al.*, 2009; McNee *et al.*, 2009), or only small improvements in kinematic variables, such as knee angle (Engsberg *et al.*, 2006),

or a combination of hip, knee and ankle angle (Unger *et al.*, 2006). To assess the effectiveness of stretching interventions, in research studies, often ROM is the only outcome measure used (Wiar *et al.*, 2008). To the authors' knowledge, we are the first to describe the effect of combined stretching and strengthening but also an only-stretching intervention on gait (Chapter 5). We did not find any changes in gait parameters, ankle and/or knee kinematics or more sophisticated measures of whole body kinematics such as the Movement Deviation Profile (Barton *et al.*, 2012). In interpreting these findings, however, there are a few limitations that we need to consider. Firstly, due to methodological reasons, the pre-intervention gait analysis was conducted up to 7 months before children actually started with the intervention. Therefore, it is likely that other factors played a role when assessing the difference between the gait analysis before and after the intervention. Secondly, for most children it was not possible to collect reliable kinetic data, which may have better captured any improvements. However, it must be accepted that the changes in muscle properties, elicited by the intervention, did not transfer to detectably improve gait characteristics. Reasons for this could be that our intervention focussed only on one unilateral muscle group, ankle plantar flexors, whereas an altered gait pattern is caused by an interaction of impairments in more and possibly different muscles. Alternatively, it could be that the change in muscle structure of a few millimetres is not sufficient to change the gait pattern. Furthermore, it is likely that ankle ROM was not the main factor limiting gait in our participants, hence an intervention targeting the most affected muscle group might be more effective in improving the gait pattern. When gait analysis is used to assess surgical interventions, the interventions are targeted specifically to the individual, the magnitude of change is much larger and many surgeries target multiple levels. In our intervention, a random sample of patients was selected, within the inclusion criteria, after which they all followed the same intervention. An intervention better targeted at the individual patient might yield different results.

Possibly, to assess the effectiveness of interventions such as physiotherapy treatments, we need a better tailored assessment method. Gait analysis is performed in a controlled environment, and might not be sensitive enough to reveal any genuine improvements in function after training (when gait is not specifically trained). Other measures aimed for example at higher levels of function, such as running, jumping or stair climbing, might be more suitable to detect improvements. Also, more local measures of for example muscle fatigue and endurance might give a better representation of the effectiveness of an intervention. Finally, it is worth mentioning that a lack of functional improvements after an intervention does not necessarily mean that the intervention is not effective. In terms of physiotherapy interventions that focus on changing muscle structure, the achieved change in muscle structure could have long term benefits that are not captured in any direct functional improvements. It is well known that muscle function

deteriorates over time in children with CP and muscle contractures develop as children grow (Herskind *et al.*, 2016). Therefore, an increase in fascicle length, which was the primary outcome measure of the intervention, achieved at a young age could prevent or delay the developments of contractures at a later age.

## Use of ultrasound to assess muscle properties

Ultrasound is a promising tool to be used in clinical assessment of musculoskeletal disorders. It has been suggested that ultrasound would be beneficial for the diagnosis of amyotrophic lateral sclerosis (Arts *et al.*, 2012) and monitoring of disease progression in Duchenne muscular dystrophy (Jansen *et al.*, 2012). However, in the clinical diagnosis and decision making of cerebral palsy, ultrasound is rarely used. We have shown that assessment at the level of the joint, as typically done in CP, is not necessarily representative of what happens at the muscle. Furthermore, we observed a large variability in the amount of stiffness and spasticity in children with CP that was not apparent from standard clinical measurements such as the modified Ashword or Tardieu scale (Appendix C). Therefore, we argue that it can be beneficial for treatment planning to include measurements of muscle architecture in the clinical assessment of CP. To achieve this within the routine care for every child, clinically applicable ultrasound-based muscle stiffness tools needs to be developed.

Within a research setting ultrasound has been used extensively to study muscle structure and muscle-tendon interaction (Cronin & Lichtwark, 2013) in clinical populations. Recent developments of 3D ultrasound techniques (Barber *et al.*, 2009; Cenni *et al.*, 2016b) can improve the accuracy of measurements of muscle architecture. Furthermore, automated feature tracking algorithms (Farris & Lichtwark, 2016; Cenni *et al.*, 2017) increase the usability of these methods.

Apart from muscle architecture, ultrasound is a great tool to study lengthening properties of muscle and tendon. However, to calculate the mechanical properties of the Achilles tendon and gastrocnemius muscle is more difficult. The passive torque measured around the ankle is a combination of different muscles and passive structures, of which the gastrocnemius is just one. Changes in moment arm (Chapter 2), joint capsule, co-contraction and force transmission would all influence the force acting within a given muscle. However, we can make an approximation of the relative stiffness between muscular and tendinous tissue by comparing muscle and tendon lengthening because these tissues act anatomically in series.

Furthermore, we need to develop our understanding of the underlying mechanisms behind the muscle alterations that we observe with ultrasound studies in children with CP. One of the most important questions in this regard is how fascicle length relates to sarcomere length, and whether we can model fascicles simply as the product of a number of sarcomeres in series. Does the increase in fascicle length as observed after 10 weeks of combined resistance training and stretching (Chapter 5) relate to increases in sarcomere number or length? This question is relevant because an increase in sarcomere number would increase the range of lengths at which a muscle can produce force. An increased sarcomere length would not have such an effect and might have different consequences for passive force in the muscle. Correspondingly, we do not know what causes the increases in muscle stiffness we observe. According to previous studies, this could be explained by overstretched sarcomeres (Mathewson *et al.*, 2014a), structural tissue alterations in the extracellular matrix (Booth *et al.*, 2001) or differences within individual sarcomeres, for example remodelling of titin (Foran *et al.*, 2005). Recently technological developments provide new promising techniques such as microendoscopy or resonant reflection spectroscopy, which are developed to assess sarcomere lengths directly in *in vivo* muscles. Using these techniques, it has been shown, at least in healthy muscle, that there is a strong relation between sarcomere length changes and changes in fascicle length (Chen & Delp, 2016; Lichtwark *et al.*, 2017). It is not known whether this relation exist as well in spastic muscles where we know resting sarcomere lengths to be greater than typical. Furthermore, it is still difficult however, to predict sarcomere numbers from the passive length of muscle fascicles (Lichtwark *et al.*, 2017). Which indicates that muscle fascicles length is not purely representative of sarcomere number.

## Future research

In this Chapter, some directions of future research have been mentioned already. However, a few other issues that warrant further investigation should be stated. The present thesis, and many other studies that use ultrasound to study muscle structure in children with CP, focus only on the calf muscles, and aim to isolate the medial gastrocnemius. There are some important reasons why this is the case, the medial gastrocnemius muscle is important for functional tasks such as walking, it is often affected in children with CP and because it is a superficial and pennate muscle, easy to scan with ultrasound. However, in a condition as CP, there is a large heterogeneity between muscles (Bar-On *et al.*, 2014b). Therefore, what we measure in the medial gastrocnemius and the calf muscles does not necessarily translate to other muscles groups. Further studies should additionally aim to apply the protocols that are in place to assess

muscle properties to other muscles such as the hamstrings, quadriceps and adductor muscles that are also often affected by spasticity.

As mentioned above, further research should seek to quantify mechanical advantage during gait and predict the change in mechanical advantage after surgery. The moment arm of the ground reaction force can be estimated from basic kinematic and kinetic data obtained during gait analysis. Measurements of the  $MA_{AT}$  during gait are a bit more complicated. However, there are techniques either using ultrasound (Rasske *et al.*, 2016) or by placing markers (Stosic & Finni, 2011) that can quantify  $MA_{AT}$  during dynamic activities. Predicting the change in mechanical advantage after surgery would allow for appropriate screening tools to be developed to assess whether children have enough muscle strength pre-surgery to overcome this decrease in mechanical advantage.

Finally, larger trials investigating the combined effect of strengthening and stretching are needed. In these studies the emphasis should lie on extrapolating our results to multiple muscle groups, for example, hamstrings and quadriceps are also commonly affected by CP and significantly affect gait. Also, potential improvements need to be assessed with more appropriate outcome measures, in which the long-term effect of the intervention should also be investigated.

## Conclusions

The main findings from the four experimental studies in this thesis show that fundamental knowledge of muscle and tendon biomechanics can be applied to a clinical setting to explain movement disorders in a cohort of children with CP. The findings conclude that the mechanical properties of the medial gastrocnemius muscle are altered, with the muscular component of the plantarflexor muscle being as stiff as the tendon in children with CP. These properties are not affected acutely or in the long term by stretching interventions. However, we showed that strengthening exercises can decrease the relative stiffness between muscle and tendon, by increasing tendon stiffness. Thus, when performed before the application of stretching exercises, the stretch seen by the muscle was increased and we established that a remodelling of muscle structure in children with CP is possible, a notion that has been questioned previously.

