AN EXAMINATION OF MUSCLE AND TENDON PROPERTIES IN CHILDREN WITH SPASTIC CERBERAL PALSY AND THEIR RESPONSE TO STRETCH: A THEORETICAL BASIS FOR EVIDENCE-BASED CLINICAL PRACTICE

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"Nothing worthwhile has ever really been achieved all that easily. But it certainly has been worthwhile, regardless how difficult it first seemed"

(Robert Fanney)

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THESIS ABSTRACT

Cerebral palsy (CP) is a heterogeneous disorder in which movement and posture are affected. Increased excitation of the central nervous system leads to neural symptoms, which can cause spasticity and muscle weakness. These neural abnormalities result in secondary CP-related mechanical adaptations of muscles and tendons, which can lead to muscle contracture, joint deformities and pain. Therapeutic interventions are therefore essential to treat CP-induced abnormalities. Passive stretching in particular is a popular treatment method in clinical practice. However, due to a lack of scientific evidence, clinicians often have to make assumptions about the mechanical adaptability of muscles and tendons. Currently, the mechanical properties of muscles and tendons in children with CP and their adaptability are not well understood, which makes it difficult to implement evidence-based practice in clinical settings. Therefore, the overall purpose of this research was to examine the mechanical properties of the medial gastrocnemius muscle and Achilles tendon in children with spastic CP, and the adaptations of the muscle and tendon to acute and long-term passive stretching.

The first experimental Chapter (3) was carried out in healthy adults, to assess the agreement between two methods of deriving Achilles tendon stiffness (i) active contraction of the *triceps surae* muscles to elongate the Achilles tendon, or (ii) passive rotation of the ankle joint. Taking into consideration the tendon's viscoelastic response, the effects of strain-rate on Achilles tendon stiffness were also described. Results revealed that tendon stiffness measured using the "active method" was 6% greater than the "passive method". There was also a significant increase in Achilles tendon stiffness in response to increased strain-rate. As the more commonly used active method is problematic to be used in children with CP, due to muscle weakness and excessive co-

contraction, the passive method of deriving tendon stiffness was used in subsequent experimental studies. In experimental Chapter 4, differences in the mechanical properties of the Achilles tendon and *triceps surae* muscles between children with CP and their typically developing (TD) peers, were investigated. The results revealed that estimates of *triceps surae* muscle stiffness were significantly greater in children with CP compared to TD children. The results also showed that despite a smaller tendon cross-sectional area in children with CP, Achilles tendon stiffness was not different between groups. In addition, children with CP had a steeper tendon stiffness-strain-rate relationship compared to TD children. These results have significant clinical implications regarding the diagnosis of spasticity using the current clinical methods.

Experimental Chapters 5 and 6 examined the muscle's and tendon's response to stretch. Passive stretching, implemented by a clinician or by the children themselves, is a commonly used intervention for children with CP with the aim of inducing structural alterations in muscles and tendons to improve function. In order for these alterations to take place, elongation of the muscle and fascicles would presumably need to occur with acute stretching. To date, this assumption has not been tested. Thus, the purpose of Chapter 5 was to investigate the medial gastrocnemius and muscle fascicle response to acute stretching, using two commonly used stretch techniques. Results of this study revealed that 100 s of stretching caused a transient increase in tendon (1.0 cm), muscle (0.8 cm) and fascicle lengths (0.6 cm). This effect was independent of stretch technique. These results provide evidence that the muscle and fascicles are capable of elongating in response to stretch in children with spastic CP. They provide a basis for the hypothesis that the spastic muscle may be able to adapt in response to long-term stretching. Thus, the purpose of the final experimental Chapter (6) was to assess the effects of a six week

passive stretching intervention (four days per week, 15 minutes per day) on muscle and tendon properties, and gait parameters in children with CP. Results revealed there was a significant reduction in joint stiffness in the experimental group following six weeks of stretching. This was accompanied by a reduction in muscle stiffness, but with no alterations in Achilles tendon stiffness. Additionally, there were no positive effects of passive stretching on gait parameters. Together, the results of the present series of investigations demonstrates how fundamental knowledge of muscle and tendon mechanics in children with spastic CP, can be implemented to support evidence-based clinical practice.

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LIST OF ABBREVIATIONS

CP	Cerebral palsy
TD	Typically developing
ROM	Range of motion
EMG	Electromyography
$Stiffness_{COM}$	Stiffness measured in a common force region
$Stiffness_{REL}$	Stiffness measured relative to peak force
CRP	Continuous relative phase
PT-stretch	Physiotherapist-stretch
GMFCS	Gross Motor Function Classification system

CHAPTER 1: GENERAL INTRODUCTION

Cerebral palsy is the most common movement disorder in children, with an incidence of 1.5 to 2.5 cases per 1000 live births (Cans, 2000). The exact cause is unclear, but it is thought to occur most commonly during pregnancy or birth (Rosenbaum, 2003). In patients with CP, aspects of normal function are disturbed by damage to the motor cortex and descending tracts, and several aetiologies may occur as a result. The heterogeneity of symptoms is vast, and the degree of the resultant disability can also span a wide spectrum, ranging from mild to severe. The most prevalent form is spastic CP, affecting around 80% of all children diagnosed (Cans, 2000).

During the time course of CP, neurological symptoms are initially dominant, causing inhibitory effects on the central nervous system resulting from the extinction of many spinal reflex responses (Sheean & McGuire, 2009). As interrupted and disused descending nerve cells degenerate, there is an emergence of abnormal and excessive reflex responses, shifting the central nervous system towards a state of increased excitation; suggesting some neuronal plasticity of the central nervous system (Sheean, 2002). In particular, increased excitation of the tonic stretch reflex may cause spasticity, which is defined as "a velocity-dependent increase in muscle tone" (Burke, Gillies & Lance, 1970; Burke, Levine & Zajac, 1971; Lance, 1980, pp. 485). This can lead to excessive co-contraction (Stackhouse, Binder-Macleod & Lee, 2005) and also muscle weakness, which are common neurological symptoms caused by altered neural activation of the muscle (Rose & McGill, 2005) and impaired motor unit recruitment patterns (Macefield, Fuglevand & Bigland-Ritchie, 1996).

CP-related neurological changes can lead to secondary musculoskeletal adaptations. Since the antagonist muscles are often too weak to counteract contraction of the spastic muscle, it remains in a constantly shortened state, preventing it from stretching during daily functional tasks (Smith *et al.*, 2009). A lack of stretch stimulus to the spastic muscle prevents it from adapting in line with bone growth (Hägglund & Wagner, 2011; Rang, Silver & de la Garza, 1986). This can lead to musculoskeletal alterations including a reduced muscle belly length (Wren *et al.*, 2010), accompanied by fewer in-series sarcomeres (Smith, Lee, Ward, Chambers & Lieber, 2011), as well as alterations in the structure and integrity of intra- and extra-muscular connective tissue (Gagliano *et al.*, 2013; Smith *et al.*, 2011). These secondary CP-related musculoskeletal changes can lead to further increases in joint stiffness for children with spastic CP (Alhusani, Crosbie, Shephard, Dean & Scheinberg, 2010; Barber, Barrett & Lichtwark, 2011a).

Currently, treatment interventions for CP mainly focus their attention towards children, as opposed to adults. The reason is that secondary musculoskeletal alterations get progressively worse during the time of maturation because of accelerated bone growth (Miller, 2007, pp. 218). However, bones and musculature do not usually become fully established until adolescence, which provides a window of opportunity in which interventions can influence musculoskeletal adaptations. These interventions are vast, and with no consensus with regards to durations, frequencies and intensities (Pin, Dyke & Chan, 2006; Wiart, Darrah & Kembhavi, 2008). This demonstrates that a significant gap exists between clinical rationale and research evidence. As such, some of the assumptions made in clinical practice with regards to CP are questionable. One such assumption is that the muscle-tendon unit adapts atypically in children with CP compared to TD children (Alhusani *et al.*, 2010; Barber *et al.*, 2011a) It becomes clear

that a fundamental knowledge of the mechanical properties of the muscle and tendon in children with CP is necessary, in order to implement effective and evidence-based clinical practice.

Over recent years, researchers have started to describe the mechanical abnormalities of the muscle in children with spastic CP (Barber *et al.*, 2011a; Barber, Hastings-Ison, Baker, Barrett & Lichtwark, 2011b; Wren *et al.*, 2010). Some of these changes include a reduced muscle belly length (Malaiya *et al.*, 2007; Wren *et al.*, 2010), reduced muscle volume (Barber *et al.*, 2011b; Malaiya *et al.*, 2007) and increased intra- and extra-muscular connective tissue (Booth, Cortina-Borja & Theologis, 2001). Results from these studies have increased our understanding of the spastic muscle dramatically, and provide the basis for several lines of research. These include CP-related abnormalities in the mechanical properties of the tendon and the muscle's mechanical response to clinical interventions such as stretching, which are the focus of this thesis.

A large gap exists in the literature, relating to the tendon's adaptations in response to abnormal mechanical properties of the spastic muscle. The stimuli through which tendon stiffness develops during maturation in TD children, may be different in children with spastic CP due to a lack of mechanical loading (Samson-Fang & Stevenson, 1998). In children with CP, where excessive muscle weakness and reduced force producing capabilities are prevalent, the stiffness of the tendon is likely to be even more central to the production of movement (Fonseca, Holt, Fetters & Saltzman, 2004; Tedroff, Knutson & Soderberg, 2008). Thus, the CP-related changes and mechanical properties of the muscle and tendon should not be considered independent to one another, and this

warrants further investigation of how the tendon, in particular, adapts in children with spastic CP.

The tendon itself has viscoelastic properties (Le Veau 1992, pp. 33-37), which implies that tendon stiffness increases with an increase in the rate at which the tendon is stretched (Pearson, Burgess & Onambele, 2007), although this has not been demonstrated in the Achilles tendon. A velocity-dependent increase in tendon stiffness would raise questions with regards to the clinical test of spasticity. For example, spasticity, defined as a "velocity dependent increase in tone" (Lance, 1980, pp. 485), is currently assessed using the Modified Ashworth Scale (Bohanon & Smith, 1987). Here, an increase in tone in response to repeated joint rotations at different angular velocities is considered to represent spasticity. However, it could be the case that any velocity-dependent increase in stiffness using this method is the result of the tendon's mechanical properties such as viscoelasticity, and not a neural response (spasticity). Such knowledge would have important implications for the current clinical test of spasticity.

Our current lack of understanding with regards to CP-related muscle and tendon abnormalities makes it difficult to determine what the aim of clinical interventions should be. Currently, increased muscle and joint stiffness represent the target of several clinical interventions. Specifically, increased stiffness is considered to impair function for children with CP by restricting joint range of motion (ROM) (Ward & Bandi, 2010, pp. 370). The clinical assumption is that increasing muscle length and decreasing joint stiffness may restore joint ROM and improve function; whilst delaying or preventing muscle contracture. For this purpose, passive stretching has been commonly and

routinely prescribed for children with spastic CP for a number of years and still continues to be advocated, often in conjunction with other interventions (Damiano, 2009; National Institute for Health and Care Excellence, 2012). Passive stretching can be delivered by physiotherapists and/or prescribed as part of a home therapy programme, delivered by parents and care givers. In theory, passive stretching may stimulate an increase in the number of in-series sarcomeres, (Coutinho, Gomas, França, Oishi & Salvini, 2004; Lieber, Steinman, Barash & Chambers, 2004) and/or alter the intra- and extra-muscular connective tissue, thereby reducing passive stiffness within the muscle. However, the clinical assumption that passive stretching can alter muscle mechanics is not obvious. For example, different relative stiffness's of the muscle and tendon could cause different magnitudes of stretch in each component of the muscle-tendon unit for a given force. If the tendon is more compliant than the muscle, any stretch applied acutely to the joint could be taken up solely by the tendon, with no alterations in the length of the muscle or fascicles. If this were the case, the muscle would not be expected to respond to long-term stretching, and this would bring into question the efficacy of passive stretching as an intervention to induce muscle length and muscle stiffness changes.

Despite the lack of understanding with regards to CP-related muscle and tendon abnormalities, there is still widespread use of passive stretching as a long-term intervention in CP (Wiart *et al.*, 2008). The main issue with stretching studies to date are the relatively global outcome measures used (Pin *et al.*, 2006; Wiart *et al.*, 2008). For example, maximal joint ROM and joint stiffness are commonly reported following weeks or months of stretching (Guissard & Duchateau, 2004; McNair & Stanley, 1996; Rosenbaum & Hennig, 1995) but these do not provide information on the constituent

components i.e., muscle and tendon changes. This makes it difficult to assess the effectiveness of current treatment outcomes.

In clinical practice, the assumption made is that long-term stretching can reduce muscle stiffness, which will lead to overall reductions in joint stiffness and improved function. The research on long-term passive stretching is largely inconclusive (Miedaner & Renander, 1987; O'Dwyer, Neilson & Nash, 1994). Evidence from animal studies (Coutinho *et al.*, 2004; Tabary, Tabary, Tardieu, Tardieu & Goldspink, 1972) suggests that stretching may be able to alter muscle length and stiffness, but it is not clear to what extent stretching could alter the properties of the tendon. Crucially, previous research rarely represents frequencies or durations commonly used in clinical practice (Wiart *et al.*, 2008), and clinicians often use much shorter duration stretches than has previously been studied. Thus, the effectiveness of these clinically relevant stretching interventions on the properties of the muscle and tendon must be investigated more thoroughly.

Finally, it is assumed that reducing joint stiffness and increasing joint ROM will lead to improvements in functional movement for children with spastic CP. The clinical assumption is that increased joint stiffness is a direct result of neurological damage to the central nervous system, causing alterations in movement patterns, and leading to movement dysfunction. However, studies of populations, whose ability to perform voluntary movements is impaired (i.e., aging, Stroke, Down's syndrome), suggest the idea that observed movements are different, but not necessarily dysfunctional (Latash & Anson, 1996). This view would suggest that muscle and tendon adaptations in children with spastic CP may be, at least in part, a compensatory mechanism through which

some degree of function is maintained. Thus, the effects of long-term passive stretching on function will also provide important information for clinicians.

The overall purpose of this research was to investigate *triceps surae* muscle and Achilles tendon alterations in children with spastic CP, and the response to passive stretching, with the goal of informing evidence-based clinical practice. For this purpose, four experiments were performed. The first two experimental Chapters (3 and 4) were designed to gain a fundamental knowledge of the relevant muscle and tendon mechanical properties. Specifically, the purpose of the Chapter 3 was to determine the agreement between two methods for measuring tendon stiffness. Commonly, Achilles tendon stiffness is derived by activating the muscle and recording associated changes in tendon elongation and force during this time. Previous observations have demonstrated that this method may be problematic for children with CP due to excessive muscle weakness and co-contraction (Sheean, 2002; Stackhouse *et al.*, 2005; Tedroff *et al.*, 2008). This may also affect the ability of children with CP to standardise the rate at which force is developed. Thus, the agreement between passively rotating the ankle joint to elongate the Achilles tendon in healthy adults, and the commonly used "active method" of deriving Achilles tendon stiffness was investigated. In addition, the strain-rate response of the Achilles tendon to stretch was also described. The purpose of the Chapter 4 was to describe the material and mechanical properties of the *triceps surae* muscles and Achilles tendon in children with spastic CP compared to TD children, using the "passive method" of determining stiffness. Additionally, the strain-rate response of the Achilles tendon in these groups was investigated.

The final two studies Chapters (5 and 6) were designed to apply this fundamental knowledge in clinically relevant contexts. Specifically, their goal was to understand the muscle and tendon response to stretch in CP. Chapter 5 examines the effects of acute stretching on medial gastrocnemius muscle belly and fascicle lengths, with the goal of establishing whether the muscle is capable of receiving a stretch during passive exercises. Following on from the findings of this study, the purpose of the final Chapter (6) was to examine the adaptability of the *triceps surae* muscles and Achilles tendon to long-term repeated stretching over six weeks. The effect of stretching on gait parameters in children with spastic CP was also investigated. Before these experimental Chapters are presented, the literature relevant to the background of the experimental research is critically reviewed. The topics of interest describe the nature of CP and the associated symptoms related to their effect on muscle and tendon properties. Research related to acute and long-term effects of stretching in CP are also reviewed.

CHAPTER 2: CRITICAL REVIEW OF THE LITERATURE

2.1 Control of voluntary movement

Voluntary motor commands that reach the spinal cord, are the result of distributed activity in several areas of the brain. The primary motor cortex (Broadmann area 4), situated in the pre-central gyrus, makes the majority of connections with motor neurons in the spinal cord to produce voluntary movement. Also involved is Broadmann area 6, which can be sub-divided into two main areas; the pre-motor area and supplementary motor area. Besides sending fibres to the spinal cord, these two areas send fibres to the primary motor cortex and thus, are thought to instruct it of what to do. Upper motor neurons from Broadmann areas 4 and 6 give rise to most fibres of the corticospinal tract, which together with the corticobulbar tract; form the pyramidal tract. The intactness of this pathway is considered to be crucial in our ability to perform voluntary movement. From here, the axons of upper motor neurons descend through the internal capsule, the cerebral peduncle, the pons and the medulla, and finally establish synaptic connections in the spinal grey matter (Figure 2.1). Here, upper motor neurons synapse onto lower motor neurons and interneurons in the ventral (anterior) horn. Other efferent fibres from the primary motor cortex reach other cortical and sub-corticol nuclei also involved in movement control. From these nuclei arise the reticulospinal and vestibulospinal tracts, which collectively form the parapyramidal tract. Further modification of the motor commands occurs at the spinal cord level by spinal reflex mechanisms. These may be simple monosynaptic connections involving only one synapse between a peripheral sensory receptor and the central motor neurons, or more complex involving several synapses and interneurons.

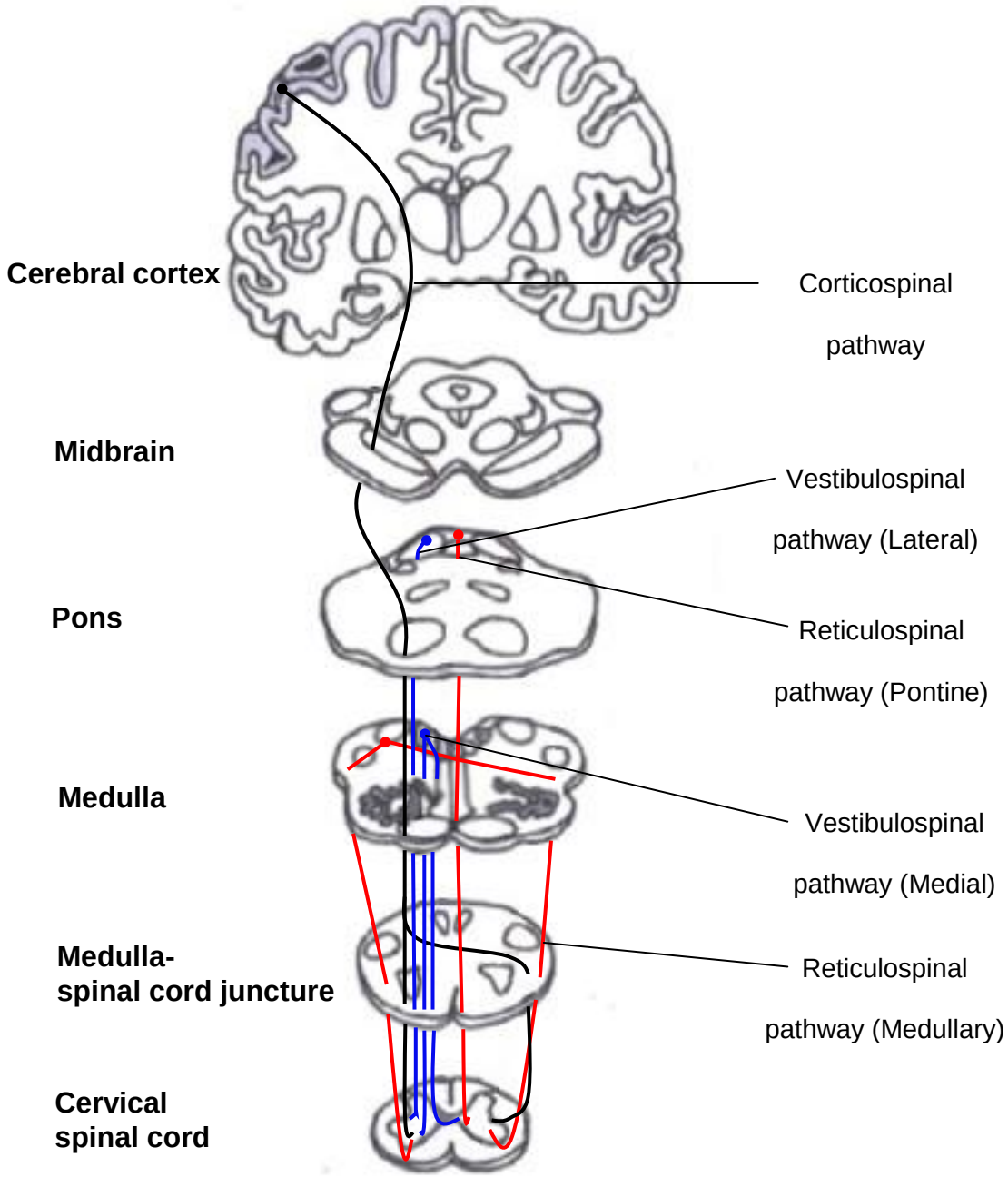

Figure 2.1. Diagram of the cerebral cortex and the main descending tracts involved in the control of voluntary movement.

2.1.1 Stretch reflex

The spinal reflex responsible for the control of muscle length and tone is termed the "stretch reflex", and is important for the regulation and modification of movement.

Many other synapses also exist to control more complex changes in length and tone, for example during joint motion. These synapses are moderated by several pre- and post-synaptic mechanisms. The stretch reflex arc is initially regulated by sensory muscle spindles. These are proprioceptive stretch receptors, which lie in the muscle belly and transmit information regarding muscle length and the rate of change in muscle length. Depending on the velocity of stretch, fast, slow or no phasic response at all can be produced. For example, a muscle stretched at high velocity will evoke a strong phasic stretch reflex response. Here, type Ia sensory fibres, which surround specialised intrafusal muscle fibres (nuclear bag and nuclear chain fibres) within the spindle are excited due to mechanical stimulation. When a muscle is stretched and held at a stretched length, a tonic stretch reflex will also be elicited. Stretch of the spindle receptors in this situation elicits excitations in type II fibres. These fibres initially respond by resisting the stretch due to their passive elastic properties. At a particular length, termed the "tonic reflex threshold", the muscle spindle is activated.

Type Ia sensory afferent fibres enter the spinal cord via the dorsal (posterior) roots, and make monosynaptic connections with alpha motor neurons of the origin muscle. A single alpha motor neuron innervates a varying number of extrafusal muscle fibres, which can cause contraction, and resistance against the stretch. The Ia afferent fibres also connect with inhibitory interneurons, which are under supraspinal influence. These project directly to the alpha motor neurons of both agonist and antagonist muscles, and may cause pre- or post-synaptic inhibition through the release of neurotransmitters: Gamma-aminobutyric acid and Glycine. Consequently, when the agonist muscle is excited, antagonists are inhibited simultaneously; a mechanism called reciprocal inhibition.

The stretch reflex can also be modified via other mechanisms. Gamma motor neurons transmit impulses to intrafusal muscle fibres from supraspinal structures, thereby influencing the responsiveness of the spindle afferents. Specialised interneurons located in the ventral (anterior) horn, Renshaw cells, are excited by recurrent collateral branches of alpha motor neurons before they exit the spinal cord. Renshaw cells inhibit the alpha motor neuron in order to limit and stabilise the discharge frequency (recurrent inhibition).

Finally, Golgi tendon organs located in the muscle-tendon junction, detect changes in tension exerted by the muscle (Ivanhoe & Reistetter, 2004; Sehgal & McGuire, 1998). They supply feedback to the central nervous system via type Ib afferents. Together muscle receptors (spindles and the Golgi tendon organs) can influence the control of movement via the regulation of muscle length and tone. At the same time, interneurons receive a wide range of inputs from several different sources, both peripheral and supraspinal. As a consequence, spinal cord reflex responses depend upon ongoing activity in the surrounding interneurons. Excitation of these interneurons reduces neurotransmitter release, thereby maintaining a tonic inhibitory influence on the reflex arc. Overall, the activation of motor neurons via the reflex arc, leads the muscle to develop an active force, which further opposes the stretch (Figure 2.2).

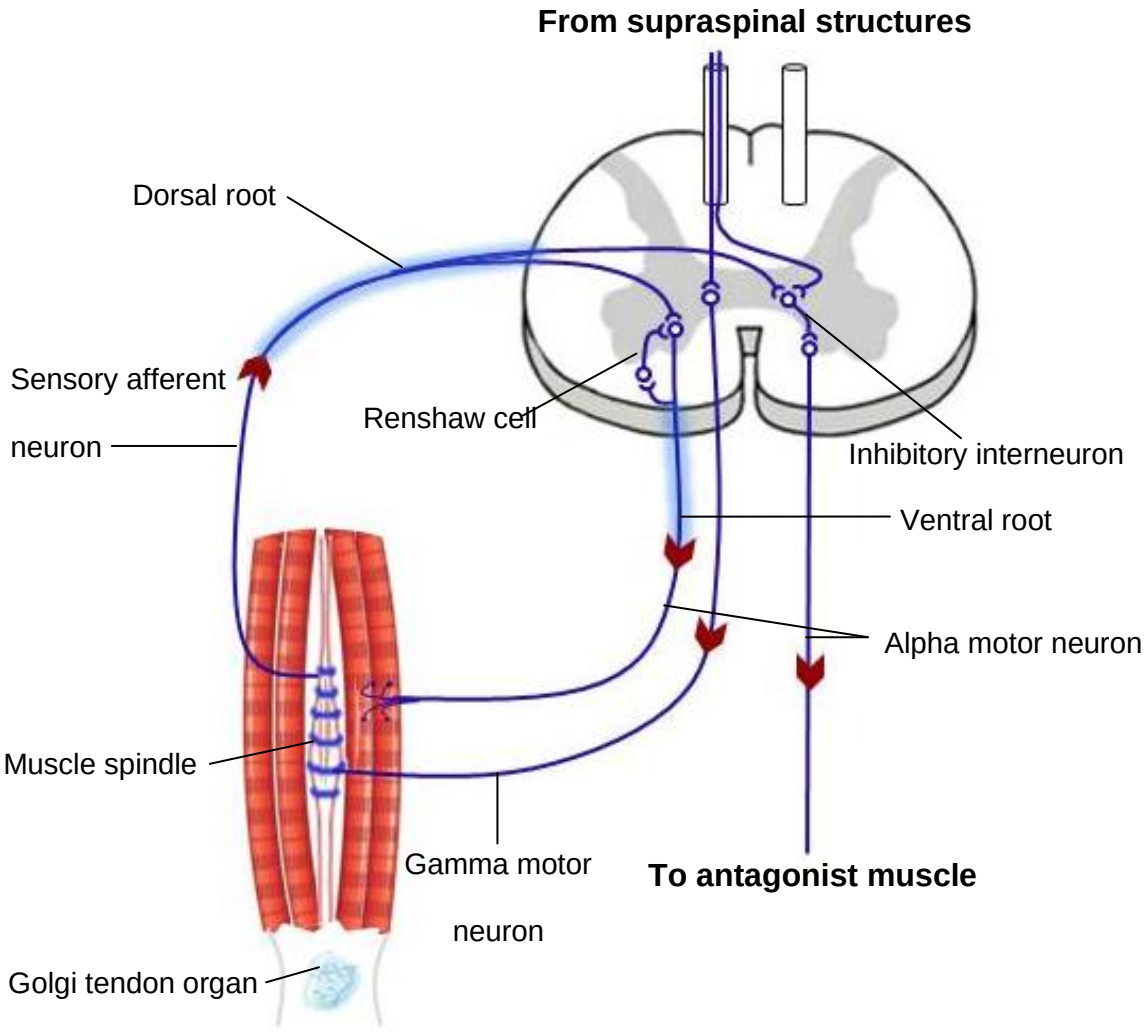

Figure 2.2. Polysynaptic spinal reflex.

In patients with CP, aspects of this normal pattern of cortical and spinal control are disturbed by damage to the motor cortex and descending tracts, along with other areas of the cerebrum (sensory, memory, learning, language). Some of the primary impairments include abnormal muscle tone, excessive co-contraction, sensory deficits, spasticity and muscle weakness. As a result of these impairments, children with CP have one of the most sedentary lifestyles across paediatric disability (Longmuir, 2000). This cycle of inactivity may become progressively worse leading to the development of

secondary impairments in the musculoskeletal system, and a further loss of function (Bottos, Feliciangeli, Sciuto, Gericke & Vianello, 2001; Damiano, 2006).

2.1.2 Classifications of CP

The exact symptoms and the extent to which these affect movement are dependent on the type of CP (Figure 2.3). Three main types exist: ataxic, dyskinetic and spastic, with each being characterised by the area of the brain which is damaged. In patients with ataxic CP, impairments of coordination are dominant, often with hypermetric movements in the extremities. Dyskinetic CP can be divided into athetoid, characterised by involuntary movements, or dystonic, where powerful contractions of the agonist and antagonist muscles occur simultaneously. These types of CP are considered "whole body involvements". Spastic CP is the most common affecting approximately 85% of patients (Cans, 2000). It is caused by an interruption of descending input to the spinal cord from the brain, and is characterised predominantly by spasticity and muscle weakness (Damiano, Vaughan & Abel, 1995).

Spastic CP is further classified with regard to localisation. Hemiplegia predominantly affects one side of the body, and as such is defined as a unilateral involvement where damage to the upper motor cortex (affecting the foot and hip) and lower motor cortex (affecting trunk, arm and hand) on one side of the brain will cause a deficit in the contralateral side of the body. Diplegia is a bilateral involvement in which only the lower limbs are affected, or are more affected than the upper limbs. It is caused by damage to the upper motor cortex of the brain. Finally, quadriplegia is defined as a bilateral involvement, in which the upper extremities are equally, or more affected than the lower extremities.

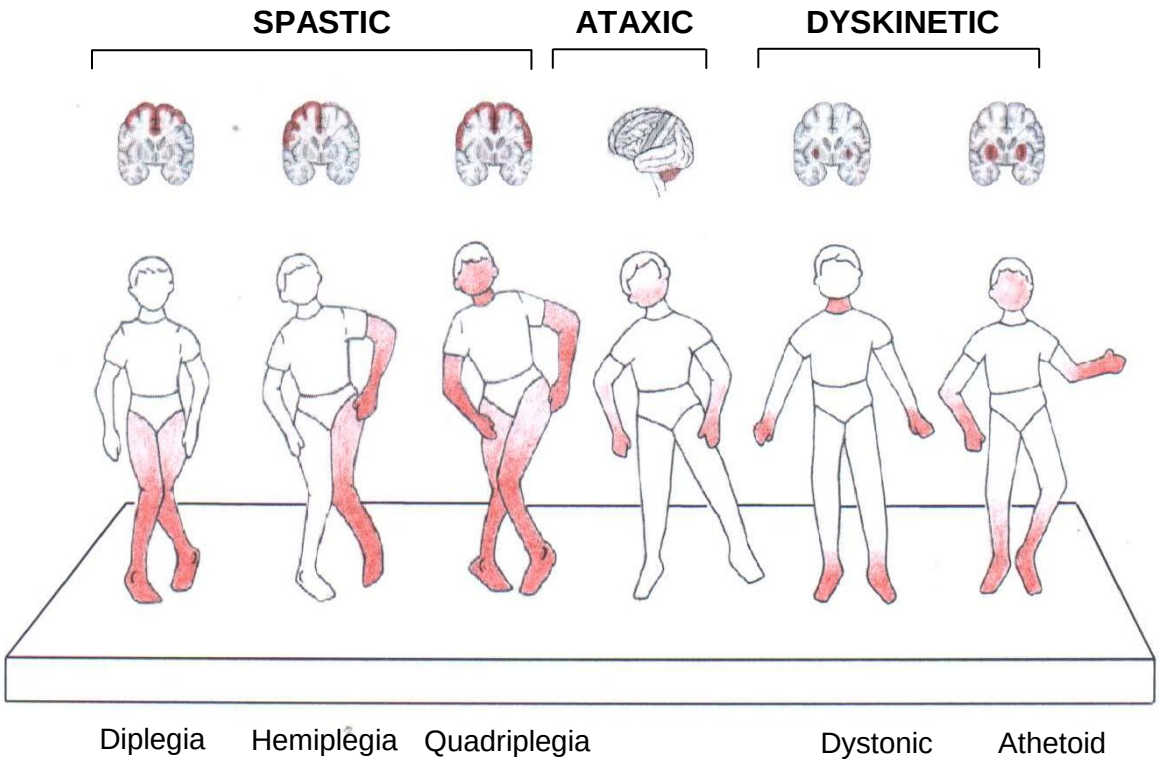

Figure 2.3. Classification of CP. Ataxic and dyskinetic CP have "whole body involvement", whilst spastic CP is classified according to which limbs are affected and is a "partial body involvement".

2.2 Neural symptoms of spastic CP

Neurological symptoms that contribute to a loss of function are the first to develop after a lesion to the brain and upper motor neurons (Sheean & McGuire, 2009). This lesion commonly results in the immediate extinction of many spinal reflex responses. As interrupted and disused descending nerve cells degenerate, extensive sprouting occurs at the level of the spinal cord. The physiological result is the gradual emergence of abnormal and excessive reflex responses. There may also be rearrangement in higher centres, such as new strategies for eliciting movement and an increased reliance on undamaged descending pathways (Gracies, 2001). These mechanisms result in a

complex pattern of atypical movement, muscle weakness and altered spinal reflex activity.

The symptoms of upper motor neuron syndrome are described as either “positive phenomena”, characterised as excess symptoms, which are additional to normal motor behaviour, and “negative phenomena”, characterised by deficits in motor behaviour (Figure 2.4). Spasticity is one positive sign associated with upper motor neuron syndrome, but is often used as a generic term for all features (Sheean, 2002). Upper motor neuron syndrome can occur following any lesion affecting some, or all of the descending motor pathways.

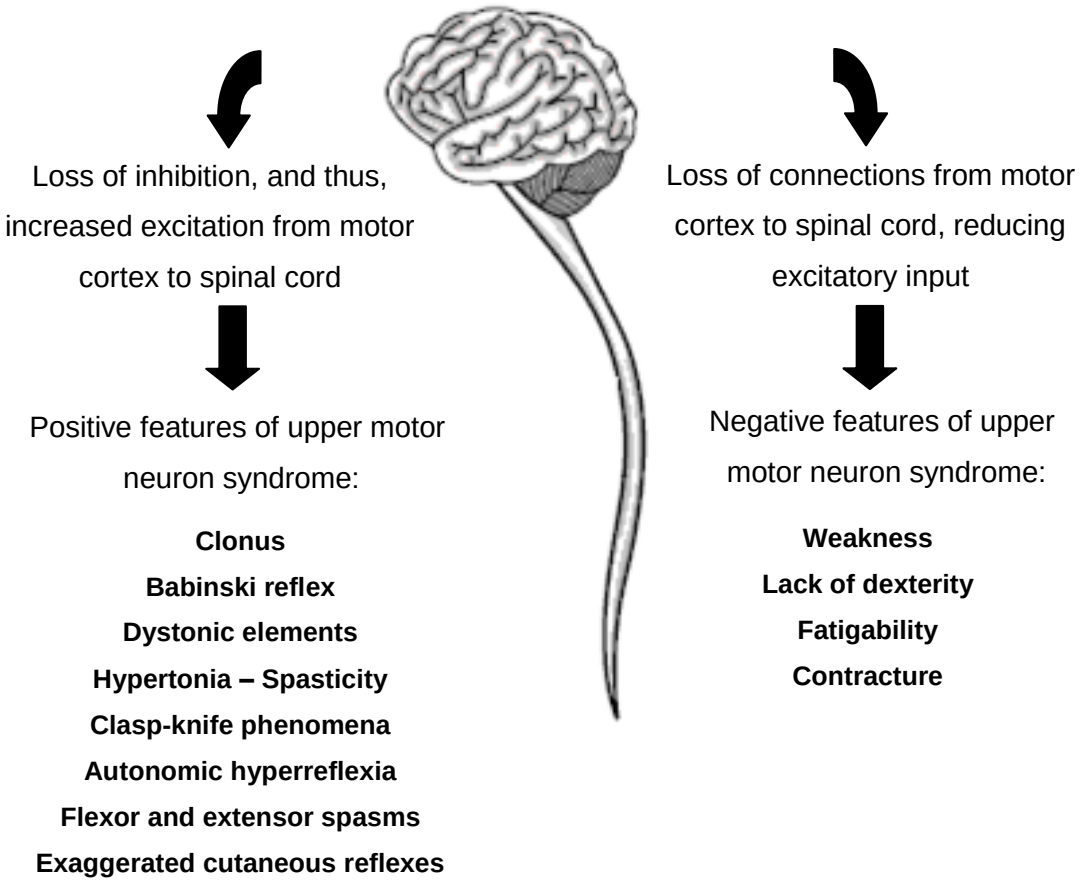

Figure 2.4. Positive and negative signs of upper motor neuron syndrome (Adapted from the work of Jackson, 1958).

