Neuromuscular determinants of muscle strength and passive range of motion in men with spastic cerebral palsy

Ayser Hussain

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Contents

Publications

The following sections of the thesis have been accepted or submitted for publication:

Chapter 3 (Submitted): **Hussain AW**, Williams AG, Onambele GL, Morse CI. The effect of seated and prone testing postures on passive stiffness of the gastrocnemius muscle. Manual Therapy

Chapter 4 (Accepted): **Hussain AW**, Onambele GL, Williams AG, Morse CI (2013) Passive stiffness of the gastrocnemius muscle in athletes with spastic hemiplegic cerebral palsy. European Journal of Applied Physiology

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Full published manuscripts of Chapter 4 and 5 can be located in the Appendices.

Other publications associated with the thesis:

Morse CI, Spencer J, **Hussain AW**, Onambele GL (2013) The effect of the oral contraceptive pill on the passive stiffness of the human gastrocnemius muscle in vivo. Journal of Musculoskeletal and Neuronal Interactions 13(1):97-104.

Abbreviations

Anatomical cross sectional area (ACSA)

Cerebral palsy (CP)

Coefficient of variation (CV)

Cross sectional area (CSA)

Dorsiflexion (DF)

Electromyography (EMG)

Gastrocnemius medialis (GM)

Gross Motor Function Classification System (GMFCS)

Gross Motor Function Measure (GMFM)

Intraclass correlation (ICC)

Limits of agreement (LoA)

Magnetic resonance imaging (MRI)

Maximal voluntary contraction (MVC)

Maximal voluntary isometric contraction (MVIC)

Muscle-tendon unit (MTU)

Myotendinous junction (MTJ)

Physiological cross sectional area (PCSA)

Plantarflexion (PF)

Range of motion (ROM)

Spastic cerebral palsy (SCP)

Standard deviation (SD)

Standard error of measure (SEM)

Tibialis anterior (TA)

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Table list

Thesis outline

Chapter 1; an introductory section to cerebral palsy (CP), which explains common terms used to describe the range of CP impairments. A rational is provided for impairment-specific selection of the participants to be examined in the present thesis along with a brief description on how spastic CP (SCP) can affect function.

Chapter 2; a literature review that discusses the neural and muscular factors associated with impaired strength and range of motion in individuals with SCP. The impairments are also related to how motor function is affected in individuals with SCP. At the end of the review, the aims of the thesis are presented.

Chapter 3; the reliability of different testing postures in the determination of passive muscle stiffness is assessed. A range of reliability tests are conducted and discussed to establish whether a seated or prone posture should be used when assessing passive muscle stiffness.

Chapter 4; the assessment of passive muscle stiffness and Young's modulus is discussed in adults with SCP using the seated posture form Chapter 3. The determining factors related to changes in the paretic muscle is discussed along with the functional implications.

Chapter 5; the factors underlying muscle weakness in adults with SCP are assessed using and interpolated twitch technique during an isometric maximal voluntary contraction. The effects of neural activation, coactivation and muscle size are discussed.

Chapter 6; the assessment of the intrinsic material force-producing capacity of SCP muscle *in vivo* was conducted using similar procedures to those discussed in chapter 5. Moment arm length, muscle size and neural factors are accounted for in the assessment of specific force.

Chapter 7; the elastic properties of the tendon in adults with SCP was conducted using a similar protocol to chapter 5 and 6. The different methods of quantifying the elastic properties of the tendon in individuals with SCP are appraised.

Chapter 8; A summary of the findings from the preceding chapters are discussed and suggestions for future research are provided.

Abstract

Spastic cerebral palsy (SCP) relates to a specific movement-related impairment characterised by a velocity-dependent resistance to stretch. Muscle weakness and decreased range of motion (ROM) are characteristics of the paretic limb in individuals with SCP. However, there are no data on the *in vivo* determinants of strength and ROM in adults with SCP. The aim of the thesis was to examine the factors associated with impaired plantarflexion, maximal voluntary isometric contraction (MVIC) and joint ROM in the paretic limb of physically active men with SCP compared to the contralateral non-paretic limb and individuals without neurological impairment. Passive stiffness, myotendinous junction displacement and ROM of the paretic gastrocnemius medialis (GM) were not different from the control muscles. However, the elastic modulus of the paretic GM was two times stiffer than the control GM muscles. MVIC torque of the paretic plantarflexors was 42% and 52% less than the non-paretic ($P = 0.007$) and control limbs ($P < 0.001$), respectively. The paretic gastrocnemius ACSA was 20% smaller than the control group ($P = 0.004$) only. Paretic agonist activation was 36% and 39% less than the non-paretic ($P < 0.001$) and control groups ($P < 0.001$), whereas paretic antagonist coactivation was 3-fold higher compared to the non-paretic ($P < 0.001$) and control group ($P < 0.001$). Agonist muscle activation accounted for 57% of variation in paretic plantarflexion MVIC torque ($P = 0.007$). When accounting for GM architecture, neural properties and moment arm length, no difference in GM specific force was established. Finally when the tendon elastic properties and Young's modulus were calculated at a standardised force, no difference was observed in tendon stiffness properties across all experimental groups. These findings suggest that in active adults with SCP, weakness is due to a reduction in

muscle size and impaired muscle activation. Furthermore, in the presence of no decline in ROM there remained an alteration in the passive elastic properties of the muscle, but not the tendon.

Chapter 1

Introduction to cerebral palsy

The term cerebral palsy (CP) describes a range of postural and movement disorders that result from a non-progressive lesion sustained to the developing brain (Bax et al. 2005). Although the damage is non-progressive, it is possible for the clinical manifestations in motor function and posture to change throughout maturation (Hanna et al. 2009; Essex 2003). Variations in the presentation of physical impairment has been suggested to be due to the location, size and severity of the lesion (Koman et al. 2004). Across all the variants of diagnoses, individuals with CP suffer from peripheral anomalies including; limited range of motion (ROM), weakness, poor balance and sensory deficits (Graham and Selber 2003; Aagaard et al. 2001; Alhusaini et al. 2010; Damiano et al. 1995b; Elder et al. 2003). The spectrum of disablement can range from individuals who are independent and ambulant with subtle impairments, to severely impaired patients that are non-ambulatory requiring care.

In order to distinguish and understand the differences between the constituent CP groups, it is common practice for clinicians to diagnose the specific movement limitation and if appropriate, its peripheral location (Koman et al. 2004). The movement limitation ranges from increased tonicity in the muscles, often referred to as hypertonia or spasticity, through to impaired voluntary coordination and balance of muscle movements (ataxia), and hyper excitable and involuntary muscle contractions during rest (athetosis). It is also common for individuals to have a mixture of these symptoms. When individuals with CP are diagnosed with ataxia or athetosis, such deficits in sensory control affect the whole body. Whereas the topography of hypertonia/spasticity is described using the following terms:

hemiplegia (involvement of the arm and leg on the same side of the body); diplegia (involvement in both legs); quadriplegia (involvement in all four limbs); and double hemiplegia (involvement in all four limbs, with impairment more substantial in two limbs).

Approximately 2 per 1000 individuals have CP (Cans et al. 2008; Odding et al. 2006) and it is considered to be the most common paediatric disability (Kuban and Leviton 1994). The number of people that have particular movement impairments in a specific location is considerably reduced when referring back to the estimated prevalence statistics. Odding and colleagues (2006) reported that out of all the various combinations of CP impairment, spastic hemiplegic CP were the most commonly diagnosed in paediatric units. Therefore, for the purpose of consistency within the thesis and acknowledging the prevalence of the diverse range of CP impairments in relation to participant recruitment, active and ambulant men with spastic hemiplegic CP were considered for inclusion.

The majority of the research conducted to assess the neuromuscular physiology of individuals with spastic CP (SCP) is conducted in paediatric groups (Friden and Lieber 2003; Malaiya et al. 2007; Marbini et al. 2002; McNee et al. 2009; Mohagheghi et al. 2007; Rose et al. 2008; Stackhouse et al. 2005; Vaz et al. 2006). Children with CP are largely sedentary (Longmuir and Bar-Or 2000) and it is possible that the degree of impairment observed in such individuals may be accentuated through reinforced sedentary behaviour. In individuals without

neurological impairment, it has regularly been reported that reduced usage of the muscle-tendon unit (MTU) through a range of antigravity models resulted in reduced force generation during maximal voluntary contraction (Adams et al. 1994; de Boer et al. 2007; Reeves et al. 2002; Sargeant et al. 1977), atrophy of skeletal muscle (Alkner and Tesch 2004; Narici and Cerretelli 1998), reduced fascicle length and pennation angle (Kawakami et al. 2000; de Boer et al. 2008; Narici and Cerretelli 1998; Reeves et al. 2002), and decreased stiffness of the tendon (Kubo et al. 2000; Maganaris et al. 2006; Reeves et al. 2005). In addition to these observations, decreases in neural voluntary activation have also been recorded as a result of disuse (Ruegg et al. 2003; Stevens et al. 2006). As it is possible that sedentary lifestyles may accentuate the severity of impairment, it is important to address the neuromuscular and tendon properties of active individuals with SCP. Furthermore, as the physical manifestations of the lesion proceed to evolve throughout maturation, the findings identified in children may not apply to fully matured physically active adults. Indeed, it is well established that the MTU alters throughout maturation in regard to aspects of muscle recruitment, muscle size, moment arm and tendon properties (Morse et al. 2008b; O'Brien et al. 2010a, b; Pang and Ying 2006; Waugh et al. 2012).

In order to assess the degree of functional impairment, the Gross Motor Function Classification System (GMFCS) was developed, which involved rating the quality of movement whilst a child with CP completed a series of tasks (Palisano et al. 1997). In total the child is required to complete 66 tasks of; a) lying and rolling, b) sitting, c) crawling and kneeling, d) standing, and e) walking, running and jumping.

This in turn helps the classifier to rank the child into one of 5 levels, level 1 being the most able children that show little impairment through to level 5 who struggle with anti-gravitational postural control. Although such systems provide a coherent template to rate the impairment of such individuals, the inter- and intra-reliability of these measures are somewhat questionable (Damiano et al. 2006). Moreover, the level of information obtained in relation to factors effecting movement is negligible. Therefore a number of studies have used objective tests to establish what the main determinants of motor function are. In paediatric patients with SCP, muscular strength and joint ROM in the paretic limb have been linked with impaired functional performance (Alhusaini et al. 2010; Damiano and Abel 1998; Elder et al. 2003; Stackhouse et al. 2005; Vaz et al. 2006). Therefore the aim of the present review will be to establish what the determinants of MTU strength and joint ROM are in the paretic limb of individuals with SCP.

Chapter 2

Literature review

2.1 Introduction

Spasticity is a specific movement disorder which is classically defined as a velocity-dependent resistance to stretch in the paretic limb of individuals with cerebral palsy (CP; Lance 1981). The aetiology of spastic cerebral palsy (SCP) originates from damage to the immature brain, where the subsequent neural communication between the central nervous system and paretic muscles have been demonstrated to have secondary impacts on the morphology of the paretic contractile tissue (Friden and Lieber 2003; Lieber et al. 2003). These neuromuscular impairments have been regularly linked with two characteristic functional impairments; these are: 1) lower muscle strength (Blundell et al. 2003; Damiano and Abel 1998; Damiano et al. 1995a; Damiano et al. 2001; Damiano et al. 1995b; Elder et al. 2003; McNee et al. 2009; Stackhouse et al. 2005), and 2) restricted joint range of motion (Alhusaini et al. 2010; Barber et al. 2011a; Vaz et al. 2006). Therefore in this review it is explored and discussed what underlying mechanisms and determinants contribute to the functional limitations in individuals with SCP.

2.2 Muscle weakness

The degree of paretic skeletal muscle torque generated during maximum voluntary isometric contraction (MVIC) has consistently been reported to be lower than the non-paretic contralateral limb in individuals with hemiplegic SCP and age-matched controls without neurological impairment (Table 2.1). Based on the available literature the MVIC torque is between 42-73% lower in individuals with SCP

compared to individuals without neurological impairment. This weakness in SCP muscle has been suggested to be more prominent in the distal musculature (i.e in the plantarflexor muscles) as opposed to proximal musculature such as the hip extensors (Wiley and Damiano 1998). Although the detriments in strength may not seem significant when considered in isolation, high correlations have been identified between MVIC force and walking velocity (r = 0.71; Damiano and Abel 1998), and isokinetic torque and the Gross Motor Function Measure (GMFM; r = 0.70 - 0.83; Damiano et al. 2001) in children with SCP. This suggests that greater strength in individuals with SCP is associated with improved motor performance and gait. In contrast to clinical measures which usually reflect the degree of spasticity within the joints of the lower limb there is no relationship between ROM and GMFM (Damiano et al. 2001).

Although the focus of the present review is to examine muscular weakness in individuals with SCP, stroke populations share similar clinical manifestations a result of damage to the brain (Sheean 2002). It has been reported that in the paretic limb of stroke individuals ranging from 42-85 years MVIC elbow flexion and extension were up to 53 and 47% lower compared to age-match controls without neurological impairment (Ada et al. 2003; Canning et al. 1999). Similarly the MVIC torque generated by elderly individuals with stroke in their quadriceps and hamstring musculature was up to 44 and 61% weaker, respectively when compared to individuals without neurological impairment (Newham and Hsiao 2001). Although the deficit in strength observed across extensor and flexor muscle groups, it is apparent that the flexor muscles are the most impaired based upon the results presented. Moreover, the variation in strength data observed in stroke

individuals has also been suggested to depend on how long the participants were examined after the insult to their brain (Newham and Hsiao 2001). Even though similarities in MTU function exist between stroke and SCP individuals compared to controls without neurological impairment, there are a number of factors that may not relate to SCP cohorts. Within the aforementioned data reported on patients with stroke (Canning et al. 1999; Ada et al. 2003; Newham and Hsiao 2001), age-related confounding factors such as sarcopenia and physical inactivity could influence the MVIC data obtained (Morse et al. 2007b; Morse et al. 2005b; Morse et al. 2005c) and potentially overestimate the severity of the impairment. Thus as a result of such confounding factors, the application of this data within SCP is questionable; therefore data on individuals with SCP will be addressed only.

As the majority of the information currently available in SCP muscle weakness is based on children (Elder et al. 2003; Stackhouse et al. 2005) or adolescents with unspecified levels of maturation (Barber et al. 2012), such findings may not be applicable to adult populations, in fact, the clinical manifestations associated with SCP are acknowledged to change during maturation (Hanna et al. 2009; Koman et al. 2004). Furthermore, throughout maturation MVIC strength is known to increase considerably with age in indviduals without neurological impairment (O'Brien et al. 2010a). Therefore, any weakness identified in children with SCP may not reflect the neuromuscular impairments experienced in adults with SCP. Nevertheless, based on the determinants of muscle strength, weakness in individuals with SCP should in fact be considered in relation to neural factors (activation and coactivation) and morphological factors (muscle size).

Table 2.1. Percentage difference in MVIC between the paretic (P) and non-paretic (N-P) limbs of individuals with SCP, and the limb of controls without neurological impairment (C).

Author	Age (CP vs. C)	Muscle	Topography	P vs. C (%)	P vs. N-P (%)
Barber et al. (2012)	15-21 & 15-20	PF	DNC	-55	NA
Elder et al. (2003)	5-12*	PF	Hemiplegic	-56	-25
		DF		-42	-30
Stackhouse et al. (2005)	7-13 & 8-12	KE	Diplegic	-56	NA
		PF		-73	

DNC, did not confirm; NA, not applicable; PF, plantarflexors; DF, dorsiflexors; KE, Knee extensors; C, Control. *Age range was reported for both groups.

2.3 Determinants of muscle weakness

2.3.1 Neural function

The ability to recruit skeletal muscle is positively related to MVC torque; in contrast, the level of antagonist muscle coactivation during activation of the agonist is inversely related to the net MVC torque (Macaluso et al. 2002). In individuals with upper motor neuron impairment and spinal cord lesions, weakness

may be associated with either a decrease in agonist activation and/or an increase in antagonist coactivation, stemming from impaired efferent and afferent inhibition and excitation pathways of the extrapyramidal and pyramidal tracts (Brown 1994; Nielsen et al. 2007; Sheean 2002; Walshe 1935). Although these findings are from individuals with upper motor neuron impairment and spinal cord lesions, these findings may also contribute to muscle tendon unit (MTU) weakness in the paretic limbs of individuals with SCP (Damiano et al. 2000; Wiley and Damiano 1998; Ikeda et al. 1998).

2.3.2 Coactivation

Common findings in SCP studies have reported increased coactivation of the antagonist muscle during gait and MVC (Damiano 1993; Ikeda et al. 1998; Damiano et al. 2000; Elder et al. 2003; Stackhouse et al. 2005). Levels of antagonist coactivation as measured by electromyography (EMG) during plantarflexion (PF) MVC in the paretic limb of children with SCP have been reported to be between ~35-50%, which was 10-35% higher than the control groups (Elder et al. 2003). In addition, Stackhouse et al. (2005) reported similar values in the paretic tibialis anterior coactivation during PF MVIC (~40%), which was reported to be ~20% higher than age-matched controls. Coactivation of the paretic semitendinosus muscle during knee extension MVC was also reported to be 83% higher than in the control group. This increased level of coactivation during knee extension may be a protective reflex response as a result of increased relative torque (torque/body mass) generated by the quadriceps femoris. In individuals without neurological impairment, such contributions from the antagonist

muscle have previously been shown to reduce the torque measured during contraction of the agonist (Hassani et al. 2006; Macaluso et al. 2002). Whereas in individuals with SCP, the greater level of antagonist coactivation may act to stabilise the joint, by increasing joint stiffness and limit the degrees of freedom during motor performance (Damiano 1993; Damiano et al. 2000; Graham and Selber 2003). Macaluso et al. (2002) reported that increased antagonist coactivation has been associated with muscle weakness in healthy and active elderly women, and although such data may apply to individuals with SCP, a direct association has yet be demonstrated. Furthermore, no information is currently available regarding the paretic antagonist coactivation properties during MVC in adults with SCP.

2.3.3 Agonist activation

In addition to impaired antagonist activation, individuals with spastic hemiparesis also exhibit impaired agonist activation during MVC (Elder et al. 2003; Stackhouse et al. 2005). This has been linked to the individuals' inability to discharge certain motor units in the paretic muscle (Gemperline et al. 1995). By superimposing an electrical stimulation through the muscle during MVIC *in vivo*, adults suffering from stroke were found to have a 25-40% deficit in quadriceps femoris voluntary activation (Newham and Hsiao 2001). This is consistent with the deficits reported in children with SCP, where the neural activation was 49% and 33% lower in the triceps surae and quadriceps femoris, respectively (Stackhouse et al. 2005). Whether this also applies to adults with SCP remains to be seen, as maturational differences have demonstrated that children without neurological impairment have

an underdeveloped ability to maximally activate the agonist musculature during MVIC (O'Brien et al. 2010a; Paasuke et al. 2000).

It is apparent that children with CP have a decreased voluntary ability to maximally activate their agonist musculature compared to children without neurological impairment (Elder et al. 2003; Stackhouse et al. 2005). Although such research has not reported the activity status of their participants, children with CP are suggested to live a sedentary lifestyle compared to age-matched counterparts without neurological impairment (Longmuir and Bar-Or 2000). Such information may provide invaluable information when interpreting the results as the neural deficits associated with muscle spasticity may be impaired further as demonstrated in disuse models (Stevens et al. 2006; de Boer et al. 2007; de Boer et al. 2008). Therefore, previous increases in the strength of the paretic muscles in children with SCP after physical training interventions (Andersson et al. 2003; Blundell et al. 2003; Damiano and Abel 1998; Damiano et al. 1995b; McNee et al. 2009) may be due to potential increases in muscle activation as a result of the participants increased physical activity over the intervention period. To eliminate the effects of disuse when assessing the paretic muscle activation properties, further research should assess physically active individuals with SCP to assess the neural impairment and minimise the effects of disuse.

2.3.4 Muscle size

It is well established that muscle size is a key determinant of strength in individuals without neuromuscular impairment (Fukunaga et al. 1996). In individuals with SCP, there is a lack of information in the assessment of muscle weakness, relative to the size of the agonist muscle(s). The only study to quantify muscle size and MVIC torque was Elder et al. (2003) where the paretic plantarflexor volume measured using magnetic resonance imaging (MRI) was ~39% lower (Figure 2.1) compared to the non-paretic muscle and individuals without neurological impairment. This deficit in muscle volume was associated with lower paretic muscle torques during MVIC. Because of the sedentary lifestyle of SCP children (Longmuir and Bar-Or 2000), muscle atrophy is likely to result from environmental disuse, as well as muscle disuse resulting from the neural impairment (Lieber et al. 2004).
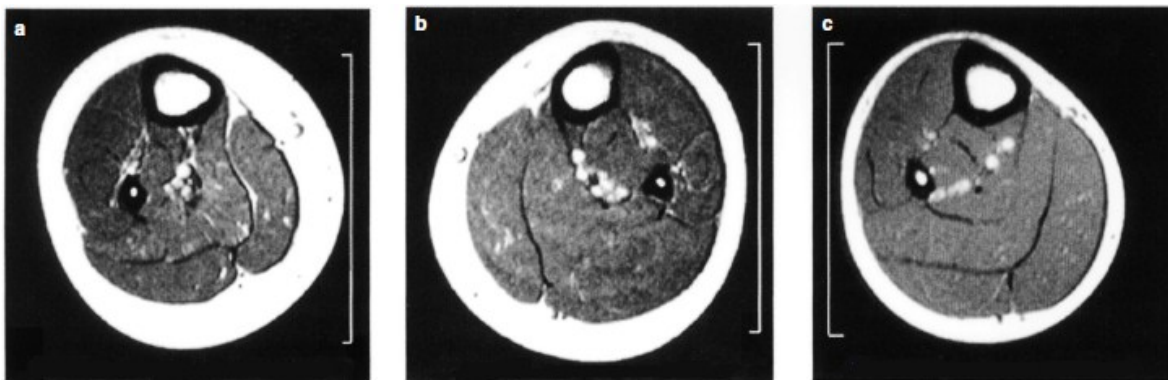
Figure 2.1. MRI scans from the lower limbs of a child with hemiplegia (a) paretic leg, (b) non-paretic leg, and (c) leg of an age-matched child without neurological impairment. Calibration bars are 8 cm (Elder et al. 2003).

Although muscle atrophy is likely to be a major determinant of the weakness associated with SCP, when differences in muscle mass are accounted for, there remains a difference between individuals with SCP and age-matched controls (Elder et al. 2003). For example, Elder et al (2003) calculated the amount of torque relative to muscle ACSA of the PF ($Nm^{.}cm^{-2}$) and reported that the relative torque of the paretic limb was up to ~40% lower than the non-paretic limb and the limb of age-matched controls. Given the large decrement in muscle activation capacity this decrease in MVC/ACSA is not surprising. Indeed, Urbancheck et al. (2001) has previously demonstrated how 'unrecruitable' muscle mass contributed to declines in MVC/ACSA in the elderly. Therefore, it would be expected (although it remains unreported) that neural factors such as decreased agonist activation and increased coactivation contribute to reductions in MVC/ACSA in SCP. It should be noted that the measurement of ACSA in pennate muscle may result in an underestimation of the true physiological CSA (PCSA) of the muscle (Figure 2.2; Narici et al. 1992). This is due to the fact that pennate muscle measured via ACSA (perpendicular to the longitudinal axis of the muscle) does not reflect the true physiological contractile area of a pennate muscle (the area perpendicular to the angle of pennation). To date, no studies have specifically assessed PCSA and muscle specific force in individuals with SCP. Without any data addressing the properties of the whole muscle specific force in the paretic limb, it is impossible to understand its intrinsic force-producing capacity. A paucity of such studies may be due to children with SCP being assessed, as the muscle in the paretic limb has been suggested not to be developed enough to obtain clear architectural data using ultrasonography or other scanning methods (as discussed by Elder et al. 2003).
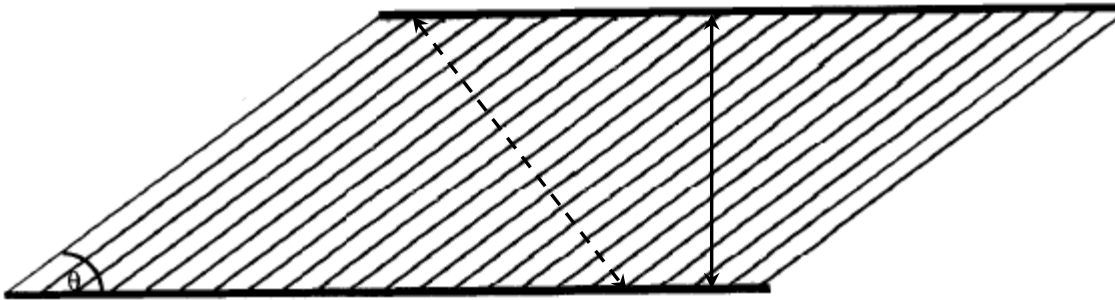
Figure 2.2. Schematic diagram of pennate muscle illustrating the difference between ACSA (solid black line) and PCSA (dashed black line) measures (adapted from Narici et al. 1992).

2.3.5 Muscle morphology

Although neural factors are likely to contribute primarily to the observed decrease in MVC/ACSA in individuals with SCP, it is possible that as with disuse and ageing, some changes in morphology may also contribute. There is evidence that fibre type may have an influence on muscle specific force or MVC/ACSA. Some studies have reported increased prevalence of type I muscle fibres in individuals ranging from 6-18 years with SCP (Ito et al. 1996; Marbini et al. 2002). Such findings may contribute to muscle weakness as the ability to generate force relative to fibre CSA in type I has been reported to be approximately 2-fold lower compared to type IIa and type IIx fibres (Bottinelli et al. 1999). This may be a reflection of progressions in the manifestations of SCP, as the impairment in children and adolescents are suggested to progress until full maturation (Hanna et al. 2009). Furthermore, variations in fibre type distribution may reside in what

muscle the biopsy was taken from, as the muscles such as the soleus are known to have an increased proportion of type I fibres (Schiaffino et al. 1989). In adults with spasticity following stroke an increase in type II fibres was reported (Sjostrom et al. 1980). Increases in type II fibre expression have been observed in individuals with spinal cord lesion, where all fibres in the paretic muscle are converted to type II fibres as a result of impaired neural input to the musculature (Grimby et al. 1976). Although the leg of individuals with SCP are only partially paretic, the observed decrease in type II muscle fibre size may be due to impaired neural communication, or disuse (Lieber et al. 2004; Marbini et al. 2002).