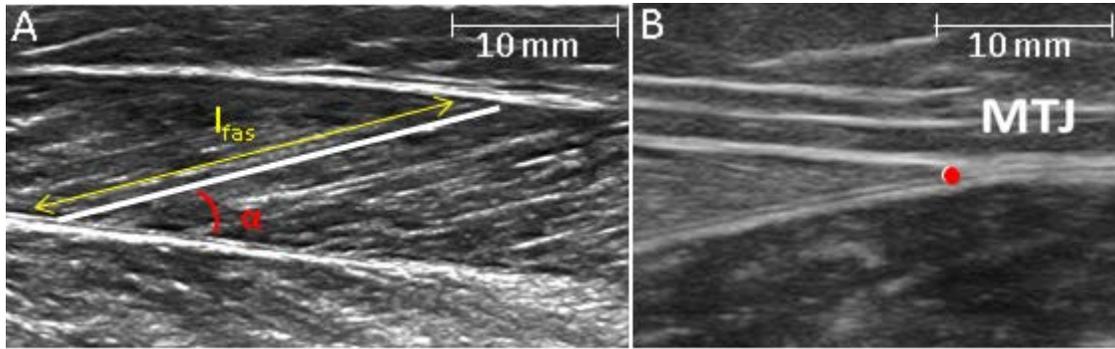
# APPENDICES

## Appendix A: Analysis of ultrasound images

For the last 20 years B-mode ultrasound has become more popular to visualise human skeletal muscles in various applications including, anatomy, engineering and human movement science. Knowledge about the length of the muscle fascicles is of great importance if we aim to understand muscle function. Fascicles, or bundles of muscle fibres, can be visualised with ultrasound and measured post processing. Similarly, the position of the muscle-tendon-junction (MTJ) can be visualised and identified in an ultrasound image. We can then study the change in muscle and fascicle length during dynamic tasks such as an isometric contraction, muscle stretch or more dynamic tasks such as walking. Typically, the digitisation process of the feature of interest is done manually, which makes it an extremely time-consuming procedure and prone to human error. Recently, different algorithms have been developed that partly or completely automate this digitisation process. In this section, the most significant algorithms will be evaluated and discussed with reference to their suitability for use in this research. The two features that we focus on are fascicle length and MTJ displacement.

### ***Fascicle length***

Fascicle analysis can be performed by placing a minimum of two points per image that represent the proximal and distal fascicle ends (figure A.1 A). Curvature of the fascicle can be taken into account by identifying more points along the line of the fascicle. However, It has been shown that when not contracted the influence of curvature of the fascicles on fascicle length measures is minimal (Muramatsu *et al.*, 2002). Hence, in this thesis fascicles are always measured as straight lines and we acknowledge that we therefore slightly underestimate fascicle length in all our measurements. Pennation angle is measured as the angle between the fascicle and the deep aponeurosis. Manual tracking of muscle fascicles is very time consuming and prone to human error. Therefore, several automated or semi-automated tracking methods for muscle fascicles have been introduced. Since this field of work is still relatively new, there is no real gold standard method. Different algorithms might work better for different muscles and/or tasks.



**Figure A.1:** Definition of fascicle length ( $l_{fas}$ ), pennation angle ( $\alpha$ ) and the myotendinous junction (MTJ)

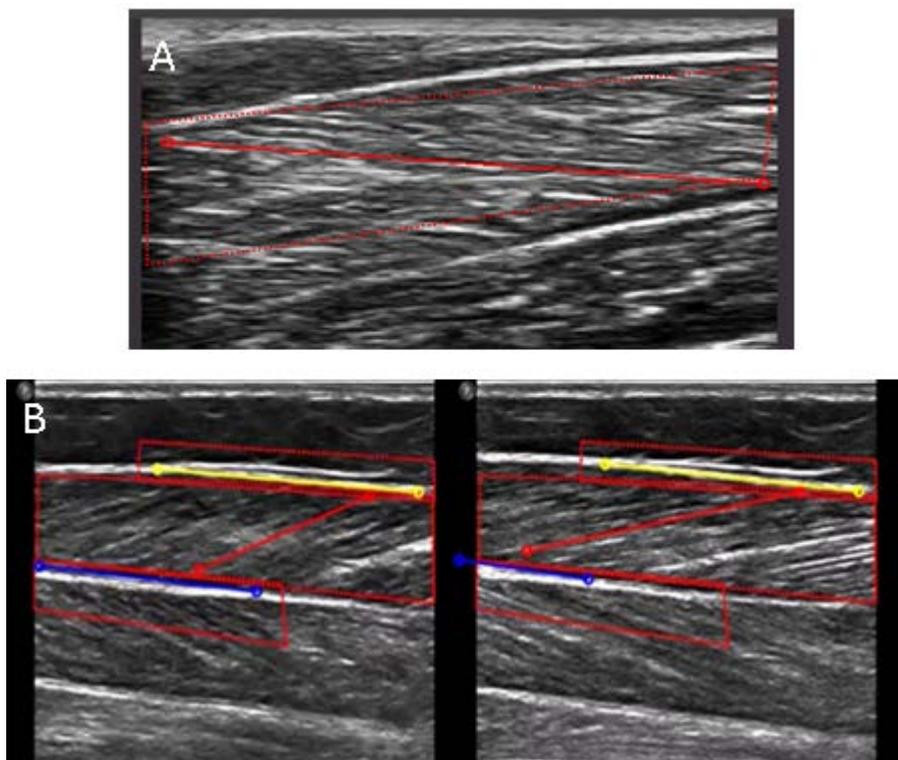
One type of automated tracking of muscle fascicles is based on the Lucas-Kanade optical flow algorithm (Lucas & Kanade, 1981). When applied to ultrasound images, it is possible to use this algorithm to determine the global movement of the visible muscle from frame to frame (Cronin *et al.*, 2011). More specifically, the optical flow algorithm computes the pixel velocities between each pair of successive frames and these are used to calculate the displacement of a specific feature. In the first frame of a sequence of ultrasound images a region of interest (ROI) is selected as the area between the superficial and deep aponeurosis and the start and end point of a fascicle are identified. The model has 6 parameters:  $v_{tx}$ : optic flow at origin of the image in x direction,  $v_{ty}$ : optic flow at origin in y direction,  $d$ : rate of dilation,  $r$ : rate of rotation,  $s_1$ : shear along the main image axis,  $s_2$ : shear along the diagonal axis. These affine flow parameters are estimated within the whole ROI based on a least square fit. These parameters are then used to calculate a flow vector at points  $(x,y)$  in the image according to the following equation:

$$(v_x, v_y) = [x \ y \ 1] \times \begin{bmatrix} d + s_1 & s_2 + r \\ s_2 - r & d - s_1 \\ v_{xt} & v_{yt} \end{bmatrix}$$

The change in position of the  $x,y$  coordinates of the endpoints of the fascicles as identified in the first image can then be calculated.

The advantage of this method is that it takes into account global movements in a large area. An important assumption however with this method is that the point in the muscle that is tracked moves according the prescribed algorithm of translation, rotation, dilation, shear and skew. With other words, we assume that there are no local shape changes within the muscle. This method has been validated in ultrasound videos of the medial gastrocnemius during different dynamic tasks (Gillett *et al.*, 2013), and a user-friendly, open-source, software application (UltraTrack) was also introduced (Farris & Lichtwark, 2016).

In this thesis however, we study fascicle excursion from maximal plantar to maximal dorsiflexion. Therefore, large changes in length need to be captured by the algorithm. We found that the proposed algorithm did not accurately track the end points of the fascicles at these extreme lengths. The rate of rotation ( $r$ ), was still tracked very accurately, but the rate of dilation ( $d$ ) was tracked with less accuracy (figure A.2). For this reason, we propose an adaptation to the proposed algorithm (Cronin *et al.*, 2011; Gillett *et al.*, 2013). We propose to track the orientation of the muscle fascicles as well as the orientation of both the upper and the lower aponeuroses. The length of the fascicles is then calculated from the extrapolated intersection points of the fascicle with the lower and upper aponeurosis. This methodology is used throughout chapter 3-5 in this thesis

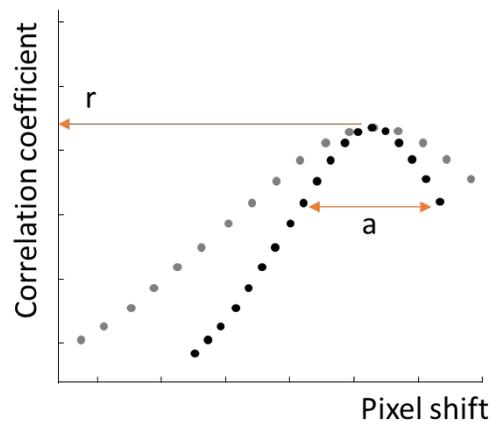


**Figure A.2:** Example of automated tracking. A) error associated with the original version of the fascicle tracking software. Underestimation of fascicle length at extreme lengths. B) The fascicle and the aponeuroses are tracked with their own region of interest. Fascicle length is defined between the intersection points.