2.2.1 Aetiology of positive signs

Upper motor neurons include supraspinal inhibitory and excitatory pyramidal fibres, which descend into the spinal cord and exert a balance of inhibitory and excitatory input to spinal reflexes. Parapyramidal fibres, under cortical control, arise from the pre-motor cortex and facilitate the medullary reticular formation - a powerful inhibitory centre to regulate muscle tone. From this descends the medullary reticulospinal tract. Higher up

in the brainstem, the hypothalamus and sub-hypothalamus are thought to be the origin of excitatory inputs, which descend through the pontine reticulospinal tract. Additionally, the vestibular nucleus gives rise to the vestibulospinal tract, which is also involved in excitation and facilitates spinal reflexes (Sheean, 2002) (Figure 2.1).

Research has shown that damage to just the pyramidal tract produces only minimal neurological deficits (Bucy, Keplinger & Siqueira, 1964). There may be some hand and foot weakness, a mild exaggeration of the deep tendon reflex and a Babinski sign, but spasticity and other forms of muscle overactivity do not occur. Instead, there are also parapyrimidal fibres, which run close to the pyramidal tract that must also be affected for spasticity to be present (Burke, 1988). Specifically, these are thought to be lesions affecting the reticulospinal tracts and the vestibulospinal tract. Damage to these gives a net loss of inhibitory control in the spinal reflexes (Brown, 1994; Burke, 1988; Pandyan *et al.*, 2005). Since these fibres run in different areas of the spinal cord, lesions may affect one fibre tract but not another. It is the variations in severity and the location of lesions, which leads to a variety of clinical syndromes. Consequently, different patients with a lesion in the same area can show vast variations in the clinical pattern of spastic CP.

2.2.2 Positive signs

Abnormal processing of spinal reflexes contributes to a great deal of the positive phenomena seen in upper motor neuron syndrome, including spasticity (Sheean, 2002). These symptoms may be a result of disturbed efferent or afferent drives, which can affect both nociceptive (cutaneous) reflexes and proprioceptive reflexes. The mechanisms underlying these abnormal reflexes are not clear but may result from

inhibitory changes such as Ia pre-synaptic inhibition, Ib non-reciprocal inhibition, impaired recurrent and/or reciprocal inhibition and increased alpha motor neuron excitability (Sheean, 2008, pp. 40-50). The clinical symptoms associated with disturbed efferent and afferent drives are briefly described below.

Efferent drives from the motor neurons during reflex activity are not only dependent upon peripheral afferent feedback, but may be driven by reflex activity higher in the central nervous system. One positive symptom of upper motor neuron syndrome, spastic dystonia, may arise from efferent mediated sources such as a tonic supraspinal drive to the alpha motor neurons, although the underlying cause is unclear. Children with CP may sometimes adopt a posture, not related to voluntary movement or to reflex action, and can be considered to display the symptom of spastic dystonia.

2.2.2.2 Nociceptive reflexes. These reflexes are mediated by non-proprioceptive afferents from the skin, and other tissues, which sub-serve the sensory modalities. These lead to the clinical phenomena of flexor spasms, extensor spasms, and the Babinski sign. Both flexor and extensor spasms occur in the TD brain to withdraw a limb away from a stimulus. In a spastic muscle, these spasms represent a disinhibited and distorted flexor withdrawal reflex, probably due to a loss of supraspinal control. Additionally, the Babinski sign is also best considered a disinhibited flexor withdrawal reflex. It is present in newborn babies and disappears shortly after. However, in upper motor neuron syndrome, the Babinski reflex returns and can inhibit aspects of movement for children with CP.

2.2.2.3 Proprioceptive reflexes. These reflex arcs relay sensory information about movement and position, and are mediated by muscle spindles. A stretch of the spindles

causes a discharge of their sensory afferents, which synapse directly with, and cause excitation, of alpha motor neurons. As previously described (Section 2.1.1 Stretch reflex), this stretch reflex arc may be phasic or tonic. Hyperexcitability of the phasic reflex causes clinical signs including hyperreflexia and clonus. The traditional view is that percussion of the tendon causes a brief muscle stretch, and a synchronous discharge of muscle spindle and Ia afferent activity, which excites alpha motor neurons. These signs may be difficult to extinguish, occurring spontaneously after only minor movements of the limb. In addition, the clasp-knife phenomenon can also be observed in clinical practice as an initial resistance to stretch, which then suddenly diminishes. This also arises because of hyperexcitability of the phasic stretch reflex and also the tonic stretch reflex.

The positive phenomenon of hypertonia describes an increase in muscle tone, but does not distinguish between the neural and mechanical components. Muscles may become stiff, as a result of secondary musculoskeletal changes (discussed in section 2.3), which manifests as an increase in tone in children with spastic CP. Additional increases in tone are thought to be due to hyperexcitability of the tonic stretch reflex, thus, compromising a neural component (spasticity). For some time, the accepted definition of spasticity was that proposed by Lance (1980), "a motor disorder characterised by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyperreflexia of the stretch reflex as one component of the upper motor neurone syndrome" (Lance, 1980, pp. 485). This describes spasticity with two main features; increased resistance to stretch that increases above a threshold, and increased resistance to stretch with increased speed of stretch, due to alterations in the tonic stretch reflex.

Evidence for spasticity seems to favour a decrease in the threshold of the tonic stretch reflex. Specifically, less afferent input is necessary to trigger stretch reflex activity in the presence of an upper motor neuron lesion. This would mean that patients with spasticity can have the muscle triggered by a smaller stimulus than in people with no spasticity. The shift of the stretch reflex threshold has been based on the "equilibrium point hypothesis" (Jobin & Levin, 2000). Briefly, this theory emphasises that in a healthy muscle, central commands from the brain use stretch reflexes to change the muscle length threshold at which motor neurones are recruited. For a fixed descending command, all equilibrium points will form a force-length curve. Typically, a muscle can relax at any angle. This means that the force-length properties of the muscle can be shifted outside the range of anatomical muscle length. A person can also produce high force even when the muscle is at its shortest length. In a spastic muscle, patients lose the ability to shift the threshold over the whole range, perhaps due to a lack of sensory feedback (Latash, 2008, pp. 314). A constricted range of voluntary threshold shifts may be associated with excessive muscle activity, while afferent signals may trigger threshold shifts that lead to uncontrolled spasms.

Also described in the definition of spasticity, is the velocity-dependent aspect, which describes that slow movements would not reveal hypertonia but fast movements would. Thilmann, Fellows and Garms (1991), showed the stretch reflex threshold to occur at 200 $deg \cdot s^{-1}$ in a healthy population. This was an important finding because it indicated that at the lower velocities of movement used to test muscle tone, there is no stretch reflex. The situation was different in spastic muscles where a stretch reflex could be elicited in movements as slow as 35 $deg \cdot s^{-1}$. In this context, spasticity may be considered as a new reflex rather than the disinhibition of an existing one.

A positive correlation between velocity and stretch reflex activity also confirms the velocity-dependent aspect of spasticity (Burke *et al.*, 1970; Burke *et al.*, 1971; Burke & Ashby, 1972; Powers, Campbell & Rymer, 1989). However, this velocity-dependence is not exclusive to spasticity, and there is insufficient evidence to support the theory that the abnormal muscle activity results exclusively from hyperexcitability of the stretch reflex, as other afferents (nociceptive and proprioceptive pathways) may also be implicated. For this reason, the SPASM consortium has recently given spasticity a wider definition, "disordered sensori-motor control resulting from upper motor neuron lesion presenting an intermittent or sustained involuntary activation of muscles" (Pandyan *et al.*, 2005). This incorporates all positive aspects of upper motor neuron syndrome.

Spasticity is the most widely studied of all positive phenomena in upper motor neuron syndrome, because for some time, spasticity was considered the main cause of muscle contracture and atypical movement in a spastic muscle. However, this assumption has been built largely on circumstantial evidence, with no human study supporting it. In a key study by Cosgrove, Corry and Graham (1994), one group of spastic newborn mice were injected with Botulinum toxin-A (to relax the muscle), and a second (control) group with saline. After a two month growth period, the Botulinum Toxin-A group showed no signs of muscle contracture compared to the group injected with saline. The authors concluded that their findings provided evidence that spasticity was the cause of muscle contracture. However, since then it has been demonstrated that in mice, symptoms of tremors and spasms are prevalent, which differs from the clinical picture of spasticity in humans (Gough, Fairhurst & Shortland, 2005). In addition, O'Dwyer, Ada and Neilson (1996) also demonstrated that in a spastic muscle, contracture was

present even in the absence of an abnormal stretch reflex. They argued the abnormal stretch reflex associated with spasticity was insufficient to explain the increased muscle tone (Hufschmidt & Mauritz, 1985; O'Dwyer *et al.*, 1996). Thus, other non-neural factors, such as musculoskeletal alterations, must also play a role.

2.2.3 Negative signs

The neurological mechanisms that contribute to symptoms of spasticity are also thought to play a role in the muscle weakness seen in CP (Cowan, Stilling, Naumann & Colborne, 1998; Leonard, Moritani, Hirschfeld & Forssberg, 1990; O'Sullivan *et al.*, 1998; Sheean, 2002; Stackhouse *et al.*, 2005; Tedroff *et al.*, 2008). A reduced/altered neural activation of the muscle has been demonstrated in children with CP during a maximal voluntary contraction, compared to controls (Rose & McGill, 2005), which may contribute to muscle weakness. This abnormal neural activation is thought to be caused by an incomplete motor unit recruitment pattern (Macefield *et al.*, 1996), a reduced motor neuron firing rate (Harrison & Connolly, 1971) and/or a reduced ability to recruit higher threshold (fast) motor units (Rose & McGill, 1998). This inability to produce high firing rates could also be responsible for structural abnormalities, including a predominance of Type I muscle fibres, and fibre size variability (Rose *et al.*, 1994), further contributing to muscle weakness.

During agonist contraction, the role of inhibitory neurons is to prevent the excitation of alpha motor neurons to the antagonist muscle. Without this mechanism, the contraction of both muscles occurs simultaneously. In TD children, a degree of co-contraction is used within the muscles as a basic motor control strategy for stability and improved motor control accuracy (Tedroff *et al.*, 2008). Children with spastic CP however,

exhibit an inability to control the reciprocal inhibition of agonist and antagonist muscles (Leonard, Hirschfeld & Forssberg, 1991) due to increased excitation, coupled with the reduced function of inhibitory neurons. This often results in excessive co-contraction in children with CP. Stackhouse *et al.* (2005) demonstrated that children with spastic CP had significantly greater co-activation than TD children. This was also demonstrated by both Elder *et al.* (2003) and Ikeda, Abel, Granata and Damiano (1998) who observed significant reductions in agonist torque, as a result of antagonist overactivity. Thus, co-contraction is thought to be the main neurological contributor to muscle weakness and reduced force output in children with spastic CP.

In summary, several positive and negative phenomena occur as a result of a lesion to the brain and upper motor neurons. In the case of positive signs, spinal reflexes are usually tightly regulated. Therefore, if inhibitory control is lost, the balance is shifted in favour of excitation (Sheean, 2002), resulting in hyperexcitability of spinal reflexes. However, if the symptoms of spastic CP were simply a case of imbalance, then spinal reflexes should become hyperactive very quickly after the lesion. However, in most cases there is a delay particularly in humans, which suggests some neuronal plasticity or change in receptor sensitivity. Coupled with reductions in neuromuscular activation, children with spastic CP often suffer a range of debilitating symptoms, which prevent typical musculoskeletal growth.

2.3 Musculoskeletal symptoms

It is generally acknowledged that secondary CP-related alterations in the muscle and tendon, which cause further functional deficits, occur after the development of neural symptoms such as spasticity (Lieber *et al.*, 2004). With the help of animal models and

clinical studies, a number of secondary musculoskeletal alterations to neurological impairment have been proposed, which may contribute to an increase in muscle-tendon unit stiffness and a reduction in muscle strength in children with spastic CP. These secondary changes are considered to be the main cause of muscle contracture (Wilson-Howle, 1999).

In TD children, maturational changes occur in the muscle, in line with changes in the skeletal system. As bone growth occurs, there is a stimulating effect on protein synthesis through regular load bearing. Muscle cross-sectional area increases as the muscle fibre splits lengthways, and there is a synthesis of new myofibrils within the existing muscle fibres (McComas, 1996, pp. 66-67). Muscle length also increases in line with bone growth, through regular stretching of the muscle and fascicles during daily movement. This stretch stimulus is thought to provoke the process of myofibrillogenesis, causing the addition of in-series sarcomeres to the ends of myofibrils.

In children with spastic CP, reduced functional movement will reduce load bearing and muscle stretch. As such, muscles develop atypically in children with spastic CP. For example, neurological weakness of the antagonist muscle prevents it from counteracting hypertonia of the spastic muscle. Thus, it is constantly in a shortened state and is prevented from stretching during daily activity (Smith *et al.*, 2009). As a result, the muscle does not lengthen in line with bone growth. This causes secondary structural changes within the muscle, which may contribute further towards movement inefficiencies in children with spastic CP (Rose, Gamble, Burgos, Medeiros & Haskell, 1990).

2.3.1 Fibre type

One such adaptation is a change in muscle fibre type. Skeletal muscle consists of different fibre types that can be classified into type I (slow oxidative fibres), type IIa (fast oxidative fibres) or type IIb (fast glycolytic fibres) based on their contractile and metabolic properties (Engel, 1998; Schiaffino *et al.*, 1989). These muscle fibres are present in different proportions and are typically regarded as being genetically determined (Bouchard *et al.*, 1986). However, research has shown that the proportion of fibre types is capable of changing depending on the muscle's function (Pette & Staron, 1997).

In children with spastic CP, morphologic changes in contractile and non-contractile elements of the muscle have been described (Fridén & Lieber, 2003). Ito *et al.* (1996) found that muscle obtained from children with CP had almost twice the expected number of type I muscle fibres. In spastic CP, muscle activity is almost continuous and firing frequencies are never high, as a consequence the muscles contract at a much slower rate (Rose & McGill, 1998). This, in turn, produces a compensatory shift to a higher proportion of type I muscle fibres, which can produce only relatively small amounts of tension, but over a long period of time. Smith *et al.* (2011) identified this shift to slower fibre types from a significant increase in type I myosin heavy chain in spastic CP muscles. This may also partially explain the muscle weakness observed in children with spastic CP.

2.3.2 Change in muscle size

Muscle structure consists of repeating sarcomere units, which form myofibrils and are enclosed by a sarcolemma membrane, which receives and conducts electrical signals to

initiate myofibril contraction. Large numbers of myofibrils assemble together to form muscle fibres, and fibres are bound together into fascicles by perimysium to form the muscle. In TD children, it is well established that determinants of muscular strength include muscle size and fibre composition, although the main influencing factor is thought to be the muscle's physiological cross-sectional area (Close, 1972; Ikai & Fukunaga, 1968; Maughan, Watson & Weir, 1983), which defines the number of sarcomeres in parallel. Most muscles have fibres that run at an angle (pennate) to the longitudinal axis of the muscle. Pennation angle allows a greater number of fibres to run in parallel, increasing the muscle's physiological cross-sectional area and allowing the velocity of shortening to be higher (Woittiez, Huijing & Rozendal, 1984). Muscle pennation also allows a greater quantity of contractile tissue to attach to the tendon, and overall, affects the force-generating potential of the muscle.

In the absence of measures of muscle physiological cross-sectional area, previous studies have reported muscle volume to make inferences about the reduced force-producing capabilities of the muscles in children with CP (Fry, Gough & Shortland, 2004; Lampe, Grassl, Mitternacht, Gerdesmeyer & Gradinger, 2006; Malaiya *et al.*, 2007; Mohagheghi *et al.*, 2007). Given that physiological cross-sectional area can be computed from the ratio of muscle volume to fascicle length, and the differences in muscle volume tend to be more pronounced than differences in observed fascicle length (discussed in section 2.3.3), it seems reasonable to suggest that muscle volume is a major determinant of reduced physiological cross-sectional area in CP. Thus, in children with spastic CP, muscle weakness, secondary to a deficiency in motor unit activation, may also be related to reductions in muscle volume. Several studies have revealed that children with CP have muscle bellies up to 50% smaller compared with their TD peers

(Fry *et al*., 2004; Lampe *et al*., 2006; Malaiya *et al.*, 2007; Mohagheghi *et al.*, 2007). This reduction in volume has been attributed to a loss of in-series (Fridén & Lieber, 2003; Fry *et al.*, 2004; Tabary *et al.,* 1972; Tabary, Tardieu, Tardieu & Tabary, 1981; Tardieu, Huet de la Tour, Bret & Tardieu, 1982; Williams & Goldspink, 1973) and/or in-parallel sarcomeres (Shortland, Harris, Gough & Robinson, 2002), which may contribute to muscle weakness and motor dysfunction.

Due to the high correlation between muscle thickness and pennation angle (Ichinose, Kanehisa, Ito, Kawakami & Fukunaga, 1998; Kawakami, Abe & Fukunaga, 1993), it may be expected that the muscle volume reductions in spastic CP would also be accompanied by a reduction in pennation angle. However, this has not been conclusively demonstrated. Zhao *et al.* (2011) found that pennation angle was indeed reduced in patients with CP, due to a reduced muscle volume. However, both Mohagheghi *et al.* (2007) and Shortland *et al.* (2002) observed no change in pennation angle in CP. Mohagheghi *et al.* (2007) suggests that although pennation angle may be smaller due to a reduced muscle volume, other mechanisms may exist, which offset the negative effect of atrophy on pennation angle. In a non-disabled population, muscle fascicles rotate about their insertion points during contraction which increases pennation angle (Maganaris, Baltzopoulos & Sargeant, 1998a; Narici *et al.*, 1996). In CP patients, hypertonia may cause this same fascicle rotation, thus increasing pennation angle. The conflicting results may be explained by different levels of hypertonia in patients. That is, with lower levels of hypertonia the muscle will not undergo the same degree of shortening or fascicle rotation. If patients with CP do indeed have no change in pennation angle, coupled with shorter muscle fibres, this will further affect the force-generating capacity of the muscle (Maganaris, *et al.*, 1998a).

2.3.3 Muscle length

One of the most predominant alterations to disuse or immobilisation is a reduction in muscle belly length (Wren *et al.*, 2010), and alterations in its constituent components. In animal studies, it has been shown that immobilisation of a muscle can lead to atrophy, which is shown to be the result of a reduced number of in-series sarcomeres. This was largely based on the work of and Tabary *et al.* (1981) and Williams and Goldspink (1973), who investigated the effect of immobilisation on rodent and cat muscle. When the muscle was immobilised in a shortened position it adapted by shortening muscles fibres through a significant reduction in the number of in-series sarcomeres. Human models of disuse, such as unloading (De Boer, Maganaris, Seynnes, Rennie & Narici, 2007; Seynnes, Maganaris, De Boer, di Prampero & Narici, 2008), bed rest (De Boer *et al.*, 2008) and even ageing (Narici, Maganaris, Reeves & Capodaglio, 2003), have been shown to result in reduced muscle size, fascicle length and pennation angle, indicating a loss of in-series and in-parallel sarcomeres.

Evidence for shorter fascicles in children with spastic CP is less conclusive (Barber *et al.*, 2011b; Gao, Zhao, Gaebler-Spira & Zhang, 2011; Malaiya *et al.*, 2007; Mohagheghi *et al.*, 2007; Mohagheghi *et al.*, 2008; Shortland *et al.*, 2002). Tardieu *et al.* (1982) observed an increase in the hypoextensibility of the *triceps surae* muscles compared with muscles of TD children. They concluded this reduction in extensibility was the result of an adaptive response through a loss of in-series sarcomeres. More recently, ultrasonography measurements have allowed for more direct estimations of fascicle length. Mohagheghi *et al.* (2007) observed a reduced fascicle length in the medial gastrocnemius in the affected leg of hemiplegic patients, which supported a reduction of in-series sarcomeres. However, using a similar method, Shortland *et al.* (2002) noted

that although muscle thickness was reduced compared to the control group, fascicle length did not differ. This was supported by Malaiya *et al.* (2007) who demonstrated no difference in fascicle lengths in children with CP. Moreau, Teffey and Damiano, (2009) supported both findings by demonstrating a reduced fascicle length in the rectus femoris but not in the vastus lateralis muscle. Thus, the evidence for a reduced fascicle length is inconclusive and may be dependent on the muscle of interest, or the methods of calculating absolute or relative fascicle length (Mohagheghi *et al.*, 2008).

Further research has reported an adaptation of the sarcomere itself (Carey & Burghardt, 1993; Smith, *et al.,* 2011). Lieber and Fridén (2002) used an intraoperative laser technique on the flexor carpi ulnaris muscle and revealed first, that sarcomere lengths were increased in spastic muscles, which may be the reason for an increase in observed passive stiffness. Second, sarcomeres in spastic muscle operate at lengths greater than the optimal sarcomere length, and on a different portion of the length-tension curve, inhibiting force generation (Gordon, Huxley & Julian, 1966). In TD children, the optimal sarcomere length is thought to be around 2.5-2.7 μm (Walker & Schrodt, 1974). It is at this point that there is an optimal overlap between actin and myosin filaments and the maximum number of crossbridges can be formed. With CP patients operating at longer lengths of the passive length-tension curve, the muscle will be experiencing higher levels of stiffness, and less crossbridges will be formed. This difference is likely to become more pronounced at extreme flexion and extension and may limit joint ROM (Smith *et al.,* 2011). It should however be noted that findings of a longer sarcomere length have previously been confounded (Fridén & Lieber, 2003). Fridén and Lieber (2003) actually demonstrated a shorter sarcomere length compared to healthy muscles, and an increase compliance of the fascicle. However, in both studies biopsies were

taken from a range of different muscles in both CP and healthy groups. It has since been demonstrated that different muscles have significantly different mechanical properties (Ward *et al.*, 2009), which may explain the differing results. More recently, Smith *et al.* (2011) showed sarcomeres to be longer in the spastic gracilis and semitendinosus muscles in children with CP, compared with sarcomeres from the same muscle in TD children. They report that fewer in-series sarcomeres cause each of the remaining sarcomeres to be stretched, which could help to explain the excessive passive tension and muscle weakness in a spastic muscle. Smith *et al.* (2011) argued that based on measured human filament lengths (Walker & Schrodt, 1974) and the 0.5 μm increase they reported in CP patients, the decrease in force from a TD child operating on the plateau of the length-tension curve, compared to a CP child operating on the descending limb would be 33%. Therefore, this structural change within the sarcomere itself would lead not only to an increase in muscle stiffness, but could also contribute to muscle weakness.

2.3.4 Changes in non-contractile proteins

Muscles consist of both contractile and connective tissue. This connective tissue primarily consists of the endomysium, perimysium and epimysium, which encapsulates the muscle and has a significant effect on passive stiffness. Each element of connective tissue is composed of both collagen and elastic fibres. The collagen fibres are arranged in bundles, and each molecule contains three polypeptide chains, which form a triple helix. These are overlapped to form a myofibril tubule around which is wound a surface band. In children with CP, there is thought be a substantial remodelling of intra- and extra-muscular connective tissue, contributing to the observed increased muscle stiffness (Smith *et al.*, 2011). This remodelling may occur in line with immobilisation

causing fibro-fatty connective tissue to proliferate and encroach into joint space. If this is long-term, fibrous adhesions may occur, and joints lose connective tissue extensibility (Farmer & James, 2001). Smith *et al.* (2011) demonstrated an increase in passive muscle tension due to changes in the extracellular matrix in children with CP. They found that collagen content was significantly increased compared to TD children. In spinal-cord injured patients, Lamontagne, Malouin and Richards, (1997) suggested a lack of mechanical stress may influence the synthesis of collagen fibres, such that the organisation of fibres may impair the materials tensile strength; thus increasing the extensibility of the extracellular matrix.

Collagen is considered the primary load bearing structure within the muscle's extra-cellular matrix (Peter, 1989) and makes up a large proportion of the tendon's material, a change in the type, or organisation of fibres would be expected to influence passive stiffness. Moreover, Goldspink and Williams (1990) reported that during immobilisation, connective tissue is lost at a much slower rate than contractile tissue which, in turn, results in a change in the stiffness of the muscle. One other hypothesis for increased muscle stiffness is alterations in titin, which is considered to be the major passive load-bearing structure within the muscle fibre (Prado *et al.,* 2005). However, alterations of this type have not been conclusively shown. Smith *et al.* (2011) found no change in the size of titin isoforms in the gracilis or semitendinosus muscle of children with spastic CP. This would indicate that for the human hamstring muscles at least, increased passive stiffness in the muscle is due to a change in extracellular matrix stiffness and perhaps, an increase in the previously described length of sarcomeres. Although traditionally, this change in connective tissue has been considered detrimental to function, research has suggested that children with CP may actually exploit the

increase in connective tissue as a functional spring (Hufschmidt & Mauritz, 1985; Tardieu *et al.*, 1982). This allows them to store and release elastic energy particularly during the early stance phase of walking, in the absence of adequate muscle force (Fonseca *et al.*, 2004).

2.3.5 Muscle stiffness

The CP-related abnormalities in the morphology of the spastic muscle are considered to be the source of increased spastic muscle stiffness. However, despite increased muscle stiffness being the basis for several therapeutic interventions, relatively few studies have quantified the mechanical properties. Those studies which have confirm the clinical assumption that children with CP have increased muscle fascicle (Barber *et al.*, 2011a) and joint stiffness (Alhusani *et al.*, 2010; Barber, *et al.*, 2011a) compared to TD children. For example, Fridén and Lieber (2003) demonstrated that muscle cells of the flexor carpi ulnaris of children with CP were twice as stiff as the muscle cells of patients without neurological impairment. In the upper limbs, Vaz, Mancini, Fonseca, Vieira and De Melo Pertence, (2006) also demonstrated increased resistance against passive movement, in the absence of muscle activity. They suggest this reflects changes predominantly in the mechanical properties of the spastic muscle. More recently, Alhusani *et al.* (2010) demonstrated that the ankle joint of children with spastic CP was significantly stiffer than TD children, reporting a difference in stiffness of 242%. This was supported by Barber *et al.* (2011a), who reported a smaller, but significant 51% group difference in ankle stiffness between CP and TD children. This study also found the muscle fascicles underwent 47% less strain in the spastic CP group compared with the TD group, in the absence of group differences in peak torque. The steeper slope of

the torque-fascicle length curve for the spastic CP group could therefore be suggestive of increased stiffness of the muscle fascicles.

2.3.6 Tendon stiffness

In children with CP, the properties of the tendon are less well reported compared to the spastic muscle. The gross tendon structure is made of up of water and collagen fibres, which form fibrils that run longitudinally. Several parallel fibrils are also embedded together within a tendon's extracellular matrix to form fibres (Benjamin & Ralphs, 1996; Kastelic, Galeski & Baer, 1978; O'Brien, Reeves, Baltzopoulos, Jones & Maganaris, 2010; Vogel, 2003). This gives the tendon both elastic and viscous properties, which are central to its role in movement. The basic role of the tendon is to transmit tensile forces from muscle to bone, storing and releasing elastic energy and thereby reducing the work of the muscle (Lichtwark & Wilson, 2008; Maganaris & Paul, 1999). The tendon also allows muscle fibres to operate on an optimal portion of the force-length curve to maximise force generation.

In children with CP, it has been suggested that the tendon may develop atypically. For example, an abnormally long Achilles tendon(Barber, Barrett & Lichtwark, 2012; Gao *et al.*, 2011) and smaller cross-sectional area (Gao *et al*., 2010) in CP has been reported compared to TD children, but with no alterations in tendon stiffness (Barber *et al.*, 2012). According to Hooke's law (that states the deformation of an elastic body is proportional to its deforming load) longer tendons are more compliant because stiffness decreases as more material is arranged in series. Additionally, tendons with a smaller cross-sectional area are also more compliant because less material is arranged in parallel. Therefore, the results of a longer tendon with no changes in stiffness may

suggest some alterations in the material properties of the tendon in children with CP, although this has not been investigated.

The mechanical properties of both muscle and tendon have important implications for understanding atypical movement in children with spastic CP. A longer, or more compliant tendon compared to the muscle, may explain movement inefficiencies in CP (Rose *et al.*, 1990).The tendon's mechanical properties will govern its function and its interaction with the muscle. Therefore, the development of the tendon in CP compared to TD children warrants further investigation.

2.3.7 Measuring muscle and tendon stiffness

The majority of previous studies investigating changes in muscle and tendon mechanical properties in CP have often used a measure of joint stiffness. From this it is not possible to determine which structure, be it the muscle or the tendon, contributes most to the increased muscle-tendon unit stiffness. More recently, studies in non-disabled populations have attempted to determine the stiffness of the muscle and tendon (Morse, Degens, Seynnes, Maganaris & Jones, 2008). This is relevant given that passive joint stiffness will originate from combined stiffness's of both the muscle and the tendon, and both structures could potentially develop atypically in CP. Therefore, understanding the individual contributions of these components is valuable in assessing or predicting appropriate treatment outcomes.

Several methodologies exist to measure different aspects of stiffness (e.g., series elastic component stiffness, musculo-tendinous or musculo-articular stiffness). In addition to *in vitro* methodologies, there are several *in vivo* techniques that are commonly used to

measure tissue-specific stiffness. Prior to the 1990s, the elastic properties of biological tissues were estimated by means of mechanical testing on excised tissue (Abrahams, 1967; Bennett, Ker, Imery & Alexander, 1986; Butler, Grood, Noyes & Zernicke, 1978; Ker, 1981; Rigby, Hirai, Spikes & Eyring, 1959), or on anaesthetised animals (Morgan, Proske & Warren, 1978; Rack & Westbury, 1984). Methods to characterise the stiffness of the series-elastic component used the quick release method, and other methods have quantified stiffness from a limb-system's response to sinusoidal perturbations (Cannon & Zahalak, 1982; Winters & Stark, 1988).

More recently, advances in imaging technology have allowed accurate, non-invasive methods of assessing muscle and tendon properties *in vivo* (Fukashiro, Rob, Ichinose, Kawakami & Fukunaga, 1995; Henriksson-Larsen, Wretling, Lorentzon & Oberg, 1992; Maganaris & Paul, 1999). For estimating tendon stiffness using ultrasonography, the change in tendon length attributable to an applied muscular load is usually measured by tracking the displacement of the muscle-tendon junction during a voluntary maximal isometric muscle contraction. The corresponding tendon force is then calculated as the ratio between joint moment and a tendon's moment arm (Ito, Akima & Fukunaga, 2000; Maganaris, Baltzopoulos & Sargeant, 2000; Spoor & van Leeuwen, 1992). Tendon stiffness is calculated as the gradient of the slope produced from plotting the relationship between tendon force and muscle-tendon junction displacement (Fukashiro *et al.*, 1995; Maganaris & Paul, 1999) (Figure 2.5). Despite several methodological considerations that must be taken into account when using this method (Arampatzis *et al.*, 2005; Maganaris, 2005; Magnusson, Aagaard, Dyhre-Poulsen & Kjaer, 2001; Reeves, Maganaris & Narici, 2003), ultrasound-based measurements of tendon stiffness *in vivo* has greatly enhanced our understanding of human tendon stiffness and its

adaptation with growth, aging, loading and immobilisation (Kubo, Kanehisa, Ito & Fukunaga, 2001a; Kubo, Kanehisa, Kawakami & Fukanaga, 2001b; Maganaris *et al.*, 2006; O'Brien *et al.*, 2010; Reeves *et al.*, 2003; Seynnes *et al.*, 2008).

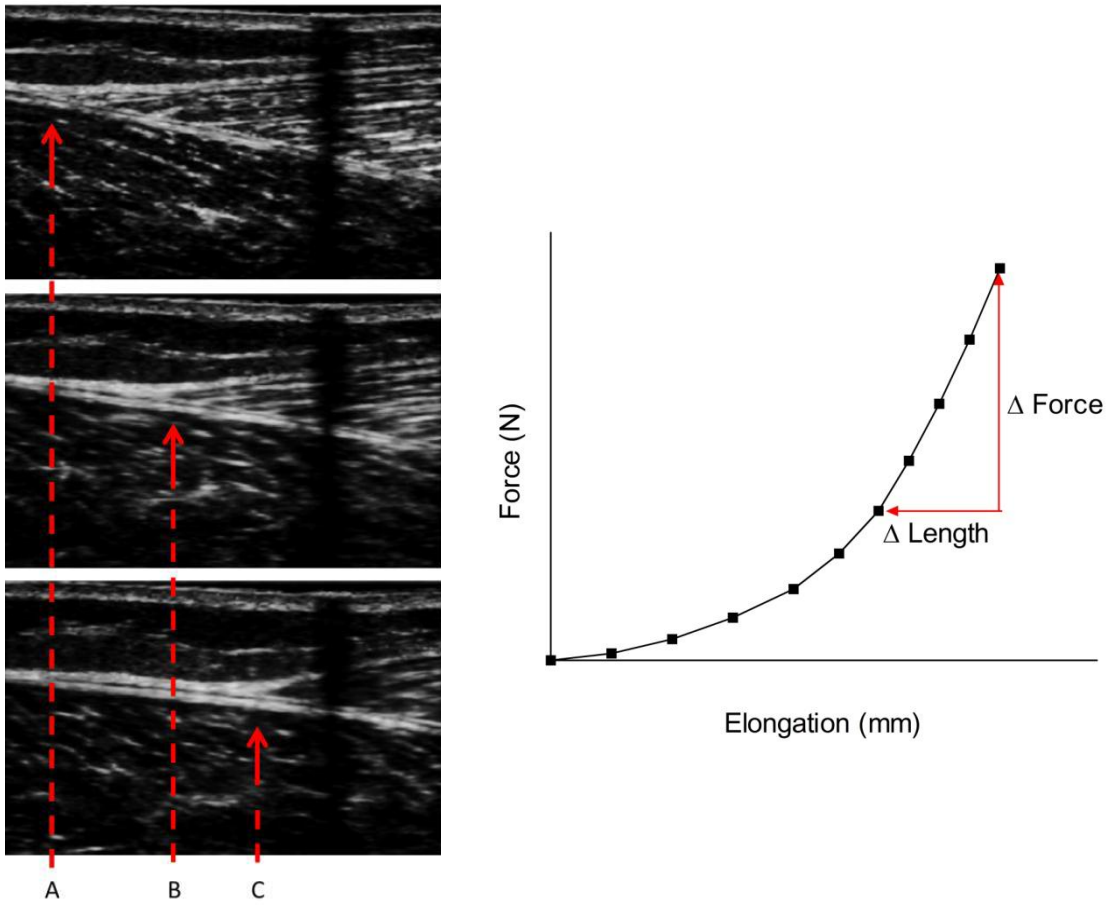

Figure 2.5. Ultrasound images visualising the displacement of the muscle-tendon junction during a ramped isometric muscle contraction at rest (A), mid contraction (B) and at maximal force (C). Muscle-tendon junction displacement is plotted against the corresponding force to produce a force-elongation graph. Tendon stiffness is measured as the slope, or the ratio between the Δ force and Δ length.