In addition to the possible contribution of fibre type to lower MVC/ACSA, the increase of non-contractile material within the muscle may also contribute. Indeed, muscle fibre packing has theoretically been linked with differences in specific force measurements, where fibre density is hypothesised to change as a result of deviations in intramuscular fat, extracellular matrix or the 'tighter' packing of muscle fibres (Jones et al. 1989). A consistent finding observed in children with SCP is that the intrinsic material of the paretic muscle contains elevated lipid and extracellular matrix content relative to contractile tissue compared to age-matched controls (Booth et al. 2001; Lieber et al. 2003; Marbini et al. 2002). This has pronounced effects including an inducement to overestimating muscle size and underestimating MVC/ACSA. Lieber et al. (2003) reported that *in vitro* samples of SCP muscle from various locations in the arm were comprised of approximately 60% extracellular matrix and only 40% contractile tissue, whereas the muscle of individuals without neurological impairment comprised of 95% muscle and 5% extracellular matrix (Figure 2.3). Given these compelling findings, it still needs to

be considered that biopsy studies are not necessarily representative of the whole muscle (Lexell et al. 1986, 1984). As biopsy samples are so small there is high chance of obtaining a subsection of the muscle where there is a high collagen and fat region, though this caveat is widely acknowledged.
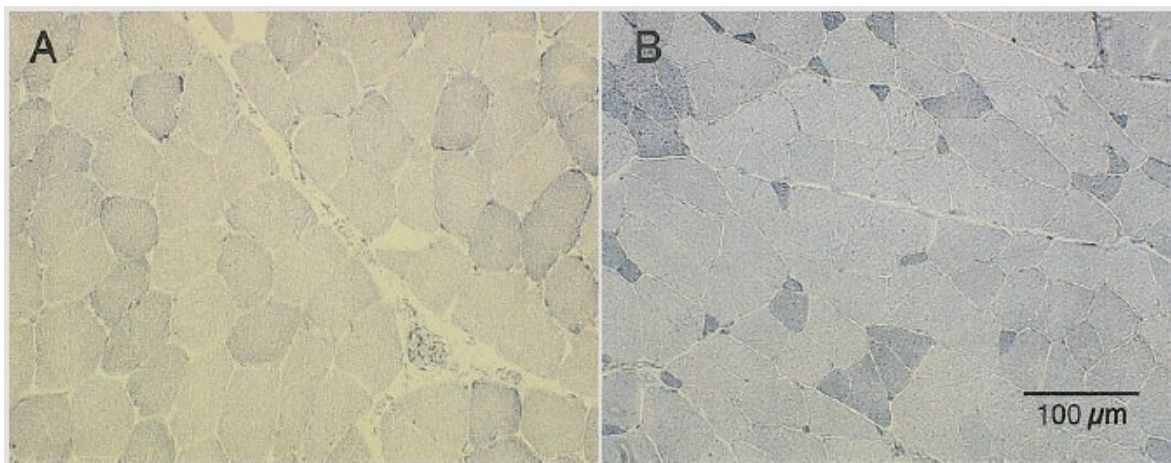
Figure 2.3. Muscle fibre size variation in (A) non-paretic extensor carpi radialis brevis muscle, and (B) paretic flexor carpi ulnaris muscle (Lieber et al. 2004).

2.4 Joint range of motion

The flexibility of the paretic limb when stretched to end range of motion (ROM) is regularly reported to be limited in individuals with SCP (Alhusaini et al. 2010; Barber et al. 2011a). Although many studies comment on the limited ROM in the paretic limb, very few studies have quantified the deficits compared to limbs of individuals without neurological impairment. Alhusaini et al. (2010) reported that whilst children (age = 6-11 years) with SCP laid supine with their foot attached to a

custom-made potentiometer footplate, the passive dorsiflexion end ROM achieved was 13.4 deg, which was 5.5 deg less than age-matched controls without neurological impairment. Similarly whilst assessing males and females with SCP (age ranging from 15-21 years), Barber et al. (2011a) reported that passive end dorsiflexion ROM was 6 deg, whereas age-matched controls achieved an ankle angle of 21 deg. The joint ROM in the ankle has received considerable attention regarding flexibility because individuals with SCP often have equinus foot positioning during stance and gait. Given that measuring end ROM around a joint can provide an indirect measure of MTU stiffness, tests such as the touch-toe and/or sit-and-reach tests are convenient and quick methods to assess MTU stiffness (Magnusson et al. 1997; Cornbleet and Woolsey 1996). Both the touch-toe and sit-and-reach tests require individuals to maintain full extension of the knee joint whilst maximally flexing around the hip and vertebrae, extending both arms and reaching as far as possible to obtain a distance achieved from the step or board, respectively. As the hip and vertebrae contribute to the single distance attained by the individuals completing the test, it is impossible to determine specifically where the ROM resides. As both of these tests are typically employed to test hamstring flexibility, Cornbleet and Woolsey (1996) measured the hip joint angle using an inclinometer during the sit-and-reach test in children without neurological impairment, which was suggested to provide a more accurate and reliable prediction of hamstring flexibility. Similar findings were also reported in young men and women where hip flexion angle was correlated (r = -0.79) with the distance achieved during the touch-toe test (Kippers and Parker 1987), conversely such tests have also been suggested to be unreliable and have a high level of variability in test-retest assessments (Merritt et al. 1986; Moran et al. 1979). As the

data obtained from such assessments of flexibility do not account for a number of confounding variables impacting on the measurement including pain, stretch tolerance and reflex responses of the MTU; thus reducing the reliability and meaningfulness of such tests (Magnusson et al. 1996a; McNair and Stanley 1996; Toft et al. 1989).

2.5 Passive stiffness measurements

The passive stiffness of material being extended is indicative of the resistive mechanical properties of the tissue to stretch (Magnusson 1998). Previously the passive torque-angle relationship has been used to calculate the stiffness of the MTU, whilst accounting for the neural and psychological shortcomings associated with end ROM measurements in individuals without neurological impairment (Gajdosik et al. 2005; Gajdosik et al. 1999; Magnusson et al. 1996a; Magnusson et al. 1996b). Such measurements are typically conducted in various postures on an isokinetic dynamometer with the joint being passively rotated at a predetermined velocity until volitional end ROM, whilst simultaneously measuring muscle electromyography (EMG), joint angle and passive torque (Alhusaini et al. 2010; Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a). Few studies have assessed the passive properties of the paretic MTU (Table 2.2). Stiffer MTUs have been reported to improve walking and jogging economy in individuals without neurological impairment (Gleim et al. 1990). However, in individuals with hypertonic CP, increased MTU stiffness of the paretic forearm musculature along with muscle weakness has been associated with impaired motor function during the Jebsen-Taylor hand function test (Taylor et al. 1973; Vaz et al. 2006), which

comprised of placing objects into a can, stacking wooden discs and picking up five containers as quickly as possible. Similar findings have also been reported in the PF muscles during gait (Dietz et al. 1981). These observations suggest that the relationship between MTU stiffness and motor function describe a parabolic curve, such that there is an optimal level of MTU stiffness to maximise motor function. Beyond this optimal point, any further increments in MTU stiffness in fact impede motor function.

Table 2.2. Relative difference in MTU stiffness between the paretic (P) limb of individuals with SCP and the limb of controls without neurological impairment (C).

Author	Age (CP vs. C)	Muscle	P vs. C (%)	Passive velocity
Vaz et al. (2006)	6-11*	WF	+24	5 deg/s^{-1}
	15-21 vs.			
Barber et al. (2011a)		PF	+34	20 deg/s^{-1}
	15-21			

DNC, did not confirm; NA, not applicable; PF, plantarflexors; WF, wrist flexors. *Age range was reported for both groups.

As the functional properties of the muscle and tendon are different, it is difficult to distinguish the difference in material properties between the contractile and/or elastic components. In recent years the *in vivo* elastic properties of the muscle have been assessed in individuals without neurological impairment using

ultrasonography to monitor the displacement of the myotendinous junction (MTJ), whilst simultaneously collecting passive torque, angle and EMG data (Abellaneda et al. 2009; Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a). The apparatus and procedures have been shown to be a valid and reliable in the assessment of passive muscle/MTU properties (Morse 2011; Morse et al. 2008a). However, very little is understood about the influence, if any, the variation in testing postures/positions have on the passive measures (Kay and Blazevich 2009; Magnusson 1998; Magnusson et al. 1997; Morse 2011; Morse et al. 2008a).

In relation to the assessment of the paretic muscle passive stiffness in individuals with CP and stroke, a number of studies imply that they have assessed the stiffness of the muscle, when they have in fact assessed MTU stiffness (Vaz et al. 2006; Harlaar et al. 2000; Alhusaini et al. 2010). Currently no information addresses the *in vivo* properties of the paretic muscle, despite the fact that differences are regularly reported between children with SCP and age-matched controls without neurological impairment. Furthermore, understanding the neural, MTU and architectural determinants associated with mechanical stiffness of a tissue is fundamental to understanding the limitations in paretic muscle flexibility.

2.6 Determinants of passive stiffness

2.6.1 Neural stretch reflex

As a result of a lesion to the central nervous system, spasticity is often associated with hyperexcitability of the stretch reflex (Sanger et al. 2003). In the assessment

of passive stiffness it is imperative that any neural reflexes are suppressed in order to examine the mechanical properties of the muscle in isolation. In relation to the definition of spasticity (Lance 1981), the most common method of ensuring that monosynaptic and/or polysynaptic reflexes are limited is by rotating the joint at an angular velocity that is slow enough not to elicit a neural response. In the paretic biceps muscle of individuals with spasticity and hypertonia, the relationship between angular velocity and muscle EMG is suggested to be linear (Figure 2.4; Sheean and McGuire 2009; Given et al. 1995). Whether this characteristic applies to muscles in the lower extremities of individuals with SCP has yet to be determined. Nevertheless when the passive mechanical properties of the paretic MTU have been studied in individuals with SCP, no quantification of EMG activation has been reported (Barber et al. 2011a; Vaz et al. 2006). Conversely in individuals without neurological impairment, resting EMG signal during passive stretch has been assessed and is reported to be no higher than 3% (Gajdosik et al. 2005; Morse 2011; Morse et al. 2008a); such minimal activation is considered to be a 'normal' level of activation during rest, so whether this would be similar in individuals with SCP has yet to be determined.
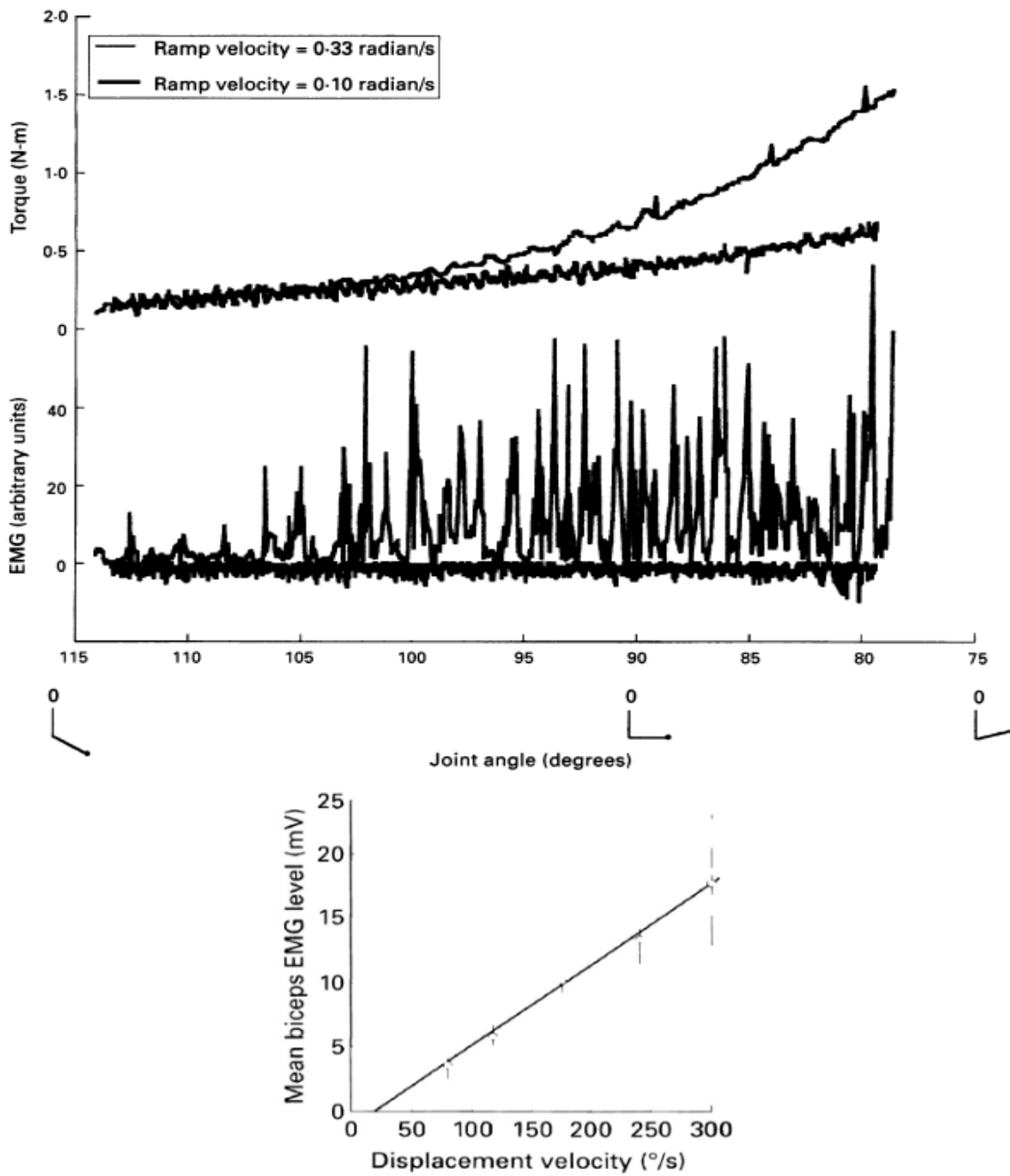
Figure 2.4. Velocity-dependent resistance to stretch in the biceps of individuals with spastic hemiplegia (Above; Given et al. 1995) and hypertonia (Below; Sheean and McGuire 2009).

2.6.2 Muscle structure and intrinsic properties

It is well established that the size of the muscle influences the passive MTU/muscle stiffness (Magnusson et al. 1997; Morse 2011; Morse et al. 2008a). In individuals without neurological impairment, larger hamstring muscles have been associated with increased passive MTU stiffness (Klinge et al. 1997; Magnusson et al. 1997). In light of these findings, it would be expected that the smaller paretic muscle of children with SCP would be more compliant in comparison to the larger muscles of age matched controls. However, increased stiffness in the paretic muscle has been reported consistently in individuals with SCP (Barber et al. 2011a; Vaz et al. 2006), which may relate to alterations in architectural and/or the intrinsic properties of the SCP muscle.

As individuals with SCP typically have smaller muscles (Elder et al. 2003; McNee et al. 2009), decreases in fascicle length may in part explain why such a difference occurs in the size of the contractile material (Malaiya et al. 2007; Mohagheghi et al. 2007). Indeed whilst children lay prone with their ankles at a relaxed angle hanging off the end of a plinth, ultrasonography was used to assess the length of the gastrocnemius medialis muscle fascicles in children ranging 4-13 years. Architectural images from the mid-belly of the muscle were obtained, and the SCP fascicles were found to be 8% shorter compared to children without neurological impairment (Malaiya et al. 2007). However, when the participants ankle angle was secured into a fixed position on an isokinetic dynamometer, Barber et al. (2011a) reported no difference between resting fascicle length of individuals with SCP and age-matched controls without neurological impairment. Whilst in the prone

position, the ankle was passively stretched at 20 deg·s^{-1} until volitional end ROM, whilst fascicle elongation was recorded using ultrasonography. Subsequently findings showed that the passive fascicle strain of the paretic limb was 47% less than age-matched controls without neurological impairment at an equivocal peak ankle torque. This suggests that paretic muscle fibres have a diminished ability to elongate with increased torque. This is consistent with *in vitro* findings demonstrating that SCP sarcomere lengths are 20% shorter and the elastic modulus of paretic muscle fibres is 49% stiffer than that of individuals without neurological impairment (Friden and Lieber 2003). Such findings may reside in the cellular properties of the paretic SCP muscle when compared to individuals without neurological impairment.

At a cellular level, titin has been suggested to influence the passive properties of the contractile tissue (Labeit and Kolmerer 1995; Neagoe et al. 2003). Titin is a large protein that spans over 1 µm in length, running from the Z discs to the M lines of the sarcomeres (Labeit and Kolmerer 1995). The titin protein has previously been reported to account for nearly all of the passive load placed on a single muscle fibre from a frog (Magid and Law 1985); demonstrating that the protein is a key determinant of the muscle fibre elasticity *in situ*. Although, no information is available on the influences of titin in the passive properties of the skeletal muscle in individuals with SCP, a number of different isoforms are suggested to exist in skeletal and cardiac muscle (Neagoe et al. 2003). It is thought that the differences in isoforms are predominantly due to the size of the PEVK domain within the titin protein (Figure 2.5), where it has been shown that cardiac isoforms are far smaller and stiffer than other variants of the protein found

in skeletal muscle. Indeed as the neural activation and subsequent environment of the skeletal and cardiac muscles are very different, this affects the expression of the titin protein isoform. Based on this concept of a changed environment, individuals with SCP may also have a different isoform expression within the paretic musculature.
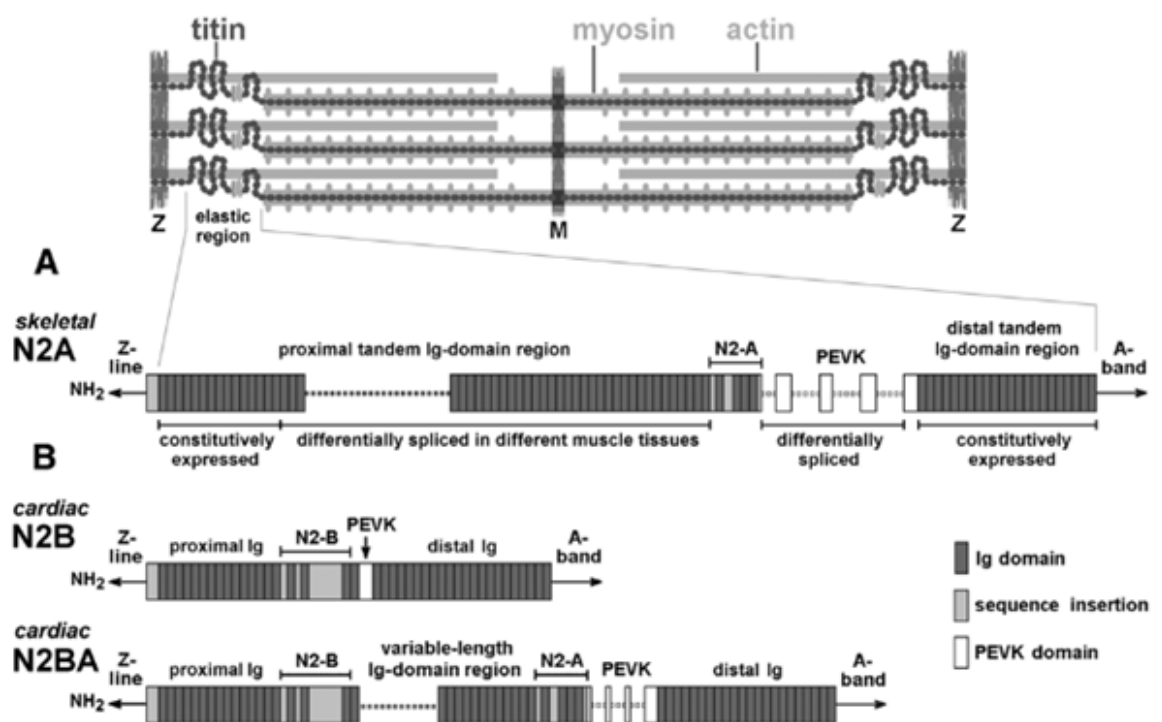
Figure 2.5. Schematic diagram of the various titin isoforms and how they are arranged in (A) skeletal muscle and (B) cardiac muscle (Neagoe et al. 2003).

Any difference in muscle stiffness that remains after accounting for differences in muscle (Blundell et al. 2003; Andersson et al. 2003; Damiano and Abel 1998; McNee et al. 2009; Damiano et al. 1995b; Dodd et al. 2003), must be attributable

to the intrinsic elastic properties of the muscle in individuals with SCP (Friden and Lieber 2003; Lieber et al. 2003). Indeed clear differences have also been reported in the intramuscular content of the extracellular matrix within SCP muscle when compared to the muscle of individuals without neurological impairment (Lieber et al. 2003). Muscle samples were obtained from paediatric patients with and without SCP from a variety of muscles in the upper extremities. The samples were then passively elongated to assess the material properties of the muscle bundles with the extracellular matrix intact, which was then compared to passive properties of single SCP and control muscle fibres. Inevitably the stiffness of the muscle bundles in both SCP and control groups were stiffer than their respective single fibre counterparts. However when the SCP and control muscle bundle were compared, the control bundle was reported to be ~40 times stiffer than the SCP bundle. This was reported to be due to the differences in material composition of the muscle. The spastic muscle sample was found to consist of 40% extracellular matrix, whereas the control sample only contained 5% extracellular matrix. Whether these findings are representative of the material properties of the whole SCP muscle is yet to be confirmed (Lexell et al. 1986, 1984). Although the quantity of extracellular matrix was greater in the SCP muscle, it is unlikely to contribute to greater fascicle stiffness, as the control muscle had a lower quantity of extracellular matrix and greater fibre stiffness. Moreover, Ito et al. (1996) established that there was no observable difference in the histological structure of SCP muscle compared to samples from neurologically unimpaired fibres. The apparent contention in the existence, and contribution, of greater extracellular matrix to passive stiffness in SCP may be due to the population sampled, where the muscle biopsy was obtained from, or the relative degree of impairment within

the SCP participants. However, there remains no data on the intrinsic passive properties of SCP muscle under stretch. In order to determine the influence of the intrinsic properties of the muscle on passive stiffness, muscle size, architecture and activation would need to be accounted for whilst the SCP is stretched.

Additionally, it is possible during muscle contraction that from the propagation of the action potential and the subsequent release of calcium from the sarcoplasmic reticulum may be impaired in CP. In the elderly populations and after 10-weeks of disuse, there is evidence for impairment in release and reuptake of calcium that contributes to sarcopenic weakness (Hunter et al. 1999; Thom et al. 2001). However, there remains no published data on calcium in individuals with SCP.

2.7 Tendon properties

During passive elongation and MVIC, the tendon is known to contribute to the degree of fascicle lengthening and shortening respectively (Maganaris and Paul 1999, 2002; Morse et al. 2008a). Furthermore, the tendon elastic properties have been linked to a number of functional measures, such as balance (Onambele et al. 2007; Onambélé et al. 2008) and rate of force development (Morse et al. 2005a). It is well established that disuse leads to an increase in the compliance of the tendon (de Boer et al. 2007), and combined with the sedentary nature of individuals with SCP (Longmuir and Bar-Or 2000), it could be predicted that any change in the passive or contractile properties of the muscle may be linked to deviations in tendon compliance. However, as the passive properties of the MTU under stretch

show a resistance to stretch (however this remains contentious), it is possible that an increase in stiffness of the tendon may contribute to the increased passive torque. Again, as with both weakness and a reduced ROM, the measurements in children may not reflect the mature MTU, and there is a scarcity of data on the elastic properties of the tendon in individuals with SCP.

2.8 Effects of maturation in individuals with SCP

All of data investigating the active and passive neuromuscular properties of individuals with SCP have been conducted on children and/or adolescent cohorts (Alhusaini et al. 2010; Vaz et al. 2006; Barber et al. 2011a; Elder et al. 2003; Malaiya et al. 2007; Mohagheghi et al. 2007; Stackhouse et al. 2005). Although these studies may apply to adult populations with SCP, there are a number of differences that have been shown to exist between children and adult populations (Falk et al. 2009; O'Brien et al. 2010b). For example, it is has been established that neural activation, muscle size and moment arm is lower by up to 13, 66 and 26%, respectively in children compared to adults without neurological impairment (Morse et al. 2008b; O'Brien et al. 2010a). This is particularly relevant when inferring SCP impairments as it is likely that the neural impairment is the main determinant of skeletal muscle strength between adults with SCP and individuals without neurological impairment, though no information currently quantifies activation/coactivation properties at present. It is likely that there is a difference in strength between children and adult populations with SCP; though no data currently addresses the magnitude and what variables account for these differences.

With regards to the material properties of the tendon, children without neurological impairment are known to have more compliant tendons compared to adults, as identified by stiffness and Young's modulus measures (O'Brien et al. 2010b). O'Brien et al. (2010b) reported that tendon stiffness could be accounted for by the children having a 34% smaller tendon ACSA compared to the adults, whereas the differences in Young's modulus are due to differences in the intrinsic tendon structures. This may be due to deviations in collagen fibril packing (Parry et al. 1978), collagen fibril diameter (Dressler et al. 2002), concentration of decorin (Del Santo Jr et al. 2000) and/or cartilage oligomeric matrix protein (Smith et al. 2002). Nevertheless, whether these differences in maturation apply to individuals with SCP has yet to be determined and requires further research.

2.9 Passive muscle and tendon stiffness methods

In order to assess the previously discussed muscle and tendon properties accurately and reliably, it is imperative that the level of measurement error is limited. Two predominant factors constitute measurement error; systematic bias, and random error, both of which account for the learning and/or fatigue effects on the tests, and the variability of the biological and/or mechanical properties of the variable measured, respectively (Atkinson and Nevill 1998). In order for the method, or its constituents to be suggested as reliable and valid, it is essential that the protocol is sensitive enough to detect a true change in performance.