### **Muscle tendon junction**

The MTJ is usually defined as a single point at the most distal point of the muscle where the upper and the lower aponeurosis join each other (figure A.1 B). During dynamic movements, this point can be tracked in

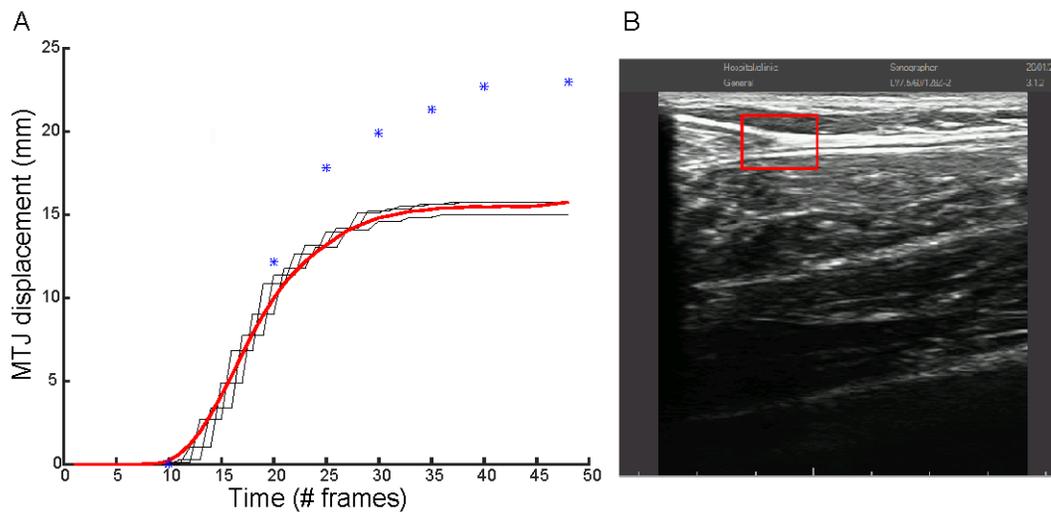
subsequent images, assuming that the probe does not move relative to the skin. For the MTJ, automated methods are also proposed in literature (Magnusson *et al.*, 2003; Korstanje *et al.*, 2010; Pearson *et al.*, 2013), but without a validation analysis in a pathological population and without providing a software application for replication the reported performances. A template matching approach has been applied to MTJ tracking (Pearson *et al.*, 2013). This approach is based on finding the relevant pixel template between subsequent images. A normalised cross correlation function is typically used to compare these templates between subsequent images. First, a region of interest (ROI) is selected in the first image. In the subsequent image, this ROI will be shifted by one pixel at a time. For each pixel shift the correlation coefficient is calculated. The pixel shift that gives us the highest correlations corresponds to the movement between the two frames. The reliability of the pixel shift can be assessed by looking at two different parameters (figure A.3). Firstly, the maximal correlation coefficient tells us something about the quality of the fit. Secondly, the narrowness of the quadratic equation as assessed by 'a' in:  $C = ax^2 + bx + c$ , where the polynomial is an equation that fits through the pixel shift-correlation coefficient relationship (figure A.3).



**Figure A.3:** relationship between pixel shift between frames and the correlation coefficient. Both a higher maximum correlation coefficient ( $r$ ) and a narrower fit ( $a$ ) indicate a better performance of the algorithm.

Automated tracking of the MTJ appears to be much more challenging than tracking of the fascicles, Since the MTJ changes shape significantly during movement. Figure A.4 A shows an example of the manual tracking of the MTJ as well as the automated tracking performed with a cross-correlation algorithm. This analysis was performed in a custom made Matlab script. The tracking algorithm calculated a normalised

cross correlation coefficient of the template (figure A.4 B) with a same-sized region within a search window in the following frame. This was repeated until the end of the movement



**Figure A.4:** **A)** Results of the manual tracking (blue stars) and the automated tracking (red line) of the MTJ. **B)** Example of the area that was selected as a template in the ultrasound image.

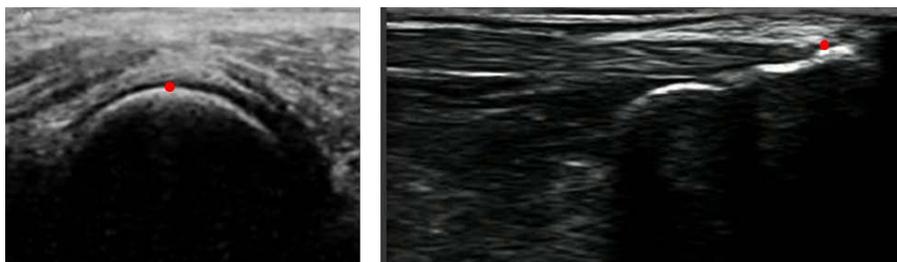
Because of the large discrepancies that were observed in all attempts, it was decided that within the scope of this thesis automated tracking of the MTJ was not feasible. Since manual tracking of the MTJ is much less time consuming than manual tracking of the fascicles, it was decided to perform the tracking of MTJ displacement manually.

Recent developments have shown promising results with regard to applying the Lucas-Kanade Optical flow algorithm to MTJ tracking during passive movements (Cenni *et al.*, 2017). However, there is still a need for open source software to perform MTJ tracking automatically.

In conclusion, to improve the speed and accuracy of feature tracking within a sequence of ultrasound images, automated tracking algorithms have been developed. In this thesis, an adapted version of a previously validated automated tracking algorithm (Gillett *et al.*, 2013) was used to dynamically track fascicle lengths. However, it was decided best to manually track MTJ displacement.

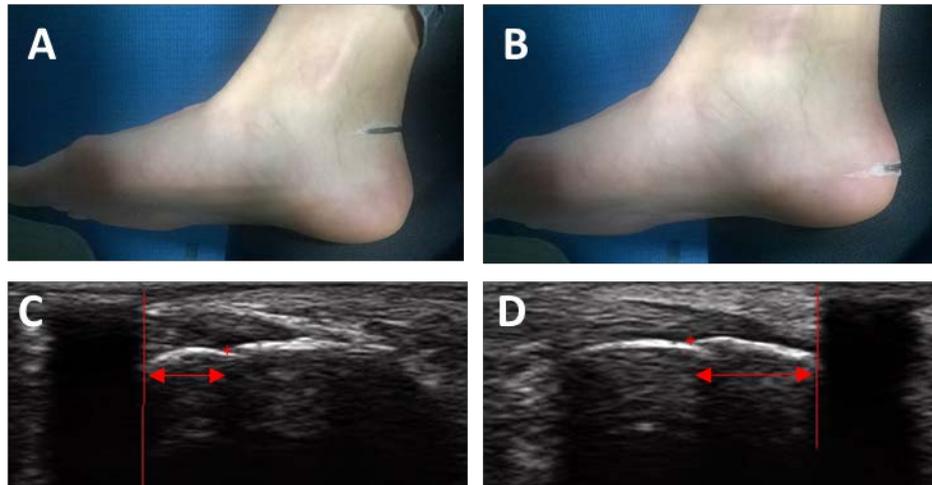
## Appendix B: Calcaneus tracking

Change in muscle-tendon-unit (MTU) length is an important parameter when assessing muscle/tendon properties of the medial gastrocnemius muscle during stretch. The location of the proximal attachment of the MTU is estimated as the most superficial point of the medial femoral condyle (figure B.1 A). Using the ultrasound probe as a spatial pointer (Cenni *et al.*, 2018) this point is identified within a local reference frame of a cluster of markers attached to the shank. In all the experiments performed for this thesis, the knee is fixed during the movement, therefore this point will not move during a stretch and can be identified in a static position. The distal end of the medial gastrocnemius MTU is defined as the attachment of the Achilles tendon on the calcaneus. Statically, this point can be identified with ultrasound (figure B.1 B), but it is not feasible to track this point during the movement as the small surface on the back of a child's calcaneus prohibits stable fixation of the ultrasound probe.



**Figure B.1:** **A)** Identification of the most superficial point of the medial femoral condyle, **B)** Identification of the most distal insertion of the Achilles tendon onto the calcaneus.

As an alternative, in the experiments performed in this thesis a reflective marker was placed on the back of the calcaneus. The position of this marker was tracked relative to the cluster of markers on the shank and the distance of the marker to the virtual marker on the medial femoral condyle was calculated throughout the range of movement. To assess the validity of this approach, the following experiment was performed. An echo reflective tape was placed over the calcaneus (figure B.2). The tape reflects the behaviour of the reflective marker during passive ankle movement. The aim of the experiment was to validate the position of the tape relative to the insertion of the Achilles tendon during movement with ultrasound.