Previous research using this method for the determination of tendon stiffness, has interchangeably used maximal or ramped voluntary contractions of different shortening velocities, and passive rotations (Kubo, Kanehisa & Fukunaga, 2002a; Morse *et al.*, 2008), which may present a problem when comparing across studies. For example, the tendon has been shown to possess viscoelastic properties, specifically, its mechanical properties, such as stiffness, will be dependent on the rate at which a load is applied (Fung, 1993; Pioletti, Rakotomanana, Benvenuti & Leyvraz, 1998; Sanjeevi, 1982). The result is that the tendon will be stiffer when stretched at high velocities, compared to if it is stretched slowly, thus a maximal voluntary contraction compared to a slower ramped contraction may impact the results of stiffness values obtained using this method. This ultrasound method has also recently been used to gain an estimate of "global" muscle stiffness in addition to tendon stiffness (Morse *et al.*, 2008). Here, the joint is passively rotated at a constant speed to stretch the muscle and tendon components. The main limitation of this method for estimating muscle stiffness is the inability to quantify the actual force contribution from individual muscles. In this estimate of stiffness several other passive structures will also contribute to the passive torque produced at the joint (Morse *et al.*, 2008).

This method of passive elongation of the tendon to determine stiffness may be more appropriate than the active method for use in children with CP. For example, Barber *et al.* (2012) showed that tendon stiffness in children with CP was not significantly different to TD children, which was mainly explained by lower peak torques in the CP group. Here, the authors used a slow, ramped maximal voluntary contraction for the subsequent determination of stiffness. It would be logical that as a result of muscle weakness and excessive co-contraction, children with CP may have difficulty activating

the correct muscle, and producing enough force to elongate the tendon. In this respect, the passive method may be a more appropriate choice for deriving estimates of tendon, and "global muscle", stiffness.

2.4 Treatment intervention

For children with spastic CP, a variety of treatments and interventions exist, although there is no cure. These treatments have tended to focus primarily on spasticity and the associated neural symptoms. Anti-spasticity drugs such as Botulinum toxin-A, Intrathecal Baclofen and Benzodiazepines have been shown to be somewhat effective in reducing some symptoms of muscle overactivity and spasticity (Verrotti, Greco, Spalice, Chiarelli & Iannetti, 2006). However, as previously described, children with spastic CP also develop mechanical changes in the muscle-tendon unit, which may contribute to movement dysfunction and contracture more than spasticity (Dietz, Quintern & Berger, 1981).

The progressive muscle weakness and increased tone (both neural and muscular) leads to a lack of weight-bearing movement, and prevents the joint from moving through its full ROM. This eventually leads to a loss of ROM and can result in subsequent muscle contracture (Gracies, 2005). A contracture arises when the fibres become too functionally short and extremely stiff. It has been shown that contracture makes a significant contribution to clinical ratings of resistance to passive movement (Vattanasilp, Ada & Crosbie, 2000), and further prevents functional movement, secondary to muscle and joint pain. The treatment for contracture is most often orthopaedic surgery, to lengthen the muscle-tendon unit and reduce joint deformities. However, research has shown that surgery usually makes the muscle significantly

weaker (Delp, Statler & Carroll, 1995) and requires intense physical therapy to improve strength and function. Moreover, depending on the age of the child it is likely that repeated surgery in the future will be needed. Therefore, given the effects and the irreversible nature of surgery, less invasive long-term intervention strategies are extremely important to slow the process, or even reverse some of the dysfunctional musculoskeletal changes in children with spastic CP. Studies which investigate the efficacy and underlying mechanical changes as a result of these long-term interventions are therefore critical.

Long-term Interventions such as strength training, splints and casting have demonstrated some positive effects towards treating musculoskeletal adaptations. Despite less conclusive evidence for the use of long-term passive stretching, it is still widely used in clinical practice as a treatment for children with spastic CP. The rationale for passive muscle stretching is twofold. First, it is thought that the main effect of long-term stretching could be to induce the addition of in-series sarcomeres, which may be lacking from an immobilised muscle. Second, long-term passive stretching may also reduce spasticity via inhibition of the tonic stretch reflex.

The neural mechanisms in response to muscle stretch are mostly studied at the level of the spinal cord to assess modulation of reflex activity, commonly through a measure of the Hoffman (H) reflex. The H-reflex can provide information about the state of excitability of the stretch reflex, which is under the influence of higher centres; hence, it gives indirect information about the state of the central nervous system. Modification of spinal reflexes has been shown to occur in response to long-term stretch in healthy individuals. For example, passive stretching of the *triceps surae* muscles has been

associated with a decrease in H-reflex amplitude (Avela, Kryöläinen & Komi, 1999; Guissard & Duchateau, 2004; Guissard, Duchateau & Hainaut, 2001), demonstrating reduced excitation of the motor neuron pool through reduced sensitivity of Ia afferents. In addition, longer duration stretching interventions have also been shown to reduce both H-reflex and tendon-reflex amplitudes (Guissard & Duchateau, 2004).

There are several mechanisms proposed, which may contribute to reduced tonic reflex activity. Pre- and post-synaptic spinal mechanisms might be involved in the inhibition of reflexes, by reducing motor neuron excitability. This may also depend on the magnitude of stretch. For example, pre-synaptic inhibition via Ia afferents might be involved in reducing motor neuron excitability during small magnitude stretches. Similarly, Golgi tendon organ type Ib afferents, Renshaw loops and supraspinal interneuronal circuitry may be involved in large magnitude stretches (Guissard *et al.*, 2001).

In a clinical population, several studies have demonstrated a significant short-term reduction in spasticity, through depression of the stretch reflex (Al-Zamil, Hassan & Hassan, 1995; Tremblay, Malouin, Richards & Dumas, 1990; Tsai Yeh, Chang & Chen, 2001; Zhang *et al.*, 2002). These studies demonstrated that after 30 minutes of passive stretching, EMG activity, H-reflex amplitude and the H_{max}/M_{max} ratio were significantly reduced, illustrating reduced excitability of the stretch reflex.

Conversely, studies have also demonstrated no change in spasticity following manual stretching, likely due to large individual and methodological differences. Richards, Malouin and Dumas (1991) demonstrated that 30 minutes of standing on a tilt-table, did

not alter EMG activity during standing or walking. This was supported by Bakheit, Maynard and Shaw (2005) as well as Chung, Bai, Rymer and Zhang (2005), who failed to identify any changes in reflex components after 30 minutes of stretching. In a longer duration study, Kunkel *et al.* (1993) found that positioning in a standing frame did not alter the latency and amplitude of the H-reflex during a six month study protocol of standing for 45 minutes twice daily. The role of stretching in spasticity is therefore not conclusive. Furthermore, in all studies showing a positive effect of stretching on spasticity, effects were short-lived. It should also be noted that different upper motor neurone syndromes may have different responses to stretch, as a result of cause and location of lesion. Thus, observed responses to stretch might be different for different pathologies. The neural changes associated with spasticity are not within the scope of this thesis, but provide an interesting direction for future research.

The effect of stretching on mechanical muscle and tendon changes are the focus of this thesis. For example, research on animal models has shown the adaptable nature of muscles and tendons, and their ability to remodel in response to long-term stretching (Goldspink, 1977; Goldspink, 1978; Williams & Goldspink, 1973). Days or weeks of continuous stretching has been shown to stimulate the addition of in-series sarcomeres, and change the collagen concentration and arrangement within the muscle (Goldspink, Tabary, Tabary, Tardieu & Tardieu, 1974; Tabary, Tardieu, Tardieu, Tabary & Gagnard, 1976; Williams & Goldspink, 1978). This has been used as a basis to assume stretching may increase muscle length, thereby increasing joint ROM, and preventing or delaying the need for orthopaedic surgical intervention (Holt, Baagøe, Lilllelund & Magnusson, 2000b). In addition, these studies largely use healthy animals, whilst here we are dealing with a pathological condition that is not completely understood. In a

human model, the effectiveness of passive stretching is limited for two reasons. First, the mechanisms and aetiology of muscle contracture are not well understood, making it difficult to know if the theory underlying stretching is correct. Second, clinical research investigating its effectiveness is inconclusive, due to large individual differences and heterogeneity of symptoms (Wiart *et al.*, 2008). Further, responses to stretch may be muscle-specific, as different muscles have been shown to have different mechanical characteristics (Ward *et al.*, 2009). Therefore, conclusions on passive stretching for spastic muscles are inconclusive. Thus, a significant gap currently exists between research evidence and clinical practice.

2.4.1 Stretching

Passive stretching is defined here as a static stretch, applied to a joint by either a physiotherapist, parent/guardian, or by the child. The effects of acute (short-term) stretching, involving a single session, and long-term stretching, involving repeated sessions over weeks or months, must be investigated. If long-term stretching is to be effective to induce structural and lasting adaptations in the muscle, then acute, albeit transient changes in muscle and fascicle lengths would presumably need to be present. If transient changes are not present, then the efficacy for long-term stretching to cause structural changes to the muscle may not be valid.

2.4.1.1 Acute stretching. Passive stretching in healthy adults has previously been shown to bring about increases in joint ROM accompanied by decreases in the stiffness of the muscle-tendon unit (Evetovich, Nauman, Conley & Todd, 2003; Halbertsma, van Bolhuis & Göeken, 1996; Wilson, Elliott & Wood, 1992; Witvrouw, Mahieu, Danneels & McNair, 2004). A decrease in muscle-tendon unit stiffness may be achieved by both a

reduction in tendon stiffness (Herbert, Moseley, Butler & Gandevia, 2002; Kubo, Kanehisa, Kawakami & Fukunaga, 2001c) and/or a reduction in muscle stiffness (Kawakami, Kanehisa & Fukunaga, 2008; Kay & Blazevich, 2009; Mizuno, Matsumoto & Umemura, 2011; Morse, *et al.*, 2008). During passive stretching, both the muscle and tendon are thought to elongate, although the fascicles have been shown to undergo smaller length changes than the whole muscle-tendon unit (Herbert *et al.*, 2002). This would suggest the tendons are responsible for the majority of the length change. This was supported by Kubo, Kanehisa and Fukunaga (2002b) who found that after five minutes of stretching, tendon stiffness decreased by 8% with a 29% decrease in hysteresis. Conversely, Morse *et al.* (2008) determined the stiffness of tendon and muscle during passive dorsiflexion. They reported a 56% decrease in muscle stiffness after stretching, but no significant change in tendon stiffness. The inconsistency between study results may be explained by the different methods employed. Kubo *et al.* (2002b) measured tendon stiffness under high torques generated by isometric contractions, whereas Morse *et al.* (2008) measured stiffness of muscle and tendon under relatively low torques. Regardless, these two studies demonstrate that stiffness of the muscle-tendon unit decreases after acute stretching, although it remains unclear whether this change is due to alterations in muscle stiffness, tendon stiffness or some combination of the two.

Only two studies have attempted to quantify the acute stretch response in CP, which both found no changes in muscle activation (Richards *et al.*, 1991; Tremblay *et al.*, 1990). No study to date has investigated whether there are alterations in muscle and/or tendon length in CP following acute stretching. If there is no elongation of the muscle or fascicles during an acute bout of stretching, this would suggest that no muscle

alterations are likely to occur with long-term stretching. Therefore, research which assesses the effects of acute stretch on muscle and tendon structures in spastic CP is needed.

2.4.1.2 Long-term stretching. The purpose of long-term stretching differs to that of acute stretching. In long-term stretching, the aim is to develop structural adaptations within the muscle, which are not readily reversible upon removal of the stretch (Folpp, Deall, Harvey & Gwinn, 2006). Tabary *et al.* (1976) evaluated the response to long-term stretching in cat muscle. When the soleus was initially immobilised in a shortened position, it adapted by shortening muscle fibres, through a 40% reduction of in-series sarcomeres. When the muscle was then positioned in an elongated position, it responded by gaining sarcomeres. This structural adaptation was considered responsible for reducing passive stiffness in the muscle, so that less force was required to stretch it to a given length. In the rat soleus muscle, Coutinho *et al.* (2004) showed that stretching, applied every three days for three weeks, resulted in increased muscle length, and a 4% increase in serial sarcomere number. The results of these animal studies largely suggest the addition of in-series sarcomeres is possible following long-term stretching, although there has not been rigorous evaluation of this assumption in human muscles (Lieber *et al.*, 2004). One recent case study which does provide some evidence towards this, showed a 10 cm increase in vastus lateralis fascicle length following femoral lengthening, with a concurrent increase in serial sarcomere number (Boakes, Foran, Ward & Lieber, 2007), demonstrating the adaptive nature of the muscle in humans. Thus, as previously outlined, if one main mechanism for increased muscle stiffness in children with spastic CP is a reduced number of in-series sarcomeres, then this may lend some support the use of passive stretching.

For obvious reasons, investigating sarcomere number pre- to post-stretch in humans is too invasive, but studies have attempted to quantify the change in muscle length indirectly. In a non-disabled population, Weppler and Magnusson (2010) generalised the findings of a review on the muscle and tendon alterations to stretching. They suggest 3-8 weeks of stretching caused similar increases in serial sarcomeres, as those seen in animal models. The addition of sarcomeres should be accompanied by a rightward shift in the torque-angle curve, that is, for a given angle less torque should be produced in a passively stretched muscle with a greater number of in-series sarcomeres. This has been demonstrated in one study of ankle plantarflexors. Guissard and Duchateau (2004) observed a right shift in the torque-angle curve following six weeks of stretching for 20 minutes per day, five days a week. Similarly, Halbertsma, Göeken, Hof, Groothoff and Eisma (2001) demonstrated that two 10 minute bouts of stretching the hamstring muscles, five days per week, over a four week period on the hamstring muscles increased torque levels. However, no direct measures of muscle or fascicle length were made. Joint ROM has also been shown to increase as a result of long-term stretching (McNair & Stanley, 1996; Rosenbaum & Hennig, 1995). Magnusson, Simonsen, Aagaard, Sørensen and Kjaer, (1996a) reported that a three week intervention induced a greater hamstring ROM, but the mechanism for this change is unclear. Despite the lack of clarity, the findings have been used as a justification for stretching in children with spastic CP. However, to date, the assumption that stretching can reduce muscle-tendon unit stiffness and increase ROM in CP has not been clearly demonstrated, despite the use of long-term stretching as a management strategy (Harvey, Herbert & Crosbie, 2002; Pin *et al.*, 2006). The minority of studies that have attempted to document the

effectiveness of stretching in CP show large methodological variation and inconclusive findings (Table 2.1).

2.4.1.2.1 ROM. Children with spastic CP have a reduced joint ROM compared to their TD peers (Barber *et al.*, 2011b; Malaiya *et al.*, 2007). Several authors have investigated the effect of stretching interventions on joint ROM in patients with reduced joint range (Fragala, Goodgold & Dumas, 2003; Lespargot, Renaudin, Khouri & Robert, 1994; McPherson, Arends, Michaels & Trettin, 1984; Miedaner & Renander, 1987; O'Dwyer *et al.*, 1994), but the results obtained from these studies are equivocal, possibly due to methodological variations. Two of these studies showed an increase in ROM post-stretching (McPherson *et al.*, 1984; Miedaner & Renander, 1987). McPherson *et al.* (1984) showed a significant increase in knee ROM and a reduction in knee flexion contracture after a two year stretching intervention. Miedaner and Renander (1987) also showed a significant increase in two out of 14 joint measurements following stretching for five days per week, for five weeks, in children with exaggerated lower limb joint stiffness. One further study reported no change in joint ROM following stretching, and also demonstrated a loss of ROM after the cessation of stretching (Fragala *et al.*, 2003). Similarly, studies by Lespargot *et al.* (1994) and O'Dwyer *et al.* (1994) demonstrated no change in hip or ankle ROM following long-term passive stretching. It is evident that the effectiveness of stretching interventions on ROM is not straight forward. It seems to depend on the joint or muscle of interest and the type and duration of the stretching intervention. As most of the authors in these studies did not state acceptable cut-off points for clinical significance, it is difficult to judge if improvements in ROM were clinically relevant (Pin *et al.*, 2006).

2.4.1.2.2 Muscle-tendon unit stiffness. Measuring stiffness in children with CP should aim to identify the contributions of both the muscle and tendon. However, studies often use a global measure of joint stiffness. The first study to attempt to identify stretching in spastic muscle was conducted by Tardieu, Lespargot, Tabary and Bret (1988). The researchers investigated the amount of time required for the soleus to be lengthened in 24 hours, in order to prevent contracture. They concluded that six hours a day of active movement training, functional movement and positioning; prevented contracture. This contrasts with most passive stretching interventions where the muscle is in a lengthened position for only a few minutes at a time. Four further studies were found which investigated the effects of shorter duration interventions on muscle tone (McPherson *et al.*, 1984; Miendaner & Renander, 1987; O'Dwyer *et al.*, 1994; Zhao *et al.*, 2011). Two of these studies, which employed a more representative stretch duration (McPherson *et al.*, 1984; Miendaner & Renander, 1987) along with daily positioning, also observed a reduction in clinically assessed muscle tone. Miendaner and Renander (1987) demonstrated that there was no difference between passive stretching two times a week versus five times a week for six out of seven joint motions. Since both studies included daily positioning (30 minutes) along with manual stretching it difficult to assess what the contribution of manual passive stretching was. One study which did look at the effects of manual stretching only (O'Dwyer *et al.*, 1994) also demonstrated a decrease in muscle tone of the *triceps surae* muscles. The ability to generalise findings from these studies is limited due to small sample sizes and a lack of homogeneity of patients. In many of these studies, the intervention exceeded what is feasible for patients, and in none of these studies was it possible to determine whether stiffness was reduced in the muscle structure, tendon or both.

In a more recent study, Zhao *et al.* (2011) found an increase in fascicle length and decreased pennation angle in both the soleus and gastrocnemius muscles of children with spastic CP. They also observed that Achilles tendon length decreased, which was accompanied by an increase in tendon stiffness. Passive stretching was implemented using an ankle stretch device for 30 minutes, three sessions per week, for six weeks. They also incorporated active movement for 30 minutes in each of the sessions. Again, it is difficult to determine whether the observed changes were from active movement training or passive stretching. It is also unclear whether the observed changes led to any improvement in functional movement.

2.4.1.2.3 Gait. From a developmental perspective, children with spastic CP and TD children present similar early gait patterns when learning to walk. In TD children, this gait pattern matures into a pendulum pattern (Figure 2.6), with the correct timing of plantarflexion activation to provide the majority of propulsive force (Francis, Lenz, Lenhart & Thelen, 2013). In children with spastic CP the maturation of this gait pattern does not develop. Due to muscle weakness, children do not have adequate power through the ankle during the push-off phase of walking (Fonseca, Holt, Saltzman & Fetters, 2001). In addition, alterations in the phases of gait are also different including a plantarflexed foot at initial contact, resulting in a pattern of atypical gait (Fonseca *et al.*, 2004). Research on the spatiotemporal parameters of gait in children with spastic CP reveal a reduced gait velocity, and a shorter stride length. In addition, a deterioration of gait stability also occurs over time, which increases the time spent in the double support phase of gait (Johnson, Damiano & Abel, 1997).

This abnormal gait pattern is thought to result largely from increased muscle and joint stiffness. As such, the clinical assumption is that stiffness impairs gait, by reducing foot contact time and impairing foot positioning (Salem, Lovelace-Chandler, Zabel & McMillan, 2010). In addition, clinicians may assume that reducing stiffness would result in improved movements such as gait in children with spastic CP. Conversely, there is also a view that atypical gait patterns emerge as a result of neurological and mechanical alterations in the muscle-tendon unit to muscle weakness. In this view, alterations such as increased muscle stiffness, co-contraction and a plantarflexed foot at initial contact allows energy to be generated, where sufficient muscle force cannot. Thus, allowing for the emergence of an atypical, but functional gait pattern (Holt, Fonseca & LaFiandra, 2000a).

The pattern of gait in CP has been compared to that of running in a non-disabled population, perhaps by using greater elastic energy, which can be stored and released in the tendon (Figure 2.6). This is supported by low levels of muscle activation observed in CP during gait (Berger, Quintern & Dietz, 1982). In addition, a plantarflexed foot on initial contact would facilitate a vertical spring by creating a longer external moment arm at the point of ground contact compared to a heel strike. This, in turn, would load the tendon to store elastic energy.

These conflicting views of gait in CP make it difficult to determine if gait parameters would potentially be expected to improve or deteriorate with stretching. With long-term stretching in CP, Salem *et al.* (2010) demonstrated 45 minutes in a standing frame, three days per week for three weeks significantly improved gait parameters including speed, stride length, stride time, stance phases and maximum ankle dorsiflexion angle. These

improvements returned to baseline three weeks after the cessation of stretching. Similarly, Wu, Hwang, Ren, Gaebler-Spira and Zhang, (2011) showed that a combination of passive and active stretching over a six week period, improved clinical tests of walking speed using the six minute walk test and timed up and go. Conversely, in patients with limited dorsiflexion ROM, three weeks of passive stretching for five minutes each day, improved maximum dorsiflexion, but did not decrease stance time during gait (Johanson *et al.*, 2006).

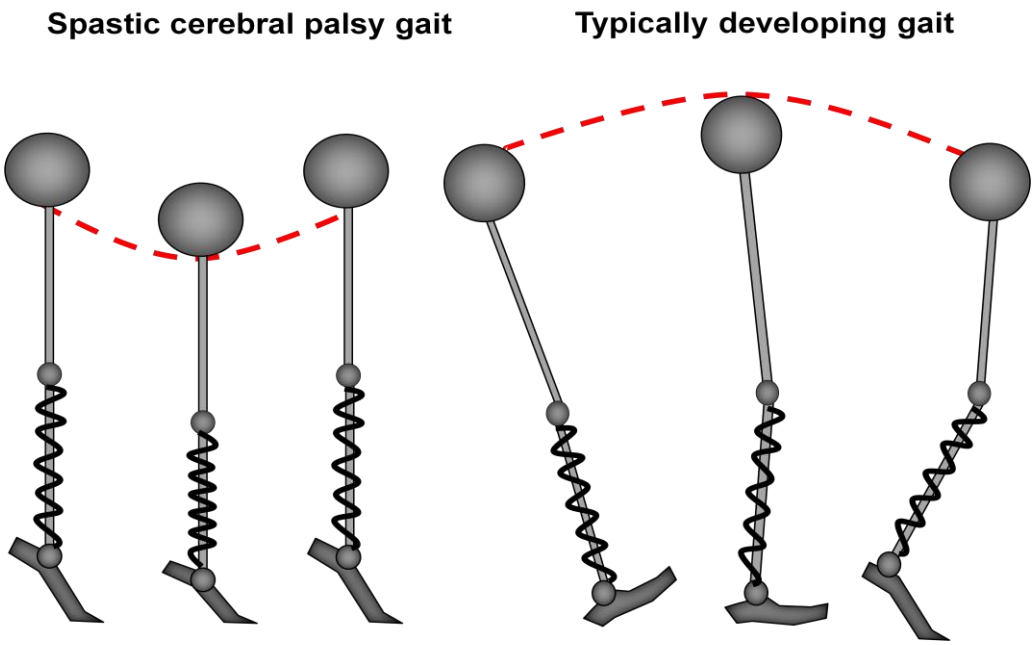

Figure 2.6. Varying gait patterns in children with CP and TD children.

2.5 Summary

A number of complex CP-related neural and musculoskeletal changes take place in response to damage to the developing brain in children with spastic CP. Although a number of studies have reported the properties of the spastic muscle, the developmental changes of the tendon are less well reported. Understanding the properties of both

muscle and tendon in children with spastic CP compared to TD children has important implications for understanding atypical movement.

Stretching is a commonly used intervention to treat a spastic muscle, despite a lack of research evidence. Due to large individual differences and heterogeneity of symptoms, results from intervention studies are difficult to assess (Tedroff *et al.*, 2008). Many studies also use long durations of stretching, which are not representative of those used in clinical practice. Both Pin *et al.* (2006) and Wiart *et al.* (2008) emphasise the inconclusiveness of the current body of research. They also highlight methodological shortcomings in the current literature, such as lack of randomised control trials and statistical power, as well as lack of homogeneity within the experimental groups.

There is some evidence to suggest that passive stretching may reduce muscle-tendon unit stiffness and increase ROM, but how the constituent components of the muscle-tendon unit are affected remains an unanswered question. There is theoretical evidence to suggest muscles may adapt to stretch and increase in length, as illustrated in animal models. This may serve to reduce muscle-tendon unit stiffness and increase joint ROM (Pin, *et al.*, 2006). However, on the other hand, the effect of any potential muscle and tendon changes on function has not been investigated. It could be the case that increased stiffness, in part, compensates for muscle weakness, creating some stability during certain movements for children with spastic CP. Most importantly however, since passive stretching is still widely used and advocated in clinical practice (National Institute for Health and Care Excellence, 2012), its effects on muscle and tendon mechanics and functional movement must be thoroughly and correctly established.

These results will help clinicians to make more informed decisions about treatment interventions for children with spastic CP in the future.

Table 2.1. Research findings from stretching in CP

Study	**Participants**	**Method of stretch**	**Outcome measure**	**Result**
Fragala *et al.* (2003)	7 children (4-18 y) with CP GMFCS IV and V	Passive stretching and positioning 40-60 s, 3 repetitions, 1-2 times per week, and positioning in the classroom	Knee and Hip extension ROM	No change in ROM
Lesparagot *et al.* (1994)	10 children (9-13 y) with spastic CP	Manual stretch: 15-20 minutes and wedge sitting 5-7 hours daily	Passive hip abduction angle	No change
McPherson *et al.*(1984)	4 children (10-18 y) with severe spastic quadriplegic CP	Passive stretching and positioning Year 1: 60 s knee extension stretch, 5 repetitions, 3 times per day, 5 days per week Year 2: Prone or supine standing for 1 hour per day	Hip extension ROM Muscle tone with special device	Knee extension increased (4-9º) Decreased by 5-10º during non-treatment periods
Miedaner *et al.* (1987)	13 children (6-20 y) with CP (severe cognitive and physical impairment)	Passive stretching 20-60 s, 5 repetitions, 5 days per week and 2 days per week	Passive hip, knee, ankle and forefoot ROM	No change in 6 of 7 joint angles. Popliteal angle increased in higher frequency intervention

Study	Participants	Method of stretch	Outcome measure	Result
O' Dwyer *et al.* (1994)	15 children (6-19 y) with spastic CP	Sinusoidal stretch: 30 minutes, 3 times per week, for 42 days	*Triceps surae* contracture (Ankle joint passive torque)	No change Spasticity reduced
Richards *et al.* (1991)	8 children (3-13 y) with spastic diplegia or hemiplegia	Acute passive stretch: standing on a tilt table with varying ankle positions for 30 minutes	Muscle activation of tibialis anterior and *triceps surae* Gait analysis	Reduction in pre-/post-ratio of tibialis anterior during initial gait cycle
Tardieu *et al.* (1988)	4 children with spastic CP	Positioning for different durations up to 6 hours	Ankle dorsiflexion ROM	6 hours of stretching in 24 hours prevented contracture
Tremblay *et al.* (1990)	21 children (3-14 y) with spastic CP	Acute passive stretch: standing on a tilt table for 30 sec to stretch dorsiflexors	Quality of passive ankle movement Quality of *triceps surae* contraction	Decreased resistance to passive movement up to 35 minutes post-stretch Decreased EMG response up to 35 minutes post-stretch

Study	Participants	Method of stretch	Outcome measure	Result
Zhao *et al.* (2011)	7 children (5-15 y) with spastic diplegic or hemiplegic CP	Passive and active stretching using an ankle rehabilitation robot 30 minutes of passive, 30 minutes of active, 3 sessions per week for 6 weeks	Medial gastrocnemius and Soleus fascicle length, pennation angle, and fascicle and Achilles tendon stiffness	Fascicle lengths increased, pennation angle decreased, fascicle stiffness decreased and tendon length decreased

CHAPTER 3: METHOD AND STRAIN-RATE DEPENDENCE OF ACHILLES TENDON STIFFNESS

3.1 Introduction

Muscle and tendon stiffness are important parameters when performing daily motor activities or sporting movements (Hof, Vanzandwijik & Bobbert, 2002; Fukunaga *et al.*, 2001). The stiffness of the body's elastic tissues governs the storage and release of elastic potential energy, and humans take advantage of this to maximise movement efficiency (Lichtwark & Wilson, 2008; Maganaris & Paul, 1999). Within this context, tendon stiffness has been widely studied in athletic (Kubo *et al.*, 2001c; McNair & Stanley, 1996) and clinical populations (Vaz *et al.*, 2006; Tardieu *et al.*, 1982). The findings of such studies have led to an enhanced understanding of how tendon stiffness influences force production (Reeves, 2006) as well as how tendon stiffness adapts to changes in loading (Kubo, Ikebukuro, Yata, Tsunoda & Kanehisa, 2010; Seynnes *et al.*, 2009).

Tendon stiffness is calculated by dividing the estimated tendon force by the tendon's elongation (Maganaris & Paul, 1999; Morse *et al.*, 2008). For this purpose, participants are commonly asked to perform a maximal isometric contraction to shorten the muscle and thereby elongate the tendon ("active method") (Kubo *et al.*, 2002b). An alternative method is to record tendon force and elongation from a passive stretch, applied by an isokinetic rotation ("passive method") (Morse *et al.*, 2008).

The choice of method is often driven by the specific purpose of an experiment and by the population of interest. For example, in clinical populations where patients with neuromuscular or musculoskeletal disorders may be unable to perform a maximal voluntary contraction reliably (Tedroff *et al.*, 2008), it may be more appropriate to use

the passive method. The primary advantage of this method is that it allows for both tendon stiffness and muscle stiffness to be estimated (Morse *et al.*, 2008). The disadvantage with this method is that that tendon stiffness can only be calculated at relatively low levels of force. The force-stiffness relationship has been shown to be non-linear (Rigby *et al.*, 1959), as at lower tendon stiffness values the un-crimping of collagen fibrils causes significant tendon elongation. As a result, tendon stiffness is greater at high compared to low force levels (Mizuno *et al.*, 2011).

A further variable, which may affect the comparability between stiffness obtained from the two methods, is the tendon's strain-rate. Tendons exhibit viscoelastic behaviour in response to stretch, meaning that tendon stiffness increases with an increased strain-rate (Le Veau, 1992, pp. 33-37; Pearson *et al.*, 2007). Thus, strain-rate needs to be taken into consideration when comparing different methods of obtaining tendon stiffness.

A range of methods, which are likely to result in different strain-rates have been used in the literature to obtain tendon stiffness, including the passive method (Mizuno *et al.*, 2011; Morse *et al.*, 2008), and several variations of the active method (e.g., fast maximal voluntary contraction manoeuvres - Kay & Blazevich, 2009; Muraoka, Muramatsu, Fukunaga & Kanehisa, 2005; or slow, ramped maximal voluntary contractions - Kubo *et al.*, 2002b; Peltonen, Cronin, Avela & Finni, 2010; Waugh, Blazevich, Fath & Korff, 2012). Such differences in strain-rates could potentially explain the relatively large range of tendon stiffness values reported across these studies. Thus, it is important to understand whether the passive and active methods are comparable to interpret findings from the literature appropriately and to compare results from studies employing different methodologies. Such an understanding would also enable researchers to make more informed decisions about the most appropriate method

to use within a specific research context. Therefore, the purpose of this study was to compare tendon stiffness obtained from the active and passive methods across different strain-rates.

3.2 Methodology

3.2.1 Participants

With institutional ethical approval, 20 healthy adults participated in this study (11 male, 9 female; age 24 ± 4 y; stature 1.8 ± 0.1 m; mass 74.9 ± 13.0 kg) (For sample size calculations see Appendix III). All participants were recreationally physically active and free from known neuromuscular or musculoskeletal problems. Written consent was obtained from all participants prior to participation.

3.2.2 Procedure

Participants attended the laboratory on one occasion. They were seated in the isokinetic dynamometer (Biodex Medical Systems, New York, USA), which was adjusted for each participant. To remove the compliance of the dynamometer, which was evident during maximal plantarflexion manoeuvres, the chair was adjusted so the right knee was initially flexed with a relative joint angle of approximately 20 deg (with 0 deg being full knee extension). The knee was then straightened to full extension, which locked the knee joint and allowed the leg to act as a passive strut (Cannavan, Coleman & Blazevich, 2012). Subsequent ankle plantarflexion manoeuvres deformed the dynamometer system only minimally. The relative hip angle was set to 85 deg. The lateral malleolus of the right ankle was aligned with the centre of rotation of the dynamometer arm. The dynamometer footplate was positioned perpendicularly to the tibia, which was the start position for all trials. To isolate the ankle movement, stabilisation straps were firmly tightened over the foot, thigh and chest, and participants were instructed to cross their arms over their chest. A gravity torque correction was performed by means of an automated correction procedure as part of the Biodex system software. Briefly, the measurement of total gravitational torque was made during a passive weighing of the segment in a mid-range plantarflexion position, approximately

50% of maximum plantarflexion ROM. The torque values recorded during the experimental trials were subsequently adjusted by either subtracting or adding the gravitational torque, depending on dorsi- or plantarflexion.

Participants were familiarised with the procedures by performing five sub-maximal isometric plantarflexions on the dynamometer, and were instructed to keep the heel in contact with the footplate throughout the contraction. After these practise trials, participants performed three to five maximal voluntary contraction manoeuvres until plantarflexion efforts were reproducible within 5%. All participants achieved this within five trials. These trials also provided pre-conditioning of the *triceps surae* muscle-tendon unit to ensure minimal variation in the load-deformation curves (Maganaris & Paul, 1999). To avoid fatigue, a 10 minute passive rest period was given before data collection. Participants then performed the active and passive trials, the order of which was randomised.

3.2.2.1 Passive method

First, the participant's maximum dorsiflexion angle was determined by slowly, manually dorsi-flexing the foot, until the onset of electrical activity (EMG) in the medial gastrocnemius muscle or until the participant reported any discomfort. This occurred between 21.5 ± 3.7 deg dorsiflexion. The ROM for the subsequent tests was defined by this maximum dorsiflexion angle and a plantarflexion angle of approximately 20 deg (20.3 ± 1.4 deg). We then applied an angular rotation of the right ankle joint at constant angular velocities of 1, 10 and 30 deg·s^{-1} throughout the ROM. EMG of the medial gastrocnemius and tibialis anterior muscles were monitored to ensure no muscle activity was invoked by the passive rotations. Participants were instructed to relax the muscles of the lower limb. Three rotations were recorded at each

angular velocity, and the order of these was randomised. EMG data of the tibialis anterior and medial gastrocnemius muscles (Telemyo 2400R, NorAxon U.S.A Inc, Arizona) as well as torque and position data from the dynamometer were collected at 1000 Hz.

3.2.2.2 Tendon elongation

Tendon elongation was measured by tracking the displacement of the gastrocnemius muscle-tendon junction during the passive and active trials using B-mode ultrasonography (Megas GPX, Esaote, Italy; 45 mm Linear array probe, 10 MHz transducer scanning). The video transmission was digitally captured at 25 Hz using a video converting frame grabber (ADVC-55, Grass Valley, France). A layer of water-based gel (Henley's Medical, Hertfordshire, UK) was applied between the ultrasound probe and skin, which enhanced acoustic transmission. The probe was placed perpendicularly to the skin surface above the muscle-tendon junction and orientated to reveal a line running between the aponeuroses of the medial gastrocnemius and soleus muscles. The probe was then fixed in position using a custom made holder. A 2 mm wide strip of echoabsorptive tape placed on the skin in contact with the probe provided a reference to which any probe movement could be identified. 2D coordinates of the muscle-tendon junction were obtained by manual digitisation (Peak Performance, Cambridge, UK). The relative change in muscle-tendon unit length with respect to change in ankle angle was estimated using a cadaveric regression model (Grieve, Pheasant & Cavanagh, 1978).