The agreement between measures of muscle MVC torque have consistently been reported as reliable in individuals without neurological impairment (Alfredson et al. 1998; Impellizzeri et al. 2008), patients that have suffered from a stroke (Hsu et al. 2002) and individuals with CP (Ayalon et al. 2000). Conversely limited information is available when referring to reliability of passive muscle and tendon properties *in vivo*. The assessment of passive muscular properties originally consisted of the indirect testing procedures such as the touch-toe (Magnusson et al. 1997), sit-and-reach tests (Cornbleet and Woolsey 1996). Using these methods, various studies have identified that estimations of passive MTU properties are not reliable, due to the influence of psychological and neural factors increasing the error in the measure (McHugh et al. 1998; McNair and Stanley 1996). With the use of surface EMG to account for muscle activation and ultrasonography to monitor myotendinous junction displacement during stretch protocols, numerous studies have directly measured the passive properties of the gastrocnemius medialis *in vivo* (Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a). The assessment of the passive muscle properties utilise two predominant postures, the prone (Morse 2011; Morse et al. 2008a) and seated postures (Kay and Blazevich 2009), however a limitation within the literature at present is that no study has addressed whether the two postures are comparable and reliable.

Although little information is currently available in the effect of posture on the passive muscular properties during stretch, Morse (2011) suggested that the ultrasonography component of the assessment is a valid tool over small distances, by reporting a mean error of 0.06 mm, and R^2 of 0.999 over 1 mm increments

using a micrometer calliper. Whilst the measurement of displacement during passive stretch manoeuvres has not been validated directly, numerous studies have consistently shown that ultrasonography is a reliable and valid tool for assessing tendon elongation (Maganaris and Paul 1999), muscle fascicle length (Narici et al. 1996), muscle cross sectional area (Reeves et al. 2004a) and muscle volume (Esformes et al. 2002). However, there are no data regarding the validity or reliability of ultrasonography when used to record the passive properties of skeletal muscle *in vivo*.

More definitive information is available in individuals without neurological impairment on the passive properties of the tendon where two predominant postures have been utilised in the assessment of Achilles tendon stiffness, prone (Maganaris and Paul 2002; Muramatsu et al. 2001) and seated (Morrissey et al. 2011). Morrissey et al. (2011) reported that in 6 physically active adults ranging from 18-40 years, tendon stiffness and tendon modulus test-retest assessment was reliable (ICC = 0.85 and 0.72, respectively). Comparatively, no study assessing tendon properties in the prone position have addressed the reliability of the measure. Therefore the seated posture should be utilised in the assessment on Achilles tendon stiffness as a result of the lack of data on the reliability of the prone posture. Caution should be drawn to the specific set up of the seated position as impaired flexibility in SCP cohorts (Alhusaini et al. 2010) may impact on the set up of the chair and isokinetic dynamometer in order to maintain consistency in the testing procedures of any future assessment.

2.10 Summary

Muscle weakness and reduced flexibility has been reported to influence functional tasks and gait performance in individuals with SCP. The paretic muscle has been shown to be smaller than the muscle of individuals without neurological impairment. The SCP-induced size decrement is counter-intuitively associated with a reduction in the flexibility of the paretic joint as measured by the passive torque-angle relation. However, the passive properties of the muscle or tendon have yet to be established *in vivo*, for instance through the use of ultrasonography during passive stretch trials. Nevertheless, the impaired flexibility of the paretic limb is likely to be due to disuse and/or impaired neural communication of the paretic muscle, resulting in muscle atrophy. In addition, intrinsic changes within the paretic muscle including increased sarcomere stiffness and extracellular matrix tissue have been suggested to impact not only on joint ROM, but also strength of the paretic muscle of individuals with SCP.

In specific relation to the strength of the paretic muscle, no study has accounted for the neural and material determinants associated with strength. Elements of muscle weakness have been assessed individually in separate studies with MVC torque. However, the calculation of the force generating capacity of the paretic muscle of individuals with SCP has yet to be addressed. Lastly, no study has examined the properties of the paretic tendon in individuals with SCP. It is not understood how the elastic structural and functional properties of the tendon contributes, if at all, to the observations related to weakness and flexibility in the paretic limib of inividuals with SCP.

Thesis aims

Based upon the presented literature, a number of factors have been associated with reduced muscle strength and MTU flexibility in individuals with SCP. The determinants include muscle size, neural signalling, muscle morphology and intrinsic muscle composition, all of which have been associated with weakness and reduced ROM in children with SCP. Although these factors have been brought together in the present literature review, very little work has been conducted into muscle flexibility and strength whilst accounting for all of the respective determining factors. Moreover, in the assessment of flexibility, two predominant postures have been utilised, however, very little is understood about how/whether testing posture can affect the measurements of passive stiffness. In addition, it is unclear from previous data, the degree to which the effects ascribed to SCP are mainly owing to sedentary behaviour as opposed to the condition itself. Therefore the aims of this thesis were:

1. To assess whether testing posture has an effect on the measured passive flexibility and stiffness measurements of the muscle.
2. To determine the passive flexibility and stiffness properties of the paretic muscle, and examine whether anatomical CSA and/or muscle length influence the passive muscle properties.
3. To ascertain the degree of muscle weakness in adults with SCP, and to assess whether muscle size, agonist activation and/or antagonist activation can account for the deficits in strength.
4. To identify whether there is a difference in paretic muscle specific force *in vivo.*

5. To assess the *in vivo* structural and mechanical properties of the paretic tendon in individuals with SCP.

The objective was also to utilise a habitually physically active hemiplegic SCP men, in order to minimise the impact of sedentarism *per se*, on the descriptive magnitude of SCP impairment on the parameters of interest.

Chapter 3

The effect of seated and prone testing postures on passive stiffness of the gastrocnemius muscle

3.1 Abstract

Introduction. The displacement of the myotendinous junction (MTJ) and passive stiffness of the human gastrocnemius medialis (GM) are frequently quantified *in vivo* using ultrasonography as they relate to the material properties of the muscle. It is however, unclear how body position impacts on the measurements. Thus this study compared the impact of seated versus prone testing postures on recorded MTJ and GM displacement measurements.

Methods. Eight men (28 (5) years) completed four randomised passive-stretch sessions; two prone, and two seated. During each stretch session, the ankle was passively dorsiflexed at 1 $deg \cdot s^{-1}$ until volitional end range of motion (ROM). Throughout each stretch session GM MTJ displacement, passive torque and ankle angle were measured. Passive GM MTJ displacement and GM stiffness were measured at 22 Nm and end ROM.

Results. GM stiffness at 22 Nm was 43% stiffer during the prone testing posture compared to the seated posture. GM MTJ displacement was 0.69 and 0.65 cm greater in the seated position at 22 Nm and end ROM, respectively. Relatively lower intra-postural reliability was identified in the prone position across all measures at 22 Nm and end ROM, compared with that in the seated position.

Conclusion. Where multiple repeat assessments are required, the seated posture should be employed to reliably and accurately assess the passive properties of the GM MTJ displacement and GM stiffness. Conversely the prone posture was identified to be an unreliable method to test the passive properties of the GM.

3.2 Introduction

Stretching is a common strategy prescribed to athlete and patient populations to increase flexibility (Dadebo et al. 2004) and decrease passive joint stiffness (McHugh et al. 1998). Flexibility is classically determined by measuring the volitional end range of motion (ROM) around a joint (Halbertsma et al. 1996; McHugh et al. 1998), using tests such as touch-toe (Magnusson et al. 1997), sit-and-reach (Cornbleet and Woolsey 1996) and other related tests (Chapter 2). Although such tests are commonly adopted in the assessment of flexibility, the reliability of end ROM data has been criticised due to several confounding factors influencing the quality of the measurement (McHugh et al. 1998; McNair and Stanley 1996). As described in Chapter 2, these limitations are reported to be due to several psychological and neural determinants, including; stretch tolerance (Magnusson et al. 1996a), pain (Toft et al. 1989), and reflex response of the muscle and tendon sensory organs (McNair and Stanley 1996). A more reliable method to overcome these limitations is to assess the passive torque-angle relationship (Magnusson et al. 1997; Toft et al. 1989).

Muscle-tendon unit stiffness is expressed as the relationship between the change in passive torque and joint angle when assessing flexibility throughout a passive stretch manoeuvre (Kubo et al. 2001a; Magnusson et al. 1997). However, this measurement does not allow the discrimination of elastic properties between the individual muscular and tendinous components. Recently, several studies have used ultrasonography to measure the *in vivo* displacement of the gastrocnemius medialis (GM) myotendinous junction (MTJ) throughout a passive stretch

manoeuvre at pre-determined increments of torque (Kay and Blazevich 2009) or ankle angle (Morse et al. 2008a) as a measure of passive muscle stiffness (Chapter 2). Such techniques are typically employed to determine and quantify whether an intervention affects the passive properties of the muscle and/or tendon (Abellaneda et al. 2009; Burgess et al. 2009; Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a).

During passive dorsiflexion (DF) trials in the prone posture, the average distal GM MTJ displacement in healthy male participants was 1.04 cm, when rotating the ankle at 1 deg·s^{-1} to end ROM (Morse et al. 2008a). Such small displacement measures require sensitive apparatus and a reliable testing protocol to identify genuine differences in elastic properties of the muscle. Recently, Morse (2011) suggested that ultrasonography is a valid tool over these small distances by reporting a mean error of 0.06 mm, and R^2 of 0.999 over 1 mm increments using a micrometer calliper. Although the measurement of the GM MTJ displacement during passive stretch manoeuvres has not been validated directly, numerous studies have consistently shown that ultrasonography is a reliable and valid tool for assessing tendon elongation (Maganaris and Paul 1999), muscle fascicle length (Narici et al. 1996), muscle cross sectional area (Reeves et al. 2004a) and muscle volume (Esformes et al. 2002). However, there are no data regarding the validity or reliability of ultrasonography when used to record the passive properties of skeletal muscle *in vivo*. Two testing postures have been utilised within the literature to examine the distal GM MTJ displacement under stretch conditions: prone (Morse et al. 2008a) and seated (Kay and Blazevich 2009). Though no

information exists on whether testing posture affects the bias and reliability within the measure of flexibility and passive muscle stiffness. Therefore, the aim of the study is to assess whether data from seated and prone postures agree and are comparable, and to establish which posture is the most reliable measure of GM MTJ displacement, muscular stiffness and ankle angle.

3.3 Methods

3.3.1 Participants

Eight healthy males volunteered to participate in the study (age = 27.6 (5.0) years, stature = 1.81 (0.07) m, mass = 86.6 (13.3) kg) and provided written informed consent. All participants were free from lower extremity injury and were physically active. The study conformed to the latest revision of the Declaration of Helsinki and the ethics committee at Manchester Metropolitan University. Each participant completed one familiarisation session and four testing sessions. Sessions were conducted on separate days (two prone and two seated) in a randomised order to assess the reliability and validity of each testing procedures. A minimum of 24 hours separated each testing session and all procedures were performed on the left leg. Previous research identified that the effect of leg dominance during passive plantarflexor stretch trials was negligible (Moseley et al. 2001). Additionally, Guette et al. (2005) reported no difference in maximal voluntary isometric contraction (MVIC) torque achieved between the dominant and non-dominant quadriceps femoris. Equivocal findings have also been found when assessing the plantarflexsion (PF) strength of adult males without neurological

impairment (Damholt and Termansen 1978), demonstrating that it is not necessary to test both limbs during such simple motor tasks (Guette et al. 2005).

3.3.2 Passive stretch set-up

Participants were secured to an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA) in the seated and prone posture (Figure 3.1; Kay and Blazevich 2009; Morse et al. 2008a). While in the seated posture, three Velcro straps were used to limit extraneous movement: one strap was used to secure the knee in full extension, and two straps were used to secure the foot to the footplate (Figure 3.1). The participant's hips were secured to the seat which was reclined at 65 deg to further limit extraneous movement throughout the passive stretch manoeuvre. Similarly, whilst in the prone posture, two Velcro straps were used to secure the foot to the footplate and the participants' hips were also secured to the bench. Consequently, whilst heel displacement was not visually identifiable, the experimenter relied on the participants to report loss of contact between heel and the footplate throughout the passive stretch, in which case a new trial would be conducted. In both postures, the medial malleolus was visually aligned with the dynamometers' central axis of rotation. Passive end ROM was identified by passively DF the participants' ankle at 1 deg·s^{-1}, from +20 deg plantarflexion, until volitional end ROM. Ankle angle was recorded using an electrogoniometer (K100, Biometrics Ltd, UK) attached to the distal aspect of the lateral malleolus. Two passive stretches were conducted in each testing session, which involved the dynamometer passively rotating the individual's ankle at 1 deg·s^{-1} through the predetermined ROM. Throughout all of the passive procedures, participants were

regularly instructed to remain relaxed (i.e. to not resist the movement of the footplate).

A multi-channel, analog-digital converter (Biopac Systems Inc., USA) recorded ankle angle, torque and GM electromyography (EMG) data at 2 kHz onto a Macintosh computer during each passive trial and analysed offline using AcqKnowledge software (MP100, Biopac Systems Inc.).

3.3.3 MTJ displacement during passive stretch

B-Mode ultrasonography (AU5, Esaota, Italy) was used to determine the GM MTJ displacement during the two passive stretches. The MTJ was identified as a continuous sagittal plane ultrasound image using a 5-cm, 7.5-MHz linear-array probe, which was time locked with torque, EMG and goniometer outputs (as described previously by Maganaris and Paul 1999; Morse 2011). MTJ displacement was measured relative to an acoustically reflective marker secured to the skin proximal to the GM MTJ. Images were recorded onto a desktop computer at 30 Hz and analysed off line at 2-Nm intervals from 0 Nm to 22 Nm (the highest passive torque reached by all participants) and end ROM using digitising software (Dartfish, Friburg, Switzerland). Subsequently, muscle stiffness was calculated as the instantaneous slope of the relationship between passive DF torque and passive distal GM MTJ displacement (Morse 2011).

Figure 3.1. Experimental set up of the seated and prone passive stretching postures (left). Schematic diagrams of the specific foot positioning in both experimental postures (right).

3.3.4 Maximal voluntary contraction

All participants were allowed 3 min to perform sub-maximal warm-up contractions prior to the MVIC testing procedure. After the warm-up, three maximal isometric PF MVICs were performed with the ankle at 0 deg. Participants were verbally encouraged throughout each isometric contraction, with consecutive efforts

separated by a 60-s rest period. The highest MVIC value that was recorded during each testing session was used in the analysis of passive muscle activation.

3.3.5 EMG

The EMG activity of the GM was recorded throughout the passive stretch using two pre-gelled, unipolar, 10-mm, Ag-AgCl percutaneous electrodes (Medicotest, Denmark). The area where the electrodes were positioned was shaved and cleaned with an alcohol swab. The electrodes were placed medially on the upper quartile of the GM. Electrodes were placed 25 mm apart and a reference electrode placed over the femurs' medial epicondyle. EMG data were recorded at 2000 Hz, with a high and low pass filter set at 500 and 10 Hz respectively, and a notch filter at 50 Hz. The integral of the root mean square of the raw signal 0.5 s either side of the specific torque values was used to quantify EMG activity. EMG stretch data is presented relative to the EMG measured during the MVIC and analysed at 25, 50, 75 and 100% of peak passive torque.

3.3.6 Statistics

Inter-postural comparisons were made on the average data from days 1 and 2. Ankles angle, GM MTJ displacement and GM stiffness data was determined by averaging the data from days 1 and 2 for each participant in the collective group, which was presented in the results section of this chapter. The agreement between seated and prone GM MTJ displacement stiffness and ankle angle data

was assessed using a two-way mixed effects intra-class correlation coefficient (ICC), using statistical analysis software (SPSS, version 16.0; Chicago, IL). Intra-posture reliability (day 1 vs. day 2) was analysed with an ICC, using a one-way random effects model. The output from an ICC can range from 0-1 and has been discussed by Atkinson and Nevill (1998) where an ICC ranging from 0.7-0.8 is suggested to be questionable, whereas values > 0.9 is high. The coefficient of variation (CV), systematic bias and 95% limits of agreement (LoA) were reported to assess the agreement between postures and the intra-posture reliability of each posture (Bland and Altman 1986). The CV was calculated by; (SD x 1.96) / mean x 100, which covers 95% of the repeated measures (Bishop 1997). It has been suggested that the variability of the CV outputs being < 10% is reliable, whereas anything over this value is not reliable (Stokes 1985), however this should be used with caution and relative to the variable being assessed (Atkinson and Nevill 1998). In order to calculate the LoA, the systematic bias and random error has to be calculated. To calculate systematic bias, the average difference from the tests conducted on day 1 and 2 were obtained. This systematic bias indicates a trend in the measurement, either in a positive/negative direction between tests, which could be due to factors such as learning effects. Whereas random error requires the SD for the data collected on day 1 and 2, which is then multiplied by ±1.96. The random error indicates the range of biological and/or mechanical variation in the measure (Atkinson and Nevill 1998). Differences between GM MTJ displacement, stiffness, ankle angle, passive torque and muscle activity were assessed by two-tailed paired t-tests. All values are stated as means (SD), unless stated otherwise.

3.4 Results

Average inter- and intra-postural measurements of muscle stiffness, GM MTJ displacement, passive torque, and ankle angle at 22 Nm are displayed in Table 3.1 and end ROM in Table 3.2. No difference was detected between inter- or intra-postural passive torques at end ROM (Table 3.2). Average muscle activity during the passive stretch was 0.11 (0.11), 0.12 (0.08), 0.48 (0.50) and 0.91 (1.20)% relative to MVIC, in the seated posture at 25, 50, 75% and end ROM, respectively. Similarly, muscle activity during the prone posture was 0.38 (0.67), 0.54 (1.09), 0.21 (0.20) and 0.17 (0.15)% in the prone posture at 25, 50, 75% and end ROM, respectively. No differences were identified between postures.

Table 3.1. Distal displacement of the GM MTJ and stiffness of the GM muscle and ROM of the ankle from 0-22 Nm.

0-22 Nm	Seated		Prone		Average	
	Day 1	Day 2	Day 1	Day 2	Seated	Prone
GM MTJ displacement (cm)	1.70 (0.31)*	1.74 (0.32)	1.07 (0.39)	0.99 (0.27)	1.72 (0.31)†	1.03 (0.23)
Stiffness (Nm cm^{-1})	13.4 (2.7)*	13.0 (2.6)	22.6 (7.1)	24.0 (7.2)	13.2 (2.6)†	23.3 (4.6)
Ankle angle (deg)	-6.91 (4.19)	-7.24 (4.05)	-10.3 (7.0)	-12.4 (6.1)	-7.08 (3.94)†	-11.3 (4.5)

Gastrocnemius (GM), Range of motion (ROM), Gastrocnemius myotendinous junction (GM MTJ). *indicates intra-postural difference ($P < 0.01$), †denotes inter-postural difference ($P < 0.05$).

Table 3.2. Distal displacement of the GM MTJ, stiffness of the GM, torque and ankle angle at passive end ROM.

end ROM	Seated		Prone		Average	
	Day 1	Day 2	Day 1	Day 2	Seated	Prone
GM MTJ displacement (cm)	1.70 (0.31)*	1.74 (0.32)	1.11 (0.39)	1.03 (0.26)	1.72 (0.31)[†]	1.07 (0.22)
Stiffness (Nm cm^{-1})	20.6 (5.9)	19.9 (6.9)	32.7 (7.2)	38.7 (15.3)	20.3 (5.9)[†]	35.7 (9.7)
Passive torque (Nm)	34.5 (9.2)	33.5 (8.2)	34.8 (9.7)	37.0 (7.9)	34.0 (8.5)	35.9 (8.6)
Ankle angle (deg)	-11.9 (5.4)	-11.6 (2.7)	-14.7 (6.8)	-16.5 (8.1)	-11.7 (3.5)[†]	-15.6 (5.8)

Volitional end range of motion (end ROM), gastrocnemius myotendinous junction (GM MTJ). *indicates intra-postural difference ($P < 0.01$), [†]denotes inter-postural difference ($P < 0.05$).

3.4.1 Inter-posture agreement

At 22 Nm and end ROM, MTJ displacement was 40 and 38% greater in seated compared to prone posture (Figures 3.2 and 3.3, $P < 0.005$), respectively. This systematic bias showed that the seated posture was 43% more compliant than the prone posture during stiffness measures at 22 Nm and end ROM (Figures 3.2 and 3.3, $P < 0.05$). The GM MTJ displacement and GM stiffness produced ICCs of 0.2 and lower (Tables 3.3 and 3.4), and CV's ranged between 59% and 76%, at 22

Nm and end ROM. The GM MTJ displacement LoA were 0.69 ± 0.82 cm and 0.65 ± 0.86 cm at 22 Nm and end ROM, respectively. At 22 Nm and end ROM, the LoA for GM stiffness were -10.1 ± 11.4 Nm cm^{-1} and -15.4 ± 21.4 Nm cm^{-1}, respectively (Bland-Altman plots are displayed in Figures 3.3 and 3.4).

The ankle angle recorded at 22 Nm and end ROM yielded ICCs of 0.409 and 0.530, respectively (Tables 3.3 and 3.4). At 22 Nm and end ROM, CV's were over 70%, with the ankle angle in the prone position being 37% greater ($P < 0.05$) than the seated angle at 22 Nm (Table 3.1). Large differences were identified between testing postures at end ROM (Table 3.2). LoA were 4.25 ± 9.27 deg and 3.93 ± 9.77 deg at 22 Nm and end ROM, respectively.
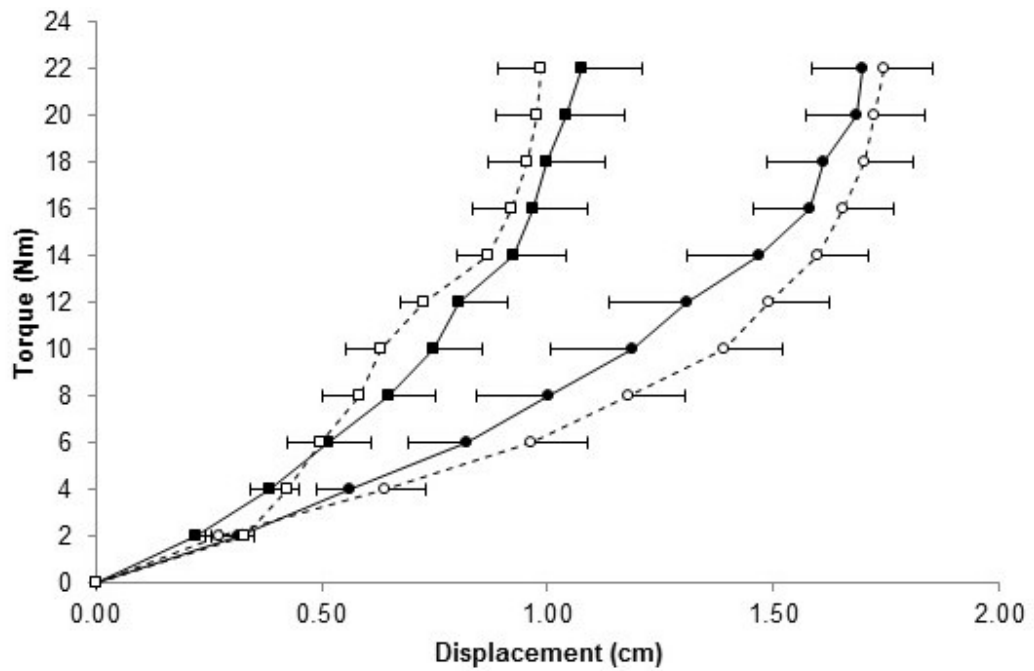
Figure 3.2. Distal displacement of the GM MTJ at 2 Nm intervals during continuous passive dorsiflexion stretch in seated (circles) and prone (squares) testing postures. Continuous and broken lines represent test day 1 and day 2, respectively. Error bars indicate SEM.

3.4.2 Prone intra-posture comparison

The prone testing posture yielded ICCs of -0.111 to 0.464, and CV's over 75% for GM MTJ displacement and muscle stiffness, at 22 Nm and end ROM (Tables 3.3 and 3.4). The GM MTJ displacement LoA were -0.09 ± 0.94 cm and -0.08 ± 0.98 cm at 22 Nm and end ROM, respectively. At 22 Nm and end ROM, the LoA for GM stiffness were 1.39 ± 21.6 Nm cm^{-1} and 5.98 ± 27.1 Nm cm^{-1}, respectively. The Bland-Altman plots (Figures 3.3 and 3.4) display the range in error between day 1 and day 2 displacement and stiffness measures. There were no differences

between day 1 and day 2 in GM displacement and stiffness data, at 22 Nm (Table 3.1) or end ROM (Table 3.2).

Ankle angle at 22 Nm (Table 3.3) and end ROM (Table 3.4) during the prone posture produced ICCs below 0.400, and CV's over 100%. The LoA at 22 Nm were -2.09 ± 18.8 deg, and -1.60 ± 18.6 deg at end ROM. No systematic bias was detected at 22 Nm or end ROM (Tables 3.1 and 3.2).

Table 3.3. ICC for all inter- and intra-postural assessments from 0-22 Nm.

0-22 Nm	Seated posture (day 1 vs. day 2)	Prone posture (day 1 vs. day 2)	Seated vs. Prone postures
GM MTJ displacement (cm)	0.992	0.038	-0.072
Stiffness (Nm cm^{-1})	0.993	-0.309	-0.057
Ankle angle (deg)	0.914	-0.067	0.409

Intraclass correlation coefficient (ICC), gastrocnemius myotendinous junction (GM MTJ).

Table 3.4. ICC for all inter- and intra-postural assessments at end ROM.

end ROM	Seated posture (day 1 vs. day 2)	Prone posture (day 1 vs. day 2)	Seated vs. Prone postures
GM MTJ displacement (cm)	0.993	-0.111	-0.181
Stiffness (Nm cm^{-1})	0.830	0.464	0.570
Ankle angle (deg)	0.594	0.390	0.530

Intraclass correlation coefficient (ICC), gastrocnemius myotendinous junction (GM MTJ), volitional end range of motion (end ROM).