**Figure B.2:** The two marker/tape positions. (A) Proximal placement. (B) Distal placement and the two corresponding ultrasound images with the measured distance visualised (C,D).

## **Method**

Data was collected from five healthy adults (age:  $30.2 \pm 3.3$ -year, 2 Male), free from neuromuscular or skeletal disorders. Participants lay prone on a bed with the foot hanging freely over the edge. A strip of echo reflective tape was placed over the back of the calcaneus. In two separate conditions, the tape was placed either proximal to the insertion of the Achilles tendon on the calcaneus (figure B.2 A), or just distal from the insertion (figure B.2 B). An ultrasound probe (Telemed-Echoblaster, Lithuania) was positioned carefully over the calcaneus and the foot was moved slowly from maximal dorsiflexion to maximal plantarflexion. Care was taken not to move the ultrasound probe relative to the skin.

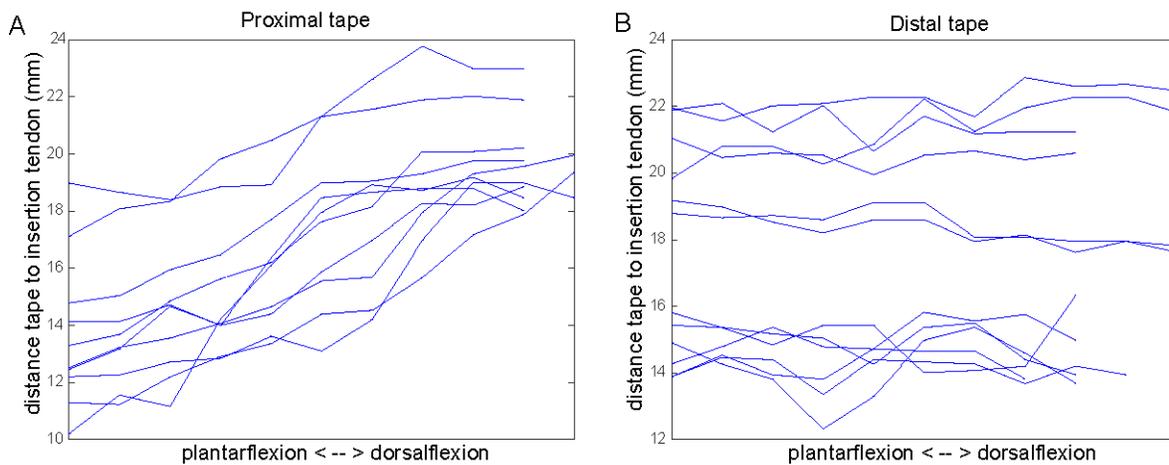
### *Data analysis*

In the sequence of ultrasound images, the perpendicular distance from the echo reflective tape, seen as a black shadow, to the insertion of the Achilles tendon to the calcaneus was measured in ImageJ (NIH, USA), see figure B.2 C and D.

## **Results**

In the proximal placement of the tape, the measured distance between the tape and the insertion of the tendon increased by  $6.1(1.5)$  mm ( $p < 0.001$ ) as the foot was moved from maximal plantar to maximal dorsiflexion (figure B.3 A). When the tape was placed distally on the calcaneus the average distance between the tape and tendon insertion does not change during the movement ( $p = 0.427$ , figure B.3 B). In

the distal condition, the random variation due to manual tracking errors was larger than the overall increase or decrease in the distance between tape and insertion.



**Figure B.3: distance of tendon insertion to marker tape relative to ankle angle. A) proximal placement of tape, B) distal placement of tape**

### **Conclusion**

These results indicate that when assessing MTU lengthening during ankle rotation with a reflective marker attached on the skin, MTU lengthening would be underestimated if the marker is placed proximal on the calcaneus. However, a marker placed on a prominent point distally on the calcaneus is considered a valid representation of the underlying attachment of the Achilles tendon to the calcaneus. In chapters 2 to 4, this method is used to assess medial gastrocnemius MTU and Achilles tendon lengthening.

## **Appendix C: The relationship between medial gastrocnemius lengthening properties and stretch-reflexes in cerebral palsy**

### ***Abstract***

Calf muscle stiffness may be more important than velocity-dependent hyperactive stretch-reflexes in impairing ankle movement in children with cerebral palsy (CP). We explore the interdependence of muscle and tendon lengthening properties with stretch-reflex hyperactivity to help explain subject variability.

Fifteen children with CP (11±3yrs, GMFCS 9I 6II, 9 diplegia, 7 hemiplegia) and 16 typically-developing (TD) children participated. Children lay prone while their ankle was passively rotated over its full range of motion slowly and as fast as possible. Ultrasound, synchronised with motion-analysis, was used to track the movement of the medial gastrocnemius (MG) muscle-tendon junction. The relative lengthening of muscle and tendon were used to infer about the tissues' material properties. Simultaneous measurement of MG surface-electromyography was used to quantify stretch-reflex hyperactivity. The effect of ankle rotation velocity on MG muscle lengthening and stretch-reflex hyperactivity were compared between CP and TD groups. Within the CP group, correlations were sought between relative MG muscle lengthening during slow and fast rotation and the timing and amount of stretch-reflex hyperactivity.

Compared to slow rotation, the muscle lengthened less and stretch-reflex hyperactivity was higher during fast rotation. These velocity-induced changes were more marked in CP compared to TD. In the CP group, MG muscle lengthening velocity 30ms prior to EMG onset was higher in those muscles that showed greater muscle lengthening during slow rotation ( $r=0.57-0.70$ ). Also in these muscles, high stretch-reflex hyperactivity ( $r=0.4$ ) and an early catch ( $r=-0.45$ ) were recorded and the latency between maximum MG muscle lengthening velocity and MG stretch-reflex onset was shortest ( $r=-0.66$ ). In general, muscle lengthening velocity had higher correlations coefficients with stretch-reflex hyperactivity parameters than joint angular velocity.

In conclusion, angular velocity was not representative of MG muscle lengthening velocity. Muscle lengthening velocity was more related to stretch-reflex parameters. Also, stiff muscles may not elongate fast enough to evoke a velocity-dependent stretch-reflex, which may suggest that muscle stiffness is protective against stretch-reflexes.

## ***Introduction***

Cerebral palsy (CP), the most common childhood disability, is an umbrella diagnosis attributed to a lesion in the developing foetal or infant brain (Graham *et al.*, 2016). Depending on the timing, location, type and extent of the lesion, the clinical manifestation is highly variable. A prevalent motor symptom directly attributed to the brain anomaly includes velocity-dependent stretch reflex hyperactivity, often defined as spasticity (Lance, 1980a).

Stretch reflex hyperactivity in the medial gastrocnemius (MG) of children with spastic CP is commonly evaluated by passively rotating the ankle joint into dorsiflexion at different velocities, such as applied in conventional clinical spasticity assessments. However, latest research using surface electromyography (sEMG) from the MG during such examination reveals unexplained heterogeneity in muscle activation between patients (Bar-On *et al.*, 2014b). High variability was noted both in the amplitude of the stretch reflex response as well as the threshold velocity at which muscle activation occurred during passive stretch, the so-called stretch reflex threshold (SRT). The reason why some muscles and some children have a lower SRT velocity is yet to be confirmed. One hypothesis is that the activation threshold is sensitive to muscle properties, such as its ability to lengthen when brought under stretch (Gracies, 2005).

Using dynamic ultrasound (US) imaging to assess muscle lengthening, it has been shown that, in comparison to typically developed muscles, the MG muscle belly in children with spastic CP has a reduced lengthening ability during slow passive ankle rotation (Barber *et al.*, 2012; Kalkman *et al.*, 2016). In addition, during the mid-stance phase of gait, muscle fascicles lengthen more in CP compared to typically developing children (Kalsi *et al.*, 2016; Barber *et al.*, 2017). These findings suggest that, in both passive and active conditions, muscle-tendon interaction during joint rotation is altered. Such a redistribution of the movement between the tendon and muscle fascicles may increase the proportion of fascicle lengthening thus triggering more muscle receptors (i.e. spindles), increasing afferent activity, and consequently increasing the stretch reflex response (Rack *et al.*, 1983).

Additionally, these particular alterations highlight that the muscle and tendon mutually modulate their behaviour when the joint is rotated. Consequently, any assessment at the joint may not represent the properties and behaviour of the muscle and tendon similarly across children. The wide inter-child variability observed in the SRT when expressed as a joint angular velocity (Jobin & Levin, 2000) may therefore be explained by differences in muscle tensile properties that alter muscle behaviour during stretch.