3.2.2.3 Active method

Participants initially performed three isometric maximal voluntary contraction manoeuvres (after the pre-conditioning contractions) to establish maximum torque. To

achieve a range of strain-rates during the active method, participants were instructed to perform ramped maximal voluntary contractions of different durations (3 s, 5 s, 8 s and 10 s). Specifically, participants were instructed to gradually increase the pressure on the footplate to gain a steady rise in torque up to their maximum. They were asked to develop this torque within 3 s, 5 s, 8 s or 10 s as determined by the experimenter. The order of these durations was randomised. A digital timer and visual display of the torque trace was positioned in front of the participant, so they could track the duration of the contraction. Verbal encouragement from the investigator was provided throughout. Two trials were initially recorded at each duration separated by a 30 s rest period. An additional measurement was allowed if the maximum torques achieved in the two trials differed by more than 5%.

3.2.2.4 Antagonistic co-contraction

To quantify antagonist co-contraction, the dorsiflexion torque-tibialis anterior EMG relationship was measured (Telemyo 2400R, NorAxon U.S.A Inc., Arizona) at 1000 Hz. Signals were amplified, digitally filtered (Spike2 v5.12a, Cambridge Electronic Design, UK) using a 10-500 Hz band pass filter, and smoothed by means of calculating the root mean square over a 100 ms sliding window. The dorsiflexor torque was estimated from the EMG-torque relationship during a ramped dorsiflexion contraction. A third-order polynomial was fitted to the EMG-torque data corresponding to the greatest level of the tibialis anterior EMG observed throughout the plantarflexion trials ($R^2 = 0.95 \pm 0.02$ across participants). The resulting regression equation was then used to estimate the antagonist torque present during the plantarflexion trials. The estimate of dorsiflexor torque was then added to the torque measured on the dynamometer to provide a corrected plantarflexor torque. One limitation to this correction is that the effect of strain-rate on the torque-angle relationship is neglected. For example, by a applying a

constant antagonistic co-contraction torque, the torque produced by the dorsiflexors at higher speeds would have been underestimated, and overestimated at lower contraction speeds. Additionally, the effect of agonist co-contraction was neglected during the dorsiflexion contractions, and therefore dorsiflexor co-contraction torque may have been overestimated.

Coordinate data were captured at 100 Hz, then filtered and downsampled to 25 Hz to match the frequency of the ultrasound data. The movement of the muscle-tendon junction attributable to ankle movement was then subtracted from tendon elongation measured during the trial. Torque and coordinate data from both methods were filtered using low-pass, fourth-order, zero-lag Butterworth filters with cut-off frequencies of 14 and 5 Hz respectively, as determined by residual analysis (Winter, 1990) (see Appendix IV). Digitised muscle-tendon junction position data for both methods were filtered using a low-pass fourth-order zerolag Butterworth filter with a 3.25 Hz cut-off frequency.

3.2.3 Data analysis

For both methods, tendon stiffness was calculated as the change in ankle torque divided by the corresponding change in tendon length. It is important to acknowledge that "tendon stiffness" as measured using the passive method is not "tendon stiffness" *per se*. The term "tendon stiffness" is referred to in this chapter and throughout the thesis, but it is acknowledged that passive tendon stiffness data is based on joint torque in passive conditions and we acknowledge that many other structures will contribute to passive joint stiffness in addition to the Achilles tendon.

To make valid comparisons between the two methods, tendon stiffness was calculated in a common torque region. This was dictated by the peak torque observed during dorsiflexion in the slowest passive trial of each participant. A range of 50-60% of maximum torque in this trial was chosen for the calculation of tendon stiffness. This same absolute range was then identified in the remaining passive and active trials for each participant. The comparable torque range in the active trials was 12.3 ± 1.6% to 15.4 ± 2.2%. For both methods, tendon strain-rate was calculated over this range, by dividing the change in tendon length by change in time. For the active trials and for each ramped duration, final stiffness was calculated using the mean of two ramped maximal voluntary contraction trials that were within ± 5% of maximum recorded torque. For the passive trials at each angular velocity, tendon stiffness was calculated using torques recorded during dorsiflexion over the torque range as described above. Final stiffness was then calculated by averaging stiffness of the two trials that were most closely matched in terms of strain-rate. For both methods, final tendon strain-rate was obtained as the average strain-rate over the two trials that contributed to the final stiffness. Figure 3.1 shows representative torque-elongation curves obtained from both active (A) and passive (B) methods for one participant. Averaged across participants, the tendon strain-rates measured during the 1, 10 and 30 deg·s^{-1} rotations were 0.09 ± 0.02; 0.45 ± 0.06; 0.84 ± 0.01 cm·s^{-1}, respectively. For the active method, the final stiffness values were chosen based on the strain-rates most closely matching these values. For all participants, this corresponded to the 5 s, 8 s and 10 s, ramped contractions, respectively. Averaged across participants, the corresponding strain-rates were 0.12 ± 0.01, 0.40 ± 0.03 and 0.80 ± 0.04 cm·s^{-1}, respectively. Within a given trial, these strain-rates did not deviate by more than 10%. Based on these results, the final stiffness values from both the active and passive methods were plotted against strain-rate, and a linear regression line was used to approximate these relationships for each

participant (R^2 = 0.98 ± 0.02 and 0.98 ± 0.03 for active and passive methods, respectively). The resulting regression equation was used to interpolate the data to give stiffness values for both methods at strain-rates 0.1, 0.4, and 0.8 cm·s^{-1}.

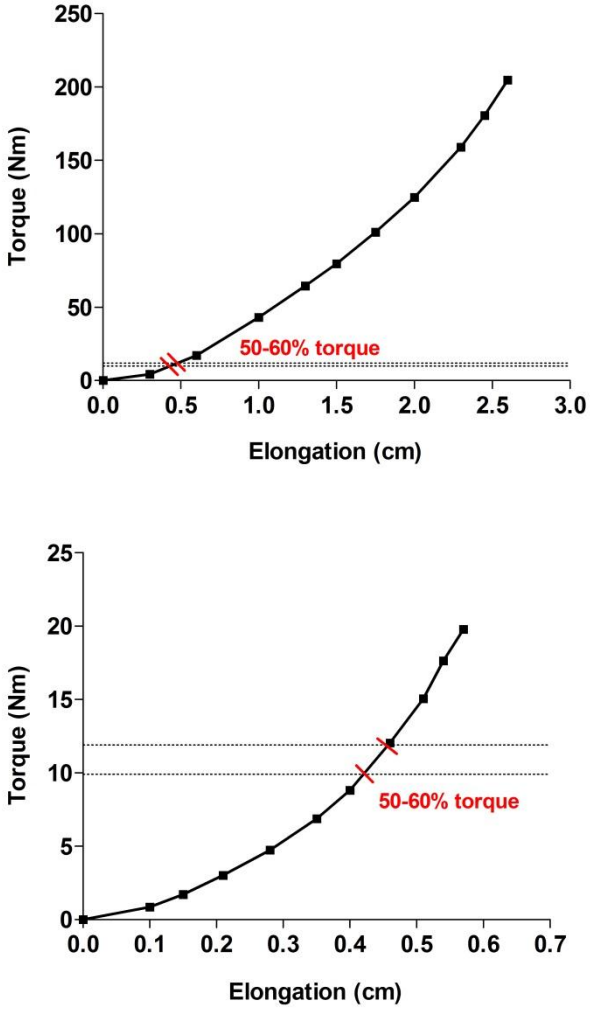

Figure 3.1. Ankle torque and Achilles tendon elongation during an isometric maximal voluntary contraction manoeuvre (top) and during passive dorsiflexion at 10 deg·s^{-1} (bottom).

3.2.4 Statistical analysis

The agreement between the interpolated active and passive tendon stiffness values was assessed in four ways. First, to determine whether systematic bias existed between the two methods across strain-rates, we performed an ANOVA. Here, we tested for a main effect of method and a method × strain interaction. In case of statistical significance, paired samples *t*-tests (Bonferroni correction) were used. Second, at each strain-rate, the Pearson's correlation coefficient was obtained to quantify the relationship between tendon stiffness values obtained from both methods. Third, an analysis of agreement was conducted according to Bland and Altman (1986). Before limits of agreement were calculated, an assessment of the normality of data (Shapiro-Wilk test) and of heteroscedastic error was conducted. As neither non-normality nor heteroscedacity existed, the absolute limits of agreement were determined using the calculated mean and standard deviation, indicating bias and random error. Standard errors and 95% confidence intervals were also calculated. Fourth, intraclass correlation coefficients with a two way random model and absolute agreement were calculated at each strain-rate. Finally, to specifically quantify the dependence of tendon stiffness on strain-rate, two statistical procedures were performed. First, using the aforementioned ANOVA, we tested for a main effect of velocity. In case of significance, follow up paired samples *t*-tests (Bonferroni correction) were performed. Second, strain-rate was correlated with tendon stiffness, and a linear regression was performed to approximate this relationship. All statistical tests were performed using SPSS statistical software (v16.0, SPSS Inc., Chicago, USA), and the *p*-value was set at 0.05.

3.3 Results

Results from the ANOVA revealed that the method-by-strain-rate interaction was non-significant ($F_{1, 19} = 1.63$, $p = 0.60$). Further, there was a significant main effect for method ($F_{1, 19} = 134.20$, $p < 0.01$). A follow up paired samples *t*-test revealed that stiffness values obtained from the active method were significantly greater compared to the passive method at all strain-rates ($t_{59} = 17.15$, $p < 0.001$). The Pearson's correlation coefficients describing the relationship of tendon stiffness obtained from methods across participants were 0.98, 0.99 and 0.99 for 1, 4 and 8 $mm \cdot s^{-1}$, respectively (Figure 3.2). The mean difference between tendon stiffness for both methods was plotted against the corresponding means (Figure 3.3). Examining the direction and magnitude of the scatter around the zero line on the Bland and Altman plots provides an indication of systematic bias and random error. This bias was consistent with the main effect for method obtained from the ANOVA. On average, the active method produced 6% greater stiffness values than the passive method.

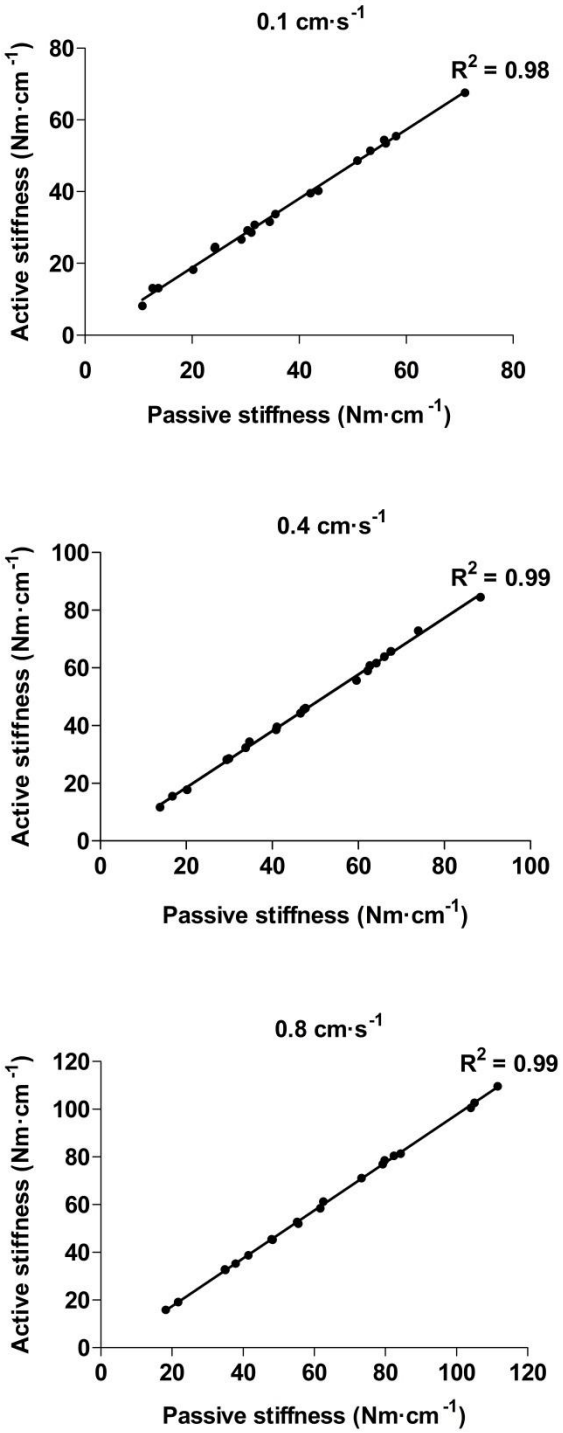

Figure 3.2. Relationship between tendon stiffness measured during the active and passive methods at 0.1, 0.4 and 0.8 cm·s^{-1} (top to bottom) measured by Pearson's correlation coefficient.

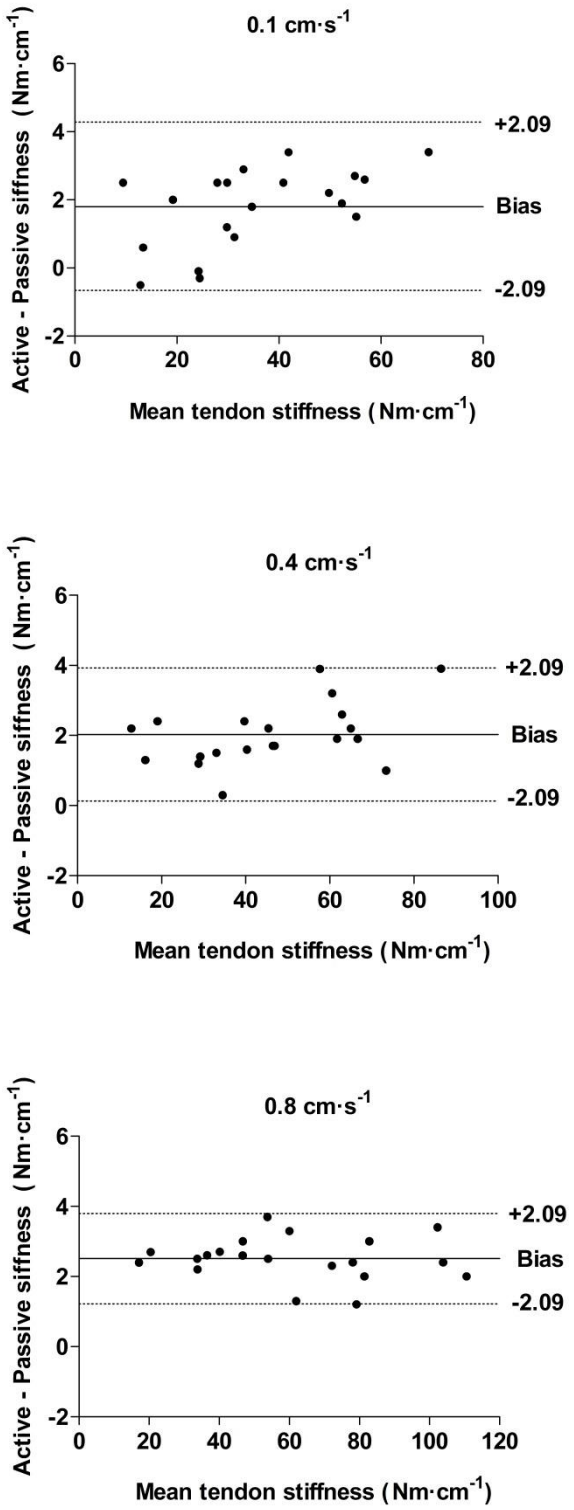

Figure 3.3. Bland and Altman plot. Differences (Active method–Passive method) plotted against the mean, at strain-rates of 0.1, 0.4 and 0.8 $cm \cdot s^{-1}$ (top to bottom).

The limits of agreement and confidence intervals are shown in Table 3.1. All data points for 0.1 and 0.8 cm·s^{-1}, and 95% of data points for 0.4 cm·s^{-1} fell within the 95% confidence intervals. The intraclass correlation coefficients were 0.99, 0.99 and 0.98, at 0.1, 0.4 and 0.8 cm·s^{-1}, respectively ($p < 0.001$ for all correlations).

Table 3.1. Limits of agreement (L of A) for strain-rates 1, 4 and 8 mm·s^{-1}

	Bias	**SE[a]**	**95% CI[b]**	**Random error**	**SE[a]**	**95% CI[b] for lower L of A[c]**	**95% CI[b] for upper L of A[c]**
0.1 cm·s^{-1}	1.81	0.26	1.26-2.36	2.47	0.46	-1.61-0.30	3.32-5.23
0.4 cm·s^{-1}	2.03	0.20	1.44-2.45	1.90	0.35	-0.61-0.86	3.19-4.66
0.8 cm·s^{-1}	2.51	0.14	2.22-2.80	1.29	0.24	0.72-1.72	3.30-4.31

[a] Standard error

[b] Confidence intervals

[c] Limits of agreement

Finally, the main effect for strain-rate on tendon stiffness was significant ($F_{1, 19} = 48.90$, $p < 0.01$). *Post-hoc t*-tests revealed that Achilles tendon stiffness was significantly greater at 0.8 cm·s^{-1} than at 0.4 cm·s^{-1} and 0.1 cm·s^{-1}. Further, this measure was greater at 0.4 cm·s^{-1} when compared to 0.1 cm·s^{-1} ($p < 0.001$). For both methods, tendon stiffness increased linearly with increasing tendon stiffness (Figure 3.4). By experimental design, both methods exhibited perfect correlations between tendon stiffness and strain-rate derived from interpolated data. However, even correlations obtained from raw data showed values close to 1. The specific relationships between

tendon stiffness and strain-rate derived from the interpolated data are given in the equations below:

Passive: Tendon Stiffness ($Nm \cdot cm^{-1}$) = 3.65 (Strain-rate ($mm \cdot s^{-1}$)) + 32.78

Active: Tendon Stiffness ($Nm \cdot cm^{-1}$) = 3.55 (Strain-rate ($mm \cdot s^{-1}$)) + 31.11

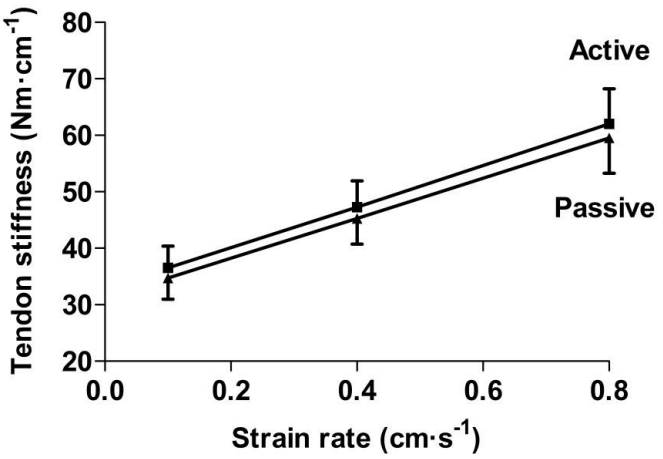

Figure 3.4. Change in tendon stiffness with increasing strain-rate at 0.1, 0.4 and 0.8 $cm \cdot s^{-1}$ for the active (squares) and passive (triangles) methods.

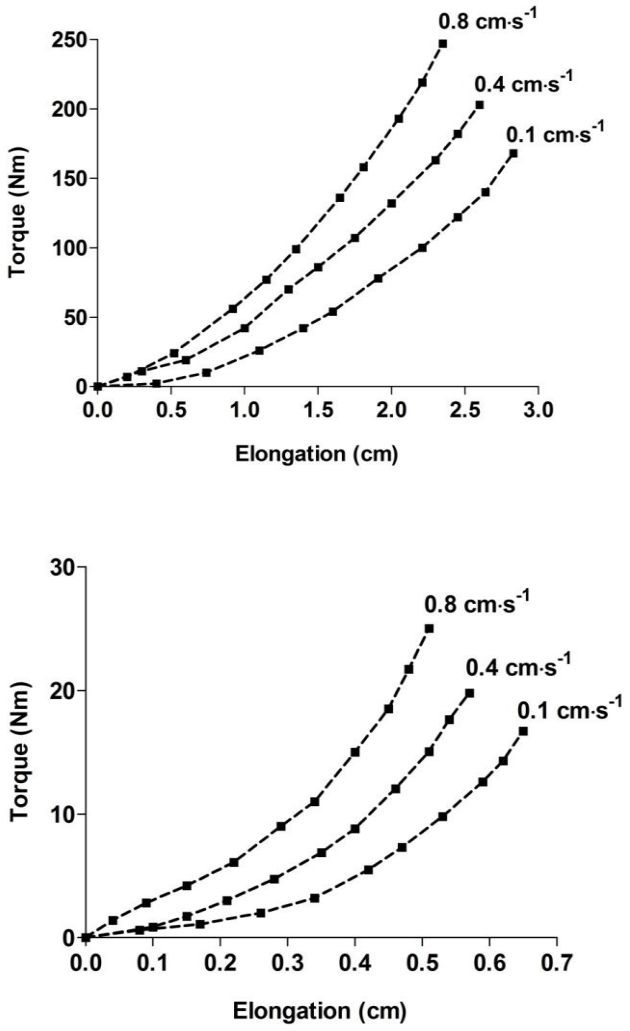

Figure 3.5. Ankle torque and Achilles tendon elongation during an isometric maximal voluntary contraction manoeuvre (top) and during passive dorsiflexion (bottom) at strain rates 0.1, 0.4 and 0.8 cm·s^{-1}

3.4 Discussion

The purpose of this study was to investigate the agreement between Achilles tendon stiffness obtained from the active and passive methods across different tendon strain-rates. We found that (1) the active method produced greater stiffness values than the passive method across all strain-rates, (2) in spite of this difference, agreement existed between the two methods across all strain-rates, and (3) tendon stiffness increased linearly across strain-rates for both methods.

Tendon stiffness is dependent on both the level of torque (Rigby *et al.*, 1959) and the tendon's strain-rate (Pearson *et al.*, 2007). Therefore, in the present study we matched both torque and strain-rate for both methods. Regardless of this, tendon stiffness values obtained from the active method were 6% higher than those obtained from the passive method at all strain-rates.

The systematic differences between methods could be explained by both stress-relaxation properties of the tendon, or the absence of moment arm measurements. First, although tendons were pre-conditioned by means of maximum voluntary contraction manoeuvres prior to the testing, the exact time course of stress-relaxation recovery for the *in vivo* tendon is not clear. A tendon not sufficiently pre-conditioned may have undergone more stress-relaxation in the passive trials as the tendon is stretched through its full dorsiflexion range, compared to the shorter duration active trials, resulting in differing torque levels and therefore stiffness values. However, both *in vitro and in vivo* studies have demonstrated that pre-conditioning alters the properties of the tendon for >30 minutes (Vidik, 1973; Kay & Blazevich, 2009), suggesting that tendons would still have been sufficiently pre-conditioned following the maximal contractions and rest period used in the current study. Second, due to the nature of the tendon stiffness

measures used in this study, moment arm calculations were neglected. As moment arm would have changed depending on joint angle and also contraction state, this may have also accounted for the systematic bias between methods. However, given that the actual difference between methods was minimal (2.1-7%) and within the coefficient of variation for tendon stiffness measures (6.5%), suggests that the physiological significance of both stress-relaxation and moment arm would also be minimal. Future research should specifically aim to determine the cause of this bias between methods.

Although there was an absolute difference in tendon stiffness between the two methods, the intraclass correlation coefficients and the limits of agreement indicated agreement between the methods. All data points for 0.1 and 0.8 $cm \cdot s^{-1}$, and 95% of data points for 0.4 $cm \cdot s^{-1}$ fell within the 95% confidence intervals. Based on this analysis of limits of agreement, our results imply that a participant's (active) tendon stiffness could be over or under-estimated by 2.1%, 4.0% and 7.0% using the passive method, for strain-rates 0.8, 0.4 and 0.1 $cm \cdot s^{-1}$, respectively. This degree of error will be acceptable in many situations in which tendon stiffness obtained from both methods is compared to each other.

The agreement between the passive, compared with the more commonly used active method for measuring Achilles tendon stiffness has practical implications. In contrast to the active method, the passive method allows for the estimation of global muscle (in addition to tendon) stiffness (Morse *et al.*, 2008). Further, the passive method may be more suitable for clinical populations who may not be able to perform maximal voluntary contraction manoeuvres reliably. Therefore our findings have implications for researchers wishing to calculate muscle and tendon stiffness independently and who seek to investigate muscle and tendon mechanics in clinical populations. In such cases,

the passive method may be used. One limitation to this recommendation is that the passive method only allows for stiffness calculations at relatively low torque levels.

Before concluding that both methods are in "good" agreement, it is important to bear in mind that the quality of agreement is context-dependent. For example, when comparing stiffness values obtained by different methods from the literature, any observed differences need to be interpreted relative to the differences reported here in our direct comparison. Similarly, when using different methods to obtain Achilles tendon stiffness (e.g., different methods for different populations), researchers will need to account for the differences reported here. It may be the case that differences in stiffness values between two populations are smaller than the difference between methods, in which case using different methods for different populations would not be advisable.

Both active and passive methods have been used to investigate tendon stiffness resulting in a large range of reported tendon stiffness values (Kay & Blazevich, 2009; Kubo *et al.*, 2002b; Mizuno *et al.*, 2011; Morse *et al.*, 2008; Waugh, *et al.*, 2012). For example, Morse *et al.* (2008) used the passive method, whilst Kubo *et al.* (2002b) used the active method. The tendon stiffness values by Kubo *et al.* (2002b) are 56% greater than those reported by Morse *et al.* (2008). Mizuno *et al.* (2011) speculated that the difference in tendon stiffness observed between the two studies was the result of methodological differences. The results of the present study support this speculation. However, whilst methodological differences partially explain these discrepancies in the literature, different torque levels used in these two studies are likely to be another contributor.

Our results further highlight that strain-rate affects tendon stiffness. Pearson *et al.* (2007) found that in the patellar tendon stiffness increased as the duration of contraction

increased. This was attributed to the composition of tendinous tissue, which includes collagen fibres that exhibit viscoelastic properties, which causes it to deform slowly in a non-linear fashion, and respond to the rate of loading. Our data extend these results on several levels. First, they demonstrate that the results by Pearson *et al.* (2007) who, investigated the patellar tendon, are transferrable to the Achilles tendon. Second, our results specifically quantify the relationship between tendon stiffness and strain-rate. In particular, we found that stiffness increases linearly by approximately 41% with an eightfold increase of strain-rate. This velocity-dependent increase in tendon stiffness is independent of the method being employed. This is similar to previously reported values *in vivo* and *in vitro*. For example, Pearson *et al.* (2009) reported an increase of approximately 43% in the patellar tendon from a slow to fast stretch. In the tibialis anterior of adult rabbits, Taylor, Dalton, Seaber and Garrett (1990) reported an increase of 40-44% with increasing stretch velocity, which supports the findings here of the *in vivo* Achilles tendon.

Our findings have implications when comparing tendon stiffness values reported in the literature, as different studies employ different protocols. Hansen, Bojsen-Moller, Aagaard, Kjaer and Magnusson (2006), for example, measured the mechanical properties of the patellar tendon under loading conditions, but used a slow ramped force rise (~10 s), which resulted in a relatively low strain-rate. Kubo *et al.* (2010) and Maganaris and Paul (1999) used shorter durations of force rise (~5 s and ~1 s, respectively). In light of our findings, the velocity-dependence of stiffness needs to be taken into consideration when comparing stiffness values reported in these studies. Our results further highlight the importance for future studies examining tendon mechanical properties to adopt a standard protocol and/or to report tendon strain-rates in order to allow comparisons between studies to be made more readily.

CHAPTER 4: MECHANICAL AND MATERIAL PROPERTIES OF THE *TRICEPS SURAE* MUSCLES AND ACHILLES TENDON IN CHILDREN WITH SPASTIC CEREBRAL PALSY AND TYPICALLY DEVELOPING CHILDREN

4.1 Introduction

Spastic CP results from damage to the developing brain before, during or shortly after birth (Reddihough & Collins, 2003). During maturation, secondary musculoskeletal adaptations occur, which can affect the mechanical and material properties of muscles and tendons. Previous research has primarily focused on the atypical development of the muscle in children with spastic CP compared to their TD peers. There is consistent evidence of a shorter gastrocnemius muscle belly length (Malaiya, *et al.*, 2007; Wren *et al.*, 2010) reduced muscle volume (Malaiya *et al.*, 2007), increased connective tissue (Booth *et al.*, 2001), and increased muscle and fascicle stiffness (Barber *et al.*, 2011a; Fridén & Lieber, 2003; Smith *et al.*, 2011). The adaptations of the tendon in children with spastic CP are less well established. However, the tendon also plays an integral role in movement, alongside the muscle. The mechanical properties of the tendon govern the transfer of muscular forces to the bone, and the storage and return of elastic energy during functional activities. It is possible that the aforementioned CP-related changes in the mechanical properties of the muscle, result in secondary mechanical adaptations of the tendon, which have implications for functional movement. Thus, the overall goal of this study was to characterise the mechanical properties of the tendon in children with spastic CP, and compare them to TD children.

Since the muscle and tendon are closely integrated in the production of movement, the mechanical properties of both structures in children with spastic CP should not be considered independent to one another. Importantly, the length and compliance of the Achilles tendon can affect the force-generating capacity of the muscles (Lichtwark,

Bougoulias & Wilson, 2007; Lichtwark & Wilson, 2008). For example, certain tendon compliance may allow muscle fibres to operate close to an optimal length and at relatively low shortening velocities, thereby aiding force production (Lichtwark *et al.*, 2007; Lichtwark & Wilson, 2006). Since movement is governed by both the stiffness of the muscle and tendon, it is important to understand how tendon mechanical properties change in concert with changes in the muscle. Therefore, the first specific aim of this study was to compare *triceps surae* muscle and Achilles tendon stiffness in children with spastic CP and TD children.

Tendon stiffness is determined by both its dimensions and material properties. Regarding the former, a long tendon with a small cross-sectional area will be more compliant than a short tendon with a large cross-sectional area. Regarding the latter, the tendon's material properties are independent of its dimensions and depend primarily on collagen fibre type, size and organisation (Silver, Freeman & Seehra, 2003). One way to differentiate between the dimensional and material properties of the tendon is to calculate Young's modulus (*E)*, which can be thought of as tendon stiffness normalised by its dimensions (i.e., E = tendon stiffness × resting length/cross-sectional area). It has previously been demonstrated that in TD children, tendon stiffness increases with maturation (Waugh *et al.*, 2012) due to changes in both dimensions and material properties. The adaptations of the material properties in particular, are thought to occur mainly in response to chronic mechanical loading, predominantly due to an increase in muscle size and force (Kubo *et al.*, 2001b). Within the context of CP, mechanical loading from increased stiffness of the spastic muscle may allow typical growth of the tendon's material properties. However, dimensional tendon differences in children with spastic CP have previously been reported. For example, the Achilles tendon has been shown to be longer than in TD children, and with a smaller cross-sectional area (Gao *et*

al., 2011), presumably as an adaptation to the atypical shortening of the muscle belly (Barber *et al.*, 2012; Wren *et al.*, 2010). Due to the aforementioned dependence of tendon stiffness on dimensions, one might expect these dimensional changes to cause a greater tendon compliance in children with spastic CP. However, Barber *et al.* (2012) did not find any differences in tendon stiffness between children with CP and TD children, which could indicate concomitant alterations in tendon cross-sectional area and maturation of the tendon's material properties, independent of dimensions. Understanding the CP-related changes in the dimensional and material properties to tendon stiffness is an important prerequisite to better understand movement efficiency and control in children with spastic CP. Therefore, the second specific aim was to compare dimensions and material properties of the Achilles tendon between children with spastic CP and TD children.

Another mechanical property of the tendon is its viscoelasticity. Tendons recoil elastically after stretch, but they also act viscously; meaning that they become stiffer at higher loading rates (Knudson, 2007, pp. 73; Le Veau 1992; Pearson *et al.*, 2007; Theis, Mohagheghi & Korff, 2012b). Previously, we have shown that the slope describing the strain-rate-stiffness relationship is lower in TD children than in adults (Theis, Mohagheghi & Korff, 2012a). This has implications for the transfer of force to the muscle, and may partly explain differences in movement efficiency between adults and children. Within the context of CP, an abnormal strain-rate response could have important implications in the interpretation of clinical tests of spasticity. For example, a widely accepted definition of spasticity is that of Lance (1980, pp. 485), which defines spasticity as a "velocity-dependent increase in muscle tone". In clinical practice, the Modified Ashworth Scale uses this definition as a basis for the identification of spasticity in the muscle (Bohanon & Smith, 1987). Implicit in this test is the assumption

that an increased joint stiffness at faster movement speeds is reflective of increases in muscle stiffness (and is therefore a neural phenomenon). However, if the aforementioned strain-rate-stiffness relationship in the tendon (Knudson, 2007, pp.73; Theis *et al.*, 2012b) was exaggerated in children with CP, then perceived abnormalities in joint stiffness may partially be explained by typical viscoelastic (i.e., passive) properties of the Achilles tendon. Thus, from a clinical perspective, it is vital to understand the strain-rate-tendon stiffness relationship in children with CP, as it could lead to more differentiated (and therefore more meaningful) assessments of spasticity. Thus, the third aim of the study was to determine the strain-rate response of the Achilles tendon in children with spastic CP, compared to TD children.

4.2 Methodology

4.2.1 Participants

Ten children with clinically diagnosed diplegic or quadriplegic spastic CP (5 males, 5 female; age 11.4 ± 3.0 y), and ten age-matched TD children (5 males, 5 female; age 12.0 ± 2.9 y) participated in this study. Six children with CP were classified as level III and four children with CP were classified as level IV on the Gross Motor Classification system (GMFCS) (Palisano *et al*., 1997) as assessed by a physiotherapist. Written consent was obtained from all parents/guardians and written assent was obtained for all children, prior to participation. The study was approved by the Research Ethics Committee at Brunel University and relevant local NHS Ethics Committees (see Appendix VI).

4.2.2 Protocol and Instrumentation

Ankle torque was measured using an isokinetic dynamometer. For the TD children, we used a Biodex dynamometer system (Biodex Medical Systems, New York, USA). For logistical reasons, children with CP were tested using a Cybex dynamometer system (Cybex Norm, Lumex, Ronkonkoma, NY, USA). To ensure that any between group differences would not be confounded by differences in the measurement modalities, we established the comparability of the data obtained from both systems. For this purpose, we compared angular velocity data obtained at 1 and 30 $deg \cdot s^{-1}$. The mean deviation between systems was 0.60% and 0.64% for 30 $deg \cdot s^{-1}$ and 1 $deg \cdot s^{-1}$, respectively. The mean deviation between systems for torque, measured under constant load conditions (22.3 N and 44.6 N), was 0.76% and 0.84%, respectively. These differences were deemed to be negligible.

To measure ankle torque, participants were seated on the dynamometer chair. The right knee was straightened to full extension for the TD group, and for the CP group the knee was straightened as much as possible, which was on average 7.0 (± 2.0) deg from full extension across participants. The relative hip angle was set to 85 deg for both groups. The lateral malleolus of the right ankle was aligned with the rotational axis of the dynamometer arm. The dynamometer footplate was positioned perpendicularly to the tibia, and this was considered to be 0 deg. Stabilisation straps were applied tightly over the foot, thigh and chest to minimise movement of the upper body or leg.

The participants' available ROM was determined by dorsi- and plantarflexing the foot at 10 deg·s^{-1}, until any discomfort was reported. This occurred between 6.3 ± 0.7 deg dorsiflexion and 20.3 ± 4.0 deg of plantarflexion for the CP group, and 19.0 ± 8.3 deg dorsiflexion and 23.1 ± 3.6 deg of plantarflexion for the TD group. The dynamometer system was then set to apply passive angular rotations to the right ankle joint at constant angular velocities of 1, 10 and 30 deg·s^{-1} within the available ROM. Participants were instructed to relax the muscles of the lower limb during the passive rotations. Three rotations were recorded at each angular velocity, and the order of angular velocities was randomised. The electrical activity of the medial gastrocnemius (EMG) was monitored throughout the rotations (Trigno wireless system, Delsys Inc., Ltd., Boston, USA). Both torque and EMG data were sampled at 1000 Hz. Torque data were filtered using a low-pass, fourth-order, zero-lag Butterworth filter with a cut-off frequency of 14 Hz as determined by residual analysis.