3.4.3 Seated intra-posture comparison

The seated testing posture produced ICCs of 0.992 and 0.993 at 22 Nm (Table 3.3), and 0.993 and 0.830 at end ROM (Table 3.4) for GM MTJ displacement and GM stiffness, respectively. The CV's of displacement and stiffness data were 4% for the two measures at 22 Nm. The CV's at end ROM were 4% and 50% for GM MTJ displacement and GM muscle stiffness, respectively. The GM MTJ displacement LoA were 0.05 ± 0.06 cm and 0.04 ± 0.07 cm at 22 Nm and end ROM, respectively. At 22 Nm and end ROM, the LoA for GM stiffness were -0.37 ± 0.50 Nm cm^{-1} and -0.68 ± 10.1 Nm cm^{-1}, respectively. Bland-Altman plots illustrate the systematic bias and random error, between the day 1 and 2 trials in the seated

testing posture at 22 Nm (Figure 3.3) and end ROM (Figure 3.4). There was a 7% increase in GM MTJ displacement (P < 0.01) at 22 Nm and end ROM, indicating systematic bias. Systematic bias was also recorded at 22 Nm, showing a 3% decrease in GM stiffness (P < 0.01) when day 1 was compared to day 2.

The ankle angle in the seated posture produced ICCs of 0.914 and 0.594 at 22 Nm (Table 3.3) and end ROM (Table 3.4), respectively. In addition, CV's at 22 Nm and end ROM were 68% and 80%, respectively. The LoA were -0.33 ± 4.80 at 22 Nm and 0.34 ± 9.36 at end ROM. No statistical difference was found between seated trials at 22 Nm and end ROM (Table 3.1 and 3.2).
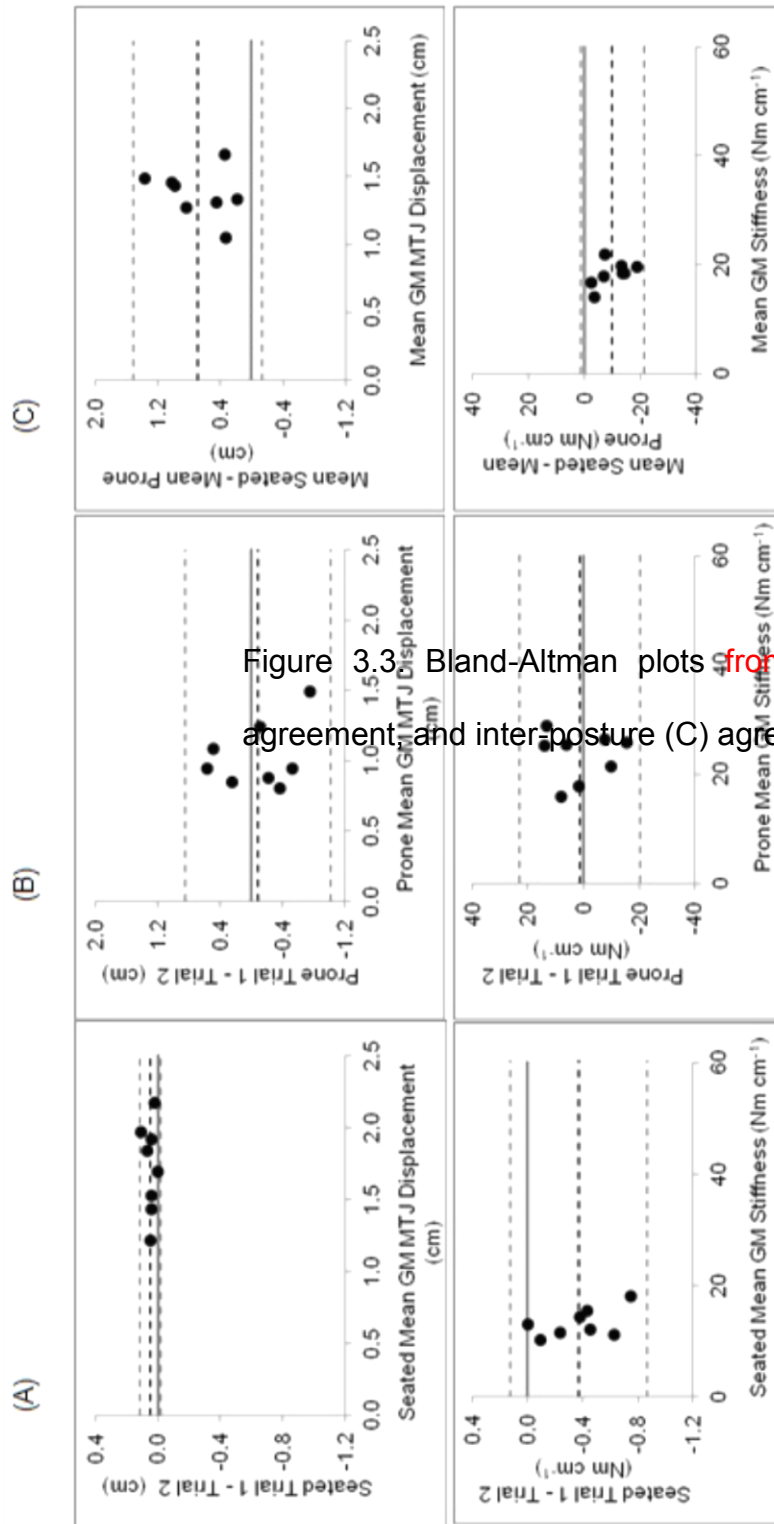
Figure 3.3 Bland-Altman plots from 0-22 Nm displaying seated (A)
agreement, and inter-posture (C) agreement.
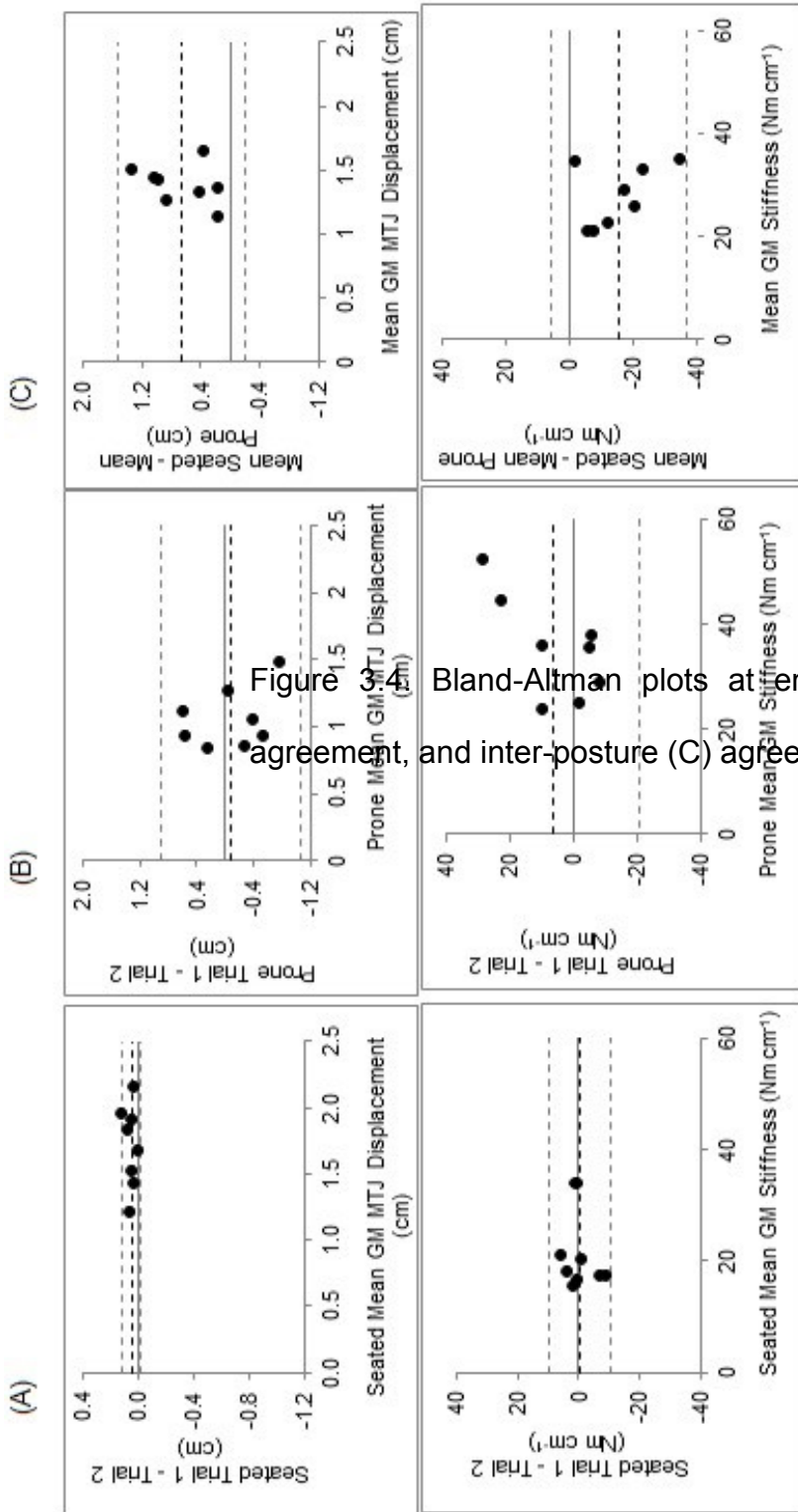
Figure 3.4. Bland-Altman plots at end ROM displaying seated (A) and prone (B) agreement, and inter-posture (C) agreement.

3.5 Discussion

The aim of the present study was to assess the level of agreement between the seated and prone postures, and to establish which posture is the most reliable measure of GM MTJ displacement, muscle stiffness and ankle flexibility. The main findings show that the seated posture was the most reliable method of measuring GM MTJ displacement, GM stiffness and ankle angle, compared to the prone posture. Furthermore, the seated and prone postures yielded poor inter-postural agreement when measuring distal GM MTJ displacement, GM stiffness and ankle angle at 22 Nm and end ROM.

The present results confirm that the seated posture is a reliable protocol when measuring GM MTJ displacement and GM stiffness at 22 Nm during a passive stretch to DF end ROM. At end ROM, distal GM MTJ displacement was the only reliable measure recorded. From the ICC analysis, the displacement assessment to compute GM stiffness and ankle angle were repeatable, however the CV and LoA revealed poor reliability and agreement between the two measures. These findings confirm the importance of using a triangulated approach (the use of three or more reliability tests to ascertain the repeatability and accuracy of a method) to obtain a more accurate interpretation of the reliability and agreement of measures (Atkinson and Nevill 1998). By using such an approach to test reliability, this also facilitates researchers to assess specific statistical reliability tests they are most familiar with implementing. Additionally, during passive stretch of the GM, it is more reliable to monitor GM MTJ distal displacement as opposed to other methods of assessing flexibility to end ROM.

GM stiffness in the present study was calculated using GM MTJ displacement and passive toque (Morse 2011; Morse et al. 2008a). At 22 Nm GM stiffness was reliable across all statistical analyses in the seated posture, though measurements at end ROM such should be used with caution as the random error and CV indicated that the measure was less reliable. Nevertheless, the systematic bias was only found to be 0.18 Nm cm^{-1} higher than that measured at 22 Nm and also the ICC rated as a reliable test. This difference in findings between end ROM and 22 Nm may be due to variability in the torque and GM MTJ displacement, as measures at end ROM are likely to be confounded by psychological factors (Magnusson et al. 1996a; Toft et al. 1989; McNair and Stanley 1996). Thus, GM MTJ displacement is a more reliable method of assessing the passive properties particularly at end ROM, however, as the measurement of GM stiffness accounts for passive torque and GM MTJ displacement, it would be expected that the level of variability would increase in the measure.

Of the two postures tested, the seated posture was the most reliable, but demonstrated a systematic bias from day 1 to day 2, with GM MTJ displacement increasing by 7% at 22 Nm and end ROM, respectively. It is questionable whether this systematic bias of 0.04 cm at end ROM would have a meaningful impact on identifying differences in the passive properties of the GM as comparatively, acute stretch intervention studies have reported GM MTJ displacement in excess of 0.3 cm at end ROM (Morse et al. 2008a). This day to day bias may also be associated with the mathematical limitations involved with the calculation of systematic bias,

such as the magnitude of variation observed (Atkinson and Nevill 1998). Alternatively, these findings may simply be due to the relatively small number of participants tested and/or the cohort being too homogenous. As displayed in figure 3.3 and 3.4, the level of variation is far smaller in the seated posture compared to the prone posture. Furthermore, the inter-postural difference in GM MTJ displacement ranged from 38-40% and showed a large variability in the relation to the LoA confidence intervals throughout the torque range. Such large variations between the seated and prone positions demonstrate that any direct comparison between the two postures would be erroneous.

Although the prone posture was unreliable it is similar to previous data; Morse et al. (2008a) reported a 1.04 cm displacement of the GM MTJ and 38.8 Nm cm^{-1} GM stiffness, whereas the present study GM MTJ displacement was 1.07 cm and GM stiffness was 35.7 Nm cm^{-1}. All of these comparisons were made at end ROM as submaximal measurements were made relative to ankle angle and not torque. Although similar to previous data, all of the prone reliability tests in the present study reported that: GM MTJ displacement, GM stiffness and ankle angle were unreliable measures at 22 Nm and end ROM. These findings could be due to several confounding factors including: heel displacement, segmental translation and neural reflexes at end ROM (Magnusson et al. 1996a; McNair and Stanley 1996).

The potential effect of heel displacement on passive torque values would result in isokinetic dynamometer moment arm assumptions being violated due to the translation of the lateral malleolus away from the axis of rotation. This excess movement of the heel could also lead to segmental translation. Indeed this translation in seated position would be minimal due to the seat being inclined, restricting the movement of the hip, whereas the prone posture had no structure limiting this extraneous movement. In contrast, neural activation was measured in the present study through EMG and expressed relative to PF MVIC. The GM was near electrical silence with prone and seated postures recording signals < 1% activity relative to GM MVIC throughout the passive stretch (at 25, 50, 75% and end ROM); confirming that the torque measured was indeed passive and that data were not confounded by voluntary or reflex contractions.

Although there are inter-postural similarities in GM activity and torque, low levels of agreement were demonstrated in GM MTJ displacement, GM stiffness and ankle angle measurements between postures. The GM at 22 Nm was 43% more compliant in the seated posture when compared to the prone position. GM MTJ displacement was also 0.69 and 0.65 cm greater in the seated position at 22 Nm and end ROM, respectively. At end ROM the average angles achieved by the seated and prone positions were 11.7 and 15.6 deg, respectively. Previous studies show a linear relationship between GM MTJ displacement and ankle angle (Morse et al. 2008a). The present study showed that lower GM MTJ displacement values are achieved in the prone posture than the seated position. This data provides indirect evidence that suggests heel displacement may have occurred during the

prone position – assuming that no heel displacement occurred during the seated posture. However, the degree of heel displacement could not be quantified in the present study, due to the structure of the footplate obstructing the recording of the foot during the testing procedure. Consequences resulting from any potential heel displacement during the prone position may have resulted in the translation of the participant away from the dynamometer, as no opposite force was applied to the resting participant during the experimental procedure to counteract the horizontal force applied during the passive procedure.

3.6 Conclusion

There are several factors that affect the measurement of ROM and the assessment of muscle elastic properties during passive stretch conditions at 22 Nm and end ROM. The present findings suggest that the inter-postural agreement between the seated and prone postures is too low to make any comparison between the two measures. The intra-postural reproducibility was highest for the seated position for GM MTJ displacement, GM stiffness and ankle angle, compared to the unreliable results obtained for the prone posture. Although the results at end ROM were subject to several confounding variables impacting on the reliability of GM MTJ displacement and GM stiffness measures, presenting these variables should be done with an understanding of the increased level of error. Thus, future studies assessing the passive elastic properties of the GM should use the seated posture at a common torque reached by all participants.

Chapter 4

Passive stiffness of the gastrocnemius muscle in active adults with spastic hemiplegic cerebral palsy

4.1 Abstract

Introduction. The passive properties of the muscle-tendon unit are regularly assessed in children with spastic cerebral palsy (SCP). However, no information is available on the passive properties of SCP adult muscle and whether any differences exist between the paretic and control muscles.

Methods. Eleven physically active ambulant men with hemiplegic SCP (21.2 (3.0) years) and controls without neurological impairment completed two and one passive stretch session, respectively. During each session, the ankle was passively dorsiflexed until end range of motion (ROM), whilst recording passive ankle angle, torque and gastrocnemius medialis (GM) myotendinous junction (MTJ) displacement. Additionally, GM cross sectional area (CSA) and length were measured. Subsequently, *in vivo* stress and strain were determined to calculate elastic modulus.

Results. Passive stiffness, MTJ displacement and ROM of the paretic GM were not different from the control muscles. However, the elastic modulus of the paretic GM was two times higher than the control GM muscles.

Conclusion. Physically active adults with SCP exhibit absolute passive muscle stiffness similar to the controls; however the elastic modulus of the SCP muscle was significantly greater. Therefore, throughout the same ROM a smaller GM CSA in physically active adults with SCP have to dissipate larger relative torque compared to the control muscles, consequently causing the muscle to elongate to the same extent as the non-paretic muscle under stretch.

4.2 Introduction

The range of motion (ROM) available to individuals with spastic cerebral palsy (SCP) around a paretic joint is often reported to be limited (Alhusaini et al. 2010; Barber et al. 2011a), consequently impacting on locomotion (Gage et al. 1995). As a result of such limitations in ROM around the paretic joint, many studies have quantified the magnitude of difference by passively lengthening the muscle-tendon unit (MTU) until volitional end ROM (Alhusaini et al. 2010; Barber et al. 2011a; Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a). As discussed in Chapter 2 and 3, this method of assessing flexibility is simple to perform and often adopted in field-based testing, however several limitations have been reported to confound such data, including psychological (Magnusson et al. 1996a; Toft et al. 1989) and neural factors (McNair and Stanley 1996). Furthermore measuring end ROM around the joint does not provide information on the constituents of the MTU that contribute to resistance to stretch.

In contrast to flexibility testing which is influenced by stretch tolerance and reflex activation of the muscle under stretch (McNair and Stanley 1996), the passive torque-angle relation has been shown to eliminate these neural shortcomings by monitoring electromyography of the muscle in order to quantify whether the stretch was passive (Magnusson et al. 1997). In individuals with hemiplegic CP, it is consistently reported that the relaxed MTU of the paretic limb is stiffer than controls without neurological impairment (Alhusaini et al. 2010; Vaz et al. 2006). This has been speculated to be due to 'spasticity', which describes a velocity-dependent resistance of the muscle to stretch, as a result of a lesion to the central

nervous system causing hyperexcitability of the stretch reflex (Sanger et al. 2003). Thus, in order to minimise muscle activation in individuals with SCP, a low velocity passive stretch is required to inhibit involuntary reflexes.

Such an increase in neural activity was previously thought to be the primary factor resisting passive movement (O'Dwyer et al. 1996). However, a number of structural factors have been suggested as determinants of passive properties of the muscle in a number of different population groups. These have included; fascicle length (Friden and Lieber 2003), fascicle stiffness (Barber et al. 2011a), muscle cross sectional area (CSA) (Magnusson et al. 1997), pennation angle (Moreau et al. 2009), extracellular connective tissue (Lieber et al. 2003), type 1 muscle fibres (Ito et al. 1996) and variation in fibre size (Marbini et al. 2002). Such information on the passive properties of the MTU in individuals with SCP has predominantly been reported in children. Where previous data is reported in adults, it does not discriminate between male and female participants and includes a range of ages (15-21 yrs) where individuals may not be fully mature (Barber et al. 2011a).

Such data may not apply to physically active adults with SCP as the brain lesion is typically non-progressive, but the resultant movement disorders may become more pronounced and progressive over time (Hanna et al. 2009). As the MTU is highly plastic, the environment the tissue is subjected to may be a key factor in how these progressions affect the passive properties of the contractile and elastic

components. In order to discriminate between the muscle and tendon structures, ultrasonography has previously been used to track the movement of the myotendinous junction (MTJ) *in vivo* throughout a passive stretch manoeuvre (Abellaneda et al. 2009; Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a). By utilising a similar technique, Barber et al. (2011a) reported that the stiffness of the paretic MTU of individuals with SCP was higher when compared to age-matched controls without neurological impairment.

As stated in Chapter 2, individuals with CP are documented to typically live a sedentary lifestyle (Longmuir and Bar-Or 2000). It is well established in individuals without neurological impairment that a reduction in physical activity results in muscle atrophy, weakness and muscle shortening (de Boer et al. 2007). Physically active adults with SCP may comparatively experience relative preservation in muscle function associated with increased physical activity. Indeed, previous data assessing the MTU properties of individuals with SCP compared to those without neurological impairment have focused on sedentary populations; there is however, no information on the impact of relatively elevated habitual physical activity of individuals with SCP compared to age-matched counterparts without neurological impairment on the passive properties of the muscle. Therefore the primary aim of the study was to assess the passive stiffness of the paretic GM in physically active adults with SCP compared to the non-paretic contralateral limb of the individuals with SCP and age-matched controls. The secondary aim was to assess whether muscle length and/or CSA have any influence on passive muscle stiffness properties. Based on previous data assessing MTU and GM stiffness in individuals

with SCP, we hypothesised that the passive stiffness of the paretic GM in physically active adults with SCP would be higher compared to the non-paretic muscle and the age match controls without neurological impairment.

4.3 Methods

4.3.1 Participants

Eleven physically active adult men with hemiplegic SCP (age = 21.2 (3.0) years, stature = 1.79 (0.10) m, mass = 70.0 (12.5) kg) and eleven males without neurological impairment (age = 21.8 (2.2) years, stature = 1.81 (0.04) cm, mass = 79.0 ± 8.4 kg) volunteered to participate in the study after written informed consent was obtained. Each participant with SCP rated between II and III on the modified Ashworth scale and had been formally classified independently by individuals from the Cerebral Palsy International Sports and Recreation Association (CPISRA). All participants with SCP completed football-specific training and stretched at least 3 times per week. All participants without neurological impairment played and trained regularly for a university soccer team. All participants were free from lower limb injury and had not received any form of medication to reduce the effects of spasticity within the last year. Both the paretic and non-paretic legs were tested in each participant with SCP, whereas the dominant limb was assessed in all individuals without neurological impairment. Informed consent was obtained from all participants involved in the study prior to the commencement of testing and approval was obtained from the local ethics committee at Manchester Metropolitan

University. Furthermore, the study conformed to the principles set out in the Decleration of Helsinki.

4.3.2 Further testing and participant recruitment information

The recruitment of healthy men without neurological impairment was completed by inviting individuals that regularly engaged in football training at Manchester Metropolitan University. Men with hemiplegic SCP were recruited from a database that The Football Association provided. The database consisted of 159 players from England and Wales that could be approached to take part in the series of studies. Due to the aims and objectives of the present thesis, the majority of the individuals on the database were unsuitable to be invited to partake in the series of studies due to their specific impairment, habitual training status and/or age. As a result of the criteria, this left 14 possible individuals that could have been recruited to partake in the series of tests. Of these 14 individuals, 11 were successfully recruited.

All of the participants that volunteered to take part in the testing protocol for the present chapter were tested on all of the parameters outlined in chapter 4-7 on the same day. Initially participants attended a familiarisation session individually where each person sampled all of the testing procedures outlined in experimental chapters 4-7. There was a period of at least 24 hours between the familiarisation session and experimental testing session where the participants completed the full testing protocol that is outlined in Figure 4.1.
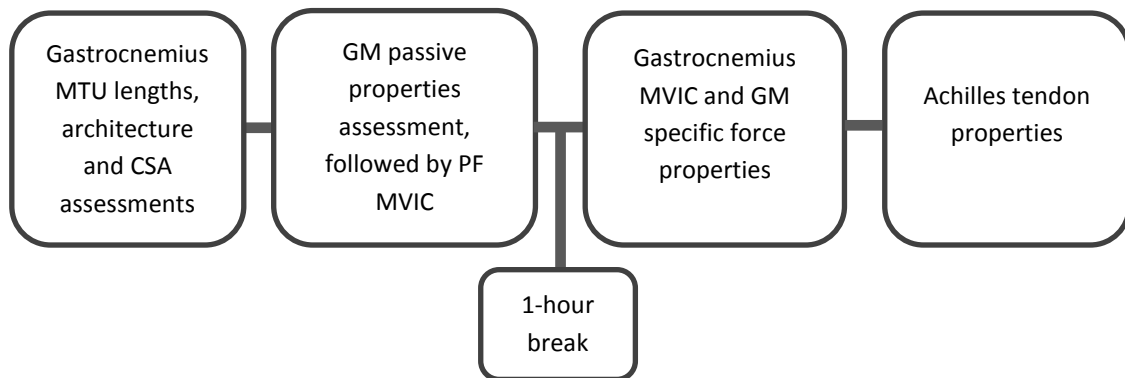
Figure 4.1. Chronological order of the testing procedures completed on all participants.

4.3.3 Passive stretch set-up

As the seated posture was found to be the most accurate and reliable test in addressing the passive properties of the muscle (Chapter 3), all tests were carried out in a seated position (for further details see Kay and Blazevich 2009; Morse 2011), using a isokinetic Cybex dynamometer (Cybex Norm, Cybex International Inc., NY, USA). The medial malleolus was visually aligned with the dynamometer's central axis of rotation. Three Velcro straps were used to limit extraneous movement: one strap was used to secure the knee in full extension, and two straps were used to secure the foot to the footplate. The participant's hips were secured to the seat to further limit extraneous movement throughout the passive stretch manoeuvre. Consequently, whilst heel displacement was not visually identifiable, the experimenter relied on the participants to report loss of contact between heel and the footplate throughout the passive stretch, in which case a

new trial would be conducted. Passive dorsiflexion (DF) end ROM was identified by the experimenter rotating the ankle at 1 deg$\cdot$s^{-1}, starting from +20 deg plantarflexion (PF), until discomfort caused the participants to cease the stretch. This velocity was chosen in relation to previous findings which elicited minimal neural activity throughout passive stretch trials in individuals without neurological impairment (Morse 2011; Morse et al. 2008a). Ankle angle was recorded by an electrogoniometer (K100, Biometrics Ltd, UK), which was attached to the distal aspect of the lateral malleolus, about the coronal axis of the ankle. Similar to Morse et al. (2008a), all angle data refer to the angle of the goniometer as opposed to the dynamometer angle. Two passive stretches were conducted in each testing session, throughout all passive procedures; participants were regularly instructed to remain relaxed and not to resist the movement of the footplate. The first passive stretch was used to identify participant end ROM, the second was used for data collection. Throughout the passive stretch used for data collection, a multi-channel, analog-digital converter (Biopac Systems Inc., USA) recorded ankle angle, torque and GM electromyogram (EMG) data at a 2-kHz sampling frequency onto a Macintosh computer. All data were analysed offline using AcqKnowledge software (MP100, Biopac Systems Inc. USA).