By combining dynamic US imaging with sEMG measurements during fast passive stretch, we can now quantify the SRT in terms of muscle lengthening velocity instead of joint angular velocity. As such, the mechanisms that trigger stretch reflexes at the level of the muscle, where the stretch receptors are located, can be directly investigated to allow better understanding of the variability in SRT sensitivity among different muscles and children. Such an investigation may also help establish the relationship between primary neural symptoms and secondary musculoskeletal alterations. Deciphering the interdependence of impairments in CP can result in a shift of the treatment focus, particularly at an early stage. Furthermore, understanding the sources of variability in clinical symptoms may help to develop personalized treatment (Taft, 1995). This is particularly important since it is debatable whether stretch reflex hyperactivity plays a dominant role in impairing gait (Dietz & Berger, 1983; Dietz & Sinkjaer, 2007; Willerslev-Olsen *et al.*, 2014).

Therefore, the aim of this study was to establish the relationship between MG muscle and tendon lengthening and hyperactive stretch reflexes recorded during slow and fast passive ankle rotations in children with spastic CP. We hypothesized that analysis at the level of the muscle, rather than at the joint, will give a better understanding of the triggers of stretch reflex hyperactivity.

## ***Materials and Methods***

### *Participants*

Children diagnosed with spastic CP and typically developing (TD) children, aged between 6 and 16 years, were recruited for this multicenter study from the gait lab of Alder Hey Children's Hospital in Liverpool and the University Hospital in Leuven. Children with CP were excluded when diagnosed with ataxia, dystonia, severe plantar flexor muscle weakness (manual muscle test <1+ (Hislop & Montgomery, 1995)), bony deformities or contractures resulting in less than 10 degrees of ankle range of motion in the sagittal plane, cognitive problems that could impede the measurements, previous orthopaedic surgery below the knee, an intrathecal baclofen pump, selective dorsal rhizotomy, or botulinum toxin-A injections 6 months prior to the measurement. All TD children were free from neuromuscular or skeletal disorders. The study was approved by the Institutional as well as the NHS research ethics committee in the UK and the University Hospital's ethics committee in Leuven. The study was conducted in accordance with the

Declaration of Helsinki. Written parental consent was obtained from the parents, and written assent was given by children in accordance with local regulations.

### *Measurement protocol*

All measurements were performed by the same trained assessor. In children with CP, assessments were carried out on the most affected calf muscle, defined by the highest clinical spasticity grade (MAS (Bohannon & Smith, 1987) and Modified Tardieu Scale (Tardieu *et al.*, 1954)). In TD children and in children with CP who were equally affected in both legs, the left leg was selected. Subject body mass and height were measured. Children lay prone on an examination table with the examined limb placed in a custom-made orthotic which allowed knee placement at 20 degrees and free sagittal plane movement at the ankle (Figure C.1). The upper leg and pelvis were fixed to the table using straps and the tibia was supported on an inclined cushion with the ankle over the edge of the table. The foot was secured to a rigid footplate with the help of an adjustable insole that ensured heel contact with the footplate during ankle rotation. A six DoF force/torque load-cell (ATI mini45: Industrial Automation) attached to the footplate was used to rotate the ankle joint and measure forces and torques at 200Hz. The point of contact of the load-cell with the footplate could be adjusted according to the foot size.

The moment arms between the lateral malleolus and the point of application of the load-cell were measured using a tape measure.

The angle of the footplate relative to the tibia was recorded using clusters of reflective markers attached to the tibia and footplate tracked by 3D motion analysis cameras (Optitrack NaturalPoint, USA) with a sample rate of 120Hz. Ankle joint calibration was carried out by pointing to anatomical landmarks on the ankle joint, thereby expressing their relative position to the cluster markers (Leardini *et al.*, 2007).

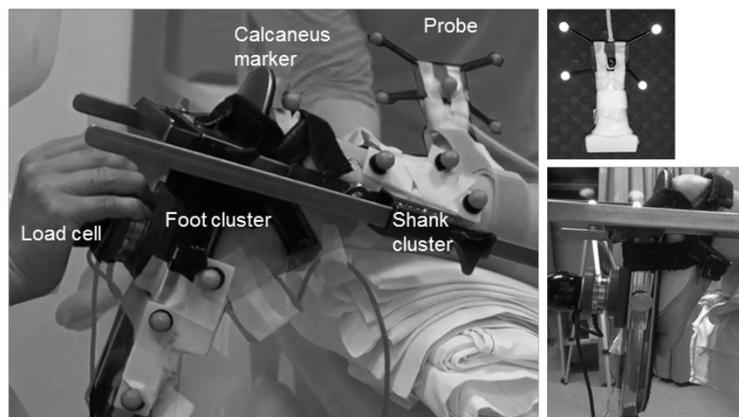
A B-mode ultrasound (US) scanner (Telemed EchoBlaster128, Vilnius, Lithuania) with a 59mm linear transducer was fitted with a cluster of reflective markers (Figure C.1 B) and used to locate and mark the most superficial point of the medial femoral condyle, the medial and lateral borders of the MG muscle and the MG muscle-tendon junction (MTJ). The MG's mid-longitudinal plane was marked from MG origin, through the muscle belly to the MTJ (Bénard *et al.*, 2009).

Surface electromyography (sEMG) electrodes location was determined using US on the muscle belly of the MG with an inter-electrode distance of 2cm (Hermens *et al.*, 2000) (Zerowire, Cometa, Milan, IT). sEMG

was sampled at 2000Hz. Three repetitions of isometric plantarflexion maximal voluntary contractions (MVC) were performed in prone for normalization purposes.

#### Muscle and tendon lengthening during passive motion

A reflective marker was placed over the distal insertion of the Achilles tendon on the most superficial part of the posterior calcaneal tuberosity. Using a custom-made holder, the instrumented US probe was fixed over the MTJ, along the mid-longitudinal plane, and secured to the orthotic to prevent it from moving. Muscle activity, US images, probe orientation, ankle torque and ankle kinematics were simultaneously recorded during passive ankle rotations manually applied across the full range of motion (ROM). Three passive rotations were performed, first at slow velocity (5s to complete full ROM) and then as fast as possible (1s). Between repeated rotations, there was at least a 7s rest interval. US images were collected at 30Hz during slow trials and at 60Hz during fast trials. A close-up video camera was used to retrospectively check heel contact with the footplate during passive rotations.



**Figure C.1 A.** Experimental design of the lower-leg placed in the custom-made orthosis to standardise the knee position and ankle movement; **B.** close-up of the ultrasound probe with reflective markers; **C.** close-up of the foot attached with an insole to the foot plate of the orthotic. A 6 DoF hand held load-cell was used to measure net ankle joint torque during passive rotation. Two clusters of reflective markers on the tibia and footplate were tracked with motion analysis and used to calculate the foot-plate angle in 3D. The ultrasound probe was placed above the muscle tendon junction, and the position and orientation of the image tracked by motion analysis by means of a cluster of reflective markers attached to the probe. Surface electromyography was collected throughout the experiments from the medial gastrocnemius.

## Data reduction

Visual scanning of the quality of the acquired data was performed in custom-made Matlab software. Poor sEMG signal quality was defined as obvious movement artifacts, or a high signal-to-noise ratio. Poor movement performance was defined when the foot was rotated more than 10° outside the sagittal plane or when the heel lost contact with the footplate. Poor imaging was defined when the probe moved 10° outside the movement plane or contact with the skin was lost. Measurements with incomplete data sets were excluded.

## Data analysis

### Muscle lengthening parameters

The position of the MTJ in the collected US images was defined as the most distal insertion of the muscle into the tendon. In a pilot study, this point, defined in a 2D image, was confirmed to be a representation of the middle of the MTJ, when comparing its location to the location identified in a 3D reconstruction (Cenni *et al.*, 2016a). The MTJ was manually tracked in consecutive US images using custom software (Cenni *et al.*, 2016b) in Python (2.7). During each slow rotation, the position of the MTJ was defined every 3 frames (on average in 25 images) and during fast rotations, in every frame (on average 60 images). The reliability of such manual tracking of the MTJ has been found to be satisfactory (Cenni *et al.*, 2016a). Muscle and tendon lengths were defined as the linear distance between the medial femoral condyle and the MTJ, and between the MTJ and the calcaneus marker, respectively. Muscle tendon unit (MTU) length was defined as the summation of muscle and tendon length. At the start of the motion, the joint was in end-range plantarflexion and all lengths were equated to zero. MTU and muscle lengthening from end-range plantarflexion to end-range dorsiflexion were calculated during slow and fast trials, and expressed in mm. Muscle lengthening was additionally expressed as a percentage of MTU lengthening. Maximum muscle lengthening velocity ( $MV_{MAX}$ ) was calculated as the first derivative of muscle lengthening

### Stretch-reflex parameters

Data analysis and parameter calculation was carried out with custom software in Matlab (R2015a). Raw sEMG signals were filtered with a 6<sup>th</sup>-order zero-phase Butterworth bandpass filter from 20 to 500Hz. The root mean square (RMS) envelope of the sEMG signal was defined using a low-pass 30Hz 6<sup>th</sup> order zero-phase Butterworth filter on the squared signal. Joint angle and angular velocity were calculated from the

marker trajectories and ankle calibration. The net ankle joint moment was calculated from the exerted moments and forces on the load-cell, the external moment arms, and the predicted torque caused by gravity on the orthotic (Bar-On *et al.*, 2013b). All kinematic and kinetic variables were filtered using a 2<sup>nd</sup> order Butterworth filter with a 6Hz cut-off frequency.