Muscle and tendon elongation were measured as the displacement of the medial gastrocnemius muscle-tendon junction throughout the passive rotations. The muscle-tendon junction was visualised using B-mode ultrasonography (Megas GPX, Esaote,

Italy; 45 mm Linear array probe, 10 MHz transducer scanning). The probe was placed perpendicularly to the skin surface above the muscle-tendon junction and orientated to clearly display the aponeuroses separating the medial gastrocnemius and soleus muscles. The probe was fixed in position using a custom-made holder. A 2 mm wide strip of echoabsorptive tape, placed on the skin in contact with the probe, provided a reference to which any probe movement could be identified. Peak Motus tracking software (Peak Performance, Cambridge, UK) was used to manually digitise 2D coordinates of the muscle-tendon junction. Extensive practise of the manual digitisation procedure during pilot testing allowed a high reliability to be obtained (coefficient of variation = 4.1%). Digitised muscle-tendon junction position data were filtered using a low-pass fourth-order zero-lag Butterworth filter with a 3.25 Hz cut-off frequency as determined by residual analysis.

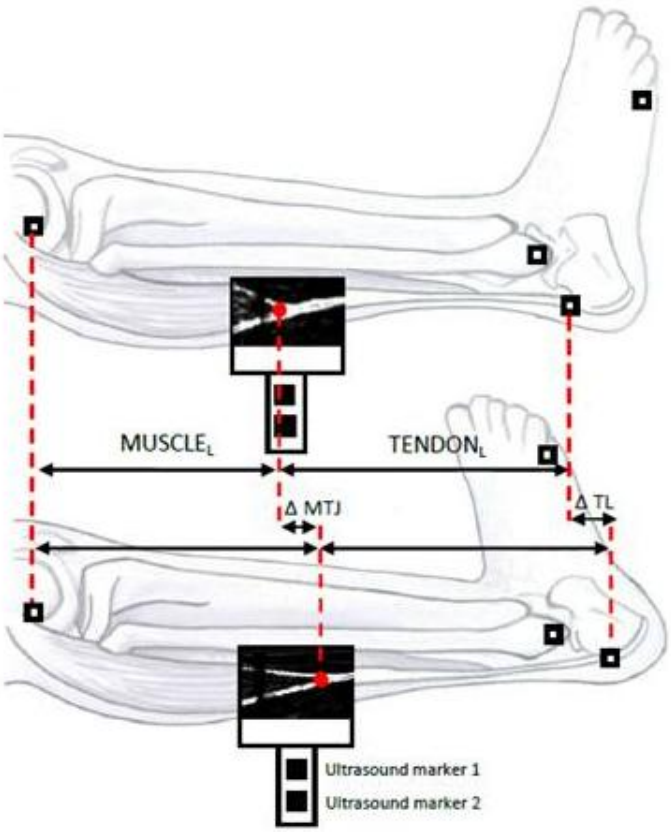

Figure 4.1. Experimental setup for measuring muscle and tendon lengths.

4.2.3 Derivation of dependent variables

Using the coordinates of two markers from the handle of the ultrasound probe, combined with the coordinates of the muscle-tendon junction in the ultrasound image, the global 2D position of the muscle-tendon junction was calculated in the sagittal plane. Tendon length was defined as the linear distance from its insertion on the calcanei to the medial gastrocnemius muscle-tendon junction. Medial gastrocnemius muscle length was defined as the distance between the medial femoral epicondyle and the global 2D coordinates of the muscle-tendon junction (Figure 4.1). Thus, both medial gastrocnemius and Achilles tendon were modelled as straight lines, using custom written analysis software (Matlab v7.14, MathWorks, Cambridge, UK) (see Appendix V). The resting length of the Achilles tendon was calculated as the length at which there

was a sustained increase in ankle torque above zero (Barber *et al.*, 2012), and thus, where tendon slack had been taken up. Tendon resting length was expressed in absolute terms at this point, and was also normalised to resting muscle-tendon unit length.

Tendon stiffness was calculated as the change in ankle torque divided by the corresponding change in Achilles tendon length. Ankle torque was calculated over the range, neutral (0 deg) to maximum dorsiflexion. An estimate of "total" *triceps surae* muscle stiffness was derived using the method described by Morse *et al.* (2008). For this purpose, the change in passive ankle torque was divided by changes in medial gastrocnemius muscle length.

Muscle and tendon stiffness for both groups was determined during dorsiflexion in the 10 deg·s^{-1} trial. Stiffness was calculated relative to each participant's maximal force, subsequently referred to as stiffness$_{REL}$. Specifically, we determined the slope of the force-elongation curve, between 20-80% of each participant's peak torque. This interval was chosen for two reasons. First, children with spastic CP have been reported to have a greater slack length, and greater "toe region". Thus, using 20% as the minimum range allowed stiffness to be calculated outside of this region. Second, it has been reported that towards maximum dorsiflexion, passive elastic structures such as ligaments, connective tissue and skin, contribute more to passive resistance (Abellaneda, Guissard & Duchateau, 2009). To minimise the influence of these structures, 80% was chosen as the maximal range. Tendon stiffness measured over the range of 20-80% gave a coefficient of variation of 6.0%. This was calculated from tendon stiffness in six children with spastic CP, on three separate occasions.

Tendon stiffness was also calculated in a common region of torque (subsequently referred to as $stiffness_{COM}$) to allow inferences to be made about the passive mechanical properties of the tendon between groups. For this purpose, we used the range, which corresponded to 20% and 80% of peak torque from the second weakest participant (corresponding to an absolute torque range of 1.1 - 4.5 Nm). For this analysis, we excluded the weakest participant, as 20% of their peak torque was lower than the minimum torque for some of the stronger participants.

The tendon's Young's Modulus was calculated by multiplying tendon $stiffness_{COM}$ by its resting length and dividing by tendon cross-sectional area. For this purpose, we used a modified silicon ultrasound gel pad (Aquaflex 2 × 9 cm, Parker Labs Inc., NJ, USA) to take three discrete ultrasound images of the Achilles tendon cross-sectional area, approximately 30 mm proximally to the tendon insertion (Magnusson *et al.*, 2001). The tendon perimeter was traced using specialist software (Esaote, Italy) (Figure 4.2), and the image with the smallest cross-sectional area was used for further analysis. The smallest cross-sectional area was chosen to represent the highest stress values of the tendon, and was compared between groups. The inter-test reliability, determined from three separate trials from three individuals, was 3.7%. The intra-observer reliability for determining the cross-sectional area, obtained by analysing 30 images three times, was 3.5%.

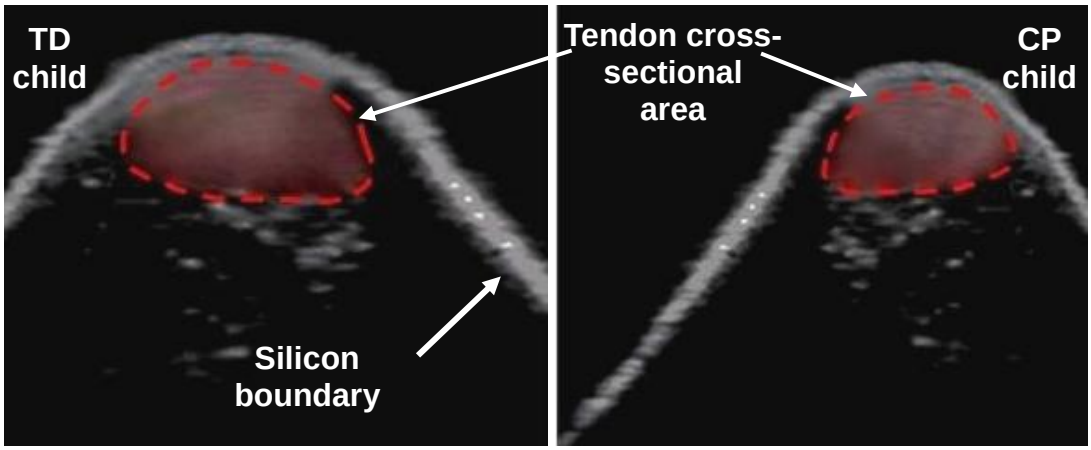

Figure 4.2. Measurement of Achilles tendon cross-sectional area

Lastly, relative tendon stiffness ($stiffness_{REL}$) was also calculated from the slope of the torque-elongation curve, corresponding to 20-80% of each participant's peak force. $Stiffness_{REL}$ was plotted against angular velocities of 1, 10 and 30 $deg \cdot s^{-1}$. The tendon strain-rates corresponding to these angular velocities were 0.12 ± 0.02, 0.43 ± 0.04, 0.81 ± 0.03 $cm \cdot s^{-1}$ for the CP group and 0.14 ± 0.03, 0.46 ± 0.10, 0.88 ± 0.09 $cm \cdot s^{-1}$ for the TD group, respectively. A linear regression line was fitted through the strain-rate-stiffness relationship to calculate the slope of the line.

4.2.4 Statistical analysis

To address the first specific aim of this study, we determined differences in muscle and tendon $stiffness_{REL}$ between groups using a mixed design repeated measures ANOVA. Here, we tested for a structure (muscle vs. tendon) × group (CP vs. TD) interaction. In case of significance, Bonferroni corrected *t*-tests were performed to locate any between group (independent *t*-test) differences and between structure (paired *t*-tests) differences.

Regarding the second specific aim, we performed a MANOVA on stiffness$_{COM}$, tendon cross-sectional area, absolute resting tendon length and resting tendon length expressed as a percentage of muscle-tendon unit length, with Bonferroni corrected *t*-tests. One further independent *t*-test was performed on Young's modulus. In addition to these statistical tests, we also determined the effect sizes (Cohen's D) to describe group differences for all dependent variables.

With regards to the third specific aim of the study, a mixed design repeated measures ANOVA was performed to test for a main effect of strain-rate on tendon stiffness$_{REL}$, and a group × strain-rate interaction. In case of main effect significance, two 1 × 3 repeated ANOVA's were performed. Following significance, paired *t*-tests with Bonferroni correction were performed to determine within-group differences in strain-rate. In case of a significant interaction, independent *t*-tests were performed to compare tendon stiffness$_{REL}$ between groups at each strain-rate. One further independent *t*-test was used to compare the slope of the strain-rate-stiffness$_{REL}$ relationship between groups.

4.3 Results

Results from the first ANOVA revealed a significant group × structure interaction effect ($F_{1, 18}$ = 8.04, p < 0.05). The main effect of structure on stiffness$_{REL}$ was non-significant ($F_{1, 18}$ = 1.88, p = 0.19). Follow up independent samples *t*-tests revealed that muscle stiffness$_{REL}$ was significantly greater in the CP group compared to the TD group (t_{18} = 2.37, p < 0.029), with no difference in tendon stiffness$_{REL}$ (t_{18} = 0.34, p = 0.74). Follow up paired samples *t*-tests revealed muscle stiffness$_{REL}$ was significantly greater than tendon stiffness$_{REL}$ in the CP group (t_9 = 2.98, p < 0.015), whilst this effect was non-significant in the TD group (t_9 = 1.03, p = 0.33) (Figure 4.3).

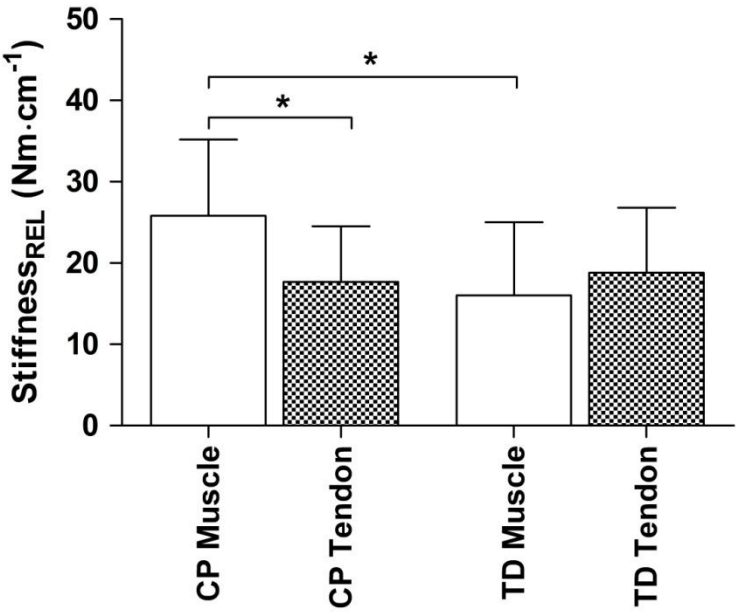

Figure 4.3. *Triceps surae* muscle and Achilles tendon stiffness$_{REL}$ in CP and TD groups (values are mean ± SD, *p = < 0.05).

The MANOVA procedure revealed significant differences in tendon cross-sectional area, resting tendon length and stiffness$_{COM}$ by group (Hotelling's T^2 = 1.60, $F_{4, 14}$ =

5.59, $p < 0.01$). Tendon cross-sectional area was significantly smaller in the CP group compared to the TD group ($t_{18} = -5.16$, $p < 0.01$). In addition, normalised resting tendon length was significantly greater in the CP group compared to the TD group ($t_{18} = 2.72$, $p < 0.05$). In contrast, absolute resting tendon length and tendon $stiffness_{COM}$ were not significantly different between groups ($t_{18} = 0.13$, $p = 0.90$; $t_{17} = -1.05$, $p = 0.31$), respectively. In addition, there were no differences in Young's modulus between groups ($t_{17} = 0.44$, $p = 0.67$, Effect size = -0.21) (Figure 4.4) (Table 4.1).

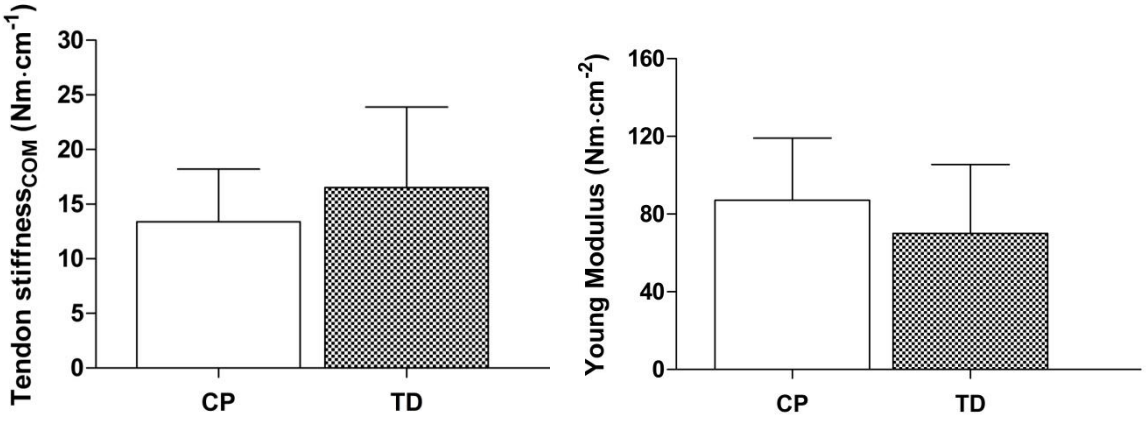

Figure 4.4. Achilles tendon $stiffness_{COM}$ (left figure) and Young's modulus (right figure) in children with CP compared to TD groups (values are mean ± SD).

The strain-rate by group ANOVA on $stiffness_{REL}$ revealed a significant interaction ($F_{2, 29} = 6.13$, $p < 0.05$). The main effect of strain-rate was also significant ($F_{2, 29} = 24.35$, $p < 0.01$). Independent samples *t*-tests revealed no significant difference between groups at any time points, 1, 10 and 30 deg·s^{-1}, respectively (1 deg·s^{-1}: $t_{18} = 0.16$, $p = 0.88$; 10 deg·s^{-1}: $t_{18} = 0.34$, $p = 0.74$; 30 deg·s^{-1}: $t_{18} = -1.25$, $p = 0.28$). To investigate the source of the strain-rate by group interaction, we determined the effect sizes (Cohen, 1988)

quantifying the group difference at each strain-rate. This analysis revealed that the group differences at the low velocities were small (effect size = 0.04 and 0.16 at 1 deg·s^{-1} and 10 deg·s^{-1}, respectively) whilst the group effect was moderate at 30 deg·s^{-1} (effect size = -0.29) (Figure 4.5).

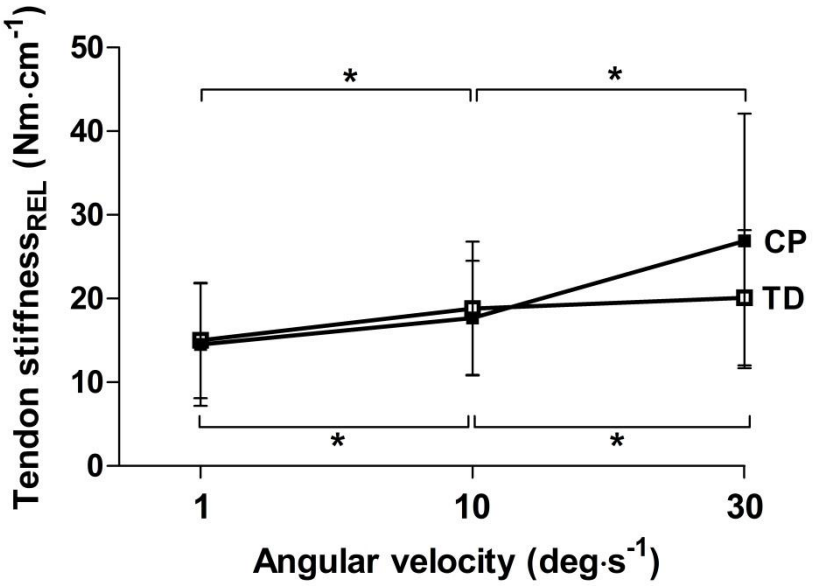

Figure 4.5. Achilles tendon stiffness$_{REL}$ measured at strain-rates 1, 10 and 30 deg·s^{-1} in CP compared to TD children (values are mean ± SD, *p = < 0.05).

The group by strain-rate interaction also expressed itself in different strain-rate effects between groups. *Post-hoc* paired samples *t*-tests revealed that in the CP group, Achilles tendon stiffness$_{REL}$ was significantly greater at 30 deg·s^{-1}, than at 10 deg·s^{-1} and 1 deg·s^{-1} (p < 0.01). In the TD group, Achilles tendon stiffness$_{REL}$ was greater at 10 deg·s^{-1} than at 1 deg·s^{-1} (p < 0.01), but not different between 10 deg·s^{-1} and 30 deg·s^{-1} (p > 0.05) (Figure 4.5). Finally, the slope of the strain-rate stiffness$_{REL}$ curve was significantly steeper in the CP group (Slope = 6.2 ± 4.1 Nm·cm^{-1}/deg·s^{-1}, R^2 = 0.85 ± 0.11) compared to the TD group (Slope = 2.5 ± 1.4 Nm·cm^{-1}/deg·s^{-1}, R^2 = 0.70 ± 0.3) (t_{18} = 2.67, p < 0.05).

Table 4.1. Descriptive characteristics of the variables associated with calculating the mechanical properties of the muscle and tendon. Calculations are taken from 10 deg·s^{-1} trial. (Values are mean ± SD).

	TD	**CP**	**Effect size (Cohen's D)**
DF angle (deg)	19.0 ± 8.3	6.3 ± 0.7	1.46
Tendon cross-sectional area (cm^2)	4.95 ± 0.7	3.54 ± 0.5	1.51
Resting tendon length (cm)	17.5 ± 2.6	17.7 ± 4.0	-0.05
Normalised resting tendon length (%)	49.9 ± 6.1	55.8 ± 2.9	-1.05
Peak ankle torque (Nm)	19.4 ± 14.0	11.1 ± 5.0	0.61
Muscle stiffness$_{REL}$ (Nm·cm^{-1})	16.0 ± 9.0	25.8 ± 9.4	-0.95
Tendon stiffness$_{REL}$ (Nm·cm^{-1})	18.8 ± 8.0	17.7 ± 6.8	0.16
Tendon stiffness$_{COM}$ (Nm·cm^{-1})	14.9 ± 6.7	12.2 ± 4.4	0.48
Young's modulus (Nm·cm^{-2})	70.1 ± 35.4	87.2 ± 32.0	-0.25

4.4 Discussion

The overall goal of this study was to characterise the mechanical properties of the tendon in children with spastic CP, and compare them to TD children. The first purpose of this study was to compare *triceps surae* muscle and Achilles tendon stiffness in children with spastic CP and TD children. For this purpose, we calculated muscle and tendon stiffness relative to each participant's maximum force. The results showed that *triceps surae* muscle stiffness was significantly greater in children with CP compared to TD children. This result is consistent with previous findings of spastic muscle stiffness (Barber *et al.*, 2011a; Friden & Lieber, 2003; Smith *et al.*, 2011). Potential mechanisms underlying these spasticity-related differences in muscle stiffness include a reduced number of in-series sarcomeres (Smith *et al.*, 2011) and remodelling of intra- and extra-muscular connective tissue (Booth *et al.*, 2001).

More interestingly, we also found that children with CP had greater muscle compared to tendon stiffness, whereas in TD children the stiffness between the two structures was not different. This difference in the muscle to tendon stiffness ratio may have important implications for movement control. For example, in a healthy system, tendon stiffness is tuned to optimise muscle fibre shortening velocity and minimise muscle activation (Lichtwark & Wilson, 2008). The tendon also has the ability to store and release elastic energy for more efficient movement during locomotion (Alexander, 1990). In children with spastic CP, a greater muscle to tendon stiffness ratio may partly explain the high mechanical energy cost and greater mean energy expenditure experienced during walking (Rose *et al.*, 1990; Olney, Costigan & Hedden, 1987).

We also showed that tendon stiffness, when expressed relative to each participant's force generating capacity, was not different between TD and CP. This finding is

consistent with Barber *et al.* (2012), who demonstrated no differences in Achilles tendon stiffness, measured in absolute terms, in children with CP compared to TD children. As such, the authors state that differences in stiffness were mainly explained by differences in ankle torque. In the present study, we also expressed stiffness relative to the individual's force capacity, which provides information about muscle and tendon stiffness as experienced in normal motor tasks. To understand differences in the mechanical properties of the tendon, however, it is necessary to also quantify tendon stiffness over a common force range (i.e., $stiffness_{COM}$).

Normalising tendon stiffness by its dimensions allows us to tease out eventual contributions of dimensions and material properties to tendon stiffness in CP. Therefore, the second specific aim was to compare dimensions and material properties of the Achilles tendon between children with spastic CP and TD children. Our results demonstrate that absolute Achilles tendon stiffness was not significantly different between children with spastic CP and TD children. This result is in contrast to the findings by Barber *et al.* (2012) and Gao *et al.* (2011), and is likely to be explained by between subject differences in limb lengths. Support for this explanation is provided by our results that when expressed as a percentage of muscle-tendon unit length, the Achilles tendon was significantly longer in CP compared to TD children. The results also revealed that children with spastic CP had a smaller tendon cross-sectional area, with no group differences in resting tendon length or Young's modulus. A smaller cross-sectional area in CP has previously been reported (Gao *et al.,* 2011), but it is not clear as to the mechanisms, which alter tendon dimensions. One speculation could be that tendon dimensions in children adapt in line with bone growth, which potentially develops slower in children with CP, due to a lack of weight-bearing activity (Samson-Fang & Stevenson, 1998). In contrast to Barber *et al.* (2012) and Gao *et al.* (2011), we

found that Achilles tendon length was not longer compared to the TD group. This may be due to small variations in limb lengths between groups, or the younger and more severely affected participants used in our study. For example, the participants used by Barber *et al.* (2012) were likely to be more ambulant and perhaps with more opportunity for maturational adaptation than the participants in the present study. In addition, a greater sample size may be needed to detect changes in tendon stiffness in this population. Theoretically, a smaller cross-sectional area should make the tendon more compliant (Effect size = 0.48). However, the reduction may not have been strong enough to elicit significant changes in tendon stiffness. These changes could have been partially compensated for by a reverse change in Young's modulus (Effect size = -0.25), suggesting a different mechanism for alterations in the tendon's material properties compared to dimensions.

On the surface, the fact that Young's modulus was not different between the groups suggests that the integrity of the tendon's material properties is unaffected in children with spastic CP. These results suggest the increased spastic muscle stiffness may provide mechanical loading of the tendon, as is the case in TD children. The proposed mechanisms, based on findings from animal studies, suggest that both collagen turnover and the density of collagen fibrils may increase with loading (Woo *et al.*, 1980). Additionally, alterations in the crimp angle of collagen fibrils have also been shown to occur following loading in animal tendons (Wood, Cooke & Goodship, 1988). Whilst our results suggest that the intrinsic tendon structure is not different between CP and TD, they do not reject this speculation irrefutably. It could be the case that concomitant alterations in the microstructure of the tendon resulted in no overall observed changes in the material properties. For example, it could be the case that alterations in the integrity of the extracellular matrix (which may decrease tendon stiffness) are accompanied by a

maturational increase in collagen fibre size and density (which may increase tendon stiffness), resulting in no overall change in tendon stiffness. Future research should specifically address these questions.

The third purpose of the study was to describe the strain-rate response of the Achilles tendon in children with spastic CP compared to TD children. Both groups showed an increase in absolute tendon stiffness with increasing strain-rate. This is consistent with previous studies of adults in both the patellar tendon (Pearson *et al.*, 2007) and the Achilles tendon (Theis *et al.*, 2012b). Specifically, we found that the slope describing the strain-response was steeper in the CP group, indicating that at higher strain-rates (i.e., 30 deg·s^{-1}), tendon stiffness was greater in children with spastic CP compared to TD children. This could again be indicative of alterations in the tendon's material properties, which would alter the viscoelastic response of the tendon, without specifically affecting tendon stiffness. These results have important implications for the clinical test of spasticity. The fact that at 30 deg·s^{-1} tendon stiffness is markedly higher in children with CP compared to TD children needs to be taken into consideration when conducting tests which make the assumption that joint stiffness is reflective of muscle stiffness. This is particularly relevant as the tonic stretch reflex is elicited at 35 deg·s^{-1} in a spastic muscle (Thilmann *et al.*, 1991), and so tests for spasticity should not be conducted exclusively below this point.

4.4.1 Limitations and future research

It is important to recognise the limitations of the present study, in particular, the derivation of "muscle stiffness". Firstly, torque measured at the ankle is not only attributable to the *triceps surae* muscle-tendon unit, but also to other passive elastic structures - the contribution of which cannot be measured *in vivo*. Second, our

calculation of stiffness does not account for potential different contributions of the gastrocnemii and soleus to the total torque. In part, it could be the case that relative differences in the cross sectional areas of the individual muscles of *triceps surae* differ between children with CP and TD children (Elder *et al*., 2003; Lampe *et al*., 2006). Future research could address this by measuring the physiological cross-sectional of all *triceps surae* muscles, so that torque measured at the ankle joint could be attributed to each muscle. Whilst this would still neglect the contribution of passive elastic structures, incorporating any differences in muscle cross-sectional area may highlight greater between group differences.

It further assumes that all *triceps surae* muscles elongate equally to that of the medial gastrocnemius muscle. In children with spastic CP, Barber *et al.* (2011a) reported that soleus elongation was similar to that of the medial gastrocnemius; however, we cannot rule out the possibility of some systematic error in the calculation of muscle stiffness used in the present study. Lastly, modelling the muscle as a straight line does not take the actual individual muscle paths into consideration. From these limitations it becomes clear that our measure of muscle stiffness is, to a certain extent, a theoretical construct. However, we believe that within the context of this study our measure of "global muscle stiffness" provides important insights into the interactions between spasticity-induced differences in muscle and tendon properties.

4.4.2 Summary

The overall purpose of the study was to characterise the mechanical properties of the tendon in children with spastic CP compared to TD children. In line with previous results, we found that children with CP have stiffer muscles than TD children, and that tendon stiffness is not different between the groups. These results provide us with a

more differentiated understanding of spasticity-induced tendon mechanical properties. This knowledge needs to be taken into consideration when interpreting and treating movement abnormalities in children with CP. The fact that strain-rate-induced increases in tendon stiffness are more pronounced in children with CP compared to TD children have dramatic consequences for the interpretability of current clinical tests of spasticity.

CHAPTER 5: DOES ACUTE PASSIVE STRETCHING INCREASE MUSCLE LENGTH IN CHILDREN WITH CEREBRAL PALSY?

5.1 Introduction

Children with CP show increased muscle stiffness and reduced muscle length, which may contribute to reduced function. Stretching is commonly used in the treatment and management of children with spastic CP and is considered to be an important part of preventing or delaying the onset of contractures (National Institute for Health and Care Excellence, 2012). The assumptions made in clinical practice are that repeated bouts of stretching over periods of weeks or months can increase muscle length and reduce stiffness (Herbert, 2004; Odeen, 1981) by providing the necessary stretch stimulus that allows the muscle to lengthen in line with bone growth.

The increased stiffness (hypertonicity) of the muscle can have both neural and mechanical components. Spasticity (neural) and reduced muscle length (mechanical) can both theoretically be addressed with stretching, although the mechanisms by which these changes occur are not fully understood (Guissard & Duchateau, 2006). Regarding the former, the reduction in neural hypertonia may be related to reduced motor neuron excitability or reduced neural input to motor neurons through both pre- (e.g., input from Ia afferents) and/or post-synaptic mechanisms (Hummelsheim, Munch, Butefisch & Neumann, 1994). The consequence of this for a spastic muscle may be a decrease in tonic reflex activity or an increase in the threshold of tonic stretch reflex, thus allowing an increase in joint ROM and muscle-tendon unit length with stretch (Calota, Feldman & Levin, 2008). Regarding the latter, chronic stretching may affect mechanical hypertonia by causing an inducing effect to increase muscle fascicle length (Coutinho *et al.*, 2004). This plasticity of muscle has been demonstrated in several animal studies,

where daily stretching over a period of several weeks was sufficient to increase the number of in-series sarcomeres (Williams, 1990).

Regardless of the mechanism involved, it seems that repeated elongation of the muscle during stretch is the key to inducing changes, both neural or mechanical (Williams, 1990). There is no consensus either in clinical practice or in the literature, with regards to the appropriate time of application, duration or frequency of stretch. However, before determining the appropriate stimuli for long-term changes in the musculoskeletal system, it should first be established whether spastic muscles are indeed able to receive a stretch during changes in joint ROM.

Acute stretching has been shown to cause short-term increases in ROM in adults (McNair & Stanley, 1996), but the underlying mechanisms are inconclusive. Whilst some studies suggest that a passive ankle dorsiflexion stretch induces length changes of the gastrocnemii (Blazevich *et al.*, 2012; Morse *et al.*, 2008) other studies suggest that increases are largely accounted for by changes in Achilles tendon length (Herbert *et al.*, 2002; Kubo *et al.*, 2002b). Muscle elongation for a given stretch intensity is governed by muscle and tendon stiffness, which are determined by their dimensions as well as material properties. In addition, muscle stiffness is also determined by neural factors (i.e., spasticity). For this reason, acute alterations to muscle length in response to stretching could be different in children with CP compared to healthy populations. Both Wren *et al.* (2010) and Barber *et al.* (2012) showed that tendons are longer in children with CP compared to their TD peers. Whilst this dimensional difference is not necessarily associated with differences in tendon stiffness (Barber *et al.*, 2012), children with CP have been shown to have a greater tendon slack length (Barber *et al.*, 2012).

During stretching, rotation of the joint may cause tendon slack to be taken up without any elongation of the muscle.

With regards to muscle properties, several dimensional and mechanical differences have been reported between children with CP and TD children. These include differences in muscle length (Malaiya *et al.*, 2007), cross-sectional area (Elder *et al.*, 2003) and alterations in connective tissue (Smith *et al.*, 2011). In addition, in a spastic muscle, the increased gain or lower threshold of the stretch reflex may cause the muscle to be activated even during low levels of stretch. If the net result of these factors was an increase in muscle stiffness, this could prevent the muscle from elongating in response to stretch. Even if muscle elongation does occur during stretch, it is not clear whether any change in muscle length will be due to the elongation of the muscle fascicles or the surrounding connective tissue. Morse *et al.* (2008) demonstrated that although acute stretching increased the length of the gastrocnemii, this lengthening was caused by changes in the connective tissue alone and not increases in fascicle length. In a hypertonic muscle this effect may be exaggerated due to a greater abundance of connective tissue in the muscle (Smith *et al.*, 2011). In contrast, Barber *et al.* (2011a) showed that although children with CP have stiffer muscle fascicles than TD children, some fascicle elongation did occur. Thus, it is not clear whether or not muscles and fascicles elongate during acute passive stretching in children with CP. If this was not the case, the effectiveness of long-term stretching to increase muscle length or reduce muscle stiffness in children with spastic CP would be questionable. Therefore, the first purpose of the study was to examine whether short-term increases in ROM in children with CP as a result of acute stretching would be due to transient changes in medial gastrocnemius muscle and fascicle length, and/or Achilles tendon length.

In addition to the knowledge of the mechanism underlying stretch-induced increases in ROM, stretch technique is another important factor to consider in clinical practice. Several techniques are used by physiotherapists and taught to parents/carers, which may be classified into two broad categories; passive stretches administered manually by a physiotherapist ("PT-stretch"), often with the patients lying supine, or standing stretches performed by the individuals themselves ("self-stretch"). These two techniques are the most commonly used stretches in clinical practice to increase ROM and muscle extensibility, and have been developed based on guidelines related to the aims of physical therapy (Bandy & Sanders, 2001).

If the primary goal of stretch is to elongate the muscle, two potential reasons exist as to why these techniques could have different effects. Due to the voluntary nature of the standing self-stretch, it might be difficult for children with CP to coordinate muscle activity to maintain the body position required for the muscle to be stretched (Rose & McGill, 2005), which could negatively impact the effectiveness of the stretch. Conversely, during the standing self-stretch proprioceptive inputs from the foot sole and/or altered input from the vestibular system, which may serve to suppress the H-reflex (Alrowayeh, Sabbahi & Etnyre, 2005), could reduce the activation level of the muscle and thereby increase the muscle's extensibility. From a basic science perspective, an understanding of the effect stretch technique has on muscle extensibility is important as it would provide further insights into these conflicting mechanisms. From an applied perspective, such an understanding would help to inform clinicians as to which technique is most effective in terms of achieving the greatest potential stretch of the muscle. Therefore, the second purpose of this study was to investigate the effect of stretch technique on muscle extensibility in children with spastic CP.

5.2 Methodology

5.2.1 Participants

Eight children with clinically diagnosed spastic diplegic CP (three males, five females; mean age 10.2 ± 3.2 y (range = 6-14 y) were recruited through the British National Health Service (NHS) paediatric physiotherapy services. Five patients were classified as Level II, and three patients were classified as Level I on the GMFCS as assessed by a physiotherapist (Palisano *et al.*, 1997). No children had received any form of orthopaedic surgery or Botulinum toxin injection prior to participation in the study. The study was approved by institutional as well as the relevant local NHS Ethics Committees. The study was conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained in addition to written assent from the children.

5.2.2 Experimental design

Participants attended the physiotherapy clinic on one occasion. During this time, each participant underwent a series of passive ankle dorsiflexion stretches using both the self-stretch and PT-stretch techniques (described below). The order of stretch techniques was administered in a random order. Both techniques were applied to the right and left legs, the order of which was also randomised. For each participant, the first stretch technique was initially performed on the ipsilateral leg, followed by the same stretch technique applied to the contralateral leg. After a period of rest, the ipsilateral leg was stretched again using the second stretch technique, followed by a stretch of the contralateral leg using the same second stretch technique (Figure 5.1). Within this context we ensured that the rest period was a minimum of 60 minute between the two different stretch techniques on the same leg. This period has previously been shown to be sufficient to eliminate the acute effects of stretch on muscle and tendon properties in healthy individuals (Magnusson, Simonsen, Aagaard & Kjaer,

1996b). The majority of this 60 minute rest period was taken up with testing the contralateral leg. For the remainder of the rest period participants were seated with the muscle in a relaxed position. Passive stretches were performed five times on each leg with each technique. Each stretch was held at maximum ROM for 20 s, followed by a 60 s rest period between stretches. Maximal ankle dorsiflexion was assessed before and after each of the five passive stretches using the PT-stretch technique outlined below.