4.3.4 MTJ displacement during passive stretch

B-Mode ultrasonography (AU5, Esaota, Italy) was used to determine the displacement of the GM MTJ throughout the passive stretch (Chapter 3). The MTJ was identified as a continuous sagittal plane ultrasound image using a 5-cm, 7.5-MHz linear-array probe, which was time locked with the torque, EMG and

goniometer outputs as described previously (Maganaris and Paul 1999; Morse 2011). MTJ displacement was measured relative to an acoustically reflective marker (a thin strip of Micropore tape) secured to the skin proximal to the GM MTJ, as previously validated by Morse et al. (Morse et al. 2008a). Images were recorded onto a desktop computer at 30 Hz and analysed off line at 2-Nm intervals from 0 Nm to 14 Nm (the highest passive torque reached by all participants) and end ROM using digitizing software (Dartfish, Friburg, Switzerland).

Subsequent to this analysis, muscle stiffness was calculated as the ratio of passive DF torque to the passive distal displacement of the GM MTJ (Morse 2011). It should be noted, as with previous investigations into the passive properties of the GM, that it is not possible to account for elongation of the proximal tendon or the contribution of other structures to passive torque, such as the joint capsule. It has been reported in rats that after a period of 14 days hind limb suspension, that 25% of the passive ankle joint tension was due to joint structures, whereas the remaining 75% was due to muscle-tendon properties (Gillette and Fell 1996), though whether this is representative of joint stiffness in humans has yet to be determined. Therefore with these caveats in mind, nominal passive muscle stiffness is defined as the distal displacement of the GM MTJ relative to passive PF joint torque ($Nm \cdot cm^{-1}$).

4.3.5 Maximal voluntary contractions

Following passive stiffness measurements, participants performed maximal voluntary isometric PF and DF contractions. All participants were given a 3 min period of time to perform sub-maximal warm up contractions prior to the maximal voluntary isometric contraction (MVIC) testing procedure. After the warm-up, three PF and DF MVICs with the ankle at 0 deg were performed. Each participant was verbally encouraged throughout each isometric contraction and each trial was separated by a 60-s rest period, which has previously been demonstrated to negate the effects of fatigue during maximal isometric testing (Binder-Macleod and McDermond 1992). The highest MVIC value was recorded, which was defined as the average torque 0.5 s either side of the instantaneous peak torque. The MVIC torque data was subsequently used to normalise EMG data during the passive stretch procedure.

4.3.6 Electromyography

The activity of both the GM and tibialis anterior (TA) muscles was recorded throughout the passive stretch using four (two each for the GM and TA) pre-gelled, unipolar, 10-mm, Ag-AgCl percutaneous electrodes (Medicotest, Denmark). The area where the electrodes were positioned was shaved, and cleaned with an alcohol swab to remove residual skin cells and oils, as well as reduce skin impedance. The electrodes were placed medially at two thirds and proximally of GM length and TA length, having first defined the boundaries of the respective muscles using ultrasonography. The upper two thirds were used for electrode placement to avoid the innervation zone (Saitou et al. 2000), and to allow ultrasound placement over the proximal MTJ. Electrodes were placed with 25 mm

distance between the centres; reference electrodes were placed over the medial and lateral epicondyle of the femur for the GM and TA, respectively. EMG data were recorded at 2000 Hz, with a low and high-pass filter set at 500 and 10 Hz respectively, and a notch set at 50 Hz. The integral of the root mean square of the raw signal 0.5 s either side of the specific torque values was used to quantify muscle activity. Integrated EMG from the stretch trials is presented relative to the integrated EMG measured during the MVIC. This allowed the quantification of muscular activity during the passive stretch, which was analysed at 25, 50, 75 and 100% of the maximum passive torque value.

4.3.7 Gastrocnemius medialis and tendon length

Although it was stated in Chapter 3 that the prone posture was an unreliable position to obtain passive data from, all measurements obtained in this posture did not required the participant or isokinetic dynamometer to move once the participant was secured into position and the participant was requested to stay as still as possible. Furthermore, whilst in the seated position, a portion of the proximal gastrocnemius was obstructed by the seat, making the accurate assessment of GM length an impossible task.

B-Mode ultrasonography was used to determine the length of the GM and the muscle tendon unit, whilst the participant lay prone. Ankle angle was fixed at 0 deg, for all individuals that stood with their heels in contact with the ground. Corrections in the fixed ankle position were made for two of the participants with

SCP, as their paretic heel did not make contact with the floor. This was completed by aligning a goniometer with the tibia, the lateral malleolus of the ankle and the fifth metatarsal until the foot made contact with the floor; using this adjusted ankle angle as 0 deg.

Whilst the participant lay relaxed in the prone position, the insertion of the GM into the medial condyle of the femur and the MTJ were marked on the skin and used to calculate GM length. Additionally the GM MTJ to the insertion of the Achilles tendon into the calcaneus (which was determined using ultrasonography) was measured using an inextensible tape to determine the length of the tendon, a method which has been previously validated (Barber et al. 2011b).

4.3.8 Gastrocnemius medialis cross-sectional area

Several axial-plane images of the GM were obtained using B-mode ultrasonography to estimate CSA (Reeves et al. 2004a). The distance between the proximal insertion and the MTJ was marked along the width of the GM at approximately 3.5 cm intervals with Micropore tape. With the probe in an axial-plane, a video recording of the probe moving from the medial to the lateral border of the GM was obtained. Individual images were extracted from the recording offline and used to construct the muscle by overlapping anatomical landmarks and external markers. Image J software (version 1.34; National Institutes of Health) was subsequently used to measure the cross-sectional area of the reconstructed GM image.

4.3.9 Elastic modulus

The GM elastic modulus, E_m, was calculated as the *in vivo* muscle stress-strain relation from 0 Nm to 14 Nm and end ROM:

$$E_m = \frac{\Delta \sigma_m}{\Delta \varepsilon_m} = \frac{(\sigma_{max} - \sigma_{\min})}{(\varepsilon_{max} - \varepsilon_{\min})}$$

Where ε_{max} and $\varepsilon_{\min}$ represent the maximum and minimum calculated strain (GM MTJ displacement/muscle length), σ_{max} and $\sigma_{\min}$ represent the maximum and minimum calculated stresses (torque/GM CSA) placed upon the GM during the *in vivo* passive stretch trial. The *in vivo* change in the muscle stress and strain (as presented in Figure 4.3) is represented by $\Delta \sigma_m$ and $\Delta \varepsilon_m$, respectively.

4.3.10 Statistics

All statistical analyses were performed using SPSS software (Version 18, SPSS Inc., Chicago Illinois). Regressions were conducted to assess whether muscle CSA and/or length were covariates of passive muscle displacement. As a result of the regression analysis, a repeated measures ANOVA was used to distinguish differences between group (3 levels; paretic, non-paretic and individuals without neurological impairment) and within-subject differences in passive muscle

displacement at each passive torque increment (7 levels; 2, 4, 6, 8 10, 12 and 14 Nm). If a significant interaction was found, independent t-tests were used to determine group differences. As assumptions of sphericity were violated, Greenhouse-Geisser correction was used to identify differences in the data.

A MANOVA was used to determine the differences between the paretic, non-paretic and individuals without neurological impairment in muscle length, CSA, ROM, MVIC and muscle activity. Independent t-tests were used to assess baseline anthropometric data between SCP and individuals without neurological impairment. All subsequent data are reported as the mean (standard deviation (SD)) and exact alpha levels are reported as published by the American Physiological Society guidelines (Curran-Everett and Benos 2004) unless $P < 0.0005$.

4.4 Results

Participant age ($P = 0.575$), stature ($P = 0.604$) and body mass ($P = 0.061$) were not different between individuals with SCP and those without neurological impairment. Table 4.1 shows that whilst the ankle was secured at 0 deg, the GM muscle and tendon lengths were not different between the paretic limb, the non-paretic limb and individuals without neurological impairment ($P = 0.222$ and $P = 0.698$, respectively). At 50% of GM muscle length, CSA of the paretic muscle was 25% and 35% smaller than the non-paretic muscle ($P = 0.029$) and the muscle of individuals without neurological impairment ($P = 0.001$), respectively. However no

difference was identified between the non-paretic muscle and that of individuals without neurological impairment (P = 0.601, Table 4.1). DF MVIC torque was not different between the paretic limb, the non-paretic limb and individuals without neurological impairment (P = 0.653, Table 4.1). PF MVIC torque in the paretic limb was less than that of the non-paretic limb (P = 0.037) and individuals without neurological impairment (P = 0.001, Table 4.1), respectively.

Table 4.1. Group comparisons of PF and DF MVIC, GM muscle and tendon lengths, and GM CSA.

	Paretic Limb	Non-paretic Limb	Control
PF MVIC (Nm)	100 (57.2)*†	152 (49.9)	187 (26.9)
DF MVIC (Nm)	17.5 (8.58)	21.3 (11.9)	20.8 (10.6)
Resting GM length (cm)	24.5 (3.75)	26.8 (3.23)	25.7 (2.00)
Resting tendon length (cm)	23.5 (2.67)	22.9 (2.29)	23.8 (2.50)
Resting GM CSA (cm^2)	12.0 (2.62)*†	15.0 (2.23)	16.2 (2.90)

Control refers to individuals without neurological impairment. *Significant difference from non-paretic group (P < 0.05). †Significant difference from control group (P ≤ 0.001). Data are expressed as mean (SD)

4.4.1 Muscle properties 0-14 Nm

Ankle angle at 14 Nm did not show a difference between any of the groups (P = 0.602, Table 4.2) The passive displacement of the GM MTJ exhibited a curvilinear

relationship with torque, but no interaction was identified between torque and group in terms of displacement between 0-14 Nm (P = 0.863, Figure 4.2). This was confirmed as GM muscle stiffness was not different between any of the groups (P = 0.169, Table 4.2). When passive displacement of the MTJ was made relative to GM muscle length, there was no group-by-torque interaction throughout the passive stretch (P = 0.965, Table 4.2). However, when passive torque was made relative to the GM CSA, a torque-by-group interaction was detected (P < 0.0005, Table 4.2). Pairwise comparisons showed no difference between the non-paretic limb and individuals without neurological impairment (P = 1.000). The passive torque relative to the paretic GM CSA was greater than the non-paretic limb (P < 0.0005) and the GM of individuals without neurological impairment (P < 0.0005) at each 2 Nm torque increment from 2-14 Nm. The elastic modulus of the paretic muscle was over 2-fold greater than the non-paretic muscle (P = 0.002) and muscle of individuals without neurological impairment (P = 0.002, Figure 4.3). However, there was no difference between non-paretic limb and individuals without neurological impairment (P = 1.000, Figure 4.3).
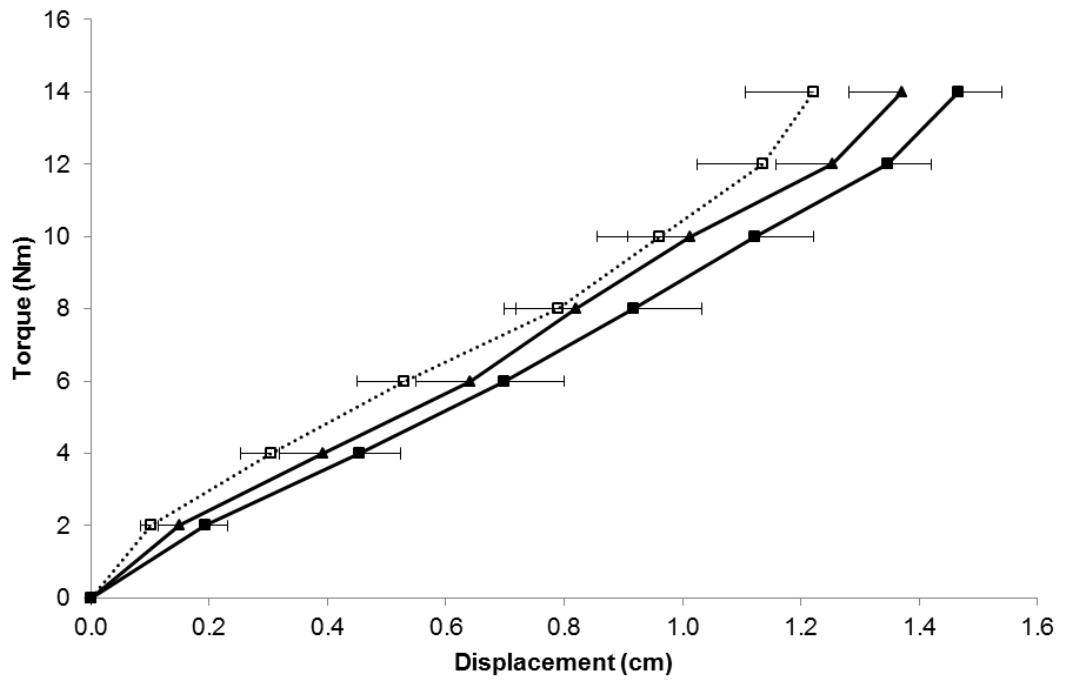
Figure 4.2. Passive torque displacement relationship in paretic (open squares) and non-paretic (closed squares) limbs and in the control group (closed triangles). Data are expressed as mean (SD).

Table 4.2. Passive properties of the GM during a passive stretch from 0-14 Nm.

	Paretic Limb	Non-paretic Limb	Control
GM muscle stiffness (Nm·cm^{-1})	13.5 (7.09)	9.98 (2.48)	10.7 (2.12)
GM MTJ displacement (cm)	1.22 (0.38)	1.47 (0.25)	1.37 (0.30)
Ankle angle (deg)	-1.84 (4.27)	-3.40 (4.02)	-1.84 (4.21)
GM MTJ displacement/L_m (%)	5.19 (1.97)	5.57 (1.23)	5.38 (1.37)
Torque/GM CSA (Nm·cm^{-1})	1.66 (0.27)[*†]	0.96 (0.14)	0.88 (0.16)
Elastic modulus (Nm·cm^{-2})	0.39 (0.21)[‡§]	0.18 (0.05)	0.17 (0.06)

Control refers to individuals without neurological impairment and L_m = muscle length. [*]Significant difference from non-paretic group ($P < 0.001$). [†]Significant difference from control group ($P < 0.001$). [‡]Significant difference from non-paretic group ($P < 0.005$). [§]Significant difference from control group ($P < 0.005$). Data are expressed as mean (SD).

4.4.2 Muscle properties at end ROM

No group differences in ankle angle at end ROM ($P = 0.527$) and passive torque ($P = 0.874$, Table 4.3). Passive GM displacement and stiffness were not different between groups ($P = 0.385$ and $P = 0.462$, respectively, Table 4.3). No difference was identified for *in vivo* strain between paretic limb, non-paretic limb and individuals without neurological impairment ($P = 0.973$), though *in vivo* stress was different between the groups ($P = 0.006$, Table 4.3). Subsequently, no differences in stress were identified between the non-paretic limb and individuals without neurological impairment ($P = 1.000$). However, the stress to which the paretic

muscle was subjected was 46% larger than the non-paretic muscle (P = 0.015) and muscle of individuals without neurological impairment (P = 0.015), respectively. The elastic modulus of the paretic muscle was 47% and 48% greater than the non-paretic muscle (P = 0.006) and that of individuals without neurological impairment (P = 0.003), respectively. No difference in elastic modulus was identified between paretic muscle and individuals without neurological impairment (P = 1.000).

Throughout the passive stretch, no EMG recording from GM or the TA exceeded 3% maximal activity across all groups. The average activity calculated at 25, 50, 75 and 100% of the passive stretch were below 2% with no between-group effect identified (GM activity; P = 0.549 and TA; P = 0.746), indicating that the stretch trials were passive and not influenced by reflex contractions (Table 4.3).
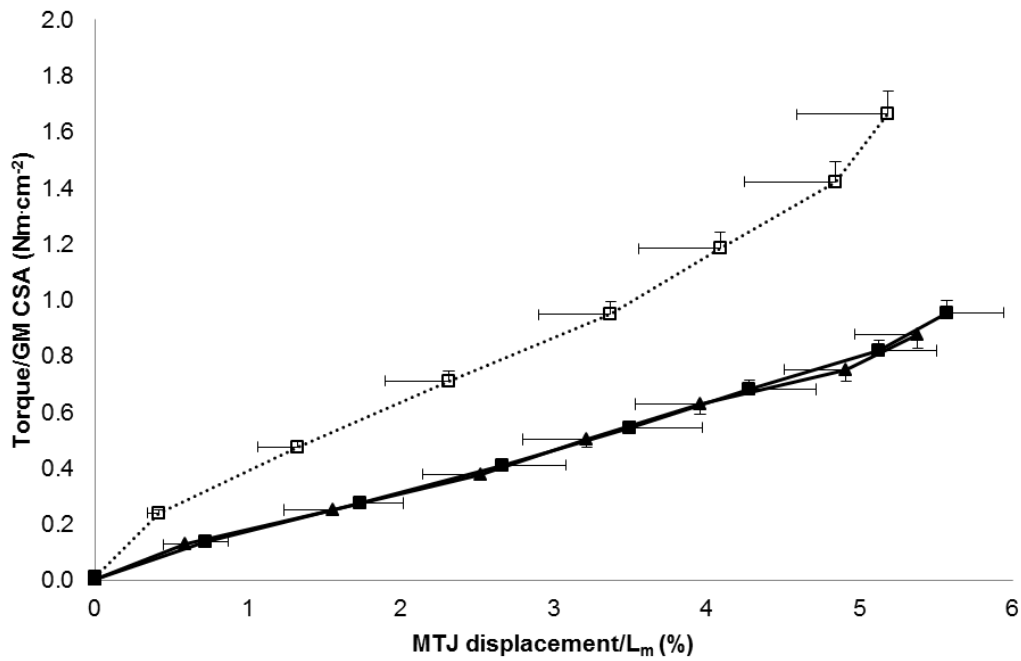
Figure 4.3. GM MTJ displacement relative to resting muscle length (strain) at 0 deg and passive torque relative to GM CSA (stress) in paretic (open squares) and non-paretic (closed squares) limbs, and in the control group (closed triangles). Data are expressed as mean (SD).

Table 4.3. Passive properties of the GM at end ROM and average muscular activity of the agonist and antagonist throughout the passive stretch.

	Paretic Limb	Non-paretic Limb	Control
GM muscle stiffness (Nm·cm^{-1})	20.2 (8.70)	16.9 (5.67)	18.8 (5.50)
GM MTJ displacement (cm)	1.73 (0.50)	1.96 (0.33)	1.94 (0.56)
Ankle angle (deg)	-8.34 (5.70)	-11.9 (4.49)	-11.6 (7.59)
Passive torque (Nm)	35.6 (16.6)	33.5 (12.5)	37.0 (18.4)
Average GM EMG (%)	0.48 (1.18)	0.09 (1.54)	0.62 (0.53)
Average TA EMG (%)	0.69 (2.53)	1.16 (1.92)	0.59 (0.56)
GM MTJ displacement/L_m (%)	7.41 (2.98)	7.42 (1.62)	7.63 (2.50)
Torque/GM CSA (Nm·cm^{-1})	4.26 (2.28)*[†]	2.29 (0.94)	2.28 (0.95)
Elastic modulus (Nm·cm^{-2})	0.58 (0.21)[‡§]	0.31 (0.05)	0.30 (0.06)

Control refers to individuals without neurological impairment and L_m = muscle length. *Significant difference from non-paretic group ($P < 0.05$). [†]Significant difference from control group ($P < 0.05$). [‡]Significant difference from non-paretic group ($P < 0.01$). [§]Significant difference from control group ($P < 0.005$). Data are expressed as mean (SD)

4.5 Discussion

The main findings of the present study show that ankle ROM, passive stiffness and MTJ displacement values of the paretic GM were not different from the contralateral non-paretic limb or the limb of individuals without neurological impairment. When these passive properties were expressed relative to structural

measurements of the GM muscle (i.e. GM CSA and muscle length), stress was found to be significantly higher at 14 Nm and end ROM. Additionally, the elastic properties of the paretic GM muscle, as measured by elastic modulus, were approximately double that of the non-paretic and control GM muscles at 14 Nm and end ROM. We also observed that there was minimal EMG activity throughout all passive trials (consistent with others at these velocities (Gajdosik et al. 2005; Morse 2011; Morse et al. 2008a)), therefore the data obtained from the GM is primarily due to the elastic properties of the muscle rather than additional neural influences.

The limited ROM around a joint or group of joints is a defining clinical characteristic of CP (Alhusaini et al. 2010; Vaz et al. 2006) and ROM is a common indicator of flexibility in athletes (Dadebo et al. 2004). In contrast to previous reports (Alhusaini et al. 2010; Barber et al. 2011a; Vaz et al. 2006), no differences were observed between the paretic limb and individuals without neurological impairment in the present study. Furthermore, no difference in ROM between the paretic and non-paretic ankle of physically active adults with SCP was observed. In the paretic limb and that of individuals without neurological impairment, it is likely that this is due to the active nature of the participants. However, a single measure of ROM does not provide information on the passive properties of the MTU due to subjective influences such as stretch tolerance (Magnusson et al. 1996a; Toft et al. 1989). Therefore, in order to understand how the elastic and contractile components behave individually, ultrasonography should be used throughout a passive stretch protocol to assess the angle/torque-displacement

relation as conducted previously in individuals without neurological impairment (Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a).

By assessing the torque-displacement relation it is possible to monitor not only the displacement of the GM MTJ or passive torque throughout a passive stretch, but also calculate passive muscle stiffness (Kay and Blazevich 2009; Morse 2011; Morse et al. 2008a). Muscular stiffness provides information on the elastic properties of the muscle *in vivo* and could be used as a monitoring tool by coaches and clinicians throughout training cycles to assess how the muscle responds to various interventions. Contrary to the hypothesis, no difference in GM MTJ displacement, passive torque or GM stiffness was identified between the paretic, non-paretic and the muscle of individuals without neurological impairment at 14 Nm and end ROM; indicating similar passive muscle properties when compared to the individuals without neurological impairment in the present study. It should be noted, however that GM stiffness at end ROM may be highly variable measure as reported in Chapter 3. Nevertheless, previous data on the torque-angle relation in children with CP reported by Vaz et al. (2006) and Alhusaini et al. (2010) found that MTU stiffness was significantly higher than aged matched controls without neurological impairment. Although MTU stiffness was not directly examined in the present study, no difference was found between torque or ankle ROM at 14 Nm and end ROM in the paretic and non-paretic limbs, and individuals without neurological impairment. As muscle activity was not reported in the aforementioned studies, the subsequent contrast in findings between the present study may be due to the increased passive velocity at which the participants were

passively stretched, which in turn may heighten neuromuscular stretch reflex responses in individuals with spasticity (Sheean 2002).

Whilst using ultrasonography and contrary to the present findings, Barber et al. (2011a) found that MTU stiffness was higher in a group of young adults with SCP as a result of an inability of the fascicles to elongate throughout a passive stretch trial when compared to age-matched controls without neurological impairment. It is likely, however, that the heterogeneous nature of the age and gender of the participants reported by Barber et al. (2011a) can account for some of the difference in the observed results from the present study. For example, it is known that gender influences the passive properties of the MTU, with stiffer MTU in males compared to females (Morse 2011). Furthermore maturation has been shown to influence the elastic properties of the MTU (O'Brien et al. 2010b). Additionally, these differences between the present study and previous findings in passive muscle extension and MTU properties may be due to the regular engagement in physical activity and flexibility training completed by the present cohort of adults with SCP as part of their training regime. It is well documented that various stretch interventions facilitate improvements in joint end ROM and passive muscle/MTU stiffness in individuals without neurological impairment (Bressel and McNair 2002; Brouwer et al. 2000; Kay and Blazevich 2009; Morse et al. 2008a). Physically active adults with SCP regularly partake in such stretch activities as an integral part of their training regime. Indeed stretching in individuals with SCP not only make the passive properties of the muscle similar to individuals without neurological impairment, but increasing passive MTU compliance has

been associated with improved gait in individuals with spasticity (Dietz et al. 1981). As no difference in ROM was observed between the paretic limb, non-paretic limb and that of individuals without neurological impairment in the present study, the training interventions that they regularly complete may have a facilitative effect on the passive muscle properties.

Although muscle stiffness, in absolute terms was not found to be different between the groups, when the dimensions of the GM were used to calculate *in vivo* stress and strain; the elastic modulus of the paretic muscle demonstrated stiffer passive properties than the non-paretic muscle and muscle of individuals without neurological impairment at 14 Nm and end ROM. As no difference in strain was identified, the observed increase in elastic modulus must be due to the increased stress the GM is subjected to during the passive stretch. Moreover, this increased *in vivo* stress in the paretic muscle results from the smaller GM CSA, as there were no differences between passive torque at end ROM. As the smaller CSA of the paretic muscle is required to dissipate the same amount of torque as that of the non-paretic muscle and that of individuals without neurological impairment; this could perhaps increase the risk of injury to the MTU, though no published information is currently available which assess the prevalence of injury in individuals with SCP.

It is evident from the present findings that the dimensions of the GM have an effect on the elastic modulus properties. Magnusson et al. (1997) found that larger CSAs

correlated with increases in passive muscle stiffness in individuals without neurological impairment. However, the present findings show that when calculated relative to CSA, paretic muscle is significantly less elastic than the non-paretic muscle and that of individuals without neurological impairment. The fact that a difference in elastic properties remains when CSA is accounted for, may reside at the single fibre level. Based on single fibre data, it is possible that shorter and stiffer sarcomeres (Friden and Lieber 2003), and a larger proportion of extracellular matrix tissue (Lieber et al. 2003), contributed to the increased elastic modulus observed in the current SCP population. However, further research is required to identify whether the previously reported differences in single muscle fibre stiffness and extracellular matrix tissue influence the elastic properties specifically in the paretic GM of physically active individuals with SCP.