Ankle flexion-extension ROM and maximum flexion-extension angular velocity ( $\omega_{MAX}$ ) were extracted from slow and fast trials. The hyper-activation (EMG gain) of the stretch reflex with increases in joint position and angular velocity was investigated in two EMG parameters following a previously validated approach (Bar-On *et al.*, 2014b). First, to investigate position-dependent increase in the EMG gain during slow trials, each rotation was divided into three equal zones between 10-90% of the ROM. The zones were defined as the time windows corresponding to: 10-36.6% ROM (P1), 36.6-63.3% ROM (P2), and 63.3-90% ROM (P3). The time windows corresponding to the extremes of the ROM (<10% and >90%) were excluded to avoid moments when the child may not have been relaxed (Bar-On *et al.*, 2013b). Average RMS-EMG per position zone was defined as the area underneath the RMS-EMG curve, divided by the duration of the corresponding position zone. Position-dependent EMG-gain during slow trials ( $EMG_{slow}$ ) was calculated as the change in average RMS-EMG between P1 and P3 (Bar-On *et al.*, 2014b). Second, the time interval in which EMG was on (EMG onset) during trials was automatically defined according to Staude and Wolf (Staude & Wolf, 1999). A manual correction was applied when automatic onset detection failed. To investigate velocity-dependent increase in EMG-gain, during fast trials, average RMS-EMG was calculated during EMG onset ( $EMG_{fast}$ ). In trials with no EMG onset,  $EMG_{fast}$  was calculated in an interval 100ms before to 100ms after  $\Omega_{MAX}$ . All EMG gain values were expressed as a percentage of the peak RMS-EMG during MVC.

When EMG onset was detected, the latency (in milliseconds) between  $\Omega_{MAX}$  and EMG-onset and between  $MV_{MAX}$  and EMG-onset were calculated. Second, the SRT was expressed in terms of the joint angular ( $FE_{SRT}$ ) and muscle lengthening ( $MV_{SRT}$ ) velocities measured 30ms prior to EMG-onset, which represents the length of the short-latency stretch reflex loop as reported in literature (Sinkjær *et al.*, 1999).

Work during slow and fast passive rotations was defined as the average area underneath the torque-angle graph from 10% until 90% ROM (Bar-On *et al.*, 2013b). For the CP group only, during fast rotations, the catch angle, expressed as a percentage of the available ROM was defined according to (Bar-On *et al.*, 2013a). Finally, the MG muscle length corresponding to the catch angle was expressed as a percentage of

the maximum muscle length. More explanation on these parameters can be found in previous literature (Bar-On *et al.*, 2013b, 2013a, 2014b).

### Statistical analysis

Parameters had a non-normal distribution (Shapiro–Wilk test) and were therefore analysed with non-parametric statistics. Comparisons of muscle lengthening during slow stretches and of stretch reflex parameters between CP and TD groups has been previously reported (Bar-On *et al.*, 2013b; Kalkman *et al.*, 2016). Therefore, here we only calculated how parameters changed between slow and fast rotations within each group and compared these change values between groups using Mann-Whitney U tests. Then, within the CP group, Spearman ranks correlation coefficients were calculated between muscle lengthening and stretch reflex parameters at each velocity and, using values averaged over available rotations per subject, between slow and fast velocity. Correlation coefficients were interpreted as poor (<0.2), fair (0.21-0.4), moderate (0.41-0.6), good (0.61-0.8), and very good (>0.8) following the guidelines by Altman, 1999 (Altman, 1999). Significance was set to  $p < 0.05$ .

## Results

Of the 38 children who participated in the study, 15 children with CP and 16 TD children had full data sets and were further analysed (Table C.1). Data were excluded from further analysis in two children due to incorrect synchronization of signals, in one child due to poor US image quality, in one child due to missing a technical cluster during calibration file, and in 3 children due to artefacts in the EMG signal. Patient characteristics (Table C.1) were not significantly different between groups.

**Table C.1:** Subject characteristics in children with cerebral palsy and typically developing children

	CP (n=15)	TD (n=16)
Average (SD) age (years)	11.3 (3.1)	10.29 (3.98)
Male/female (n)	11/4	7/9
Average (SD) Height (cm)	141 (21.3)	138.1 (19.1)
Average (SD) Mass (kg)	36 (18)	35 (15)
Average (SD) tibia length (mm)	339.7 (54.3)	329.4 (52.7)
GMFCS (I-IV) (n)	9 I, 6 II	n/a
Diagnosis (n)	8 Diplegia, 7 Hemiplegia	n/a
Modified Ashworth Score (n=7) and	MAS: 1.5 (n=6); 3 (n=1)	n/a
Average Modified Tardieu (n=8)	Tardieu: 2 (n=5); 3 (n=3)	n/a

GMFCS: gross motor functional classification scale; MAS: modified Ashworth Scale. \*Tardieu scores from children recruited at Alder Hey Children’s Hospital. MAS from children recruited at University Hospital Leuven. One participant unknown.

During fast rotations, EMG onset were automatically detected in 13 subjects of the CP group. In 2 subjects from the CP group and in 2 TD children, automatic detection of EMG onset failed since EMG gain was relatively low and onset was of short duration. In these cases, EMG onset was manually defined.

**Table C.2.** Median (and IQR) of all outcome parameters during slow and fast ankle rotations.

Parameters		CP (n= 15)		TD (n=16)	
		Slow	Fast	Slow	Fast
Joint	ROM (deg)	40.0 (26.6)	51.9 (19.6)	61.4 (20.7)	66.7 (14.7)
	FE <sub>MAX</sub> (deg/s)	12.7 (4.2)	359.5 (178.3)	15.1 (9.8)	464.9 (168.3)
Muscle	Muscle lengthening (mm)	18.3 (9.6)	17.4 (8.8)	26.5 (6.9)	29.2 (11.3)
	Muscle lengthening (%MTU)	48.6 (9.3)	41.3 (19.8)	61.8 (11.9)	54.4 (12.3)
	MV <sub>MAX</sub> (mm/s)	7.6 (2.4)	285.1 (224.8)	9.5 (5.3)	255.8 (106.5)
Stretch-reflex	EMG <sub>slow</sub> (%MVC)	1.3 (2.4)	Na	0.003 (0.2)	Na
	EMG <sub>fast</sub> (%MVC)	0.3 (0.5)	8.1 (9.9)	0.1 (0.1)	0.5 (1.0)
	Work (J)	1.0 (0.4)	4.5 (1.6)	1.0 (0.5)	2.9 (1.7)
	Catch angle (%ROM)	Na	82.24 (13.6)	Na	Na
	Catch muscle length (% max MTU)	Na	71.74 (21.47)	Na	Na
	Latency time FE <sub>MAX</sub> - EMG onset (ms)	Na	-2.5 (72.10)	Na	Na
	Latency time MV <sub>MAX</sub> - EMG onset (ms)	Na	40.00 (54.20)	Na	Na
	FE <sub>SRT</sub> (deg/s)	Na	212.11 (154.61)	Na	Na
MV <sub>SRT</sub> (mm)	Na	134.02 (186.46)	Na	Na	

MV<sub>MAX</sub>, maximum muscle lengthening velocity; TV<sub>MAX</sub>, maximum tendon lengthening velocity; FE<sub>MAX</sub>, maximum angular velocity; MTU, muscle tendon unit length; ROM, range of motion; EMG<sub>slow</sub>, average electromyography during slow rotation; EMG<sub>fast</sub>, average electromyography during fast rotation; MVC, maximum voluntary contraction; FE<sub>SRT</sub> angular velocity at the stretch-reflex threshold; MV<sub>SRT</sub> muscle lengthening velocity at the stretch-reflex threshold Na, not applicable.

Table C.2 shows median (and IQR) values of muscle lengthening and stretch reflex parameters extracted from slow and fast rotations in CP and TD. Maximum angular and tissue lengthening velocities were about 30 times higher during fast rotations than slow rotations in both CP and TD. In CP, this translated to average EMG responses that were, on average, 26 times higher during fast than slow rotations, resulting in higher work values. Higher values of EMG and work in fast versus slow rotations were also found in TD, but the increases in EMG and in work values between slow and fast rotations were significantly lower than those of the CP group ( $p=0.001$ ,  $p=0.021$ , respectively). The MTU lengthened by the same amount in slow and

fast trials, resulting in a similar ROM. During fast trials, in the CP group only, tendon lengthening showed an increased contribution to total MTU lengthening ( $p=0.026$ ).