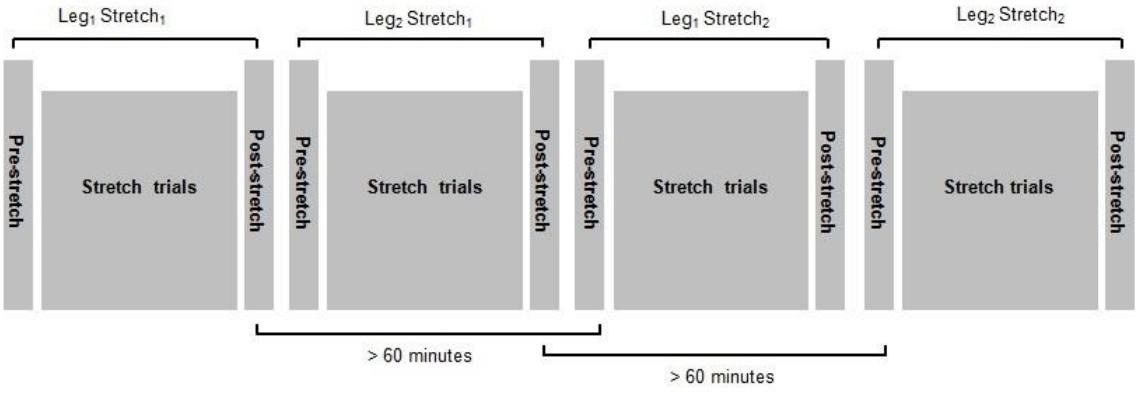

Figure 5.1. Experimental design

5.2.3 Procedure for stretching

5.2.3.1 PT-stretch

For the PT-stretch, participants lay supine on a foam mat, with the physiotherapist positioned to the side of the participant - opposite to the leg being stretched. To gain the initial stretch position, the leg was lifted with the knee flexed to 90 deg. To initiate a stretch of the gastrocnemius muscle, the physiotherapist's hand was cupped around the heel, with the palm of the hand flat against the foot. The knee was supported and slowly guided into full extension. This position was stabilised and maintained by pressure at the proximal tibia. Once the knee was locked in an extended position, the ankle was slowly dorsiflexed, with pressure from the hand and forearm on the plantar surface of

the forefoot. Joint rotation continued to be applied by the physiotherapist until feedback from the participant indicated the point of discomfort. This point was considered to be maximal dorsiflexion. Once in a maximal stretch position, the joint was held for a period of 20 s. A similar method was used to assess maximum dorsiflexion angle before and after a set of five passive stretches. However, here, the stretch was held only until maximum dorsiflexion had been reached.

5.2.3.2 Self-stretch

For the self-stretch, children were instructed to stand facing the wall. To gain the initial stretch position, participants were positioned at approximately arm's length from the wall with their hands flat on the wall at shoulder height. The leg to be stretched was placed behind the body, and the contralateral leg was flexed and placed in front of the body for support. To maintain an upright posture, participants were instructed to keep their hips facing the wall and to draw the belly button inwards. This pulled the pelvis towards the centre line of the body, maintaining a straight line between the back leg, hips and trunk. For the stretch, participants were asked to ease the back leg away from the wall keeping the knee in an extended position, and pressing the heel into the floor. Once in this stretched position as determined by the participant and physiotherapist, this position was maintained for 20 s. Each participant performed the self-stretch five times on each leg (see Figure 5.1). Maximum dorsiflexion was determined before and after the sequence of five stretches as described in the previous section. For all participants, the same physiotherapist was responsible for implementing all passive stretches, and provided all detailed instructions and demonstrations during the self-stretches.

5.2.4 Data processing

For both techniques, participants were positioned equidistantly between six infrared LED motion capture cameras (Motion Analysis, Santa Rosa, USA). The cameras were positioned on both sides of the participant. Reflective markers were placed bilaterally on the heads of the first and fifth metatarsals, the medial and lateral malleoli, and lateral and medial femoral epicondyles. Further markers were placed on the calcanei as well as the greater trochanters of the right and left femur. Finally, two markers were placed on the handle of the ultrasound probe, perpendicularly to the field of view. All kinematic data were filtered using a low-pass, fourth-order, zero-lag Butterworth filter with a cut-off frequency of 5 Hz, as determined by residual analysis (Winter, 1990). The relative ankle angle was defined as the angle between the shank and foot. To account for eventual movements out of the sagittal plane, the angle was calculated using the 3D coordinates. For this purpose, the 3D locations of the midpoints between the 1^{st} and 5^{th} metatarsals, the lateral and medial malleoli as well as the lateral and medial femoral epicondyles were calculated. The relative ankle angle was calculated from these three 3D coordinates using the law of cosines.

During each trial, muscle and tendon elongation were measured by tracking the displacement of the medial gastrocnemius muscle-tendon junction, using B-mode ultrasonography Megas GPX (Esaote, Genova, Italy; 45 mm Linear array probe, 10 MHz transducer scanning). The video transmission was digitally captured at 25 Hz using a video converting frame grabber Canopus ADVC-55 (Grass Valley, Paris, France). A layer of water-based gel (Henleys Medical Supplies Ltd., Hertfordshire, UK) applied between the ultrasound probe and skin enhanced acoustic transmission without depressing the dermal surface. The probe was placed perpendicularly to the skin surface

above the muscle-tendon junction of the medial gastrocnemius and orientated to reveal a line running between the aponeuroses of the medial gastrocnemius and soleus muscles. The probe was fixed in position using a custom made holder. Peak Motus tracking software (Peak Performance, Cambridge, UK) was used to manually digitise 2D coordinates of the muscle-tendon junction from the ultrasound images. Medial gastrocnemius muscle fascicle length was also quantified in six children, using open source digital measurement software ImageJ (NIH, Bethesda, USA) on the existing ultrasound trials. These measurements were made at approximately the mid-belly of the muscle as changes at this site have been shown to be relatively uniform (Lichtwark *et al.*, 2007). Three optimal and identifiable fascicles were selected and measured from deep to superficial aponeuroses. These fascicles were tracked in each frame of the pre- and post-stretch trials, and an average of the three fascicles was used for subsequent analysis. The ultrasound data were synchronised with kinematic data by means of an electrical trigger (Trigger module SP-U03, Delsys Inc., Ltd., Boston, USA). Digitised muscle-tendon junction position data were filtered using a low-pass fourth-order zero-lag Butterworth filter with a 3.25 Hz cut-off frequency. Filtered motion analysis data were down-sampled to 25 Hz to match the sampling frequency of the ultrasound data. Muscle and tendon lengths were calculated from a combination of motion analysis and ultrasound data. Using the positions of the two markers from the ultrasound probe, combined with the coordinates of the muscle-tendon junction in the ultrasound image, the global 3D position of the muscle-tendon junction was calculated in the inertial reference frame. During pilot testing performed on four healthy adults, the coefficient of variation for muscle-tendon junction location obtained from three separate measures was determined to be 1.35%.

Medial gastrocnemius muscle length was defined as the distance between the midpoint coordinates of the femoral epicondyles and the global 3D coordinates of the muscle-tendon junction. Achilles tendon length was calculated as the distance between the muscle-tendon junction to the calcanei. Thus, both medial gastrocnemius and Achilles tendon were modelled as straight lines. This analysis was performed using custom written analysis software Matlab v7.14 (MathWorks, Cambridge, UK). To ensure the correctness of this algorithm, we used a sample data set to confirm that the programme's outcome measures were identical to those obtained "manually" by means of spreadsheet calculations.

5.2.5 Dependent variables

Muscle length, muscle fascicle length and tendon length were expressed firstly at a reference angle (defined as the relative ankle angle of 10 deg plantarflexion). This angle was calculated from the pre-stretch trials where the ankle was slowly and passively moved from a relaxed plantarflexed position into dorsiflexion. In subsequent analyses, we identified the three data points that were closest to this reference angle for a given trial. For all participants, the ankle angle corresponding to these data points did not deviate by more than one degree from the reference angle. Muscle and tendon lengths were then averaged across these three points. Ankle angle, muscle and fascicle lengths, and tendon length at maximum dorsiflexion were then measured before and after five passive stretches. For each of these variables, we calculated the mean of right and left legs for the corresponding conditions.

5.2.6 Statistical analysis

To test whether the acute effects of the first stretch technique had diminished before the measurements of the second stretch technique with the same leg, a paired *t*-test on

maximum dorsiflexion angle was conducted. Three time (reference angle, pre-stretch, post-stretch) by technique (self-stretch, PT-stretch) repeated measures ANOVAs were performed on ankle dorsiflexion angle, muscle length, and tendon length. A further time (pre-stretch, post-stretch) by technique (self-stretch, PT-stretch) ANOVA was performed on muscle fascicle length. To examine the effects of stretching per-se, we tested for a main effect of time. To test whether stretching effects would be dependent on stretch technique, we tested for a time by technique interaction. In the case of significance, follow up paired *t*-tests with Bonferroni correction were performed. Statistical significance was accepted at $p < 0.05$.

5.3 Results

The paired samples *t*-test showed no significant difference in maximum dorsiflexion angle ($t_7 = 1.12$, $p = 0.29$) between pre-stretch trials (i.e., before the two different stretch techniques were applied to the same leg). We also found that maximum muscle length increased progressively during the five stretches (Figure 5.2), which confirmed the assumption that the muscle was indeed receiving a stretch across the five stretch trials.

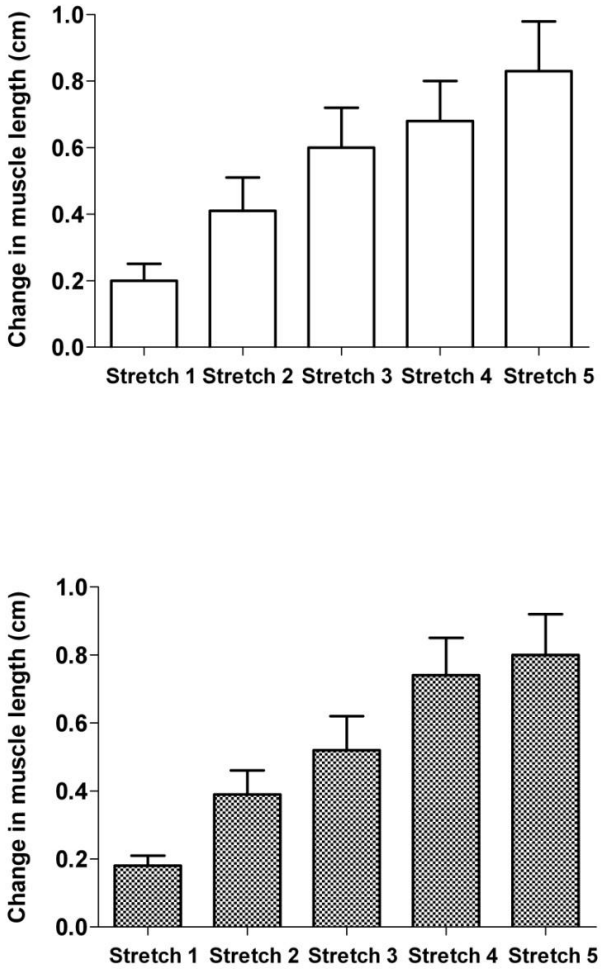

Figure 5.2. Change in muscle length across the five stretch trials for the PT-stretch (top figure), and the self-stretch (bottom figure). This change is calculated from muscle length in the pre-stretch trials (values are expressed as mean ± SD).

The ANOVAs revealed that the main effects for technique and the time by technique interactions were non-significant for ankle angle, muscle and tendon length ($F_{2, 14}$ = 0.34−2.98, $p > 0.05$) and fascicle length ($F_{2, 12}$ = 0.21−1.69, $p > 0.05$). The main effects for time were significant for ankle dorsiflexion angle ($F_{2, 14}$ = 150.0, $p < 0.001$), muscle length ($F_{2, 14}$ = 268.27, $p < 0.001$), tendon length ($F_{2,14}$ = 459.61, $p < 0.001$) and fascicle length ($F_{2, 12}$ = 640.76, $p < 0.001$). Since there was no interaction effect, data were collapsed across techniques for *post-hoc* analyses. These tests revealed that all variables were significantly greater during the pre-stretch condition compared to rest ($p < 0.05$). Further, all variables were significantly greater during the post-stretch condition compared to the pre-stretch condition ($p < 0.05$) (Table 5.1) (see Figure 5.4).

Table 5.1. Absolute and relative changes in dorsiflexion angle and muscle-tendon variables (Values expressed as mean ± SD. Asterisks represent significance, $^{**}p < 0.01$).

	Change		**Percentage change**		$t_{(7)}$
	PT-stretch	Self-stretch	PT-stretch	Self-stretch	
Maximum ankle dorsiflexion (deg)					
Rest to Pre-stretch	1.80 ± 0.27	1.84 ± 0.23	21.9%	22.5%	21.02**
Pre- to Post-stretch	9.8 ± 1.7	0.93 ± 0.16	11.9%	11.4%	16.56**
Muscle length (cm)					
Rest to Pre-stretch	1.15 ± 0.16	1.12 ± 0.18	8.2%	8.0%	-15.07**
Pre- to Post-stretch	0.83 ± 0.26	0.80 ± 0.29	5.8%	5.7%	-9.14**
Tendon length (cm)					
Rest to Pre-stretch	0.99 ± 0.18	9.0 ± 2.7	6.2%	5.7%	-12.98**
Pre- to Post-stretch	1.02 ± 0.19	9.6 ± 1.6	6.3%	6.0%	-17.38**
Muscle fascicle length (cm)					
Rest to Pre-stretch	0.58 ± 0.06	0.53 ± 0.15	14.3%	12.9%	-17.33**
Muscle-tendon unit length (cm)					
Rest to Pre-stretch	2.14 ± 0.30	2.02 ± 0.37	7.1%	6.7%	
Pre- to Post-stretch	1.85 ± 0.41	1.76 ± 0.42	5.8%	5.5%	

To illustrate the physiological significance of the effects of stretching on the dependent variables, we also report the relevant absolute and relative changes (Table 5.1). Following the stretching protocol, maximal dorsiflexion angle increased by approximately 12% pre- to post-stretch (Figure 5.3). This change was accompanied by increases of muscle and tendon length of approximately 6% and a change in muscle fascicle length of 14% pre- to post-stretch. The absolute change in maximal dorsiflexion

angle was approximately 10 deg and accompanied by an 8 mm change in muscle length and 10 mm in tendon length (Figure 5.4).

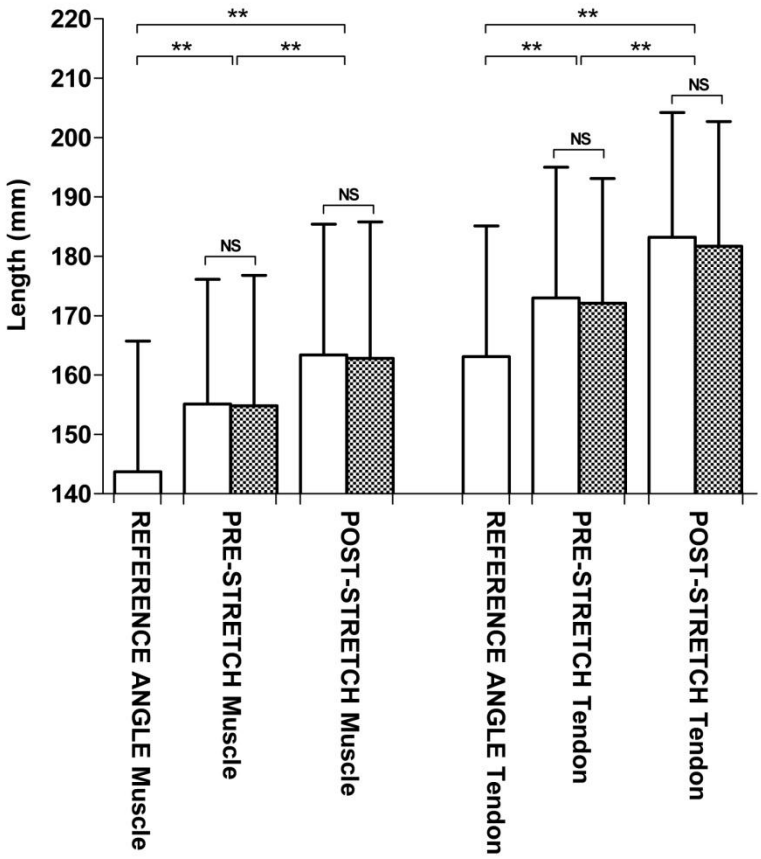

Figure 5.3. Comparison of muscle and tendon length at each testing condition for the PT-stretch technique (empty columns) and the self-stretch technique (hashed columns) (values are expressed as mean ± SD. Asterisks represent significance, $^{**}p < 0.01$).

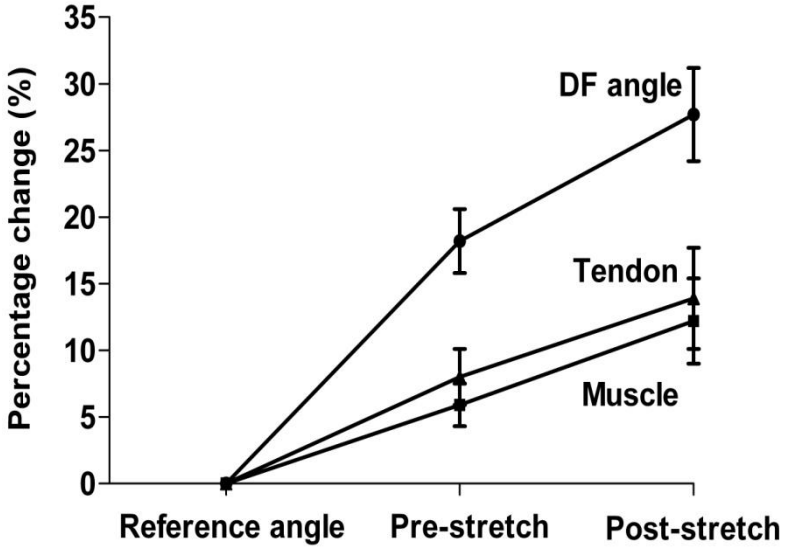

Figure 5.4. Percentage change in ankle dorsiflexion angle (DF) (closed circles), muscle length (closed squares) and tendon length (closed triangles) at each testing condition for the collapsed data (values are expressed as mean ± SD).

5.4 Discussion

The first purpose of the study was to examine whether stretch-induced increases in ankle ROM in children with CP would be due to alterations in medial gastrocnemius muscle length and/or Achilles tendon length. Following five stretches, ankle dorsiflexion angle increased approximately 10 deg, which is in agreement with previous findings (Miedaner & Renander, 1987). This increase was accompanied by relatively equal amounts of Achilles tendon elongation (1 cm), and medial gastrocnemius elongation (0.8 cm). Muscle fascicles lengthened by 0.6 cm pre- to post-stretch. This result was not necessarily expected, as children with CP have an abnormally large amount of connective tissue in their muscles (Smith *et al.*, 2011), which could decrease the muscle's extensibility. However, in our participants, this accumulation of connective tissue did seem not to affect the extensibility of muscle fascicles. A possible explanation for this result is a lack of organisation and integrity of the connective tissue in spastic muscles, which may impair the tissue's tensile strength (Lamontagne *et al.*, 1997). A consequence could be that this connective tissue is therefore weak and compliant, which would, in turn, allow the muscle fascicle to stretch. Interestingly, we also showed that the muscle fascicles underwent a smaller length change than the whole muscle belly, which is consistent with previous findings (Morse *et al*., 2008). This suggests that during a stretch, the muscle's connective tissue (endomysium, perimysium and epimysium) situated between the fascicles, is stretched, which increases the distance between the fascicle insertion points (muscle "opens up"). The result is an increase the overall length of the muscle, which is greater than the length change of the muscle fascicles".

The stretch-induced increases in muscle and fascicle lengths were not necessarily expected for two reasons. First, increased neural activation in the spastic muscle could

increase its resistance to stretch (Calota *et al.*, 2008). An increased gain or lower threshold of the tonic stretch reflex could cause the muscle to become active during periods of stretching, preventing elongation of the muscle. Second, in children with CP, rest and slack lengths of the Achilles tendon are longer, whilst gastrocnemius muscle length has been reported to be shorter than in TD children (Wren *et al.*, 2010). This was also the case in our participant population (see Figure 5.3). Longer tendons have previously been shown to be more compliant (Zhao, Ren, Wu, Liu & Zhang, 2009), and so one might have expected the tendon to take up more of the stretch than the muscle. However, our results show that despite muscle spasticity and tendon length differences, muscles and tendons elongate relatively equally in response to stretching, with smaller length changes from the fascicles.

From a clinical perspective, these results are of immense importance. Previously, inferences about a spastic muscle's extensibility have been made based on the assumption that changes in ROM would be reflective of muscle length changes. This assumption is not intuitive, as increases in ROM can be due to the extensibility of tendons and other passive structures. Our results demonstrate that in a group of children with spastic diplegic CP classified as GMFCS levels 1 or 2, passive stretching at the ankle joint resulted in a significant and acutely sustained elongation of the muscle. They thereby confirm the clinical assumption that acute stretching might increase muscle length in children with CP. Therefore, the present study is a useful and necessary prerequisite to examine the effectiveness of long-term stretching as a clinical intervention to achieve a sustained increase in muscle length in this group of children.

The acute and transient changes in muscle length in response stretching raise the question about the underlying mechanisms. Acute changes in muscle length in response

to stretch have been previously reported in healthy adults (Magnusson *et al.*, 1996b) and are thought to result from two main mechanisms. First, the Golgi tendon organs dampen the effect on the motor neuronal discharges, thereby causing relaxation of the muscle-tendon unit, which would in turn reset its resting length. Second, Pacinian corpuscles serve as pressure sensors to regulate pain tolerance. Both mechanisms lead to a change in the muscle's tolerance to stretch. Repeated stretching has also been shown to reduce passive tension and to allow greater elongation through small changes in the viscoelastic properties of the muscle-tendon unit (Ryan *et al.*, 2008). These mechanisms cause only acute changes in maximal muscle and tendon lengths, which dissipate shortly after stretching has stopped. However, they may provide the necessary stimulus for the muscle's adaptive process. Whilst the exact mechanism for sarcomere addition and longitudinal growth in the muscle is unknown, previous research has indicated that muscle stretching is a very powerful stimulant (Williams, 1990). Future research is needed to confirm the speculation that an appropriately designed stretching regime would result in long-term longitudinal muscle growth, reductions in spasticity and potential improvements in function in children with CP. Within this context, our results may provide a possible avenue to induce muscle elongation in an ethical and ecologically valid fashion. A limitation to this recommendation is that it is not clear whether the magnitude or duration of this stimulus would be sufficient to bring about long-term adaptations in the muscle. A logical question arising from these results is whether such mechanical changes in muscle structure can be achieved with an ecologically valid and clinically applicable long-term stretching protocol. Our results provide a first step towards answering this question.

The second purpose of the study was to determine whether any change in muscle length was dependent on stretch technique. The results demonstrate that changes in ankle

ROM, maximal muscle and tendon lengths were independent of stretch technique. It was hypothesised that there may be a reduction in neural activation associated with standing (Ali & Sabbahi, 2000), through inhibition of the H-reflex, which could have resulted in a greater maximal stretch. This may be because the self-stretch position required less modulation of the vestibular system as participants were asked to place their hands on the wall in front of them and were not unstable in this position. Therefore the magnitude of inhibition may not have been different between techniques. This finding has direct clinical implications, by providing parents and clinicians reassurance that as the child takes steps towards self-management, and transitions from physiotherapist led stretching to self-stretching, both techniques are equally effective. It needs to be noted that in our study, the self-stretch technique was performed under the supervision of a physiotherapist who gave verbal feedback where necessary. Thus, our findings do not rule out the possibility that the self-stretch would be less effective if performed without professional supervision.

It is important to recognise the limitations of this method for calculating muscle and tendon lengths. We modelled muscle and tendon paths as two straight lines. This approach does not take the curvature of muscle and tendon into consideration. At maximal ankle dorsiflexion this systematic error may have been minimal since any slack from muscle and tendon would have been taken up in this position. At rest, muscle and tendon lengths may have been underestimated by assuming two straight lines due to muscle, and in particular, tendon slack. Another limitation of our approach is that our estimate of "muscle length" included the length of the proximal tendon of the medial gastrocnemius. However, its role within the context of muscle/tendon dynamics is considered to be negligible (Maganaris & Paul, 2002; Morse, 2011), and therefore, we believe that this assumption does not affect our results.

In summary, we found that acute stretching causes transient increases in both muscle and tendon length in children with CP independent of stretch technique. The results thereby suggest that stretching in children with CP is a suitable treatment to gain short-term increases in muscle length, which may lead to long-term adaptations in the spastic muscle if repeated over a period of weeks or months. These findings constitute a first step towards a more refined understanding of the relationship between stretching and changes in the mechanical structure of muscles and tendons. They thereby have direct implications for clinicians treating children with CP.

CHAPTER 6: DOES LONG-TERM PASSIVE STRETCHING ALTER MUSCLE-TENDON UNIT MECHANICS AND GAIT IN CHILDREN WITH SPASTIC CERBERAL PALSY?

6.1 Introduction

Children with spastic CP experience greater overall joint stiffness compared with TD children (Alhusani *et al.*, 2010; Barber *et al.*, 2011a; Tardieu *et al.*, 1988). The common view in clinical practice is that this increased stiffness arises as a direct consequence of neurological insult (e.g., an abnormal tonic stretch reflex threshold), and/or from secondary CP-related musculoskeletal adaptations, in response to impaired control and posture (e.g., when spastic muscles are maintained in a shortened position). These secondary changes in the musculoskeletal system can lead to further increases in joint stiffness, as well as muscle contracture and joint deformities. In this context, passive stretching has been the most common method of rehabilitation for a number of years (Damiano, 2009; National Institute for Health and Care Excellence, 2012). Its use is still widely advocated for children with spastic CP, as a means of reducing neural and mechanical components of joint stiffness, with a view to improving function (Farmer & James, 2001; Lieber & Bodine-Fowler, 1993). Specifically, the clinical assumption is that passive stretching may potentially delay or prevent muscle contracture, increase joint ROM and reduce spasticity (Wiart *et al.*, 2008).

Although the clinical assumption is that increased joint stiffness impairs function, research has demonstrated that actually, excessive muscle weakness, rather than increased stiffness, negatively affects function (Burke, 1988; O'Dwyer *et al.*, 1996). This discrepancy demonstrates that the role of stiffness in functional tasks is probably not well understood in CP. This brings into question what the aim of clinical interventions should be. For example, it has been speculated that stiffness may be, at least in part, a compensatory mechanism to excessive muscle weakness (Holt *et al.*,

2000a; O'Dwyer *et al.,* 1996). Specifically, this increased stiffness in CP may act as a mechanical spring allowing greater storage and release of energy during aspects of walking, in the absence of adequate force production. In addition, increased stiffness may also provide joint stability, to counter some weakness of the muscle (Holt *et al.*, 2000a; Tedroff *et al.*, 2008). This alternative view of joint stiffness, suggests the current clinical rationale for passive stretching may not be correct (Latash & Anson, 1996). Based on this speculation, it could be the case that reducing joint stiffness does not lead to improvements in function. This highlights a current gap between research evidence and clinical rationale for long-term stretching to alter muscle and tendon mechanics and improvements in function, for children with spastic CP.

There is some evidence from animal studies that demonstrate the ability of the healthy muscle to adapt to long-term stretch by increasing its number of serial sarcomeres (Coutinho *et al.*, 2004; Salvini , Coutinho, Russo & DeLuca, 2006; Tabary *et al.*, 1976; Williams, 1990), and some evidence that healthy human muscles may respond similarly (Boakes *et al.*, 2007). In children with spastic diplegic CP, there are evidences for the potential responsiveness of the muscle to stretching (Theis, Korff & Mohagheghi, 2013), but such long-term changes are not yet reported. Few studies have demonstrated increased joint ROM in response to long-term stretching in CP (McPherson *et al.*, 1984; Miedaner & Renander, 1987), and decreased resistance of the joint to passive stretch (Kubo, Kanehisa & Fukunaga, 2002c; McPherson *et al.*, 1984; Nakamura, Ikezoe, Takeno & Ichihashi, 2012; O'Dwyer *et al.,* 1994), but none report the effects on muscle or tendon stiffness.

Current research evidence on passive stretching in CP is not adequate to support or refute the effectiveness of stretching as a management strategy (Pin *et al.*, 2006; Wiart

et al., 2008). Previous studies commonly investigate joint stiffness and ROM as outcome measures, which lack information regarding alterations in the muscle and tendon. Joint stiffness could theoretically remain unchanged, but with alterations in the relative stiffness's of the muscle and tendon. Thus, the effectiveness of long-term passive stretching on these constituent components should be investigated from a theoretical point of interest, and to provide a basis for future interventions.

The second purpose of this study was to investigate whether any potential alterations in stiffness would affect gait parameters. Conflicting findings have been reported with regards to stretching and gait in CP. For example, Salem *et al.* (2010) demonstrated improved speed, stride length, stride time, stance phases and maximum ankle dorsiflexion angle following a standing frame intervention. Similarly, Wu, Hwang, Ren, Gaebler-Spira and Zhang, (2011) showed that a combination of passive and active stretching over a six week period, improved clinical tests of walking speed. Conversely, it has also been reported that passive stretching in patients with limited dorsiflexion ROM did not decrease stance time during gait (Johanson *et al.*, 2006). Further, Crosbie Alhusaini, Dean and Shepherd (2012) demonstrated an association between stiff calf muscles and greater speed, stride length and cadence in children with spastic CP. They propose that the increased muscle stiffness may provide a stable ankle, which facilitates a more rapid gait.

The results will have important implications for the prescription of passive stretching, and whether this, or alternative interventions should be used to treat children with spastic CP in the future. Therefore, the first aim of this study was to identify whether six weeks of passive stretching altered the mechanical properties of the *triceps surae*

muscles and Achilles tendon in children with spastic CP. Second, we also investigated whether any potential alterations in stiffness affected clinical gait parameters.

6.2 Methodology

6.2.1 Participants

Thirteen children with spastic CP (seven male, six female; mean age 10.3 ± 3.0 y) participated in this study. Seven children had diplegic CP, and six children had quadriplegic CP. It was important to maintain a homogenous group so only children with both lower limbs affected were included in this study. Participants were recruited from a local school for children with disabilities. Children were identified by a physiotherapist as diplegic or quadriplegic, and with a clinical diagnosis of spasticity in the lower limbs. Children were randomly assigned to an experimental (seven participants) or control group (six participants). Six patients attained GMFCS level III and seven patients attained GMFCS level IV as assessed by a physiotherapist. None of these children had received any form of lower limb surgery, 24 months prior to participation in the study, and none had received Botulinum toxin injections to the legs 4 months prior to participation. All children were wheelchair users, but were ambulant with the use of a walking aid. The study was approved by institutional as well as the relevant local NHS Ethics Committees. The study was conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained in addition to written assent from the children.

6.2.2 Experimental set-up

The experimental group completed a six week stretching programme in addition to their normal routine. The control group did not receive the additional stretching programme but continued with their normal routine. Before and after the six week intervention period, all participants underwent two testing sessions. Pre-intervention data were collected 24 hours prior to the first stretching session. Post-intervention data were collected 48-72 hours after the final stretching session.

During the first testing session, we obtained data for the mechanical properties of the muscle, tendon and fascicles of the right leg. During the second session, we obtained data on gait parameters. Six participants (three control group and three experimental group) were also tested again in a follow-up session, four weeks after the cessation of the stretching intervention.

6.2.3 Stretching programme design

Each participant in the experimental group received an ankle dorsiflexion stretch to both the right and left legs, applied by a clinician. Stretches took place on four days per week for six weeks. The stretch was performed for a total of 15 minutes on each leg, in 60 s repetitions followed by a 30 s rest period. These stretch durations and frequencies were chosen based on durations frequently used in clinical practice (Wiart *et al.*, 2008).

The stretches were performed with the children seated in their wheelchair. To gain the initial stretch position, the leg was lifted and the knee was slowly guided into extension. To initiate a stretch of the *triceps surae* muscles, the clinician's hand was cupped around the heel, with the palm of the hand flat against the foot. The knee was supported in an extended position and the ankle was slowly dorsiflexed, with pressure from the hand. Once in a maximal stretch position, defined also as maximal ROM, the muscle was held for a period of 60 seconds. To ensure continuity of each stretch trial, an electrogoniometer (Type F35, Biometrics Ltd., UK) was placed on the ankle joint to monitor maximal dorsiflexion angle achieved during the stretch. A new maximal ankle angle was recorded at the start of each week. The free end of the goniometer was placed on the lateral part of the foot, just below the fifth metatarsal. The fixed end was placed just above the lateral malleolus. Initially the strain gauge was relaxed and the

electrogoniometer calibrated with the foot in a resting plantarflexed position. Ankle angle was displayed throughout the dorsiflexion stretch. This angle or greater was achieved in all subsequent stretches.

6.2.4 Pre, post and follow-up data acquisition

Part one of the pre, post and follow-up testing sessions, measured the mechanical properties of the medial gastrocnemius muscle and fascicles, and the Achilles tendon. For this purpose, participants were seated on an isokinetic dynamometer (Cybex Norm, Lumex, Ronkonkoma, NY, USA). Hip angle was set to 85 deg and the knee was straightened as much as possible, which was approximately 7 ± 2 deg from full extension. The lateral malleolus of the right ankle was aligned with the centre of rotation of the dynamometer arm, to ensure movement in the sagittal plane. Stabilisation straps were firmly tightened over the foot, thigh and chest. Three infrared LED motion capture cameras (Motion Analysis, Santa Rosa, USA) were positioned on one side of the dynamometer. Reflective markers were placed on the head of the first metatarsal, the medial malleoli, the calcanei, the medial femoral epicondyle, and two markers on the handle of the ultrasound probe. All kinematic data were filtered using a low-pass, fourth-order, zero-lag Butterworth filter with a cut-off frequency of 5 Hz.

We initially determined each participant's ROM, by manually dorsi- and plantarflexing the foot, until any discomfort was reported. A rotation of the right ankle joint at 10 $deg \cdot s^{-1}$ was then applied through the ROM, starting at maximal dorsiflexion. This angular velocity was chosen so as not to evoke stretch reflex activity in the spastic muscle (Thilmann *et al.*, 1991). Participants were instructed to relax the muscles of the lower limb as much as possible during this time, while three rotations were recorded. Muscle activity from the medial gastrocnemius and tibialis anterior muscles were

monitored throughout the passive rotation using EMG to ensure muscle activity was not evoked by the rotations. EMG data of the tibialis anterior and the medial gastrocnemius muscles (EMGworks, Delsys Inc., Ltd., Boston, USA) as well as torque data from the dynamometer were collected at 1000 Hz. Torque data were filtered using a low-pass, fourth-order, zero-lag Butterworth filter with a cut-off frequency of 14 Hz. Data for all participants in each condition (pre, post and follow-up) were analysed after the final follow-up testing session. To reduce experimenter bias, the experimenter was blinded to the "condition" during data analysis.

Joint stiffness was determined by plotting filtered torque data against ankle angle (expressed as a percentage of each participants maximum ROM). Specifically, average joint stiffness was calculated in the range corresponding to 20% and 80% of each participant's peak torque. This interval was chosen so as to avoid the "toe region" at the lower end of the stiffness curve, and also to minimise the contribution of passive elastic structures such as ligaments, connective tissue and skin at the upper end of the stiffness curve (Abellaneda *et al.*, 2009). This interval provided reliable stiffness data across trials in children with CP (coefficient of variation = 5.7%).

6.2.4.1 Muscle and Tendon Stiffness

Achilles tendon stiffness was calculated as the change in passive ankle torque divided by the corresponding change in Achilles tendon length. A measure of "total *triceps surae* muscle stiffness" was also derived from the data by dividing passive ankle torque and elongation of the medial gastrocnemius muscle. Muscle and tendon stiffness were calculated from the slope of the torque-elongation curves, in the same range as joint stiffness, which corresponded to 20% and 80% of each participants peak torque.