In order to assess the passive properties of the muscle, neural (McNair and Stanley 1996; O'Dwyer et al. 1996) and mechanical factors (Friden and Lieber 2003; Morse 2011; Morse et al. 2008a) have been suggested to affect the contractile component under passive stretch conditions. As the activity of the GM and TA muscles were 2 and 3%, respectively in the present study, it is unlikely that neural activation of the agonist or coactivation of the antagonist muscles confounded the passive properties of the muscle whilst the GM was passively stretched at 1 deg$\cdot$s^{-1}. Gajdosik et al. (2005) defined that if the muscle activity levels during the passive stretch did not exceed 5%, the neural responses of the stretch were deemed negligible, in which the data presented in the present study conforms to. As a result of neuromuscular activity being minimal, it can be

assumed that the stretch was passive and not confounded by spasticity or stretch reflexes. Thus, one may argue that the results in the present study are due to mechanical factors intrinsic to the GM.

4.6 Conclusion

Individuals with SCP are commonly defined by their limitations in joint ROM, whereas in the present physically active population no such impairment in ROM was observed, providing additional *in vivo* data to the clinical definition of the SCP. However, when the smaller CSA of individuals with SCP was accounted for, the passive stress of the muscle was significantly higher than the contralateral non-paretic muscle and the muscle of individuals without neurological impairment. In this particular physically active SCP population it is possible that their regular training may have facilitated these observed findings.

Chapter 5

Muscle size, activation and coactivation in adults with spastic cerebral palsy

5.1 Abstract

Introduction. Muscle weakness is present in the paretic limbs of individuals with spastic cerebral palsy (SCP). This study aims to determine what neuromuscular factors contribute to weakness in adults with SCP during maximal voluntary isometric contractions (MVIC).

Methods. Gastrocnemius anatomical cross-sectional area (ACSA), agonist and antagonist activation were measured in 11 SCP and 11 control adult males during plantarflexion MVIC.

Results. Plantarflexion MVIC torque of the paretic leg was 42% and 52% less than the non-paretic and control limbs, respectively. The paretic gastrocnemius ACSA was smaller than the control group only. Paretic agonist activation was less than the non-paretic and control groups, whereas antagonist coactivation was higher. Multiple regression analysis revealed muscle activation accounted for 57% of variation in paretic plantarflexion MVIC torque.

Conclusion. This demonstrates that in individuals with SCP, muscle weakness in the paretic limb is primarily attributed to impaired neural activation, and to a lesser degree ACSA.

5.2 Introduction

Individuals with spastic cerebral palsy (SCP) often suffer with a number of impairments including impaired and muscle weakness, particularly in the paretic muscles (Chapter 4). The maximum voluntary isometric contraction (MVIC) torque generated in this population has regularly been reported to be less in the paretic muscle when compared to controls without neurological impairment (Elder et al. 2003; Stackhouse et al. 2005) or the non-paretic leg of hemiplegic children (Elder et al. 2003). Muscle weakness in individuals with SCP occurs primarily as a result of a non-progressive lesion to the brain, impairing cortical input into the descending neural signalling pathways to the muscle, resulting in secondary adaptations in the muscle (Ito et al. 1996; Koman et al. 2004; Lieber et al. 2003; Malaiya et al. 2007; McNee et al. 2009; Mohagheghi et al. 2007). These secondary adaptations which could contribute to weakness in SCP include smaller muscle size, reduced agonist muscle activation and/or changes in patterns of coactivation (Chapter 2).

The size of the muscle in the paretic limb of children with SCP has consistently been shown to be smaller when compared to the muscles of the non-paretic limb (Mohagheghi et al. 2007) and that of individuals without neurological impairment (Malaiya et al. 2007). Smaller muscle sizes have previously been correlated with decreased strength in elderly populations without neurological impairment and children with SCP (Elder et al. 2003; Morse et al. 2004), however no such relationship has been identified specifically in adults with SCP. Increases in muscle strength with resistance training have been attributed to a corresponding

increase in size in the paretic muscle (McNee et al. 2009), with subsequent improvements being identified in functional performance in children (Blundell et al. 2003; Damiano and Abel 1998; Dodd et al. 2003) and adults with SCP (Andersson et al. 2003). Although understanding the force generation capabilities of children with SCP is fundamental to addressing clinical manifestations such as muscle weakness, its applicability to adult cohorts may be considered limited. Previous findings in individuals without neurological impairment have shown that adult males have a larger muscle size, accounting for 75% of MVC torque, when compared to prepubescent boys (O'Brien et al. 2010a). Additionally, the ability of prepubescent children with SCP to exert maximal force reliably has been questioned (Sanger et al. 2003; Verschuren et al. 2008; Ayalon et al. 2000; Allen et al. 1995; Taylor et al. 2004). This may be due to their level of maturation, lack of understanding of the requested instruction and/or inability to recruit the required motor units to exert a MVC (Stackhouse et al. 2005). Nevertheless, a recent study assessing the torque-length relationship of the plantarflexion (PF) muscles in an older SCP population ranging from 15-21 years reported lower torque values across the available range of motion in the paretic limb when compared to individuals without neurological impairment (Barber et al. 2012), These findings are consistent with those studies completed in children populations (Elder et al. 2003; Stackhouse et al. 2005), though the reasons for these differences have yet to be assessed in adult SCP populations.

In addition to muscle size being a determinant of strength, the neural factors including activation and coactivation may also have an effect. During maturation of

children with SCP there are no reported alterations to the brain lesion (Koman et al. 2004). However, clinical manifestations, such as motor function that was assessed by the Gross Motor Function Classification System, are reported to change throughout maturation, depending on the individuals gross motor function classification (Hanna et al. 2009). This may suggest that adaptations in the muscle-tendon unit may occur or even change in response to the altered neural signalling pathways. In order to assess whether individuals are able to fully activate all of their motor units *in vivo*, an electrical stimulation is superimposed onto the muscle during an MVC (Bampouras et al. 2006; Stackhouse et al. 2000). Such studies have previously been performed in children with SCP, which identified that there was approximately 50% less activation in the paretic gastrocnemius when compared to individuals without neurological impairment (Elder et al. 2003; Stackhouse et al. 2005), but no difference was identified when compared to the non-paretic gastrocnemius (Elder et al. 2003). Another factor that has been associated with muscle weakness is the increased coactivation of the paretic antagonist muscles (Ikeda et al. 1998), which has regularly been demonstrated to contribute to muscle weakness of the gastrocnemius in individuals with SCP (Elder et al. 2003; Stackhouse et al. 2005). As children with CP are suggested to typically live a sedentary lifestyle (Longmuir and Bar-Or 2000), the data presented in these previous studies may not apply to physically active adults with SCP who as a consequence may have lower levels of antagonist coactivation.

In children with SCP, it is evident that neural and muscular factors contribute towards the force generation limitations of the paretic muscle. Although chapter 4 highlighted that the paretic maximal gastrocnemius torque was lower than the non-paretic limb and control individuals without neurological impairment, no such information currently addresses whether similar neuromuscular limitations exist in adults with SCP, particularly where issues of sedentary lifestyle are controlled. Therefore, the aim of present study was to identify the magnitude of weakness and to assess whether muscle size, agonist activation and/or antagonist activation are responsible for these deficits in physically active adults with SCP. We hypothesised, that, even with elevated habitual physical activity, the size of the paretic muscle would be lower in relation to individuals without neurological impairment. Additionally it was also hypothesised lower agonist activation and higher antagonist coactivation during PF MVIC in the paretic limb of adults with SCP.

5.3 Methods

5.3.1 Subjects

Eleven ambulant males with hemiplegic SCP (age = 21.2 (3.0) years, stature = 1.79 (0.10) m, mass = 70.0 (12.5) kg) and 11 males without neurological impairment (age = 21.8 (2.2) years, stature = 1.81 (0.04) cm, mass = 79.0 (8.4) kg) volunteered to participate in the study after written informed consent was obtained. All participants with SCP had been classified by physical therapists from the Cerebral Palsy International Sports and Recreation Association (CPISRA). All

participants, SCP and individuals without neurological impairment self-reported as undertaking 3-5 hours of sport-based exercise per week and did not partake in any structured resistance training regime. The entire cohort involved in the study was free from lower limb injury and had not received any form of medication to reduce the effects of spasticity within the last year. The study conformed to the standards set by the latest revision of the Declaration of Helsinki and the local ethics committee at Manchester Metropolitan University. Both the paretic and non-paretic legs were tested in each participant with SCP, whereas the dominant limb was assessed in the control participants.

5.3.2 Protocol

Participants attended the laboratory on two occasions. During the first visit, familiarisation was carried out which included a series of six PF MVIC followed by a series of submaximal percutaneous electrical stimulations. During the second visit, participants were assessed for resting measures of muscle size, which were then followed by the MVIC testing procedures.

5.3.3 Strength measurements

Participants were secured to an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA) in a seated position with the back angle reclined at 65 deg (Chapter 3 and 4), prior to the commencement of MVC trials. The participant's knee was secured in full extension with Velcro straps, which were positioned

proximal to the knee. The medial malleolus was visually aligned with the dynamometer's central axis of rotation and two Velcro straps were used to secure the foot to the footplate in order to minimise heel displacement. The participant's hips were also secured to the seat to limit extraneous movement during PF MVIC trials. All participants warmed up by performing three submaximal isometric contractions at 0 deg (the individual's anatomical zero), each of which were separated by a 1 min rest period. In this instance, 0 deg was defined as the foot at a 90 deg to the tibia. Two out of the 11 participants with SCP could not achieve the described 0 deg in the paretic leg as their heel did not rest on the ground during anatomical stance. In these two cases, 0 deg was calculated by using a goniometer to quantify how much their resting ankle angle differed and an appropriate correction was measured on the isokinetic dynamometer. Ankle angle was corrected by 10 and 7 deg in the two participants. According to Narici et al. (1996), fascicle length would have been underestimated by 4 and 3 mm, respectively, in each of these individuals based on changes in GM fascicle length relative to ankle angle in individuals without neurological impairment. Although it is not clear whether the length changes are different between individuals with SCP equinus gait and those without, it is impossible to add a correction factor to two individuals. Furthermore, the error in the estimation in fascicle length change between the two angles is so small that it is not likely to make a difference to the mean data obtained. After the warm up, the participant's ankle remained fixed at 0 deg where two PF MVICs were obtained, separated by a 1 min rest period. Dorsiflexion (DF) MVIC was completed using the same testing posture and protocol to assess PF MVIC in order to calculate coactivation of the tibialis anterior (TA).

5.3.4 Voluntary muscle activation

To quantify the participants' ability to voluntary activate the PF muscles during MVIC, percutaneous stimuli (DSVDigitimer Stimulator; Digitimer, Herts., UK) were applied to the gastrocnemius using rubber stimulation pads (size ranging from 38 mm x 89 mm to 76 mm x 127 mm; Versastim; Conmed, NY, USA). The two stimulation pads were placed transversely distal to the popliteal crease and proximal to the myotendinous junction of the soleus. The amplitude of the stimuli was determined prior to interpolation whilst the participant was in a relaxed state; administering twitches starting from 50 mA and increasing in increments of 50-100 mA, until no further increase in twitch torque was elicited. Two supramaximal stimuli were applied to assess the activation capacity of the gastrocnemius. The first twitch was applied once a plateau in maximal torque had been reached and the second twitch was initiated approximately 2 s after the first twitch, once resting torque values (0 Nm) were attained. All participants were instructed to relax immediately after the first twitch. Throughout all MVC trials, participants were verbally encouraged to exert as much force as possible. The voluntary activation level of each participant was assessed using the trial that produced the highest contractile torque and was calculated using the following equation: percentage activation = [1 – (superimposed stimulus torque / post MVC stimulus torque)] x 100 (Allen et al. 1995; Bampouras et al. 2006; Harridge et al. 1999; Morse et al. 2004).

It is well reported that an increased number of twitches superimposed decreases the variability of the data obtained when assessing muscle activation in healthy individuals (Bampouras et al. 2006; Suter and Herzog 2001), though this finding is not always consistent (Behm et al. 1996). Nevertheless, during the familiarisation sessions, the participants with SCP were blind tested on their reaction to superimposed twitches and doublets and the majority of the participants commented that the doublet stimuli were 'unbearable' compared to the twitch procedure. Resultantly, in order to obtain agonist activation data from the participants with SCP twitches stimuli were used.

5.3.5 Coactivation

During MVIC trials, TA electromyography (EMG) activity was recorded using two pre-gelled, unipolar, 10-mm, Ag-AgCl percutaneous electrodes (Medicotest, Denmark). Boundaries of the TA were determined using ultrasonography to ensure the accurate placement of each electrode along the mid sagittal axis of the muscle and to reduce cross-talk. Two electrodes were placed at two thirds of the TA length and one reference electrode was placed over the lateral epicondyle of the femur. The area where the electrodes were positioned was shaved and cleaned with an alcohol swab to remove residual skin cells and oils, as well as reduce skin impedance. Raw EMG data were recorded at 2000 Hz, with band pass filter set at 10-500 Hz, and a notch at 50 Hz. The integral of the root mean square of the raw signal 0.5 s either side of the MVC PF torque was used to quantify the level of muscle coactivation. Coactivation was calculated as:

percentage coactivation = (TA activity during PF MVC / TA activity during DF MVC) x 100 (Klein et al. 2001; Morse et al. 2004).

5.3.6 Anatomical cross sectional area

Several axial-plane images of the gastrocnemius medialis and lateralis were obtained using B-mode ultrasonography to estimate gastrocnemius anatomical cross-sectional area (ACSA; Reeves et al. 2004a), across the greatest width of the muscle. The gastrocnemius medialis and lateralis proximal insertion and the myotendinous junction were marked. An axial line was then marked from the medial to lateral border of the gastrocnemius, perpendicular to the insertion and the myotendinous junction, which marked the path where the axial images would be obtained. Strips of Micropore tape were placed axially across the line of the gastrocnemius at approximately 3.5 cm intervals. These strips of tape were used as echo-absorptive markers that project a shadow onto the ultrasound image to provide a positional reference into the scanned structures. With the probe in an axial-plane, a recording of the probe moving from the lateral border of the gastrocnemius lateralis to the gastrocnemius medialis medial border was obtained. Individual images were extracted from the recording offline and used to construct the muscle by overlapping anatomical landmarks and external markers. Image J software (version 1.34; National Institutes of Health) was used to measure the CSA of the constructed gastrocnemius medialis and lateralis to determine gastrocnemius ACSA.

5.3.7 Statistics

All statistical analyses were performed using SPSS software (Version 18, SPSS Inc., Chicago Illinois). The Shapiro-Wilk and Levene's tests were utilised to assess the normality and variance of the data. As there were no breaches of these statistical assumptions, and to avoid a type I error (as could occur with repeated ANOVA tests), a MANOVA was used to compare the differences in: PF MVIC torque, agonist activation, gastrocnemius ACSA, antagonist coactivation, PF MVIC torque relative to body mass and PF MVIC torque relative to gastrocnemius ACSA variables of the paretic vs. non-paretic limbs vs. the dominant limb of individuals without neurological impairment (the latter which will be referred to as the control group herein). Pearson correlations were used to examine the level of association between all of the assessed variables, with corrections for multiple associations. A stepwise multiple regression was used to predict paretic limb PF MVIC torque from gastrocnemius ACSA, agonist activation and antagonist coactivation. In addition, a second multiple linear regression to predict PF MVIC torque, where data across all groups were pooled, was used to determine whether an equation that incorporates gastrocnemius ACSA, experimental group, agonist activation and antagonist coactivation, would realise a stable regression model. In addition, independent t-tests were used to assess baseline anthropometric data between SCP and control groups. Statistical significance was accepted at the $P < 0.05$ level, moreover, exact P-values are reported unless $P < 0.001$. Effect size is reported as partial eta squared ($p\varepsilon^2$) and data are presented as the mean (SD). It has been suggested that $p\varepsilon^2$ effect sizes for an ANOVA range from; small effects < 0.1, medium effects ranging from 0.1-0.25; and large effects > 0.4 (Portney and Watkins 1997).

5.4 Results

Adults with SCP and matched controls without neurological impairment were of similar age (P = 0.575), stature (P = 0.604) and body mass (P = 0.061).

5.4.1 MVC torque and gastrocnemius ACSA

The PF MVIC differed between groups (P < 0.001, $p\varepsilon^2$ = 0.461). Post-hoc pairwise comparisons (Figure 5.1) showed that the paretic limb PF MVIC torque was 52% less than the controls (P < 0.001) and 42% less than the non-paretic limb (P = 0.007). No difference in PF MVIC torque was identified between the control group and non-paretic limb (P = 0.334, Figure 5.1). Furthermore, no differences were identified in DF MVIC torque between groups (P = 0.653, $p\varepsilon^2$ = 0.028, Figure 5.1).

Gastrocnemius ACSA differed between groups (P = 0.004, $p\varepsilon^2$ = 0.306). Post-hoc pairwise comparisons showed that the paretic muscle was 20% smaller than that of the control group (P = 0.004, Table 5.1). There were no differences between the paretic and non-paretic muscle (P = 0.054, Table 5.1), or between the control group and non-paretic muscle (P = 0.925).

When MVIC PF torque was considered relative to the ACSA of the gastrocnemius muscle, differences existed between groups (P = 0.001, $p\varepsilon^2$ = 0.376). Post-hoc

pairwise comparisons (Table 5.1) revealed that PF MVC torque relative to ACSA in the paretic limb was 44% less than the control group (P = 0.001) and 34% lower than the non-paretic limb (P = 0.027). There was no difference between the control group and non-paretic limb (P = 1.000). Similarly, when PF MVIC torque was considered relative to body mass, there were again between-group differences (P = 0.001, $p\varepsilon^2$ = 0.396). Post-hoc comparisons (Table 5.1) showed that PF MVIC torque relative to body mass in the paretic limb was 46% and 42% less than the control group (P = 0.001) and non-paretic limb (P = 0.005), respectively. There was no difference between the control group and non-paretic limb (P = 1.000).
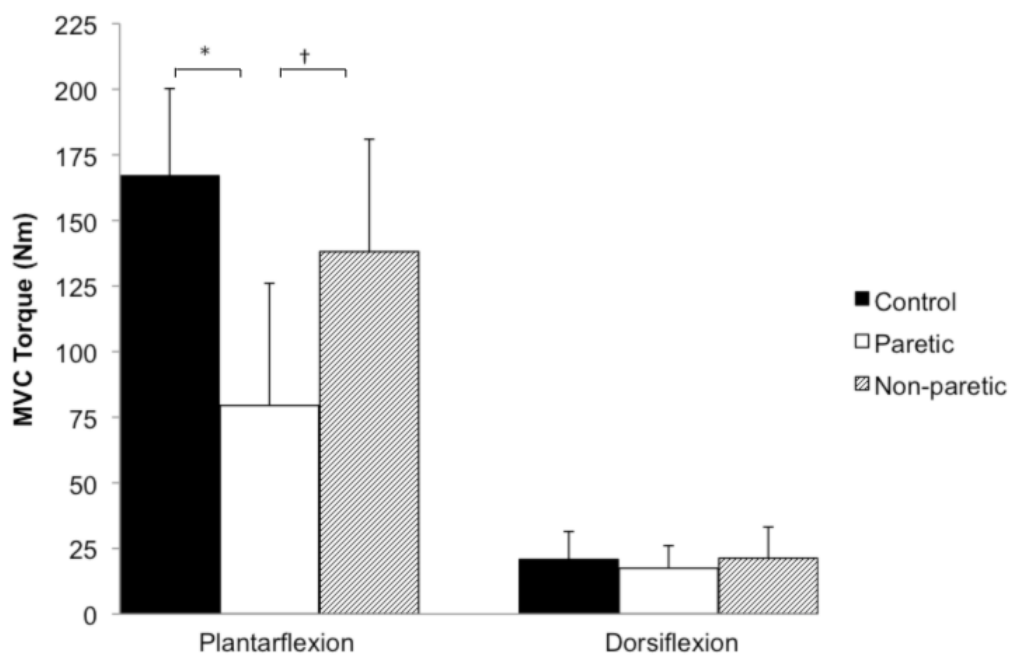
Figure 5.1. Gastrocnemius PF and TA DF MVIC torque. *Difference between paretic and control groups (P < 0.001). [†]Difference between paretic and non-paretic groups (P = 0.007).

Table 5.1. ACSA of the gastrocnemius, PF MVIC torque relative to gastrocnemius ACSA and PF MVIC torque relative to body mass in the paretic, non-paretic and control groups.

Group	Control	Paretic	Non-paretic
Gastrocnemius ACSA (cm^2)	25.9 (3.99)	20.7 (3.48)*	24.3 (2.75)
MVC torque/Gastrocnemius ACSA (Nm·cm^{-2})	6.65 (1.56)	3.71 (1.85)†‡	5.62 (1.37)
MVC torque/Body mass (Nm·kg^{-1})	2.13 (0.45)	1.14 (0.66)†§	1.97 (0.54)

*Difference between paretic and control groups (P = 0.004). †Difference between paretic and control groups (P $\leq$ 0.001). ‡Difference between paretic and non-paretic groups (P = 0.027). §Difference between paretic and non-paretic groups (P = 0.005).

5.4.2 Voluntary muscle activation and coactivation

There were differences between groups in terms of voluntary activation of the gastrocnemius muscle during the PF MVIC (P < 0.001, pε^2 = 0.496). Post-hoc pairwise comparisons (Figure 5.2A) revealed that the paretic limb was 36% and 39% less activated during an MVIC than the non-paretic limb (P < 0.001) and control group (P < 0.001), respectively. There was no difference between the control group and activation properties of the muscle in the non-paretic limb (P = 1.000).

The coactivation of the TA muscle during PF MVIC also differed between groups ($P < 0.001$, $p\varepsilon^2 = 0.741$). Post-hoc pairwise comparisons (Figure 5.2B) revealed that antagonist coactivation of the paretic muscle was over 3-fold greater when compared to the non-paretic limb ($P < 0.001$) and control group ($P < 0.001$). There was no difference between the control group and non-paretic limb ($P = 1.000$).
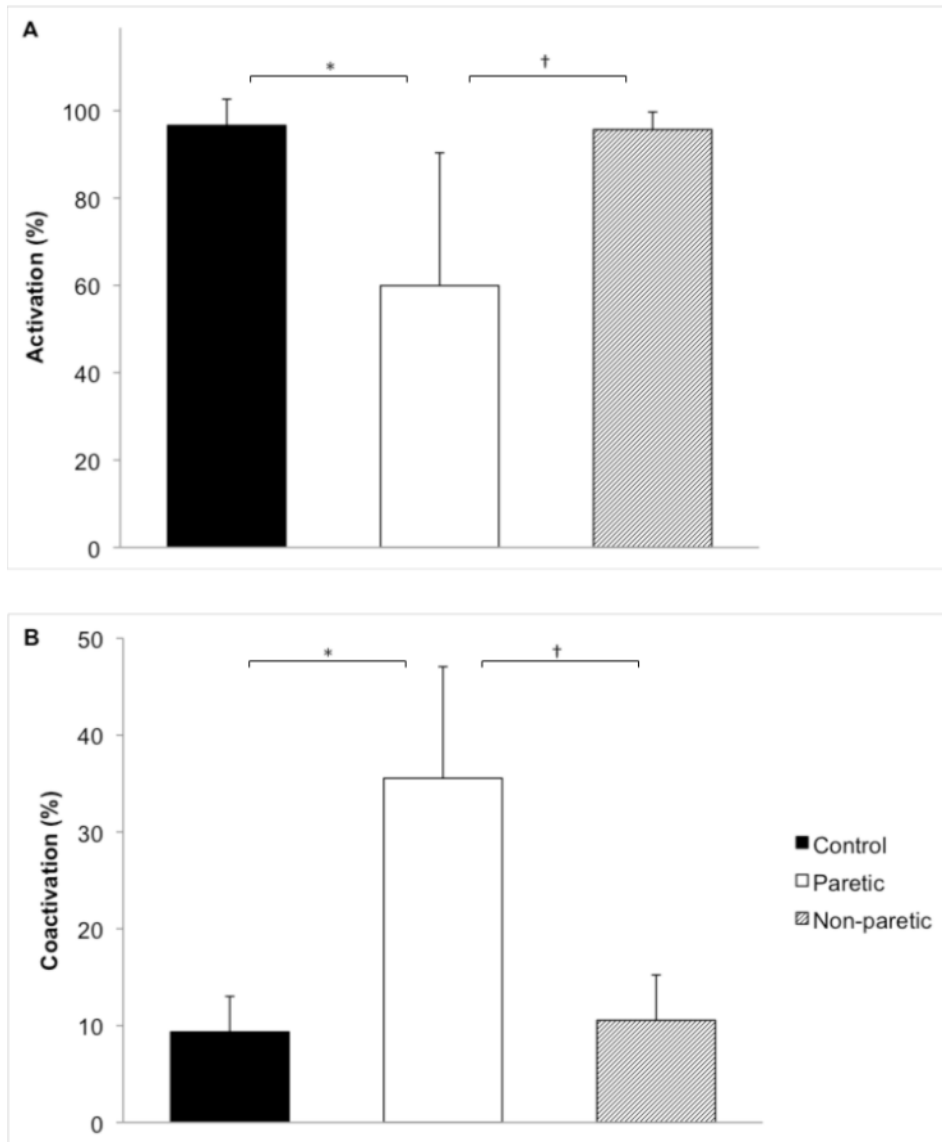
Figure 5.2. (A) Voluntary activation of the gastrocnemius during an isometric PF MVC. (B) Coactivation of the TA during an isometric PF MVC. *Difference between paretic and control groups (P < 0.001). †Difference between paretic and non-paretic groups (P < 0.001).

5.4.3 Correlations

The levels of associations between PF MVIC torque and the three candidate predictor variables (gastrocnemius ACSA, agonist activation and antagonist coactivation) in the paretic limb, non-paretic limb, control group and entire participant population are shown in Table 5.2. Gastrocnemius ACSA was positively associated with PF MVIC torque in the paretic limb (r = 0.650), non-paretic limb (r = 0.702) and entire participant population (r = 0.688). Additionally, agonist activation was positively associated with PF MVIC torque in the paretic limb (r = 0.753), non-paretic limb (r = 0.631) and entire participant population (r = 0.750). Neither gastrocnemius ACSA nor agonist activation were significantly associated with PF MVIC torque in the control group. Coactivation of the antagonist muscle was not associated with PF MVIC torque in either the paretic limb, non-paretic limb or control group. However a negative association was established between coactivation and PF MVIC torque in the entire participant population (r = -0.706).