In the CP group, during slow rotations, fair negative correlation values were found between  $EMG_{slow}$  and absolute (mm) and relative (%MTU) muscle lengthening ( $r=-0.33$ ,  $p=0.054$ ;  $r=-0.35$ ,  $p=0.04$ , respectively) indicating higher amount of position-dependent EMG gain in those muscles that were unable to lengthen (Figure C.2 A). Also during slow rotations, work showed a moderate positive correlation to relative muscle lengthening ( $r=0.43$ ,  $p=0.01$ ), but a moderate negative correlation with ROM ( $r=-0.38$ ,  $p=0.03$ ).

During fast rotations, there was a fair positive correlation of  $EMG_{fast}$  with  $MV_{SRT}$  ( $r=0.40$ ,  $p=0.02$ ), and a moderate negative correlation with catch muscle length ( $r=-0.50$ ,  $p=0.002$ ). There were moderate to good positive correlations between absolute and relative muscle lengthening during slow rotation and  $MV_{SRT}$  and  $MV_{MAX}$  during fast rotations (Figure C.2 B). Muscle lengthening during slow rotation also had a strong negative correlation with catch muscle length during fast rotation (Table C.3).

## ***Discussion***

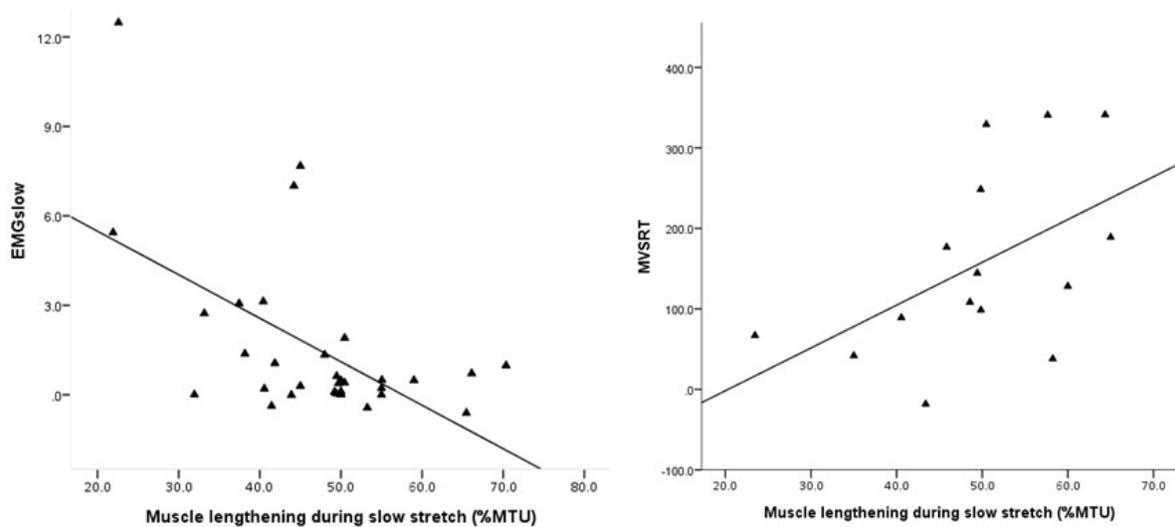
By providing detailed experimental data on the passive lengthening behaviour of muscle and tendon tissue during slow and fast passive ankle rotations, this study innovatively showed that the degree of muscle lengthening and stretch reflex hyperactivity in medial gastrocnemius muscles of children with CP is highly variable and that the two do not necessarily co-exist.

The individual forces exhibited on the tissues cannot be assessed in-vivo and therefore direct quantification of the stiffness of muscle or tendon tissue cannot be defined by means of conventional ultrasound. However, studying the relative lengthening contribution of the muscle and tendon to the lengthening of the muscle tendon unit, allowed us to make inferences about the muscle's relative tensile behaviour during passive ankle rotation. As such, we found that muscles with relatively less muscle lengthening during slow passive ankle rotation showed lower maximum muscle lengthening velocities during fast rotation. On the other hand, muscles with high relative lengthening during slow rotations reached a higher muscle lengthening velocity during fast rotation and were subsequently found to have the largest velocity-dependent stretch reflex responses.

**Table C.3: Spearman rank correlation coefficients between parameters collected during slow and fast rotations in the cerebral palsy group**

		Latency time FE <sub>MAX</sub> and EMG onset (ms)	FE <sub>MAX</sub> (deg/s)	FE <sub>SRT</sub> (deg/s)	Catch angle (%ROM)	Latency time MV <sub>MAX</sub> and EMG onset (ms)	MV <sub>MAX</sub> (mm/s)	MV <sub>SRT</sub> (mm/s)	Catch muscle length (% max muscle length)	EMG <sub>fast</sub>	Muscle lengthening slow rotation (mm)	Muscle lengthening slow rotation (%MTU)
<b>Angular parameters</b>	fast stretch	Latency time FE <sub>MAX</sub> and EMG onset (ms)										
		FE <sub>MAX</sub> (deg/s)	-0.097									
		FE <sub>SRT</sub> (deg/s)	.300	.247								
		Catch angle (%ROM)	.024	.039	.007							
<b>Muscle parameters</b>	fast stretch	Latency time MV <sub>MAX</sub> and EMG onset (ms)	.471**	-0.051	-0.013	.368*						
		MV <sub>MAX</sub> (mm/s)	.226	.313	.558**	-0.136	-0.261					
		MV <sub>SRT</sub> (mm/s)	-0.237	.188	.320	-0.307	-0.660**	.616**				
		Catch muscle length (% max muscle length)	.538**	-0.188	.083	.356*	.632**	-0.047	-0.448**			
		EMG <sub>fast</sub>	-0.199	.239	.235	-0.173	-0.490**	.031	.400*	-0.503**		
<b>Slow stretch</b>	Muscle lengthening slow rotation (mm)	.459	.261	.768**	-0.549	-0.310	.857**	.696**	-0.147	.033		.325
	Muscle lengthening slow rotation (%MTU)	-0.015	-0.025	.254	-0.187	-0.473	.204	.575*	-0.658**	.446	.325	

FE<sub>MAX</sub>, maximum angular velocity; MV<sub>MAX</sub>, maximum muscle lengthening velocity; EMG<sub>fast</sub> average electromyography during fast stretches; FE<sub>SRT</sub> angular velocity at the stretch-reflex threshold; MV<sub>SRT</sub> muscle lengthening velocity at the stretch-reflex threshold. Correlation coefficients (Altman, 1999): <0.2, poor (red); 0.21-0.4, fair (orange); 0.41-0.6, moderate (light green), 0.61-0.8, good (dark green). \*p<0.05 \*\*p<0.01

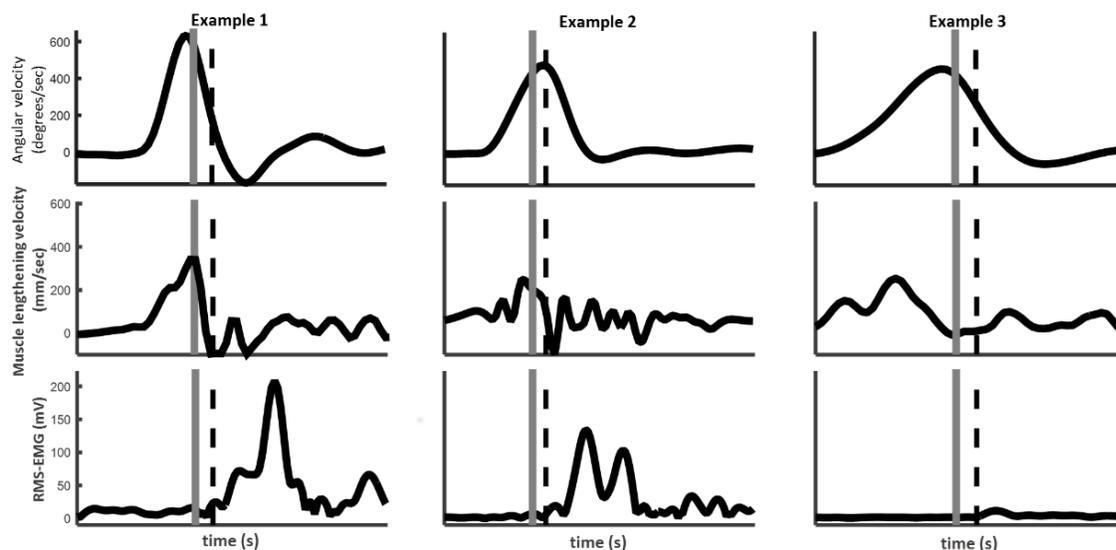


**Figure C.2:** Relative muscle lengthening during slow rotations in children with cerebral palsy versus **A.** normalised rms-EMG during slow rotations and **B.** average  $MV_{SRT}$  during fast rotations. A regression line is shown for significant relationships.