6.2.4.2 Muscle and tendon length

For the calculation of muscle and tendon stiffness, elongation of the medial gastrocnemius muscle and Achilles tendon were measured, respectively. This was done by tracking the displacement of the gastrocnemius muscle-tendon junction, using B-mode ultrasonography (Megas GPX, Esaote, Italy; 45 mm Linear array probe, 10 MHz transducer scanning), captured at 25 Hz. A layer of water-based gel (Henley's Medical, Hertfordshire, UK) applied between the ultrasound probe and skin enhanced acoustic transmission. The probe was placed perpendicularly to the skin surface above the muscle-tendon junction and orientated to reveal a line running between the aponeuroses of the medial gastrocnemius and soleus muscles. The probe was then fixed in position using a custom made holder. A 2 mm wide strip of echoabsorptive tape placed on the skin in contact with the probe provided a reference to which any probe movement could be identified. The 2D coordinates of the muscle-tendon junction were obtained by manual digitisation (Peak Performance, Cambridge, UK). Digitised muscle-tendon junction position data for both methods were filtered using a low-pass fourth-order zero-lag Butterworth filter with a 3.25 Hz cut-off frequency.

The lengths of the medial gastrocnemius muscle and Achilles tendon were combined with the ultrasound and motion analysis data. Specifically, the position of the muscle-tendon junction was calculated by combining coordinates from the handle of the ultrasound probe, with muscle-tendon junction coordinates from the ultrasound image; to give the position of the muscle-tendon junction with respect to the global coordinate system of motion analysis. Medial gastrocnemius muscle length changes were calculated as the distance from the medial epicondyle marker to the global muscle-tendon junction marker, and the Achilles tendon changes, as the distance from the muscle-tendon junction to the calcanei, using custom written analysis software (Matlab

v7.14, MathWorks, Cambridge, UK). Medial gastrocnemius muscle and Achilles tendon resting lengths were calculated at 100% plantarflexion ROM. This was used for the subsequent calculation of muscle and tendon strain, which was calculated by dividing elongation at maximum dorsiflexion, by resting length.

6.2.4.3 Fascicle strain

Medial gastrocnemius muscle fascicle strain was calculated by dividing change in fascicle length by fascicle resting length, which was quantified using open source digital measurement software (Image J, NIH, USA). These fascicle measurements were made at the mid-belly of the muscle. Three optimal and identifiable fascicles were selected and measured from deep to superficial aponeurosis. An average of the three fascicles was used for the subsequent analysis of resting fascicle length, taken with the ankle at 100% plantarflexion ROM. For each participant, fascicle strain was calculated at maximum dorsiflexion.

Mechanical properties of the medial gastrocnemius and Achilles tendon were compared pre- to post-intervention, for both the experimental group and the control group. Muscle and tendon stiffness in six participants (three control and three experimental) was also measured again during the follow-up session, four weeks after the cessation of the intervention period.

6.2.4.4 Gait analyses

For the second part of the pre and post-intervention session, each participant was asked to complete two 6 m overground walks, with the use of walking aids, at a self-selected pace. Eight infrared LED motion capture cameras (Motion Analysis, Santa Rosa, USA) were positioned around the 6 × 4 m area. Reflective markers were placed bilaterally on

the heads of the first and fifth metatarsals, the medial and lateral malleoli, the calcanei, the medial and lateral femoral epicondyles, and the greater trochanters. We focused the analysis on three key spatiotemporal parameters: stride length, stride velocity and time in double support stance. These variables were chosen on the basis that they are widely used in the assessment of CP children and are valid and reliable parameters (Dini & David, 2009), which adds to the clinical relevance of these results. In addition, spatiotemporal parameters have been reported as being more sensitive indicators of motor involvement in CP than single joint kinematics (Damiano & Abel, 1996). In addition, we calculated the internal ankle joint angle at initial contact (0%), mid-way into the stance phase (50%) and toe-off (100%). These variables were computed for both groups, and compared pre- to post-intervention.

We were also interested in inter-segmental coordination pre- to post-intervention. For this purpose we calculated continuous relative phase (CRP) during the stance phase of the right leg. Stance phase was defined as the point of initial contact to toe-off. For continuous relative phase, we first calculated the angle of the foot (represented by the distance between the fifth metatarsal and the lateral malleolus) and the angle of the shank (represented by the distance between the lateral malleolus and the lateral femoral epicondyles). These were calculated with respect to the horizontal axis using filtered coordinates (low-pass fourth order zero-lag Butterworth filter with 10 Hz cut-off frequency). For each segment, phase angle was calculated according to the following equation:

$$= \tan^{-1} —$$

(Equation 6.1)

Where φ: phase angle, *i*: data point within stance phase, ω_i = angular velocity, and θ_i = angular displacement.

The CRP between these two segments was then calculated. This is defined as the difference between the two phase angles at any point of the gait cycle (Figure 6.7). We defined the CRP between the foot and shank as follows:

$$CRP_{Foot\text{-}Shank} = \varphi_{Foot} - \varphi_{Shank}$$

For the calculation of CRP, phase angles of the foot and shank segments were ensemble averaged across three step cycles. A $CRP_{Foot\text{-}Shank}$ of 0 deg indicates that the segments move in-phase (as two constrained segments with no movement at the ankle). A positive $CRP_{Foot\text{-}Shank}$ indicates that the foot "leads" the shank in its phase trajectory, and a negative CRP indicates the foot "leads" the shank (Barela, Whitall, Black & Clark, 2000; Hamill, van Emmerik, Heiderscheit, 1999). Segment coordination patterns were analysed by calculating the mean absolute relative phase (Kyvelidou *et al.* 2009), which captured the entire relative phase in a single value. This was calculated for each participant and compared pre- to post-intervention.

6.2.5 Statistical analysis

Several group (experimental vs. control) × condition (pre vs. post-intervention) ANOVAs with repeated measures on condition, were used to determine changes in the

mechanical properties of the muscle and tendon, and parameters of gait. Specifically, for mechanical changes, ANOVAs were performed on ankle dorsiflexion angle, joint stiffness, muscle and tendon stiffness, muscle, fascicle and tendon strain and resting fascicle length. To determine changes in gait parameters, ANOVAs were performed on spatiotemporal parameters of gait, and mean absolute relative phase (pre vs. post-intervention) between the experimental and control groups.

One further group (experimental vs. control) × condition (pre vs. post-intervention) × stance (0%, 50% and 100% stance) ANOVA with repeated measures on condition and stance was used to determine changes in ankle joint angle at different phases of stance between the experimental and control groups. Follow-up *t*-tests with Bonferroni correction were used where relevant. The significance level for alpha was set at $p < 0.05$.

6.3 Results

Compliance to the stretching intervention in the experimental group was 99.4%, with just one participant from the experimental group missing one session. The first ANOVA revealed that both the main effect of condition ($F_{1, 11} = 32.30$ $p < 0.001$) and its interaction with group on maximum dorsiflexion angle ($F_{1, 11} = 38.90$, $p < 0.001$) were significant. These results show that maximum dorsiflexion angle increased from pre- to post-intervention in the experimental group, but not in the control group (Figure 6.1).

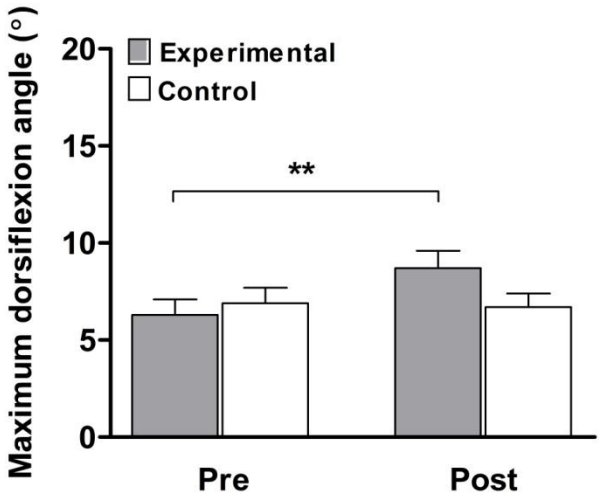

Figure 6.1. Ankle ROM in the experimental (grey) and the control (white) groups, at pre and post-intervention.

6.3.1 Joint, muscle and tendon stiffness

Results for joint stiffness and muscle stiffness indicated significant main effects for condition ($F_{1, 11} = 26.91$ $p < 0.001$; $F_{1, 11} = 11.58$ $p < 0.01$, respectively), and significant group × condition interaction effects for joint ($F_{1, 11} = 43.00$, $p < 0.001$) and muscle stiffness ($F_{1, 11} = 57.73$, $p < 0.001$). Joint (Figure 6.2) and muscle (Figure 6.3) stiffness decreased significantly post-intervention in the experimental group. In the control group

muscle stiffness increased significantly during the intervention period. No significant changes in tendon stiffness were observed in either group, pre- to post-intervention.

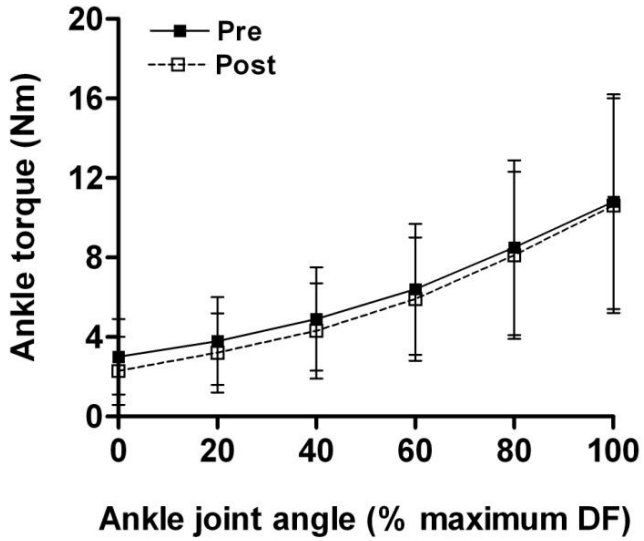

Figure 6.2. Passive torque-angle curve plotted as percentage of dorsiflexion ROM for the experimental group (values are mean ± SD).

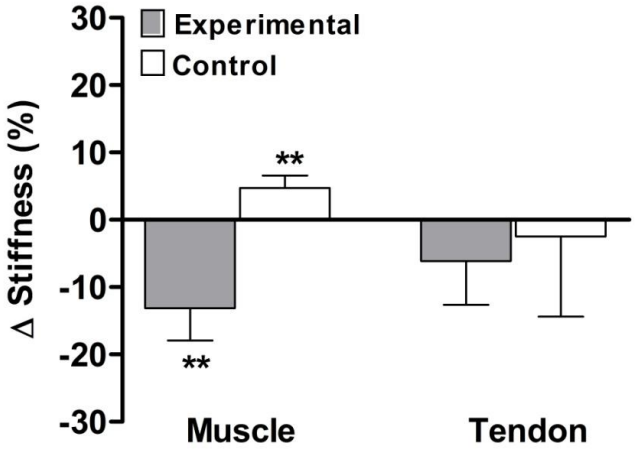

Figure 6.3. Change (Δ) in muscle and tendon stiffness pre- to post-intervention in the experimental and control groups (values are mean ± SD, $^{**}p < 0.01$).

6.3.2 Muscle, fascicle and tendon strain

There was also a significant main effect of condition on muscle strain ($F_{1, 11} = 8.89$ $p < 0.05$), and a significant interaction effect between condition and group ($F_{1, 11} = 35.54$, $p < 0.001$). Muscle strain increased from pre- to post-intervention in the experimental group. For fascicle strain there was also a significant main effect ($F_{1, 11} = 97.75$ $p < 0.001$) and a significant interaction effect ($F_{1, 11} = 235.64$, $p < 0.001$). Fascicle strain increased in the experimental group following the stretching intervention (Figure 6.4). There was no change in resting fascicle length from pre- to post-intervention ($F_{1, 11} = 0.66$, $p = 0.44$). In addition, there were no significant changes in tendon strain in either the experimental or control group, pre- to post-intervention.

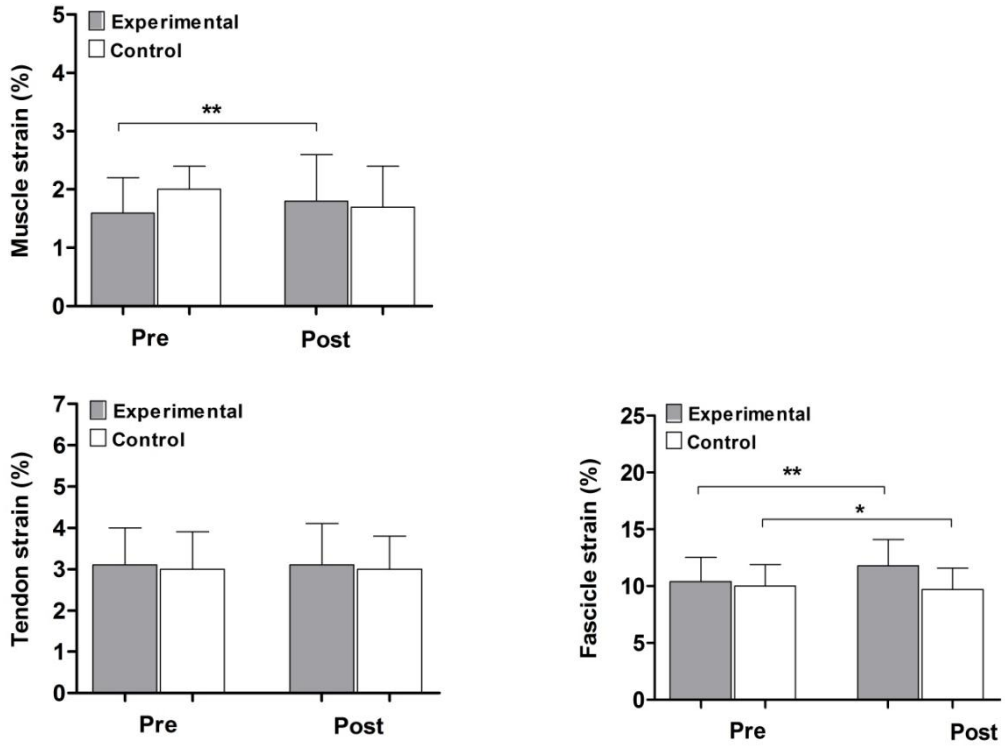

Figure 6.4. Changes in muscle strain (left), fascicle strain (centre) and tendon strain (right) in the experimental group and control group, pre- to post-intervention.

6.3.3 Findings from follow-up

To assess whether any effects of stretching were maintained after the cessation of the stretching intervention, three participants from the experimental group and three from the control group also took part in one further follow-up session, which measured muscle and tendon stiffness. In the experimental group, the percentage change in muscle stiffness was -12.7% pre- to post-intervention, and -6.3% when measured post-intervention to follow up. Although there was still a reduction in muscle stiffness four weeks after the intervention, the percentage change was smaller indicating an increase in muscle stiffness post-intervention to follow up, back towards baseline values. For tendon stiffness, percentage change measured pre- to post-intervention was -2% in the experimental group. When measured post-intervention to follow up, the percentage change was -0.2%, indicating no considerable alterations in tendon stiffness four weeks after the intervention. For the control group, there was also no considerable percentage change in muscle and tendon stiffness from pre- to post-intervention (3.9% and 1.3%, respectively), or from post-intervention to follow up (2.1% and 2.7%, respectively).

6.3.4 Gait parameters

An analysis of the spatiotemporal gait characteristics demonstrated a significant main effect of condition on stride velocity ($F_{1, 11} = 29.82$, $p < 0.01$), and a significant interaction effect between group and condition ($F_{1, 11} = 7.33$, $p < 0.05$). Stride velocity decreased in the experimental group post-intervention. In addition, no differences were observed pre- to post-intervention in either group for stride length ($F_{1, 11} = 2.14$, $p = 0.55$) or for the stance time in double support ($F_{1, 11} = 0.18$, $p = 0.68$).

For ankle angle, the main effect of condition ($F_{1, 11} = 24.09$, $p < 0.001$) and its interaction with group ($F_{1, 11} = 18.08$, $p < 0.001$) were significant, which showed that overall, dorsiflexion angle increased post-intervention and the increase was larger in the experimental group. The main effect of stance phase ($F_{2, 22} = 15.55$, $p < 0.001$) and its interaction with condition ($F_{2, 22} = 11.01$, $p < 0.001$) were significant. This meant that overall, dorsiflexion angle was not the same at all phases of stance. The results showed that at post-intervention ankle joint was more dorsiflexed in at least one phase of stance, compared to pre-intervention. The significant three-way interaction ($F_{2, 22} = 6.07$, $p < 0.001$) showed that increased dorsiflexion for at least one phase of stance was larger in the experimental group. Further, multiple *t*-tests with Bonferroni correction ($p < 0.016$) showed that post-intervention dorsiflexion angle decreased more profoundly in the experimental group at 50% ($t_6 = 6.68$, $p < 0.001$) and 100% ($t_6 = 8.43$, $p < 0.001$) of the stance phase. There were no changes in ankle angle after the intervention period, at any phase of stance in the control group (Figure 6.5).

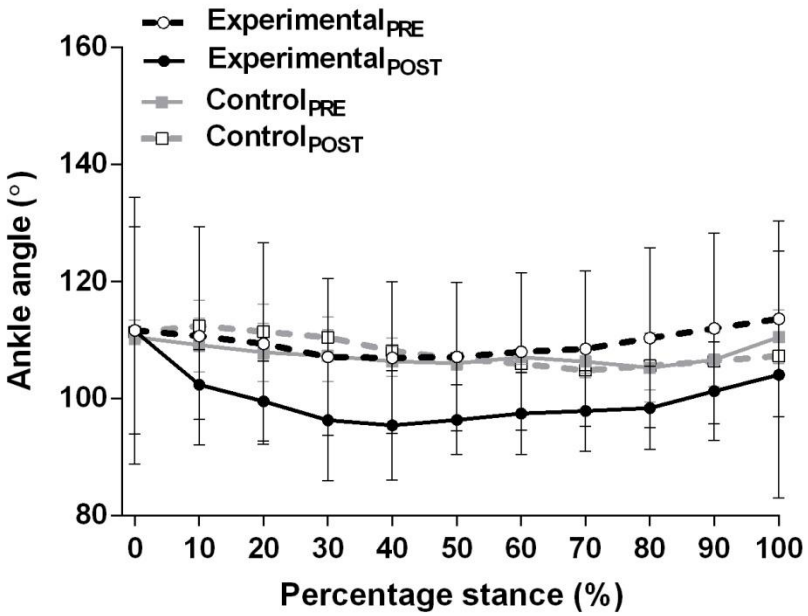

Figure 6.5. Ankle angle measured during the stance phase of gait in both the experimental and control groups, pre and post-intervention.

$CRP_{Foot\text{-}shank}$ during the stance phase, at pre and post-intervention for the experimental and control groups are illustrated in Figure 6.6. In general, $CRP_{Foot\text{-}shank}$ values close to zero imply that the foot and shank move in-phase with each other, whilst positive values imply that the foot segment "leads" the shank through its phase trajectory and *vice versa*. Larger standard deviations of the $CRP_{Foot\text{-}shank}$ in the experimental group imply that, as a group, the coordination between the foot and shank was less stable during stance - although this was not directly measured.

There was a significant main effect of condition on mean absolute relative phase ($F_{1,11}$ = 10.70, $p < 0.01$) and a significant interaction effect between group and condition ($F_{1,11}$ = 7.35, $p < 0.05$). Mean absolute relative phase was higher in the post-intervention condition compared to pre-intervention, which implied that the foot and shank segments moved more independently from each other (out of phase). In addition, mean absolute

relative phase at post-intervention was also larger in the experimental group compared to the control group (Figure 6.7).

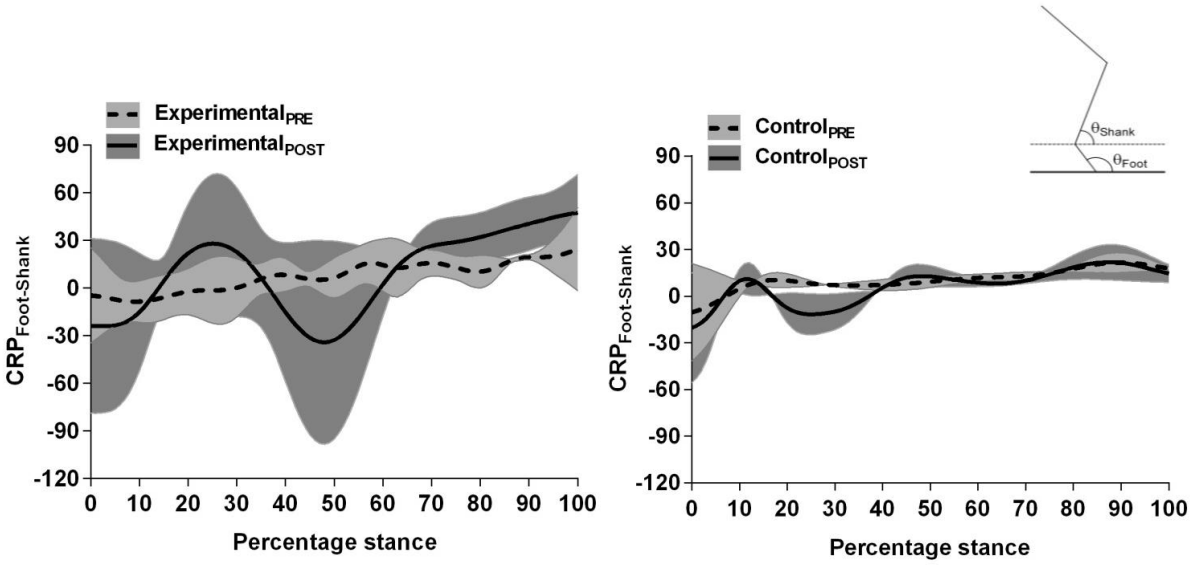

Figure 6.6. $CRP_{Foot\text{-}shank}$ during the stance phase, at pre (dashed line) and post (solid line) intervention for the experimental (left) and control groups (right). Mean ensemble CRP (± SD) of the right leg over three stance cycles, pre (dashed line) and post (solid line) intervention, for the experimental (left) and control (right) groups.

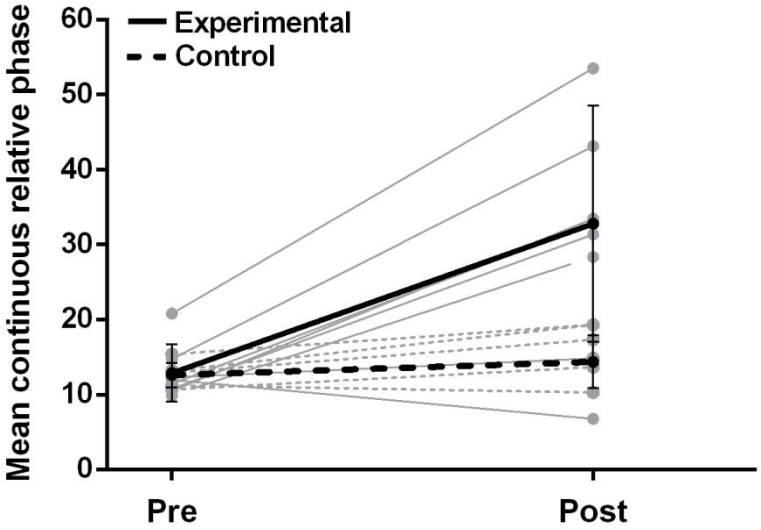

Figure 6.7. Mean absolute relative phase measured in the experimental and control groups, pre- to post-intervention (solid black lines indicate group means ± SD).

6.4 Discussion

The overall purpose of this study was to describe the mechanical and functional adaptations resulting from a six week passive stretching intervention of the *triceps surae* muscle-tendon unit in children with spastic CP. Regarding the first purpose, six weeks of passive stretching increased maximal passive dorsiflexion angle and also reduced joint stiffness. This was associated with a reduction in muscle stiffness, with concurrent increases in muscle and fascicle strain. However, no differences in tendon stiffness or strain were found following the intervention. Regarding the second purpose, six weeks of passive stretching increased ankle dorsiflexion angle at 50 and 100% of stance. In addition, foot and shank inter-segmental coordination became more variable, suggestive of the foot and shank segments moving more "out of phase" following the intervention. In addition, no positive effects of stretching, on spatiotemporal parameters of gait were found. In fact, there was a decrease in stride velocity following the intervention.

The stretching intervention elicited an increase in maximal passive ankle dorsiflexion from 6 deg to 9 deg, accompanied by a 32% decrease in passive joint stiffness. This finding has previously been reported in healthy populations following stretching (Kubo *et al.*, 2002c; Nakamura *et al.*, 2012). Nakamura *et al.*, (2012) reported a 13% reduction in passive joint stiffness following four weeks of stretching using a smaller volume (120 s per day) than in the present study. Similarly, in children with spastic CP, O'Dwyer *et al.* (1994) showed a reduction in passive joint stiffness after 30 minutes of stretching, three times per week for six weeks, which is consistent with the findings in this study.

The most significant findings in the present study were that changes in joint stiffness were associated with a 12% reduction in muscle stiffness, confirming the clinical

assumption in this regard. These are the first data to show a clear tissue-dependent response to stretching exercise in this population, and indicate importantly, that the spastic muscle can respond to long-term stretching. It should be noted that the measures of muscle stiffness made in this study are somewhat conceptual. "Muscle stiffness" as measured here, contains not only stiffness of the *triceps surae* muscles, but all surrounding tissues, the contribution of which cannot be known. Despite this limitation, the findings of "muscle stiffness" are in line with the finding of a concurrent reduction in joint stiffness and no alterations in tendon stiffness. A lack of alteration in the tendon's stiffness suggests that passive stretching does not provide an effective stimulus to alter the mechanical properties of the tendon in children with spastic CP. This is in line with previous research in a healthy population, which found that static stretch training was not sufficient to elicit changes in tendon properties (Kubo *et al.*, 2002c; Mahieu *et al.*, 2007).

The changes in stiffness were concurrent with changes in strain. There was an increase in muscle (23%) and fascicle strain (13%) post-stretch in the experimental group. The present results are of substantial importance, showing that the muscle offers a greater plasticity in response to passive stretching than the tendon. The results from the follow-up session showed a trend towards increased muscle stiffness towards baseline, four weeks after the intervention, although some reduction in stiffness compared to pre-intervention did still exist.

The factors which contribute to muscle and joint stiffness in CP are not completely understood, but given that a decrease in muscle stiffness was observed after six weeks of passive stretching, it is interesting to speculate as to the possible mechanisms underpinning the change. One mechanism is a change in the mechanical properties of

the series elastic component. Although no changes were observed in the tendon, it cannot be ruled out that changes in other tissues occurred such as changes in myofilaments or titin (Prado *et al.*, 2005). Changes may also have occurred within the parallel elastic component. The endomysium, perimysium and epimysium are thought to substantially influence passive resistance to stretch (Gajdosik, 2001), although it is not possible to directly measure these in humans. Given that muscle strain showed a greater change post-stretch, than the fascicle, changes are likely to have occurred in the parallel elastic component and intra-muscular connective tissue, which is shown to contribute to increased passive stiffness in CP (Smith *et al.*, 2011). In addition, fascicle strain did also increase suggesting some alterations in intra-fascicular structures (Prado *et al.*, 2005), fibre-based connective tissues (Prado *et al.*, 2005), or the number of serially-arranged sarcomeres within fibres (Tabary *et al.*, 1981). Of these, a change in in-series sarcomere number is often speculated to underpin changes in muscle or fascicle extensibility (Gajdosik, 2001) and, in fact, increases in sarcomere number were observed after long-term, intense muscle strain was imposed by tibial lengthening in humans (Boakes *et al.*, 2007). Nonetheless, the present data are not consistent with this finding because there was no change in absolute resting fascicle length. In the absence of firm evidence in this and other studies, it suggests that this volume of passive stretching was not sufficient to alter sarcomere number in children with spastic CP.

Neural factors could contribute to the reduction in stiffness. For example, a reduction in stretch reflex gain or an increase in the threshold of the stretch reflex could theoretically reduce muscle stiffness, although there has been no conclusive evidence of a change in these parameters in CP following stretching. More frequently, alterations in stretch tolerance has been reported involving a change in the perception of stretch. However, given that our post measurements were made at least 48 hours after the last stretch

session, and the fact that some reductions in stiffness could still be observed four weeks after the intervention, suggests that at least some of the alterations seen here were a result of changes in the mechanical properties of the muscle and fascicles.

We speculated that increased muscle and joint stiffness observed in children with spastic CP, may not solely be a result of neurological insult, but could act partly as a compensatory mechanism against excessive muscle weakness, particularly during movement such as gait. Thus, increased stiffness may not impair gait, but may aid aspects of gait, perhaps by helping to store and release elastic energy in the *triceps surae* muscles and Achilles tendon, in a spring-like fashion (Holt *et al.*, 2000a). We speculated that if CP-related muscle and tendon alterations were, in part, a compensation to increased muscle weakness then any reductions in joint stiffness would potentially not improve function (Latash & Anson, 1996).

To measure the effects of passive stretching on function and describe the corresponding alterations in movement kinematics, we measured spatiotemporal gait parameters, ankle angle during the stance phase, and inter-segmental coordination, pre- to post-intervention. Stride length and time in double support did not change following six weeks of passive stretching. However, there was a decrease in stride velocity from pre- to post-intervention. This could have been caused by reductions in muscle and joint stiffness. For example, the association between stiffer calf muscles and greater stride velocity has previously been shown (Crosbie *et al*., 2012). A stiffer muscle may provide stability at the ankle (Duan, Allen & Sun, 1997), which facilitates a more rapid gait in children with spastic CP (Crosbie *et al.*, 2012). Thus, these present findings of a reduced stride velocity with reduced stiffness are in agreement with previous findings.

In addition, stretching also altered the foot-shank inter-segmental coordination. Descriptively, rather than moving as fixed (constrained) segments, the foot and shank segments alternatively led in the phase space. This was demonstrated by larger absolute CRP values and the appearance of clear reversals (i.e., local minima and maxima) in the CRP configuration throughout the stance. For example, from approximately 50% of the stance period, foot segment velocity started to increase, and around approximately 63% of the stance phase the foot started to lead the shank in the stance phase. Overall, the coordination between the two segments appeared less stable (larger standard deviations) post-intervention supporting the notion of fewer constraints on the movement of the two segments after a reduction in muscle and joint stiffness (Figure 6.6). This further supports the speculation that reducing stiffness does not lead to improvement in gait parameters in CP. However, to fully understand the role of muscle stiffness in gait, future studies should include a physiological measure of gait efficiency, which would provide a better understanding of the changes in gait as a result of stretching.

Finally, ankle angle was also significantly smaller (increased dorsiflexion) during 50 and 100% of stance following the intervention, concurrent with improvements in joint ROM. Children with spastic CP display equinus gait where the toes provide initial contact with the ground, as opposed to the heel. In clinical practice, it is often claimed that a plantarflexed foot is the direct result of the neurological insult. However, the results here show that despite achieving a greater maximum dorsiflexion angle during stance, ankle angle at initial contact did not change. A plantarflexed foot on ground contact could be an effective biomechanical adaptation, providing a mechanism for loading the body mass in a spring-like fashion, and thereby improving the ability to load the Achilles tendon and *triceps surae* muscles (Holt *et al.*, 2000a). It has been previously shown that younger children with CP often have enough passive ROM

needed for a normal gait pattern, but they still walk with plantarflexed foot on initial contact. Thus, the plantarflexed foot on initial contact may not be a direct result of the insult but an adaptation that facilitates aspects of gait (Fonseca *et al.*, 2004). Collectively, these results demonstrate that there were no improvements in the spatiotemporal gait parameters following six weeks of passive stretching in CP. This refutes the clinical assumption that reducing stiffness improves function such as gait in children with spastic CP.

Collectively, the results of this study confirm the clinical assumption that passive stretching can reduce joint stiffness for children with CP. Moreover, the present study shows for the first time that such reductions were associated with changes in the mechanical properties of muscle but not the tendon. The alterations in muscle and joint stiffness led to no improvements in gait parameters in children with spastic CP. This lends some support to the speculation that joint stiffness may aid some aspects of function, particularly during gait. However, future research is needed to support this speculation. Interventions that reduce muscle stiffness whilst increasing tendon stiffness, such as the study by Zhao *et al.* (2011), should be investigated more thoroughly to determine if concomitant changes in muscle and tendon stiffness would affect function. Further, the results add to the evidence that muscle weakness rather than stiffness affects function in children with spastic CP (Damiano, Dodd & Taylor, 2002) Thus, strength training could be a more effective intervention to target muscle weakness, and increase tendon stiffness, which may be the key to improving gait parameters in this population.

In conclusion, we show for the first time that passive stretching as a clinical intervention in spastic CP can cause alterations in the mechanical properties of the muscle whilst

having no effect on the tendon. We suggest that future therapies might consider concurrent manipulation of muscle and tendon stiffness. Specifically, therapies should aim to reduce muscle stiffness and increase muscle strength, which may be the key to improving function in CP.

CHAPTER 7: GENERAL DISCUSSION

Children with spastic CP experience both neurological and musculoskeletal adaptations as a result of damage to the developing brain. Among these adaptations is an increase in muscle and joint stiffness (Alhusani *et al*., 2010; Barber *et al*., 2011a; Smith *et al*., 2011), which has previously been considered to be the main cause of movement dysfunction (Ward & Bandi, 2010, pp. 370). However, it has also been suggested that these CP-related muscle and tendon abnormalities may represent an energy-saving mechanism during movements such as gait, in the absence of adequate muscle force (Fonseca *et al*., 2004; Holt *et al*., 2000a; Latash & Anson, 1996). These conflicting views demonstrate the existing disconnect of clinical rationale and research evidence, and they highlight several areas warranting further investigation. For example, the adaptations of the spastic muscle are often the target of therapeutic interventions, whilst the role of the tendon is largely overlooked. From research in adults, we know that the mechanical properties of the tendon, in particular its stiffness, are important factors influencing force production (Lichtwark & Wilson, 2008). More specifically, tendon length and stiffness will affect the force-generating capacity of the spastic muscles, which has important implications for understanding atypical movement patterns and inefficiencies in children with spastic CP. The lack of understanding with regards to CP-related muscle and tendon adaptations makes it difficult to determine appropriate clinical interventions.

Passive stretching has been commonly and routinely prescribed in children with spastic CP for a number of years (Damiano, 2009; National Institute for Health and Care Excellence, 2012). Implicit in this practice is the clinical assumption that reducing joint stiffness, through reductions in muscle stiffness, improves function. However, previous research evidence is lacking, which can support or refute the use of passive stretching in

CP to improve function. Therefore, the main purpose of this research was to investigate muscle and tendon adaptations in children with spastic CP, and the response to passive stretching, with a view to informing evidence-based clinical practice.

7.1 Summary of main findings

Experimental Chapter 3 sought to identify an appropriate method for deriving muscle and tendon stiffness in children with spastic CP. Commonly, tendon stiffness is estimated by dividing changes in force by Achilles tendon elongation, measured during a maximum voluntary contraction ("active method"). Due to excessive muscle weakness and co-contraction (Rose & McGill, 2005; Stackhouse *et al*., 2005), children with spastic CP encounter problems performing maximal voluntary contraction manoeuvres (Tedroff *et al*., 2008). Therefore, the agreement between the "passive method" of deriving stiffness and the commonly used "active method" was investigated. The results showed good agreement between tendon stiffness measured using the "active method" compared to the "passive method". Specifically, there were strong correlations between methods at all strain rates ($R^2 > 0.98$). In addition, 95% of all data points across strain rates fell within 95% confidence intervals for limits of agreement. In the context of this research, this agreement was considered acceptable. The results of Chapter 3 also show a clear strain-rate dependence of tendon stiffness. More specifically, higher strain rates resulted in higher stiffness values, which has important implications for the measurement of tendon stiffness. Different protocols such as maximal voluntary contractions (e.g., Kay & Blazevich., 2009), ramped manoeuvres (e.g., Kubo *et al*., 2002a) or passive rotations (e.g., Morse *et al*., 2008) are likely to assess tendon stiffness under different strain rates. Thus the strain-rate dependence of tendon stiffness needs to be taken into consideration when comparing tendon stiffness values across studies.