Table 5.2. Level of association between gastrocnemius ACSA, agonist activation and antagonist coactivation in relation to PF MVIC torque.

Group	Control	Paretic	Non-paretic	Entire population
Gastrocnemius ACSA (cm^2)	0.224	0.650$^\parallel$	0.702‡	0.688*
Activation (%)	0.431	0.753†	0.631§	0.750*
Coactivation (%)	-0.167	-0.568	-0.176	-0.706*

Correlation with PF MVIC torque; *($P < 0.001$), †($P = 0.007$), ‡($P = 0.030$), §($P = 0.037$), $^\parallel$($P = 0.016$).

5.4.4 Multiple regressions

A multiple regression analysis was conducted to identify which variables are determinants of paretic PF MVIC torque. Gastrocnemius ACSA, agonist activation and antagonist coactivation in the regression model predicted torque ($P = 0.020$, $r = 0.858$, $r^2 = 0.736$), but only agonist activation was a significant predictor variable ($P = 0.043$). When the regression was repeated in a stepwise manner, agonist activation was indeed the only predictor variable identified, explaining 57% of PF MVIC torque in the paretic limb ($P = 0.007$, $r = 0.753$, as previously presented in Table 5.2).

When all PF MVIC torque data were pooled together (from paretic limb, non-paretic limb and control group), a regression model including all potential variables

was produced (P < 0.001, r = 0.848, r^2 = 0.719), with agonist activation (P = 0.005) and gastrocnemius ACSA (P = 0.012) being determinants of PF MVIC torque. However, participant group (P = 0.143) and antagonist coactivation (P = 0.433) were not determinants of PF MVIC torque. When the analysis was repeated in a stepwise manner, only agonist activation (P < 0.001) and gastrocnemius ACSA (P = 0.001) were included in the model, explaining 69% of PF MVIC torque variation in the entire participant population (P < 0.001, r = 0.831).

5.5 Discussion

This is the first study to assess the neuromuscular properties of ambulant adults with SCP. The purpose of this study was to identify whether muscle ACSA, agonist activation and antagonist coactivation are different in the paretic limb, when compared to the non-paretic limb and control group when assessing PF MVIC torque. The main findings are that differences in PF MVIC torque, agonist activation and antagonist coactivation exist between the paretic limb when compared to the non-paretic limb and control group. The paretic gastrocnemius ACSA was smaller than the control group muscle, though no difference in size was observed between the paretic and non-paretic muscle. Gastrocnemius ACSA and agonist activation were found to be determinants of isometric PF MVIC torque across the entire participant population, whereas agonist activation was the only predictor of paretic limb PF MVIC torque.

The PF MVIC torque of the paretic limb generated 42% and 52% less torque than the non-paretic limb and control group, respectively; however no difference between the groups was identified when assessing DF MVIC torque. The average findings in DF MVIC torque in the present study is consistent with data reported in males aged 20-40 years at 0 deg, with torque measures ranging between 17 Nm to 26 Nm (van Schaik et al. 1994). This suggests that based on the DF MVIC torque in the paretic limb of individuals with SCP alone, it can be assumed that impairment in the TA is limited or negligible. However, it should be noted that van Schaik and colleagues (1994) reported that the optimal ankle angle for testing DF peak torque in relation to the force length relation is at 20 deg PF. Whether this data from individuals without neurological impairment applies to individuals with SCP has yet to be determined. Therefore, further research is required to address whether this is the optimal angle to assess DF strength in the paretic limb of men with hemiplegic SCP. In relation to PF MVIC torque in the present study, this data is consistent with previous findings assessing muscle weakness in the PF and quadriceps of children with SCP in comparison to the non-paretic limb and age matched controls without neurological impairment (Elder et al. 2003; Damiano et al. 2001; Stackhouse et al. 2005). This difference in findings may be due to individuals with SCP employing an avoidance strategy by becoming more dependent on their dominant side, perpetuating weakness in the paretic limb (Gage et al. 1995). Additionally, as the adults in the present study are active individuals, the difference in muscle strength between both limbs may be further exacerbated as a result of non-paretic limb dominance during exercise.

In the present study, the paretic limb was weaker than both the non-paretic limb and control group. Previous findings have reported 62% of the variability in PF MVIC torque to be due to differences in the gastrocnemius ACSA (Fukunaga et al. 1996). Here a similar association between the gastrocnemius ACSA and PF MVIC torque in the paretic, non-paretic limbs and the entire participant population was identified. In agreement with the hypothesis, the paretic muscle of adults with SCP was 20% smaller than that of the control group. This finding is similar to that reported by Elder and colleagues (Elder et al. 2003), who showed that the paretic triceps surae ACSA in children with SCP was approximately 27% smaller than the control muscle of aged matched controls without neuromuscular impairment. They also reported no difference between paretic and non-paretic muscle ACSA, which is consistent with the findings from the present study. As there was a positive correlation between gastrocnemius ACSA and PF MVIC torque in the present study, it was expected that as PF MVIC torque was significantly different between the paretic and non-paretic limbs, gastrocnemius ACSA would also differ. However, this was not the case, suggesting that internal mechanical factors of the muscles may be different between the paretic and non-paretic limbs. This is reflected in the present data when the strength/size relationship is considered.

When PF MVIC torque is considered relative to the ACSA of the gastrocnemius, the paretic limb torque was 56% and 66% less than the non-paretic limb and control group torque, respectively. This finding is consistent with previous data on children with SCP, where the paretic limb was approximately 56% of the non-paretic limb and 60% of the control group (Elder et al. 2003). This decline in PF

MVIC torque relative to ACSA in the paretic limb is likely to be due to an overestimation of contractile material (Lieber et al. 2003; Urbanchek et al. 2001), or neural factors such as a reduction in agonist motor unit recruitment and/or an increase in antagonist muscle coactivation (Elder et al. 2003; Lieber et al. 2004; Stackhouse et al. 2005).

Lieber et al., (2003) reported that paretic muscle biopsy samples contained 40% contractile tissue and the remaining tissue was composed of extracellular matrix. In contrast, in control children 95% was contractile tissue and only 5% extracellular matrix. Although these data were obtained from children with SCP, the present study concerns active adults with SCP who may have a more 'normal' muscle composition due to the regular physical exercise they undertake, and perhaps an increased muscle mass through hypertrophic adaptations too.

The neural capabilities of the paretic triceps surae to fully activate motor units during a MVC is approximately 40% less in children with SCP when compared to control populations (Elder et al. 2003; Stackhouse et al. 2005). The present study reports similar observations with activation approximately 37% less in the paretic limb, when compared to the non-paretic limb and control group, which is in accordance with the hypothesis. This is likely to be due to the insult sustained to the brain, resulting in impaired efferent neurological signalling (Koman et al. 2004; Lieber et al. 2004), and sensory deficits within the paretic muscle which may impact on muscular weakness (Graham and Selber 2003). Although these

differences exist between the different groups, this study is the first to identify an association between PF MVIC torque and agonist activation in the paretic, non-paretic limbs and the entire participant population. This association between PF MVIC torque and agonist activation in the paretic limb demonstrates that muscle weakness in SCP is linked with lower levels of voluntary motor unit recruitment during PF MVIC.

The present study also identified that gastrocnemius ACSA was not associated with MVIC torque in the control group, which is contrary to previous research (Morse et al. 2004). It is apparent that the control group (torque = 167 (33.3) Nm) was more homogenous than the paretic (torque = 79.5 (46.6) Nm) and non-paretic (torque = 138 (42.9) Nm) limbs, which may provide some explanation to the non-significant findings in the present study. In addition, the small participant cohort may also be a factor affecting the outcome, particularly when considered in conjunction with the relatively lower variation in data in the control group. Indeed when the entire testing cohort was assessed, a correlation was identified between gastrocnemius ACSA and MVIC torque.

In support of the hypothesis, antagonist coactivation properties of the TA in the present study were approximately 3-fold higher in the paretic limb of adults, when compared to the non-paretic limb and control group. Previous reports in children with SCP showed that coactivation of the triceps surae was approximately 2-fold higher when compared to control populations (Elder et al. 2003; Stackhouse et al.

2005). The present study also identified an inverse association between PF MVIC torque and antagonist coactivation in the paretic limb and entire participant population. Such increases in coactivation have been previously suggested to act as a safety mechanism to increase joint stabilization and limit the degrees of freedom during gait in children with SCP (Damiano et al. 2000).

Although antagonist coactivation of the TA was associated with PF MVIC torque and was found to have a large effect size when identifying differences between the paretic limb, non-paretic limb and control groups, it was found from the multiple regression that antagonist coactivation was not a significant predictor of PF MVIC torque. Previous findings have demonstrated that increased coactivation of the antagonist muscle has been associated with decreased MVIC torque in elderly individuals without neurological impairment (Macaluso et al. 2002). Although antagonist coactivation was not found to be a predictor of muscle torque, muscle activation and gastrocnemius ACSA were found to explain 69% of PF MVIC torque from the multiple regression assessing the entire participant population in the present study. The variables contributed to a large proportion of PF MVIC torque, though 31% of variation remains unexplained. In specific relation to the paretic limb and in contrast to the present findings across the entire participant population, agonist activation was the only predictor identified to influence PF MVIC torque. It was found to explain 57% of the variation in PF MVIC torque, which negates previous findings that imply that muscle ACSA has an impact on muscle MVC torque capabilities in the paretic muscle of individuals with SCP (Elder et al. 2003; Stackhouse et al. 2005), which is consistent with the clinical origins of the

impairment. Thus, gastrocnemius ACSA is not representative of the recruitable muscle mass in the paretic limb of individuals with SCP. As ACSA is not a determinant of paretic PF MVIC torque, it must be noted that measurement of the bi-pennate gastrocnemius ACSA underestimates the physiological CSA of the muscle (Narici et al. 1992). Therefore future research should assess the impact of fascicle pennation angle (Aagaard et al. 2001), muscle fascicle length (Mohagheghi et al. 2007), and moment arm of the tendon (Maganaris et al. 2000), on PF MVIC torque.

The present study shows that agonist activation during PF MVIC in the paretic limb accounts for over half of the variation in torque. Specifically in adults with SCP an inability to successfully recruit the agonist muscle contributes significantly to the weakness observed in this population. It is likely that the capacity to improve strength from a structured training regime in individuals with SCP may be accentuated through improvements in activation capacity to a greater degree than unimpaired individuals who experience minimal agonist activation impairments. Therefore one of the primary implications for development of muscle strength in individuals with SCP is a resistance training program that emphasises the recruitment (i.e. neural factors) of the agonist muscle over and above muscle hypertrophy. In adults without neurological impairment, the improvement in agonist activation with training is accentuated in heavy load resistance training programs (Häkkinen et al. 1998; Higbie et al. 1996), however whether such findings are applicable to SCP populations needs to be determined.

5.6 Conclusion

The findings from the present study demonstrate that active adults with hemiplegic SCP have muscle weakness as a result of muscular and neural differences in the paretic limb. This was confirmed as PF MVIC torque was on average 47% weaker than the non-paretic limb and control group. The paretic gastrocnemius ACSA was smaller compared to the control group, though no difference was observed between paretic and non-paretic limbs. The paretic limb demonstrated an inability to innervate the PF muscles and increased antagonist coactivation was also evident when compared to the non-paretic limb and control group. Although these differences between the paretic limb and control group were identified, the multiple regression on the paretic limb identified that muscle activation is a significant predictor of gastrocnemius PF MVIC torque. This demonstrates that the key determinant of weakness in individuals with SCP is their ability to activate the gastrocnemius muscle, regardless of the differences identified in paretic gastrocnemius ACSA and antagonist coactivation.

Chapter 6

Gastrocnemius medialis specific force of adults with spastic cerebral palsy

6.1 Abstract

Introduction. Muscle weakness has become synonymous with the paretic muscles of individuals with spastic cerebral palsy (SCP). The aim of the present study was to establish whether the specific force of the paretic muscle was similar to the non-paretic and dominant muscle of individuals without neurological impairment.

Methods. The physiological cross sectional area and specific force of the gastrocnemius medialis (GM) were assessed in both paretic and non-paretic legs of 11 physically active men with SCP, and the dominant limb for 11 age-matched individuals without neurological impairment. GM fascicle force was calculated from the plantarflexion isometric maximal voluntary contraction (MVIC) torque with interpolated singlet stimuli, whilst moment arm length (estimated from tendon excursion), muscle architecture (measured using ultrasonography) and antagonist coactivation (estimated from electromyography) were all accounted for. Physiological cross sectional area (PCSA) was estimated as GM volume divided by fascicle length. Specific force was calculated as fascicle force divided by PCSA.

Results. Paretic fascicles were 28% longer than that of the control group. Pennation angle of the paretic GM was 31% smaller than the non-paretic limb and 41% smaller than the control group. PCSA of the paretic GM was 41% and 47% smaller than the non-paretic and control group, respectively. No difference was identified in GM specific force between the groups when accounting for neural differences, however, paretic GM fascicle force was 41% less than the non-paretic limb and 52% lower than the control GM fascicle force.

Conclusion: Based on the difference in findings between the paretic GM fascicle force and specific force, contractile weakness is not due to the intrinsic properties of the muscle being impaired. Therefore the deficit in strength in relation to the material properties of the contractile tissue in the paretic limb of active individuals with SCP is due to decreased muscle PCSA.

6.2 Introduction

Muscle weakness (as presented in Chapter 5) in individuals with spastic cerebral palsy (SCP) have been shown to originate from impaired neural signalling to the paretic muscle, consequently impacting on muscular size and architecture (Elder et al. 2003; Malaiya et al. 2007; Mohagheghi et al. 2007; Stackhouse et al. 2005). Such weakness of the paretic muscles have been shown to contribute to differences in gait patterns (Damiano and Abel 1998), and limit motor control performance (Damiano et al. 1995b; Wiley and Damiano 1998). Although muscle weakness may limit the performance of daily tasks, only a few studies have addressed the underlying determinants of weakness specifically in adults with SCP.

It has been reported that larger deficits in weakness exist in the more distal paretic musculature of the lower limbs in individuals with SCP (Brown et al. 1991). With this in mind, Elder et al. (2003) reported that the isometric plantarflexion (PF) torque of the paretic limb relative to the anatomical cross sectional area (ACSA; $Nm^.cm^{-2}$) in children with hemiplegic SCP was ~40% lower than either the non-paretic limb or individuals without neurological impairment. Although such findings are crucial to furthering our understanding of the determinants of muscle weakness, Chapter 2 discussed how ACSA measurements have been well documented for underestimating the true physiological cross sectional area (PCSA) of pennate muscles (Morse et al. 2005c; Morse et al. 2008b; Narici et al. 1992). In support of these findings, correlations between muscle force during PF isometric maximal voluntary contraction (MVIC) and PCSA have been shown to be

considerably higher   than correlations with ACSA (r = 0.72 vs r = 0.92, respectively) (Fukunaga et al. 1996).

The architectural characteristics of pennate muscles are also known to influence contractile function as well as muscle size. Changes in architecture as a result of resistance training (Aagaard et al. 2001) and bed rest interventions (de Boer et al. 2008; Reeves et al. 2002) have been suggested to impact on the force output capabilities of a muscle in individuals without neurological impairment. In children with spastic hemiplegic SCP, the resting fascicle lengths of the paretic muscle in the gastrocnemius have been reported to be 8% smaller when compared to the muscle of children without neurological impairment (Malaiya et al. 2007) and the contralateral non-paretic limb (Mohagheghi et al. 2007). These differences in fascicle length and muscle ACSA indicate that there are fewer sarcomeres in series and in parallel, respectively, in the paretic muscle of individuals with SCP. Additionally, Malaiya et al. (2007) showed that fascicle pennation angle of the paretic gastrocnemius medialis (GM) did not differ when compared to the non-paretic muscle of individuals with SCP and the dominant limb of control participants without neurological impairment. Although architectural parameters reported at rest provide information to the understanding of fundamental differences in the paretic muscle, and in Chapter 5 the neural influence on MVC/ACSA was identified, no information is currently available on the contribution of architectural factors or PCSA to weakness in adults with SCP.

In order to calculate specific force, tendon force is calculated from the measured torque during MVIC and moment arm length (Maganaris et al. 2001; Morse et al. 2005c; Morse et al. 2008b). With moment arm being one of several identified determinants of muscle specific force (García-Morales et al. 2003; Rassier et al. 1999), the comparison of strength between individuals with SCP and individuals without neurological impairment, may be influenced by differences in moment arm length. However at present there remains no information on moment arm lengths in individuals with SCP.

Given the established weakness of the paretic limb and the subsequent limitations in motor function, the aim of the present study was to identify whether there is a difference in paretic muscle specific force *in vivo*, when compared to the non-paretic muscle and the dominant limb of individuals without neurological impairment. Therefore it was hypothesised that the GM specific force of the paretic limb would be lower than the non-paretic limb and the dominant limb of individuals without neurological impairment (form herein this group will be referred to as 'controls').

6.3 Methods

6.3.1 Participants

Twenty-two active and ambulant males gave written informed consent to participate in the study. Eleven of the participants had spastic hemiplegic CP (age

= 21.2 (3.0) years, stature = 1.79 (0.10) m, mass = 70.0 (12.5) kg) and the 11 control participants had no history of musculoskeletal or neurological impairment (age = 21.8 (2.2) years, stature = 1.81 (0.04) cm, mass = 79.0 (8.4) kg). Each participant with SCP rated between II and III on the modified Ashworth scale and had been formally classified independently by individuals from the Cerebral Palsy International Sports and Recreation Association (CPISRA). Both the paretic and non-paretic legs were tested in each participant with CP, whereas the dominant limb was assessed in the control participants. All participants were free from lower limb injury and had not received any form of medication to reduce the effects of spasticity within the last year. The study was approved by the local ethics committee at Manchester Metropolitan University and conformed to the standards set by the latest revision of the Declaration of Helsinki.

6.3.2 Protocol

Participants attended the laboratory on two occasions. During the first visit, familiarisation was carried out which included a series of six PF MVIC followed by a series of submaximal percutaneous electrical stimulations. During the second visit, participants were assessed for resting measures of muscle size, and moment arm length, which were then followed by the MVIC tests.

6.3.3 Strength measurements

The PF MVIC torque was recorded with the participants secured to an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA) in a seated position with the back angle reclined at 65 deg (Chapter 3-5). The participant's knee was secured in full extension with Velcro straps, which were positioned proximal to the knee. The medial malleolus was visually aligned with the dynamometer's central axis of rotation and two Velcro straps were used to secure the foot to the footplate in order to minimise heel displacement. The participant's hips were also secured to the seat to limit extraneous movement during PF MVIC trials. All participants warmed up by performing three submaximal isometric contractions with the ankle angle at 0 deg (the individual's anatomical zero), each of which were separated by a 1 min rest period. In this instance, 0 deg was defined as the foot at 90 deg to the tibia. Two out of the 11 participants with CP could not achieve the described 0 deg in the paretic leg as their heel did not rest on the ground during anatomical stance. In these two cases, 0 deg was calculated by using a goniometer to quantify how much their resting ankle angle differed and an appropriate correction was measured on the isokinetic dynamometer. After the warm up, the participant's ankle remained fixed at 0 deg where two PF MVICs were obtained, separated by a 2 min rest period. Throughout all MVIC trials, participants were verbally encouraged to exert as much force as possible and online visual feedback was provided on a monitor. Dorsiflexion (DF) MVIC was completed after the PF MVICs using the same testing posture and protocol in order to calculate tibialis anterior (TA) coactivation.

6.3.4 Agonist Activation

In order to account for any deficit in MVIC torque in the quantification of specific force, two supramaximal stimuli were applied to the muscle (with a pulse width of 50 μs), the first of which was applied during MVIC and the second approximately 2 s after the first stimulus when the muscle was fully relaxed, in order to quantify the individual's ability to voluntary activate the gastrocnemius (Chapter 5; Maganaris et al. 2001; Morse et al. 2005c). The stimulus was delivered by applying two percutaneous stimuli (DSVDigitimer Stimulator; Digitimer, Herts., UK) to the gastrocnemius using rubber stimulation pads (size ranging from 38 mm x 89 mm to 76 mm x 127 mm; Versastim; Conmed, NY, USA), both of which were placed transversely distal to the popliteal crease and myotendinous junction of the soleus. The amplitude of the stimuli was determined prior to interpolation whilst the participant was in a relaxed state; administering twitches starting from 50 mA and increasing in increments of 50-100 mA, until no further increase in twitch torque was quantified. The voluntary activation level of each participant was assessed using the trial that produced the highest contractile torque. Agonist activation was calculated by dividing the supramaximal twitch torque during MVIC by the post MVIC twitch torque consistent with Morse et al. (2005c). If there was a deficit in muscle activation (a value below 100%) and assuming a linear relationship between MVIC torque and agonist activation (Gandevia 2001; Herbert and Gandevia 1999), a correction was made to PF MVIC if there was a deficit in agonist activation to the measured torque, which was calculated as; (PF MVIC torque / 100) x deficit in voluntary activation. This value was subsequently added to the MVIC torque along with torque contributions in coactivation to estimate PF MVIC net torque.

6.3.5 Coactivation

In physically active men with hemiplegic SCP, the level of coactivation in the paretic limb was 3 times higher than the non-paretic contralateral limb, and the limb of individuals without neurological impairment (Chapter 5). With such large deviations in agonist activation during MVIC trials, TA electromyography (EMG) activity was recorded using two pre-gelled, unipolar, 10 mm, Ag-AgCl percutaneous electrodes (Medicotest, Denmark). Boundaries of the TA were determined using ultrasonography to ensure the accurate placement of each electrode along the mid sagittal axis of the muscle and to reduce cross-talk. Two electrodes were placed distally at two thirds of the TA length and one reference electrode was placed over the lateral epicondyle of the femur. Prior to placement of the electrodes, the area was shaved and cleaned with an alcohol swab to remove residual skin cells and oils, as well as reduce skin impedance. Raw EMG data were recorded at 2000 Hz, with a high and low band-pass filter set at 10 and 500 Hz, respectively, with a notch set at 50 Hz. The integral of the root mean square of the raw signal 0.5 s either side of the MVIC PF torque was used to quantify the level of muscle coactivation. The torque produced by the DF during PF MVIC was estimated by assuming a linear relationship between torque and EMG activity, as previously reported (Maganaris et al. 1998b). The relative contribution of antagonist coactivation from the DF MVIC was added to estimate PF net torque along with any correction in agonist activation as aforementioned.

6.3.6 Muscle volume

B-mode ultrasonography (AU5, Esaote, Italy) was used to obtain several axial-plane images of the GM to measure ACSA (Reeves et al. 2004a). The GM proximal insertion and the MTJ were marked to identify 50% of muscle length. Strips of Micropore tape were placed axially across the mid-line of the GM at approximately 3.5 cm intervals. These strips of tape were used as echo absorptive markers that project a shadow onto the ultrasound image to provide a positional reference into the scanned structures. With the probe in an axial-plane, a recording of the probe moving from the medial to the lateral border of the GM was obtained. Individual images were extracted from the recording offline and used to construct the muscle by overlapping anatomical landmarks and external markers. Image J software (version 1.34; National Institutes of Health) was used to measure the ACSA of the constructed GM. The volume of the GM was calculated assuming a conical volume using the constructed ACSA. This technique used to estimate muscle volume has previously been validated in young men (Morse et al. 2007a).

6.3.7 Muscle architecture

At the point of peak torque during the MVIC trials, real time ultrasonography was used to record fascicle length and pennation angle during contraction, which was synchronized with the measured PF torque values. The 5 cm, 7.5 Hz linear array probe was held on the mid-sagittal plane of the GM equidistant between the proximal and distal tendon insertions previously established by ultrasonography. Additionally, the probe was held perpendicular to the surface of the skin to obtain several visible fasciculi ranging from the superficial to the deep aponeuroses. After the PF MVIC trials were completed, the recording of the highest torque trial was

analysed offline using Image J software. Fascicle length was measured as the length between the superficial and deep aponeuroses (Narici et al. 1996). Pennation angle was defined as the insertion angle of the fascicle into the deep aponeurosis (Maganaris et al. 2001). Fascicle length and pennation angle were measured at the time point of maximum PF torque, as it has been reported that pennation angle is underestimated and fascicle length is overestimated during rest conditions by 18.1 deg and 17.0 mm, respectively (Narici et al. 1996). Thus, in order to accurately calculate the intrinsic force generating capacity of the GM, data must be obtained during contraction and not rest (Maganaris et al. 2001; Morse et al. 2005c; Reeves et al. 2004b). The dimensions of the window used for analysis was 4.15 cm x 3.5 cm; in some cases fascicle length was estimated using linear extrapolation if a whole image of the fascicle was not available for direct measurement.

6.3.8 PCSA

The PCSA was estimated as the ratio of GM muscle volume to fascicle length (Alexander and Vernon 1975; Maganaris et al. 2001; Morse et al. 2008b).

6.3.9 Moment arm length

The tendon excursion method was used to estimate moment arm length during a passive stretch trial on an isokinetic dynamometer by passively rotating the ankle to calculate tendon excursion whilst in a seated position (as previously described

in Chapter 3 and 4). The medial malleolus was visually aligned with the dynamometer's central axis of rotation. Prior to the experimental trial, end dorsiflexion end range of motion was identified by the experimenter by rotating the ankle at 1 $deg\cdot s^{-1}$, starting from 15 deg PF, until discomfort caused the participants to cease the stretch in dorsiflexion. This velocity was chosen in relation to previous findings which elicited minimal neural activity throughout passive stretch trials in individuals without neurological impairment (Morse 2011; Morse et al. 2008a). Throughout the passive stretch, B-Mode ultrasonography was used to determine the displacement of the GM MTJ throughout the passive stretch. MTJ displacement was measured relative to an acoustically reflective marker (a thin strip of Micropore tape) secured to the skin proximal to the GM MTJ, as previously validated by Morse et al. (Morse et al. 2008a).