The average EMG onset during fast stretch occurred around  $58 \pm 43$ ms after maximum muscle lengthening velocity. This duration is slightly longer than the latency time of the short latency reflex (30ms), described to be involved in the pathophysiology of hyperactive stretch reflexes (Sinkjaer *et al.*, 1996) and also recorded during gait in the MG of children with CP (Dietz & Berger, 1983). However, after excluding muscles showing an EMG response during fast rotation that was less than 10%MVC ( $n=4$ ), the latency time decreased to  $44 \text{ms} \pm 28 \text{ms}$ . Further exploration showed that when only taking muscles ( $n=4$ ) into account that had muscle lengthening that was greater than the tendon lengthening during slow rotation, the average latency time was  $30 \pm 18$ ms. Therefore, we suggest that a pure velocity-dependent stretch reflex hyperactivity occurs only when the muscle is able to lengthen fast enough. In other muscles, decreased relative muscle lengthening may prevent a large stretch reflex response. Figure C.3 shows signals obtained from three individual muscles and highlights the variability in responses.

Neither  $MV_{SRT}$  nor  $MV_{MAX}$  were in synch with the  $\omega_{SRT}$  or  $\omega_{MAX}$ , indicating that joint angular velocity was not representative of MG muscle lengthening velocity. In addition, while  $\omega_{SRT}$  showed no correlation with parameters of stretch reflex hyperactivity,  $MV_{SRT}$  was predictive of EMG magnitude during fast rotations and an earlier catch. Another indication of the discrepancy between joint angular velocity and muscle lengthening velocity was seen in the latency time between EMG onset and  $MV_{MAX}$  and  $\omega_{MAX}$ . While  $MV_{MAX}$

was closely synchronized with EMG onset,  $\omega_{MAX}$  often occurred after EMG onset. This can be explained by the joint continuing to accelerate in the latency period between the muscle reaching the velocity necessary to elicit a stretch reflex ( $MV_{SRT}$ ) and the muscle force decelerating the joint. Such disparity between angular velocity and muscle lengthening velocity may indicate that the tendon plays a compensatory role in dictating joint behaviour. In other words, in stiffer muscles, the tendon is likely to lengthen relatively more to achieve angular rotation. As a result, the muscle does not reach high lengthening velocities. This has some important clinical implications, as it is important to consider that any assessment carried out at a joint level does not reflect the underlying muscle and tendon interactions consistently across all children. For example, clinical decision making based on an assessment of the passive ROM as traditionally performed in a clinical exam, may result in a misdiagnosis of the underlying muscle length. Similarly, gait analysis data reporting only the joint kinematics, and not the underlying muscle-tendon interactions, may be misleading (Kalsi *et al.*, 2016; Barber *et al.*, 2017). Further, the effectiveness of treatment such as serial casting and stretching that aim to lengthen the muscle by applying stimulus to the joint could be questioned.



**Figure C.3:** Angular velocity, medial gastrocnemius muscle lengthening velocity, and medial gastrocnemius rms-EMG (normalised to maximum voluntary contraction- MVC) during fast passive rotation examples of muscles from three different subjects in the CP group. Timing of EMG onset (black dashed line) and the stretch-reflex threshold, 30ms prior to EMG onset (grey vertical line), are indicated.

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During fast rotations, there was a large variability in the magnitude as well as in the timing of muscle activation response, even when the latter was expressed in terms of muscle lengthening instead of angular velocity. Muscles that were found to lengthen less during slow ankle rotation, either showed very little muscle activation response during fast rotation, or, as in the majority of muscles, showed responses that occurred at latencies of more than 30ms after the maximum lengthening velocity was reached, indicating a response that was not purely velocity-dependent. The relationship between stretch reflex hyperactivity and reduced muscle lengthening is not clear, and the common belief that spasticity, as defined by Lance (Lance, 1980a), contributes to the development of stiffer muscle (Morrell *et al.*, 2002) could not be corroborated in this study. Therefore, early aggressive treatment of stretch reflex hyperactivity which has become usual care to delay and reduce the need for orthopaedic surgery (Molenaers *et al.*, 2006), may not be considered beneficial in all cases.

Interestingly, low muscle lengthening during slow rotation was associated with high levels of position-dependent activation ( $EMG_{slow}$ ). Previous literature has also indicated the presence of length-, rather than velocity-dependent muscle activation during slow passive rotation in subjects with neuromuscular disorders (Thilman *et al.*, 1991; Pandyan *et al.*, 2006; Lebedowska & Fisk, 2009; Noort van den *et al.*, 2010; Bar-On *et al.*, 2014b). Some authors have used the term *spastic dystonia* to describe a muscle that is over responsive to the degree of a tonic stretch, rather than to its velocity (Gracies, 2005). It has also been suggested that increased muscle stiffness, as observed in case of contractures, increases the spindle stimulation from, and its response to, a given amount of lengthening force (Gracies, 2005). This would imply that stiffer muscles show a greater activation response to muscle lengthening. In their research, Dietz and Berger refer to this phenomenon as a 'pseudo stretch reflex', suggesting that its development allows for better gait stability in children with CP (Dietz & Berger, 1983). Our finding that lower  $MV_{SRT}$  values are associated with muscles that lengthened relatively less than their tendon, supports this hypothesis. However, we also established the presence of muscles with reduced lengthening that showed

very limited muscle response to slow or fast rotation. In such cases, muscle stiffness may actually be considered as a protective mechanism against spasticity. This may question treatments that aim to reduce stiffness in the calf muscles. Does reducing muscle stiffness encourage increased muscle lengthening velocity during angular rotation, and consequently increase the stretch reflex response? In general, given the large variability in the amount of muscle lengthening and hyperactive stretch reflex in the subject sample included in this study, it is clear that quantification of the amount of stretch reflex hyperactivity and lengthening per individual muscle, is of paramount importance to improve our insight into the underlying mechanisms of increased resistance against stretch and may eventually lead to improved patient-specific treatment.

Some limitations of the current study need to be mentioned. Firstly, other plantarflexors that insert into the Achilles tendon influence the force in the tendon and the movement of the MG MTJ. However, in CP, we expect the MG to be the most impaired in terms of stretch reflex activation and muscle shortening (Gage *et al.*, 2009). Secondly, we used a 2D imaging technique to visualize and track the movement of 3D structures. While this is an inherent limitation of dynamic US imaging, our results of limited muscle belly lengthening in children with CP are in agreement with previous literature (Matthiasdottir *et al.*, 2014). Furthermore, data from a study in which 3D US imaging at different static ankle angles in children with CP was used to validate whether 2D tracking of the MTJ over the range of motion results in an acceptable description of its movement, indicated that this approach is valid for the purpose of the study (Cenni *et al.*, 2016a). A third limitation is the sampling frequency of the US imaging during fast rotations. In the current study, during fast rotation acquisitions we sampled at 60 fps which means that tissue lengthening velocities at 30ms prior to EMG onset were defined in only 2 frames. This may also explain why some latencies were longer than expected. Fourthly, this investigation was carried out during passive joint rotation. The assumption that stretch reflexes as assessed during passive rotation occur when the muscle is activated voluntarily or in an upright posture cannot be made. Although discerning the occurrence of stretch reflexes during active muscle lengthening is challenging, future research should attempt to investigate the impact of stiffness and stretch reflexes on performance of functional activities. Fifth, it is also possible that muscles that were found to be less stiff were stretched at higher velocities by the examiner thus eliciting a stretch reflex response. Passive rotation imposed by a robotic device would have allowed for more controlled ankle angle manipulation. However, the velocity profile achieved with a manual rotation better mimics the rotation pattern of the ankle during gait (Sloot *et al.*, 2016) and is found to more often elicit stretch reflexes (Ada *et al.*, 1998). Finally, due to foot deformations in the CP group,

we cannot exclude the possibility that the axis of the orthotic was always perfectly aligned with the ankle axis. However, since a similar method was used to secure the foot to the orthosis in all measured subjects, we do not expect this to have influenced our results.

## ***Conclusions***

We established different patterns of tensile muscle behaviour in the tested children with CP sample: 1. muscles with high muscle lengthening allowing high muscle lengthening velocities that elicit a stretch reflex; 2. muscles with little lengthening, preventing high muscle lengthening velocities, that exhibit length-dependent muscle activation at low stretch velocities; and 3. muscles with little lengthening and little to no reflex activation at either slow or fast rotation. Given this and the large variability between children with CP, treatments directed at the medial gastrocnemius that aim to decrease ankle joint hyper-resistance should ideally be based on quantification of the amount of stretch reflex hyperactivity and stiffness at the isolated muscle-tendon tissue level, rather than gross measurement of passive resistance at whole joint level.

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