Experimental Chapter 4 investigated the mechanical properties of the muscle and tendon in CP compared to TD children, with a particular focus on the adaptations of the Achilles tendon. Following the close agreement between methods in experiment 1, the passive method was chosen for the subsequent calculation of Achilles tendon stiffness and a measure of "global" muscle stiffness. This measure incorporated all *triceps surae* muscles (soleus as well as medial and lateral heads of the gasctrocnemius) and all surrounding tissues in the calculation of stiffness. CP-related abnormalities in the spastic muscle have been previously reported in CP (Barber *et al.*, 2011a; 2011b; Wren *et al*., 2010), but it was of interest to determine the mechanical properties of both muscle and tendon in individuals, due to the close interaction of the muscle and tendon to the production of movement. Despite children with CP having a smaller tendon cross-sectional area compared to TD children, tendon stiffness and Young's modulus were not different between groups. These results let us speculate that the tendon's material properties do not necessarily adapt atypically in children with CP compared to their TD peers. A possible explanation for this could be that the spastic muscle provides a mechanical loading stimulus to the tendon, similar to the stimulus arising from an increase in muscle size and force in TD children (Kubo *et al*., 2001b). The results of a smaller cross-sectional area may imply that the dimensional properties of the tendon respond to a different stimuli. For example, a slower rate of bone growth has been reported in CP, due to a lack of weight-bearing activity (Samson-Fang & Stevenson, 2008), which may prevent dimensional alterations in the tendon in CP compared to TD children. The results of this study also showed that the spastic muscle was significantly stiffer than the tendon, which may partly explain movement inefficiencies in this population (Rose *et al*., 1990). The study also expanded the results from Chapter 3, by documenting the strain-rate response of the Achilles tendon in children with spastic CP,

compared to TD children. The results showed that at angular velocities up to 30 deg·s^{-1}, there was a velocity-dependent increase in tendon stiffness, the slope of which was steeper in the CP group. This has important implications for the current clinical test of spasticity. For example, the results demonstrate that at angular velocities below 30 deg·s^{-1}, there is a clear velocity-dependent increase in tendon stiffness, independent of a neural response. Thus, the tendon's response to increasing strain-rate needs to be taken into consideration when conducting clinical tests of spasticity The assumption that a velocity-dependent increase in joint stiffness is reflective of spasticity may not be correct.

The final two experimental Chapters (5 and 6), were conducted with the goal of understanding how these CP-related changes in muscle and tendon mechanics influence the degree to which clinical interventions are effective. Based on the discussion from Chapter 4 that muscles are significantly stiffer than tendons in children with CP, it was hypothesised that any acute stretch applied to the ankle may be taken up solely by the tendon, and may not reach the muscle. Despite these CP-related changes in muscle and tendon, the results of Chapter 5 showed that elongation of the muscle-tendon unit was equally attributable to elongation of the muscle and elongation of the tendon. In particular, it was shown that tendon, muscle belly and muscle fascicles increased by 1.0 cm, 0.8 cm and 0.6 cm respectively. These results are of major significance as these are the first data to demonstrate that muscles elongate in response to stretch in children with spastic CP, which is an assumption that is commonly used by clinicians. The results were consistent across two different commonly used ("ecologically valid") stretch techniques, which are also of practical implications. Finally the results are an important prerequisite to test the hypothesis that long-term stretching may cause adaptations in the

muscle. If the muscle does not respond to acute stretch then long-term adaptations in the properties of the muscle would not be expected.

In the final study, the effects of six weeks passive stretching on the properties of the Achilles tendon and *triceps surae* muscles, as well as gait parameters were investigated. Following the results of Chapter 5, the PT-led technique was chosen to implement the six week stretching intervention. The reason for this choice was that the self-stretch is motivation-dependent, and it was important to ensure a consistent stretch during each session. The results of Chapter 6 confirmed the clinical assumption that passive stretching reduces muscle and joint stiffness in children with spastic CP. These results appear to be related to alterations in intra- and extra-muscular connective tissue. An increase in muscle stiffness towards baseline was observed four weeks after the cessation of the intervention. Interestingly, six weeks of passive stretching did not alter the stiffness of the Achilles tendon. Despite these alterations and reductions in muscle and joint stiffness, spatiotemporal gait parameters were largely unchanged, with the exception of stride velocity, which became slower in the experimental group. The results confirm the clinical assumption that muscle and joint stiffness are reduced with long-term stretching. However, this did not result in improved gait, which suggests the clinical assumption that increased muscle and joint stiffness impairs function, may not be correct. The result of a slower stride velocity after stretching may even suggest some aspects of increased joint stiffness are beneficial for certain aspects of gait in this population.

7.2 Implications

Results of this thesis have vast implications both on a basic science, as well as an applied level. Regarding the former, the results presented here extend our fundamental

knowledge of muscle and tendon properties in children with spastic CP. Specifically, the result that *triceps surae* muscles are stiffer than the Achilles tendon, and that the strain-rate dependence of tendon stiffness is different in children with spastic CP is an important clinical finding. In addition, in contrast to the spastic muscle, the mechanical properties of the tendon have not received much attention by the scientific community. Thus, the results from this thesis relating to the mechanical properties of the Achilles tendon, add a significant contribution towards fully understanding muscle and tendon properties in children with spastic CP.

Regarding the latter, the results presented here have important implications for clinical practice. Specifically, the spastic muscle does elongate in response to both acute and long-term stretching, which confirms the clinical assumption in this regard. In addition, both joint stiffness and *triceps surae* stiffness decreased in response to long-term stretching. However, this decrease in stiffness did not improve any aspects of gait, which does not support the clinical assumption that increased stiffness causes movement dysfunction in children with spastic CP. This may imply that clinical interventions should aim to maintain a certain level of joint stiffness, whilst aiming to alter the relative stiffness's of the muscle and tendon. In addition, there is an exaggerated strain-rate dependence of tendon stiffness in children with spastic CP. This has important clinical implications for the test of spasticity. If the joint is rotated at angular velocities up to 30 $deg \cdot s^{-1}$, there will be an appreciable increase in stiffness as a result of changes in the tendon, which makes the diagnosis of a neural velocity-dependent increase in tone, invalid.

Collectively, results from this thesis provide an example of how fundamental knowledge of muscle and tendon mechanics can be implemented in clinical practice. In

order to provide the most appropriate and effective treatments to patients, clinicians are required to implement evidence-based practice. The results such as those found in this thesis will play a role in helping clinicians to make decisions about the best ways to treat children with CP in the future, and provides future direction for the establishment of other effective interventions.

These results provide us with some directions for future research. For example, several secondary CP-related musculoskeletal adaptations occur in response to CP, the most dominant being muscle weakness and stiffness. The results demonstrate that reducing muscle stiffness did not improve function. This supports the use of interventions such as strength training, which target muscle weakness. It could be the case that an increase in joint stiffness aids some aspects of gait (Fonseca *et al*., 2001; 2004; Holt *et al*., 2000a), and interventions should therefore aim to optimise the musculoskeletal system in CP (Latash & Anson, 1996). One way to do this may be to maintain a certain level of joint stiffness, whilst altering the relative stiffness's of the muscle and tendon. This speculation may be achieved through a combination of resistance training to increase tendon stiffness, with stretching to reduce muscle stiffness. This combination of stretch and resistance training has previously been reported to cause concomitant changes in muscle and tendon stiffness (Zhao *et al*., 2011). The effect of these muscle and tendon changes on gait parameters and other functional outcomes will be important considerations for future research. Additionally, the results showed that the tendon's dimensions adapt atypically in children with spastic CP, whilst the tendon's material properties were not necessarily different to TD children. This suggests different mechanical stimuli may be responsible for the respective dimensional and material alterations in the tendon. The adaptations of the tendon to various training stimuli is still a relatively new field of research. As such, there is still much to be done to quantify the

exact stimuli, which cause dimensional and material alterations towards increasing tendon stiffness in children, and those with spastic CP.

7.3 Limitations

The main limitation to this series of investigations was in regards to the "passive method" to derive stiffness. Regarding tendon stiffness, the passive method uses a force range that is significantly lower than the more commonly used active method, in which stiffness is usually assessed in the toe region. In spite of this, it was demonstrated that the two methods correlate well with each other. The passive method was also considered to be more appropriate for use in children with spastic CP, due to co-contraction and excessive muscle weakness (Rose & McGill, 2005; Stackhouse *et al*., 2005). It was observed that children with CP had problems voluntarily activating the correct muscle group to produce torque. Although a maximal force-elongation curve is not entirely necessary for the calculation of stiffness, a force level that is too low will incorporate the toe-region of the curve, underestimating stiffness. Finally, we have shown the velocity-dependence of tendon stiffness to be an important consideration in the measurement of tendon stiffness. Due to reduced activation and firing rates (Macefield *et al*., 1996; Rose & McGill, 2005), it was considered children with CP may have problems controlling the rate at which force was developed, thus confounding the results.

With regards to muscle stiffness measured in the present series of studies; its estimation is likely to contain an appreciable contribution from ligaments, skin, epimysium, perimysium and endomysium, contractile and non-contractile components (Morse *et al.*, 2008). As such, we currently cannot specifically measure muscle stiffness, because the contributions of these other structures to force are unknown. The reason for the decision

to use this method was due to the fact that children with spastic CP have an abundance of intra- and extra-muscular connective tissue, which is thought to substantially contribute to increased stiffness. All structures, which contribute to passive stiffness, could theoretically be affected by an intervention such as stretching. Therefore, although this measure of "muscle" stiffness is somewhat conceptual, it provides important information on the changes to all passive elastic structures in CP compared to TD children, and the changes that occurred following six weeks of stretching. Thus, in the present context, it was not necessary to know the stiffness of just the muscle component, but of all other structures, which contribute to passive stiffness. Finally, to measure the length of the muscle and tendon in these studies, we combined coordinates of the muscle-tendon junction from the ultrasound image, with motion analysis coordinates of the epicondyles, calcanei and ultrasound probe. As such, muscle and tendon lengths were modelled as straight lines, which would have likely underestimated the elongation of the tendon. However, since this method was used in all participants, a systematic underestimation of tendon length would not have changed the overall results of these studies.

7.4 Conclusions

The main findings from the four experimental studies (Chapters 3-6) provide an example of how fundamental knowledge of muscle tendon mechanics can be implemented in clinical practice. The findings conclude that the properties of the Achilles tendon in children with CP are different to that of TD children. These alterations may also have contributed to the greater strain-dependence of Achilles tendon stiffness, than in TD children. These results may help to explain atypical movement patterns observed in CP. The findings also showed that muscle and joint stiffness were reduced with passive stretching, but there were no changes in the

mechanical properties of the tendon. Despite reductions in stiffness, there were also no improvements in spatiotemporal gait parameters following the stretching intervention. Collectively these results suggest that interventions should focus on optimising the properties of both the muscle and tendon. The properties of the tendon should not be overlooked as a factor contributing to movement and effective interventions in children with spastic CP. The results from this research provide a useful basis for future research, which should look at the optimising the mechanical properties of the muscle and tendon to improve movement efficiency in children with spastic CP, and second, to investigate the functional outcomes of a combination of resistance and stretching exercises, to alter the mechanical properties of the muscle and tendon, whilst maintaining a certain level of joint stiffness.

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APPENDICES

Appendix I: Peer-reviewed publication 1

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Method and strain rate dependence of Achilles tendon stiffness

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ABSTRACT

Tendon stiffness is calculated by dividing changes in tendon force by tendon elongation. For this purpose, participants are commonly asked to perform a maximal muscle contraction ("active" method). Alternatively tendon elongation can be achieved by means of a passive joint rotation ("passive" method). The purpose of this study was to compare Achilles tendon stiffness obtained from both methods across different tendon strain rates. Twenty adults performed a series of ramped maximum isometric plantarflexions of different durations. Passive ankle rotations of different angular velocities were also performed. Achilles tendon stiffness was obtained from a combination of motion analysis, isokinetic dynamometry and ultrasonography and compared across methods at three strain rates. At all strain rates, tendon stiffness obtained from the active method was 6% greater compared to the passive method. In spite of this systematic bias, there was good agreement between the methods. Intraclass correlation coefficients were greater than 0.98, and more than 95% of data points fell into the 95% confidence intervals. This agreement will be acceptable in many research contexts. We also found a linear increase in tendon stiffness with increasing strain rate, which must be taken into consideration when interpreting or reporting tendon stiffness.

1. Introduction

Muscle and tendon stiffness are important parameters when performing daily motor activities or sporting movements (**Hof et al., 2002; Fukunaga et al., 2001**). The stiffness of the body's elastic tissues governs the storage and release of elastic potential energy, and humans take advantage of this to maximise movement efficiency (**Lichtwark and Wilson, 2008; Maganaris and Paul, 1999**). Within this context, tendon stiffness has been widely studied in athletic (**Kubo et al., 2001; McNair and Stanley, 1996**) and clinical populations (**Vaz et al., 2006; Tardieu et al., 1982**). The findings of such studies have led to an enhanced understanding of how tendon stiffness influences force production (**Reeves, 2006**) as well as how tendon stiffness adapts to changes in loading (**Kubo et al., 2010; Seynnes et al., 2009**).

Tendon stiffness is calculated by dividing the estimated tendon force by the tendon's elongation (**Kubo et al., 2002; Maganaris and Paul, 1999**). For this purpose, participants are commonly asked to perform a maximal isometric contraction to shorten the muscle and thereby elongate the tendon ("active method") (**Kubo et al., 2002**). An alternative method is to record tendon force and elongation from a passive stretch, applied by an isokinetic rotation ("passive method") (**Morse et al., 2008**).

The choice of method is often driven by the specific purpose of an experiment and by the population of interest. For example, in clinical populations where patients with neuromuscular or musculoskeletal disorders may be unable to perform a maximal voluntary contraction (MVC) reliably (**Tedroff et al., 2008**), it may be more appropriate to use the passive method. The primary advantage of this method is that it allows for both tendon stiffness and muscle stiffness to be estimated (**Morse et al., 2008**). The disadvantage with this method is that that tendon stiffness can only be calculated at relatively low levels of force. The force-stiffness relationship has been shown to be non-linear (**Rigby et al., 1959**), as at lower tendon stiffness values the un-crimping of collagen fibrils causes significant tendon elongation. As a result, tendon stiffness is greater at high compared to low force levels (**Mizuno et al., 2011**).

A further variable, which may affect the comparability between stiffness obtained from the two methods, is the tendon's strain rate. Tendons exhibit viscoelastic behaviour in response to stretch, meaning that tendon stiffness increases with an increased strain rate (**Le Veau, 1992; Pearson et al., 2007**). Thus, strain rate needs to be taken into consideration when comparing different methods of obtaining tendon stiffness.

A range of methods, which are likely to result in different strain rates have been used in the literature to obtain tendon stiffness, including the passive method (**Mizuno et al., 2011; Morse et al., 2008**), and several variations of the active method (e.g., fast MVC

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Does acute passive stretching increase muscle length in children with cerebral palsy?☆

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ABSTRACT

Background: Children with spastic cerebral palsy experience increased muscle stiffness and reduced muscle length, which may prevent elongation of the muscle during stretch. Stretching performed either by the clinician, or children themselves is used as a treatment modality to increase/maintain joint range of motion. It is not clear whether the associated increases in muscle–tendon unit length are due to increases in muscle or tendon length. The purpose was to determine whether alterations in ankle range of motion in response to acute stretching were accompanied by increases in muscle length, and whether any effects would be dependent upon stretch technique.

Methods: Eight children (6–14 y) with cerebral palsy received a passive dorsiflexion stretch for 5 × 20 s to each leg, which was applied by a physiotherapist or the children themselves. Maximum dorsiflexion angle, medial gastrocnemius muscle and fascicle lengths, and Achilles tendon length were calculated at a reference angle of 10° plantarflexion, and at maximum dorsiflexion in the pre- and post-stretch trials.

Findings: All variables were significantly greater during pre- and post-stretch trials compared to the resting angle, and were independent of stretch technique. There was an approximate 10° increase in maximum dorsiflexion post-stretch, and this was accounted for by elongation of both muscle (0.8 cm) and tendon (1.0 cm). Muscle fascicle length increased significantly (0.6 cm) from pre- to post-stretch.

Interpretation: The results provide evidence that commonly used stretching techniques can increase overall muscle, and fascicle lengths immediately post-stretch in children with cerebral palsy.

1. Introduction

Children with cerebral palsy (CP) show increased muscle stiffness and reduced muscle length, which may contribute to reduced function. Stretching is commonly used in the treatment and management of children with spastic CP and is considered to be an important part of preventing or delaying the onset of contractures (National Institute for Health and Clinical Excellence, 2012). The assumptions made in clinical practice are that repeated bouts of stretching over periods of weeks or months can increase muscle length and reduce stiffness (Herbert, 2004; Odeen, 1981) by providing the necessary stretch stimulus that allows the muscle to lengthen in line with bone growth.

The increased stiffness (hypertonicity) of the muscle can have both neural and mechanical components. Spasticity (neural) and reduced muscle length (mechanical) can both theoretically be addressed with stretching, although the mechanisms by which these changes occur are not fully understood (Guissard and Duchateau, 2006). Regarding the former, the reduction in neural hypertonia may be related to reduced motor neuron excitability or reduced neural input to motor neurons through both pre- (e.g. input from Ia afferents) and/or post-synaptic mechanisms (Hummelsheim et al., 1994). The consequence of this for a spastic muscle may be a decrease in tonic reflex activity or an increase in the threshold of tonic stretch reflex, thus allowing an increase in joint range of motion (RoM) and muscle–tendon unit length with stretch (Calota et al., 2008). Regarding the latter, stretching may affect mechanical hypertonia by causing an inducing effect to increase muscle fascicle length (Coutinho et al., 2004). This plasticity of muscle has been demonstrated in several animal studies, where daily stretching over a period of several weeks was sufficient to increase the number of in-series sarcomeres (Williams, 1990).

Regardless of the mechanism involved, it seems that repeated elongation of the muscle during stretch is the key to inducing changes, both neural or mechanical (Williams, 1990). There is no consensus either in clinical practice or in the literature, with regards to the appropriate time of application, duration or frequency of stretch. However, before determining the appropriate stimuli for long-term adaptations, it should first be established whether spastic muscles are indeed able to receive a stretch during changes in joint RoM.

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Appendix III: Power analyses

Prior to the calculation of the effect size, pooled standard deviation was calculated, as depicted in the following equation below.

Pooled standard deviation =

$$SD_{pooled} = \frac{\quad}{\quad}$$

(Equation AIII 1)

SD = standard deviation

Effect size was determined using the equation below and power calculations were then used to determine the appropriate sample size required to reach statistical power.

$$\text{Effect size} = \frac{\quad}{\quad}$$

(Equation AIII 2)

M = mean

For Chapter 3, effect size was calculated based on data collected from the first 5 participants. Specifically, differences in tendon stiffness between strain rates 1 $deg \cdot s^{-1}$ and 30 $deg \cdot s^{-1}$ were calculated. No differences were expected between stretch techniques; hence, effect size was not calculated for this data. For Chapter 4, effect size was based on data collected from the first four CP participants and the first four CP participants. Data for muscle and tendon stiffness was compared between groups. For Chapter 5, effect size was calculated based on data collected from the first four

participants. Changes in muscle and tendon length were measured from rest to post stretch in the PT-led stretch technique. Finally, for Chapter 6, effect size for fascicle stiffness was calculated based on data from a previously published study (Zhao *et al*., 2011).

Sample size calculations

Chapter 3

Tendon stiffness from 1 deg·s^{-1} and 30 deg·s^{-1}:

Effect size = 0.65

SAMPLE SIZE = 21

Chapter 4

Muscle stiffness:	Tendon stiffness:
Effect size = 1.57	Effect size = 0.58
SAMPLE SIZE = 6	**SAMPLE SIZE = 26**

Chapter 5

Muscle length:	Tendon length:
Effect size = 0.97	Effect size = 1.09
SAMPLE SIZE = 9	**SAMPLE SIZE = 7**

Chapter 6

Fascicle stiffness (Zhao *et al.*, 2011):

Effect size = 0.7

SAMPLE SIZE = 15

Appendix IV: Residual analysis

The cut-off frequencies for each of the digital filters used in this thesis were determined using the residual analysis technique as described by Winter (2009). The residual between the filtered and unfiltered signal was calculated according to the following equation (A1). Residuals were summed, and plotted as a function of the filter cut-off frequency.

$$\bar{r}$$

(Equation AIV 1)

The residuals were calculated from 1 Hz to half of the sampling frequency (500 Hz in the following example). When the data consisted of random noise, the residual represented a straight line. The cut-off frequency was visually selected from the residual plot. A straight line from the "random noise line" was extrapolated to the y-axis to find its intercept (A). A horizontal line from this point was plotted to the point at which it intercepted (B) the signal curve. The x-coordinate of the point of intercept on the signal curve (C) provided the suggested cut-off frequency.

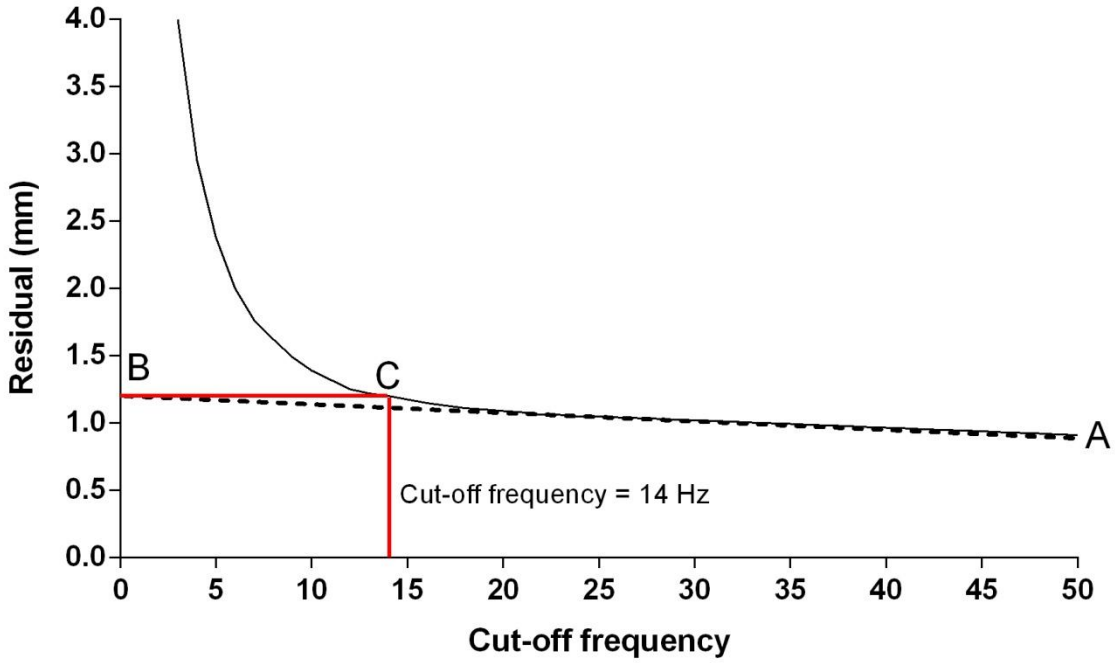

Figure AIV 1. A Plot of residual amplitude vs. 4th order low pass Butterworth filter frequency. Line A represents a straight line through the "random noise line" to the intercept of the y-axis. Line B represents the horizontal extension from Line A's point of intercept, to the signal curve. Finally, the coordinate of point C on the x-axis represents the recommended cut-off frequency, in this case 14 Hz.

Appendix V: Protocol for measuring muscle and tendon lengths

```
%Calculating muscle and tendon length
%Nicola Theis and Tom Korff
%Dec 2011


start=1;
finish=10000;

clear all; close all

    % Load motion analysis file

filename='.........' % Motion analysis file
data=dlmread(filename);
    % Filter the coordinate data

for j=1:41

[B,A] = butter(../100);
datafilt(:,j) = filtfilt(B,A,data(:,j));

end

down=(1:2:length(data));
datafiltdown=datafilt(down,:);

for i=1:length(datafiltdown(:,9)); %start:finish

     % Mid points between Malleoli, Metatarsals, Epicondyles (x)

Epicondyles_x(i)=datafiltdown(i,12)+((datafiltdown(i,9)-
datafiltdown(i,9))/2);
Metatarsals_x(i)=datafiltdown(i,39)+((datafiltdown(i,36)-
datafiltdown(i,36))/2);
Malleoli_x(i)=datafiltdown(i,33)+((datafiltdown(i,30)-
datafiltdown(i,30))/2);

     % Mid points between Malleoli, Metatarsals, Epicondyles (y)

Epicondyles_y(i)=datafiltdown(i,13)+((datafiltdown(i,10)-
datafiltdown(i,10))/2);
Metatarsals_y(i)=datafiltdown(i,40)+((datafiltdown(i,37)-
datafiltdown(i,37))/2);
Malleoli_y(i)=datafiltdown(i,34)+((datafiltdown(i,31)-
datafiltdown(i,31))/2);

 % Mid points between Malleoli, Metatarsals, Epicondyles (z)

Epicondyles_z(i)=datafiltdown(i,14)+((datafiltdown(i,11)-
datafiltdown(i,11))/2);
Metatarsals_z(i)=datafiltdown(i,41)+((datafiltdown(i,38)-
datafiltdown(i,38))/2);
Malleoli_z(i)=datafiltdown(i,35)+((datafiltdown(i,32)-
datafiltdown(i,32))/2);
```

```
% Law of cosines: a^2=b^2+c^2-2bccos(angle)
    % To work out distance 'c'

Met_Mal(i)=sqrt((Metatarsals_x(i)-Malleoli_x(i))^2+(Metatarsals_y(i)-
Malleoli_y(i))^2+(Metatarsals_z(i)-Malleoli_z(i))^2);

     % To work out distance 'b'

Epi_Mal(i)=sqrt((Epicondyles_x(i)-Malleoli_x(i))^2+(Epicondyles_y(i)-
Malleoli_y(i))^2+(Epicondyles_z(i)-Malleoli_z(i))^2);

    % To work out distance 'a'

Met_Epi(i)=sqrt((Metatarsals_x(i)-
Epicondyles_x(i))^2+(Metatarsals_y(i)-
Epicondyles_y(i))^2+(Metatarsals_z(i)-Epicondyles_z(i))^2);



    % To calculate ankle angle
    % Angle = cos (b^2+c^2-a^2)/(2bc)

Ankle_angle (i) = acos(Met_Mal(i)/Met_Epi(i));
Ankle_angle_deg (i)=Ankle_angle(i)*180/pi;

end


plot (Ankle_angle_deg)



%Next section is for calculating muscle and tendon lengths


xp1=datafiltdown(:,18); % x coordinate of distal ultrasound marker
yp1=datafiltdown(:,19); % y coordinate of distal ultrasound marker
zp1=datafiltdown(:,20); % z coordinate of distal ultrasound marker
xp2=datafiltdown(:,15); % x coordinate of proximal ultrasound marker
yp2=datafiltdown(:,16); % y coordinate of proximal ultrasound marker
zp2=datafiltdown(:,17); % z coordinate of proximal ultrasound marker


for i=1:length(datafiltdown(:,2))

US_length(i)=sqrt((xp2(i)-xp1(i))^2+(yp2(i)-yp1(i))^2+(zp2(i)-
zp1(i))^2);



% ultrasound angle1 is the angle defining the ultrasound handle with
%respect to the global horizontal line - needed for expressing
ultrasound
% origin in global coordinate system

ultrasoundangle1(i)=(acos((datafiltdown(i,20)-
datafiltdown(i,17))/US_length(i)));
```

```
% ultrasound angle 2 is the angle defining the orientation of the
probe
% with respect to the horizontal line - needed for

ultrasoundangle2(i)=(pi/2)-(acos((datafiltdown(i,20)-
datafiltdown(i,17))/US_length(i)));



end

% Load ultrasound data to get absolute coordinate of MTJ

US_data=load('.....');
answer='y'; start=0


for j=1:3
[B,A] = butter(../50);
US_datafilt(start:finish,j) = filtfilt(B,A,US_data(start:finish,j));

end


% Converting US data from cm into mm

x_US1 =US_datafilt(:,1)*10;
z_US1 =40-(US_datafilt(:,2)*10); %

for  i=start:finish

     xs(i)=xp1(i)+(50+z_US1(i))*(xp2(i)-xp1(i))/US_length(i);
     zs(i)=zp1(i)+(50+z_US1(i))*(zp2(i)-zp1(i))/US_length(i);
     ys(i)=yp1(i)+(50+z_US1(i))*(yp2(i)-yp1(i))/US_length(i);



end

close all

for i=start:finish


US_origin_z(i)=zs(i)-23.33*cos(ultrasoundangle2(i));
US_origin_x(i)=xs(i)+23.33*sin(ultrasoundangle2(i));



  ultrasoundangle3(i)=3*pi/2-ultrasoundangle2(i); % 270 deg -

% then rotate according to the angular position of ultrasound
coordinate system with respect to inertial reference system

MTJ_global_x(i)=US_origin_x(i)+cos(ultrasoundangle3(i))*x_US1(i)+sin
(ultrasoundangle3(i))*z_US1(i);
MTJ_global_z(i)=US_origin_z(i)-sin(ultrasoundangle3(i))*x_US1(i)+cos
(ultrasoundangle3(i))*z_US1(i);
```

```
Tendon_length(i)=sqrt((MTJ_global_x(i)-datafiltdown(i,27))^2+
(MTJ_global_z(i)-datafiltdown(i,29))^2+(ys(i)-datafiltdown(i,28))^2);

Muscle_length(i)=sqrt((MTJ_global_x(i)-
datafiltdown(i,9))^2+(MTJ_global_z(i)-datafiltdown(i,11))^2+(ys(i)-
datafiltdown(i,10))^2);


    MTU(i)=tendon_length(i)+Muscle_length(i); % MTU length

MTU_verified (i) = sqrt((datafiltdown(i,27)-
datafiltdown(i,9))^2+(datafiltdown(i,29)-datafiltdown(i,11))^2+
(datafiltdown(i,28)-datafiltdown(i,10))^2);

% Verify MTU length

end


plot(Tendon_length(start:finish));hold on
plot(Muscle_length(start:finish),'r');hold on


stopstop
```

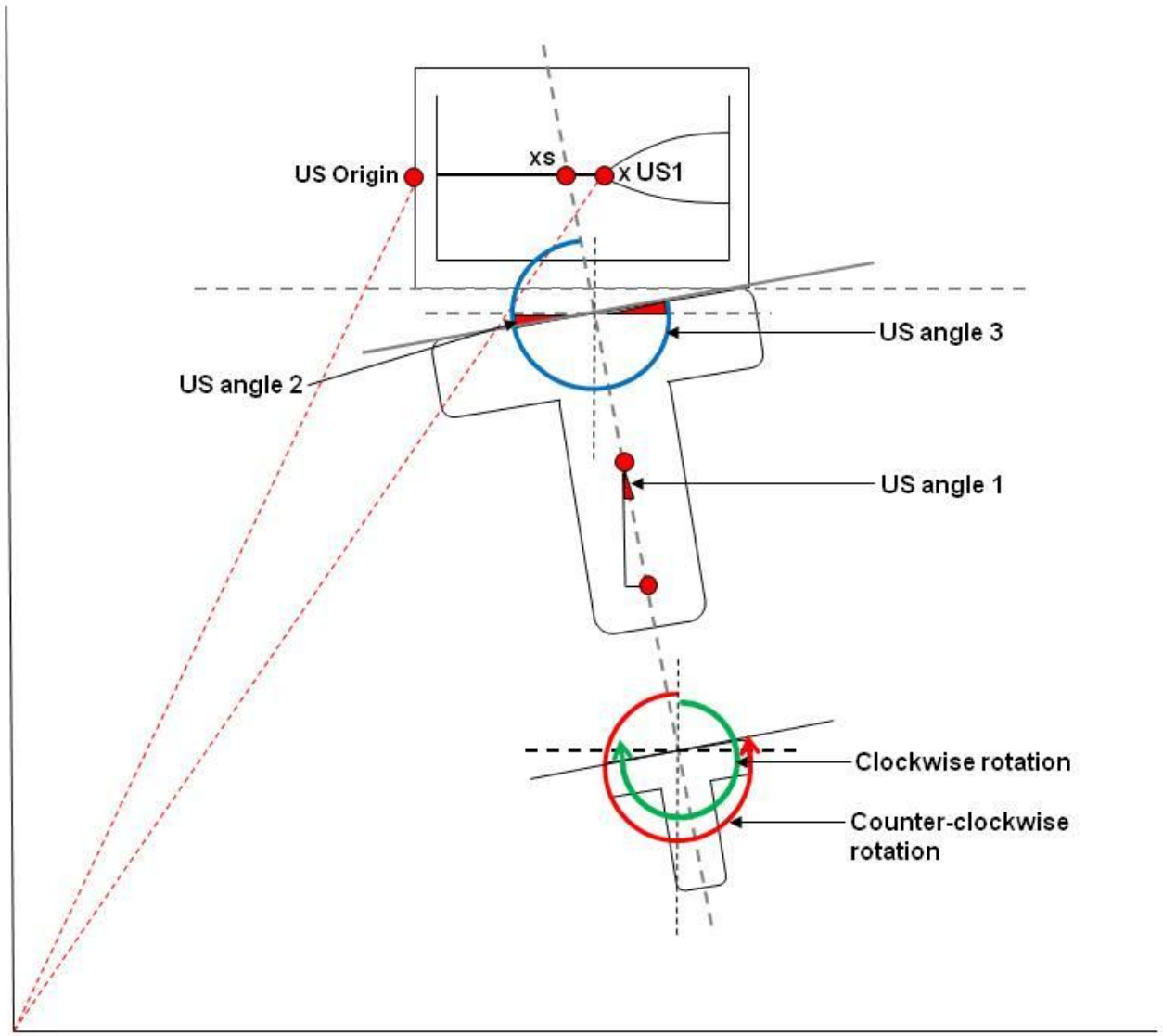

Figure AV 1: A diagram showing the rotational matrix applied to the ultrasound probe in the calculation of muscle and tendon length

Appendix VI: Ethical approval

Head of School of Sport & Education
Professor Susan Capel

Brunel UNIVERSITY WEST LONDON

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Nicola Theis
MPhil (Sport Science) Student
School of Sport and Education
Brunel University

27th May 2010

Dear Nicola

RE19-09 – In vivo examination of muscle and tendon extensibility

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application to amend the above mentioned research study. Your amendments have been independently reviewed to ensure they comply with the University Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to you amending your study as proposed.

Any further changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for further consideration.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your revised study.

Yours sincerely

Dr Simon Bradford
Chair of Research Ethics Committee
School Of Sport and Education

NHS
National Research Ethics Service
North London REC 1
Room 019, Level 7 Maternity Block
Northwick Park Hospital
Watford Road
Harrow
Middlesex
HA1 3UJ

Telephone: 020 8869 3805
Facsimile: 020 8869 5222

30 April 2010

Miss Nicola Theis
30 Morton close
Uxbridge, Middlesex
UB8 3WR

Dear Miss Theis

Study Title:	**In vivo examination of medial gastrocnemius muscle, muscle fascicles, and tendon extensibility in children with cerebral palsy**
REC reference number:	**10/H0717/10**
Protocol number:	**2.5**

Thank you for your letter of 29 March 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC.

A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated

RE03-10.

NHS

National Research Ethics Service

North West London REC 1

REC Office
Maternity, Level 7
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Harrow
HA1 3UJ

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15 December 2010

Dr Amir A Mohagheghi
Lecturer in Biomechanics
Brunel University
School of Sport and Education
Brunel University
Uxbridge, Middlesex
UB8 3PH

Dear Dr Mohagheghi

Study Title:	**The effectiveness of prolonged stretching on gastrocnemius muscle and tendon extensibility and functional movement in children with cerebral palsy.**
REC reference number:	**10/H0722/75**
Protocol number:	**Brunel University**

Thank you for your letter of 18 November 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair, in consultation with one member.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.