The total change in MTJ displacement was divided by the change in ankle range of motion (rad), to predict the moment arm length for each individual. This technique has previously been validated using cadavers when assessing the moment arm length of the quadriceps (Spoor et al. 1990).

6.3.10 Achilles tendon force

Tendon force was calculated by dividing the net plantarflexion torque by the Achilles tendon moment arm length (Morse et al. 2005c; Morse et al. 2008b).

6.3.11 Fascicle force

In order to estimate GM fascicle force, PF MVIC net torque was multiplied by the relative contribution of the GM PCSA within the triceps surae muscle group. The relative PCSA of the PF muscles have previously been used to determine the relative contribution of each contributing muscle, whereby the relative PCSA of the GM was found to account for 15.4% of the Achilles tendon force (Fukunaga et al. 1996). Therefore the force generated by the GM was calculated by determining ratio of GM contribution to Achilles tendon force. The force generated by the GM muscle was subsequently divided by the cosine of the pennation angle measured during contraction to determine GM fascicle force.

6.3.12 Specific force

Specific force was calculated by dividing GM fascicle force by GM PCSA.

6.3.13 Statistics

All statistical analyses were performed using SPSS software (Version 19, SPSS Inc., Chicago Illinois). To ensure the data were parametric, the Shapiro-Wilk and Levene's tests were utilised to assess the distribution and variance of the data. As there were no breaches of these statistical assumptions, independent t-test were used to assess baseline anthropometric data between CP and control groups. To minimise type I error with the main outcome measures (as could occur with repeated ANOVA tests), a MANOVA was used to compare the differences and

interactions in the joint torque, force, neural and architectural variables (listed in Table 6.1-6.3) of the paretic vs. non-paretic limbs vs. the dominant limb of control individuals. Statistical significance was accepted at the $P < 0.05$ level, and all data are presented as mean (SD).

6.4 Results

Adults with CP and matched control individuals were of similar age ($P = 0.575$), stature ($P = 0.604$) and body mass ($P = 0.061$).

6.4.1 Torque and moment arm properties

The PF MVIC torque produced by the paretic limb was 33% less than the non-paretic limb ($P = 0.039$), and 46% lower than the control group ($P < 0.001$). No difference in PF MVIC torque was identified between the non-paretic limb and control group ($P = 0.178$). During the PF MVIC trial, was 30% lower than the control group ($P = 0.017$). However, no difference was identified between the paretic and non-paretic net PF MVIC torque ($P = 0.892$), nor between the non-paretic and control groups ($P = 0.193$). No differences were identified in DF MVIC torque ($P = 0.653$) or moment arm length ($P = 0.281$) between groups (Table 6.1).

Table 6.1. Joint torque, moment arm and neural properties of the paretic and non-paretic limbs of individuals with SCP, and individuals without neurological impairment.

	Paretic limb	Non-paretic limb	Control group
PF MVIC (Nm)	102 (55.8)*†	153 (47.7)	190 (26.7)
Net PF MVIC (Nm)	139 (59.5)*	160 (46.9)	198 (27.3)
DF MVIC (Nm)	17.5 (8.58)	21.3 (11.9)	20.8 (10.6)
Moment arm (cm)	6.05 (1.69)	5.08 (0.98)	5.54 (1.56)

*Difference between paretic and control groups ($P < 0.001$). †Difference between paretic and non-paretic groups ($P = 0.039$). ‡Difference between paretic and non-paretic groups ($P < 0.001$).

6.4.2 GM muscle size and architecture

There was no difference between the paretic and non-paretic fascicle lengths ($P = 0.070$), and non-paretic and control fascicle lengths ($P = 0.929$; Table 6.2). However, the paretic fascicles were 28% longer than in the control group ($P = 0.005$). The pennation angle at which the fascicles joined the superficial and deep aponeurosis during PF MVIC in the paretic GM was 41% smaller than the non-paretic limb ($P = 0.001$), and 41% smaller than the control group ($P < 0.001$). No difference in pennation angle was identified between the non-paretic limb and control group ($P = 0.095$).

The ACSA of the GM was found to be 20% and 27% smaller than non-paretic muscle (P = 0.028) and control group (P = 0.001), respectively. No differences were identified between the non-paretic and control group GM ACSA (P = 0.601). The paretic GM volume was 27% smaller than that of the non-paretic (P = 0.014) and 30% smaller than the control group (P = 0.005). Similarly, the PCSA of the paretic GM was 41% and 47% smaller than the non-paretic (P = 0.002) and control group (P < 0.001), respectively. However, no difference was identified between non-paretic limb and control group when assessing GM volume (P = 1.000) and GM PCSA (P = 0.933). Furthermore, no difference between groups was identified when assessing GM length (Table 6.2; P = 0.095).

Table 6.2. Muscle size and architectural characteristics of the GM muscle in the paretic and non-paretic limbs of individuals with SCP, and individuals without neurological impairment.

	Paretic limb	Non-paretic limb	Control group
Fascicle length (cm)	3.70 (0.62)*	3.14 (0.56)	2.89 (0.47)
Pennation angle (deg)	25.7 (4.08)[†§]	37.2 (7.59)	43.4 (7.00)
GM length (cm)	24.5 (3.75)	26.8 (3.23)	25.7 (2.00)
GM ACSA (cm^2)	12.0 (2.62)[‡‖]	15.0 (2.23)	16.5 (2.90)
GM volume (cm^3)	98.0 (28.3)*[¶]	135 (31.3)	141 (26.2)
GM PCSA (cm^2)	26.3 (5.83)[†#]	44.8 (14.2)	50.0 (12.0)

*Difference between paretic and control groups (P = 0.005). [†]Difference between paretic and control groups (P < 0.001). [‡]Difference between paretic and control groups (P = 0.001). [§]Difference between paretic and non-paretic groups (P = 0.001). [‖]Difference between paretic and non-paretic groups (P = 0.028). [¶]Difference between paretic and non-paretic groups (P = 0.014). [#]Difference between paretic and non-paretic groups (P = 0.002).

6.4.3 Force measurements

Achilles tendon force (P = 0.010) and GM force (P = 0.010) of the paretic limb was 41% lower than the control limb. No difference in the non-paretic Achilles tendon force and GM force was established when compared to the paretic limb (both P = 0.100) and control group (both P = 1.000). The paretic GM fascicle force was 41% less than the non-paretic limb (P = 0.024) and 52% lower than the control GM

fascicle force (P < 0.001). No difference between the non-paretic GM fascicle force and control group was identified (P = 0.370). Lastly, no difference between the groups was established when assessing GM specific force (Table 6.3; P = 0.393).

Table 6.3. Force measurements in the paretic and non-paretic limbs of individuals with SCP, and individuals without neurological impairment.

	Paretic limb	Non-paretic limb	Control group
Achilles tendon force (kN)	2.26 (0.57)*	3.34 (1.59)	3.81 (0.32)
GM muscle force (N)	347 (88.2)*	515 (244)	586 (161)
GM fascicle force (N)	388 (104)†‡	662 (317)	814 (205)
Specific force (N$\cdot$cm^{-2})	15.0 (3.66)	14.6 (4.12)	17.2 (5.94)

*Difference between paretic and control groups (P = 0.010). †Difference between paretic and control groups (P < 0.001). ‡Difference between paretic and non-paretic groups (P = 0.024).

6.5 Discussion

This study assessed the specific force of the GM in active individuals with SCP. The purpose of which was to establish whether the specific force of the paretic GM and the variables used in its calculation, differed when compared to the non-paretic limb and control participants. Contrary to the hypothesis, the main finding of the present study was that there was no difference between the *in vivo* specific force of the paretic and non-paretic GM of active individuals with SCP, and the

muscle of control participants. Although specific force of the GM was the same across all groups, paretic fascicle force was 41% and 52% lower than the non-paretic and control group, respectively.

Consistent with the results of Chapter 5, the SCP participants demonstrated significantly lower levels of activation than unimpaired counterparts. The aim of the electrical stimulation in the present chapter was not to calculate the level of the activation deficit, but to account for any neural contribution to weakness and allow for a more accurate measure of GM specific force. By accounting for the differences in GM activation and TA coactivation across the paretic, non-paretic and control groups, the net PF MVIC torque remained 30% lower in the paretic limb when compared to the control group, but not different from the non-paretic limb. Whereas prior to this correction, the paretic PF MVIC torque was 46% and 33% lower compared to the control group and non-paretic limb, respectively. The remaining difference in net PF MVC torque is therefore attributable to morphological or architectural properties of the muscle.

The majority of research concerning muscle weakness in individuals with SCP measure the MVIC torque generated by a muscle or group of muscles (Barber et al. 2012; Damiano et al. 2001; Elder et al. 2003; Stackhouse et al. 2005). One limitation when assessing torque is that moment arm lengths between limbs and/or groups of individuals are not taken into account. As individuals with SCP may present structural deformities in the paretic limb as a result of increased tonicity of

the muscle throughout maturation (Koman et al. 2004), it is possible that the internal structures between the paretic, non-paretic and control limbs may be more prominent in adults, compared to paediatric populations. It is established that Achilles tendon moment arm increases in length with plantarflexion (Maganaris et al. 1998a), it remains to be seen however whether the sustained equinus posture of CP will alter the moment arm. In the present study, based on the consistent foot angle of 0 deg we observed no significant difference in moment arm between the SCP and control groups. Nevertheless, when the *in vivo* forces were calculated in the paretic Achilles tendon and GM, it was found to be 41% weaker than the control group only, but no difference was established between the paretic and non-paretic limbs. Although the 1 cm difference in Achilles tendon moment arm as observed in our participants does not seem substantial, with all else being equal when calculated using the mean PF MVIC net torque in the present SCP population, an increase in moment arm length by 1 cm would theoretically increase the tendon force in the SCP limb by 240 N. As a result, the assessment of specific force using joint torque rather than tendon force would underestimate the true force producing capacity of the contractile mass. Accounting for moment arm lengths, muscle architecture and neural properties facilitates the assessment of the intrinsic material force-producing capacity of muscle *in vivo* (Morse et al. 2005c; Reeves et al. 2004b).

In children with SCP, the morphology of the paretic GM during rest showed that fascicle length was 18% shorter compared to non-paretic contralateral limb (Mohagheghi et al. 2007) and 16% shorter compared to age matched controls

(Malaiya et al. 2007). Based on these previous findings, deficits in paretic fascicle length would imply that the number of sarcomeres in series is typically lower than the control comparisons. However, in contrast to previous architectural data, in the present study fascicle length during PF MVIC was 8% longer in SCP than in the control group. It is likely that the contrasting results obtained in the present study reflect the nature of the measurement technique. Where previous studies have reported muscle architecture at rest, GM fascicle length in the present study was measured at peak PF MVIC torque; as is consistent with the calculation of specific force (Maganaris et al. 2001). Due to the spasticity in the GM muscle, the paretic foot of the participant with SCP is typically in an equinus position, consistent with immobilisation studies (Williams and Goldspink 1978), where the fascicles would be expected to be shortened when measured in this 'relaxed' position (Malaiya et al. 2007). In addition, as GM fascicle length was measured during PF MVIC, any difference in the tendon properties, or force that was produced would influence the relative shortening experienced by individuals with and without SCP, as has been observed in the elderly (Reeves et al. 2004b). However, at present there remains no published data on the fascicle length-tension relation or the influence of tendon properties in individuals with SCP.

In the present study, when neural and architectural factors were accounted for, there remained a difference in GM fascicle force between SCP and control participants which was almost entirely accounted for by the difference in PCSA, as evidenced by similar values for specific force between SCP and control participants. As PCSA takes into account the volume and fascicle length of the

muscle, it provides a more accurate measurement of the true contractile area of pennate muscle (Morse et al. 2005c; Morse et al. 2008b; Narici et al. 1992). The present study identified that the paretic GM PCSA was ~45% smaller than the non-paretic limb and control group, similar to the observed difference in GM fascicle force (52% smaller). Such differences indicate that the paretic GM muscle has fewer sarcomeres in parallel compared to the non-paretic and control GM muscle and any weakness at the whole muscle level is unlikely to be influenced by a decrease in the quality of the muscle at the fascicle level.

In the present study no difference in specific force was observed between the SCP and control group, or between the paretic and non-paretic limb of the SCP participants. This would initially appear to be in contrast to previous work which has established that the size/strength relationship of muscles in individuals with SCP is reduced compared to individuals without neurological impairment (e.g. ACSA/PF MVIC torque (Elder et al. 2003)). However, as previously stated, based on the architectural differences between SCP and the control group, and the substantial neural contribution to reduced joint torque, the estimation of the size strength relation may be erroneous unless these factors are considered. Indeed, based on histological data from single fibres of individuals with SCP there appears to be no discernible difference between SCP and control individuals at the single fibre level (Ito et al. 1996).

Although this is the first study to address the intrinsic force generating capacity of SCP muscle, several factors may potentially impact on the data presented. Within the present study moment arm was estimated during rest, whereas specific force should represent the data obtained during MVIC. The difference in Achilles moment arm length during MVIC is approximately 1.5 cm longer than resting measures in individuals without neurological impairment (Maganaris et al. 1998a). In addition, fascicle length was measured at a comparable length rather than the angle at which peak torque occurred (Reeves et al. 2004b), due to the fact that in the present study, 0 deg ankle angle was used to control for the different resting angles between the participants. However, as the plantarflexors are on the ascending limb of the force length relation, it is likely that specific force is underestimated in the present investigation (Maganaris et al. 2001; Morse et al. 2007b; Reeves et al. 2004b). To compare between individuals that have limited dorsiflexion ROM a consistent joint angle was chosen for the measurement of specific force at 0 degrees. Nevertheless, the interaction of the muscle and the tendon has yet to be addressed in individuals with SCP. As the elastic component of the MTU is pivotal in the transition of force from the muscle to bone, the tendon properties in individuals with SCP should be assessed to identify whether the functional properties of the tendon are altered *in vivo*.

6.6 Conclusion

The present study has showed that the paretic GM of physically active individuals with SCP has a similar specific force generating capacity to the non-paretic muscle and the GM of control individuals. This study also demonstrates how the pennation

angle and fascicle length of the paretic muscle at MVIC is different from the control group. Nevertheless, weakness (whilst accounting for neural properties, moment arm lengths and muscle architecture) observed in the paretic GM is not due to the intrinsic material properties of the muscle, but because of the smaller PCSA of the muscle.

Chapter 7

Achilles tendon stiffness in adults with spastic hemiplegic cerebral palsy

7.1 Abstract

Introduction. The stiffness of the tendon properties have yet to be assessed in individuals with spastic cerebral palsy (SCP), despite numerous studies investigating the constituent muscle. The aim of the present study was to assess the *in vivo* structural and mechanical properties of the paretic tendon in physically active men compared to control tendons.

Methods. Eleven physically active, ambulant men with hemiplegic SCP (21.2 (3.0) years) and age-matched controls without neurological impairment, completed a plantarflexion isometric maximal voluntary contraction (MVIC) session, respectively. During each session, tendon force and gastrocnemius medialis (GM) myotendinous junction (MTJ) displacement was measured. Additionally, tendon cross sectional area (CSA), length and moment arm length were measured. Subsequently, *in vivo* stress and strain were determined to calculate Young's modulus.

Results. At MVIC, the paretic tendon force was lower than individuals without neurological impairment. Paretic tendon at MVIC was more compliant than individuals without neurological impairment as a result of decreased paretic tendon CSA. Young's modulus at MVIC in individuals without neurological impairment was double the stiffness of the paretic tendon, as a result of 30% less paretic tendon strain. However, when tendon stiffness and Young's modulus was calculated at a standardised force, no difference was observed in tendon elastic properties across all groups.

Conclusion. Active individuals with CP exhibit different stiffness and Young's modulus properties in the paretic tendon compared to individuals without neurological impairment at MVIC, which is due to large differences in tendon force and strain. Whereas the paretic tendon stiffness and Young's modulus at a standardised force, is similar in individuals without neurological impairment. This data suggests that active individuals with SCP may have a similar intrinsic tendon composition compared to the control limbs, likely as a result of regular physical exercise.

7.2 Introduction

The gait and motor performance of individuals with spastic cerebral palsy (SCP) have regularly been demonstrated to be impaired (Damiano et al. 2006; Burtner et al. 1998; Damiano et al. 1995a; Wiley and Damiano 1998), as a result of weakness (Damiano and Abel 1998; Elder et al. 2003) and increased passive muscle-tendon unit (MTU) stiffness (Alhusaini et al. 2010; Vaz et al. 2006) in the paretic musculature (as previously described in Chapters 4, 5 and 6). Although such limitations in motor function are often associated with the impairment of the paretic muscle, the tendon plays an integral part within the MTU as it deforms and transmits the contractile forces generated by the muscle to the bone, to enable joint movement (Maganaris and Paul 2002). Yet research into the properties of the tendon in individuals with SCP has yet to be considered.

The elastic properties of the tendon in individuals without neurological impairment have regularly been shown to behave in a curvilinear fashion (Maganaris and Paul 1999; Onambele et al. 2007), with compliant tendons being linked with slower rates of torque development and *vice versa* for stiffer structures (Reeves et al. 2003; Morse et al. 2005a; Pearson and Onaimbele 2006). Resistance interventions have shown that regular increased loading placed on the tendon results in stiffer material properties (Kubo et al. 2001b; O'Brien et al. 2010b; Reeves et al. 2003), whereas more compliant tendons have been suggested to result from disuse (Kubo et al. 2000; Reeves et al. 2005). This demonstrates that the elastic component responds to differing patterns of use through changes in the dimensions and/or material composition of the tendon (Kjær 2004; Maganaris et

al. 2006). Furthermore, as individuals with hemiplegic SCP often employ an avoidance strategy and use their non-paretic limbs to perform the majority of motor tasks, it may be considered that the paretic limb is subjected to disuse, and thus the associated tendon may be more compliant.

It has previously been established that differences in architectural (Mohagheghi et al. 2007) and contractile properties (Elder et al. 2003; Stackhouse et al. 2005) of the paretic muscle in individuals with SCP are altered when compared to age-matched controls without neurological impairment. This has been suggested to be due to the impaired neural communication between the central nervous system and the paretic muscle (Koman et al. 2004). Based on these neuromuscular deficits and increased passive stiffness of the paretic MTU, compared to age-matched controls (Alhusaini et al. 2010; Vaz et al. 2006), it is likely that the properties of the paretic tendon may be altered. Indeed, Malaiya and colleagues (2007) reported that length of the paretic gastrocnemius medialis relative to limb length was shorter than the non-paretic muscle and that of individuals without neurological impairment, which is in agreement with *in vitro* results (Friden and Lieber 2003). With such decreases in paretic muscle length, this may provide evidence to suggest that tendon length is increased, assuming that no discrepancies in length exist between the paretic and non-paretic limbs. Such deviations in tendon length between limbs may also result in altered intrinsic material properties of the paretic tendon as previously observed in SCP muscle (Lieber et al. 2003; Lieber et al. 2004) and in Chapter 6 where the paretic fascicle and penation angle was 28% longer and 41% smaller, respectively.

In order to measure the *in vivo* mechanical properties of the tendon, various studies have tracked the structural displacement from rest to a ramped isometric maximal voluntary contraction (MVIC) by using ultrasonography in individuals without neurological impairment (O'Brien et al. 2010b; Aagaard et al. 2001; Maganaris et al. 2000, 1998a; Waugh et al. 2012; Onambele et al. 2007; Reeves et al. 2003). Currently no data exists in relation to the tendon properties of individuals with SCP, despite previous research being conducted into the strength and passive stiffness of the paretic muscle. Therefore the aim of the present study was to assess the *in vivo* structural and mechanical properties of the paretic tendon in physically active men compared to the contralateral non-paretic tendon and that of individuals without neurological impairment.

7.3 Methods

7.3.1 Participants

Eleven ambulant men with hemiplegic SCP (age = 21.2 (3.0) years, stature = 1.79 (0.10) m, mass = 70.0 (12.5) kg) and 11 men without neurological impairment (age = 21.8 (2.2) years, stature = 1.81 (0.04) cm, mass = 79.0 (8.4) kg) volunteered to participate in the study after written informed consent was obtained. All participants with SCP had been classified by physical therapists from the Cerebral Palsy International Sports and Recreation Association (CPISRA). All participants, including SCP and individuals without neurological impairment, self-reported that they undertook 3-5 hours of sport-based exercise per week and did not partake in

any structured resistance training regime. The entire cohort was free from lower limb injury and had not received any form of medication to reduce the effects of spasticity within the last year. The study conformed to the standards set by the latest revision of the Declaration of Helsinki and the local ethics committee at Manchester Metropolitan University. Both the paretic and non-paretic legs were tested in each participant with SCP, whereas the dominant limb was assessed in control participants without neurological impairment.

7.3.2 Protocol

Participants attended the laboratory on two occasions. During the first visit, familiarisation was carried out which included a series of six PF MVIC whilst the tendon elongation was tracked using ultrasonography. During the second visit, participants were assessed for resting measures of GM tendon size, and Achilles tendon moment arm length, which were then followed by the PF MVIC tests in order to assess tendon elongation.

7.3.3 Maximal plantarflexion and dorsiflexion torque

Participants were secured to an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA) in a seated position with the back angle reclined at 65 deg prior to beginning MVC trials (Chapter 3-6). The knee was secured in full extension with Velcro straps, which were positioned proximally. The medial malleolus was aligned visually with the central axis of rotation of the dynamometer,

and 2 Velcro straps were used to secure the foot to the footplate to minimise heel displacement. The participant's hips were also secured to the seat to limit extraneous movement during plantarflexion (PF) MVIC trials. Prior to completion of the experimental trial, all participants were familiarised with the forthcoming procedure. Participants warmed up by performing 3 submaximal ramped isometric contractions at 0 deg (the individual's anatomical zero), each of which were separated by a 1-2 min rest period. In this instance, 0 deg was defined as the foot at a 90 deg angle to the tibia. Two out of the 11 participants with SCP could not achieve the described 0 deg in the paretic leg, as their heel did not rest on the ground during anatomical stance. In these 2 individuals, 0 deg was calculated by using a goniometer to quantify how much their resting ankle angle differed, and an appropriate correction was measured on the isokinetic dynamometer. After the warm up, the ankle remained fixed at 0 deg, where a PF MVIC was obtained by the participant slowly increasing their effort over a ~4 second period until torque plateaued (Onambele et al. 2007; Reeves et al. 2003). Subsequent to this procedure, ramped dorsiflexion (DF) MVICs were completed using the same testing posture and protocol in order to estimate co-contraction of the tibialis anterior (TA) during PF MVIC.

7.3.4 Tendon moment arm length

The tendon excursion method was used to estimate moment arm length during a passive stretch trial on an isokinetic dynamometer by passively rotating the ankle to calculate tendon excursion whilst in the seated position (as previously described in Chapter 3, 4 and 6). The medial malleolus was visually aligned with the

dynamometer's central axis of rotation. Prior to the experimental trial, DF end range of motion was identified by the experimenter rotating the ankle at 1 deg·s^{-1}, starting from 15 deg PF, until discomfort caused the participants to cease the stretch in DF. As reported in Chapter 4, this velocity was chosen as it has previously been found to elicit minimal neural activity throughout passive stretch trials in individuals without neurological impairment (Morse 2011; Morse et al. 2008a). Throughout the passive stretch, B-Mode ultrasonography (AU5, Esaote, Italy) was used to determine the displacement of the GM MTJ. As described in detail in Chapters 1 and 2, displacement of the GM tendon was measured relative to an acoustically reflective marker (a thin strip of Micropore tape) secured to the skin proximal to the GM MTJ, as previously validated by Morse et al. (2008a) and Morse (2011).

The range of motion used to estimate moment arm length was between 15 deg PF to 15 deg DF. The total change in MTJ displacement was divided by the change in ankle range of motion (rad), to predict the moment arm length for each individual. This technique has previously been validated using cadavers when assessing the moment arm length of the quadriceps (Spoor et al. 1990).

7.3.5 Tendon force

Tendon force was calculated by dividing the net PF MVIC torque by the Achilles tendon moment arm length (Kongsgaard et al. 2011). Net PF MVIC torque was

calculated by adding the estimated co-contraction torque of the TA to the PF torque during the ramped MVIC (Onambele et al. 2007).

7.3.6 Electromyographic activity and cocontraction

During MVIC trials, TA electromyography (EMG) activity was recorded using 2 pre-gelled, unipolar, 10 mm, Ag-AgCl percutaneous electrodes (Medicotest, Denmark). Boundaries of the TA were determined using B-Mode ultrasonography to ensure accurate placement of each electrode along the mid-sagittal axis of the muscle and to reduce cross-talk. Two electrodes were placed proximally at two-thirds of the TA length, and 1 reference electrode was placed over the lateral epicondyle of the femur (Merletti et al. 2003; Zipp 1982). The area where the electrodes were positioned was shaved and cleaned with an alcohol swab to remove residual skin cells and oils, as well as reduce skin impedance. Raw EMG data were recorded at 2000 Hz, with band pass filter set at 10-500 Hz and a notch at 50 Hz.

Antagonist coactivation was calculated over 50ms at each 10% increments of PF MVIC torque, by taking the root mean square of the TA EMG activity. These measures were made relative to the maximal activation of the TA that was determined during DF MVIC and measured over 1 s (500 ms either side of maximum torque). Assuming a linear relationship between antagonist EMG activity and torque (Lippold 1952), the relative contribution of the DF muscles during PF MVIC was calculated.

7.3.7 GM tendon elongation

Throughout the ~4 s ramped PF MVIC, B-mode ultrasonography captured the elongation of the GM myotendinous junction (MTJ) and was measured offline relative to an echo-absorptive marker (thin strip of Micropore tape) fixed distally to the GM MTJ at rest. Measurements of tendon elongation were assessed at 10% increments of PF MVIC torque, using Image J (version 1.34; National Institutes of Health), which has previously been reported as being a reliable method (ICC = 0.91) when assessing patella tendon elongation (Onambele et al. 2007). To ensure reproducibility, the mean of three measurements for each image was calculated (Reeves et al. 2003). In order to account for the deficit in force generated by the paretic limb (Chapter 4, 5 and 6), tendon elongation was also examined at 645 N (the highest force attained by the weakest participant).

7.3.8 GM tendon length and cross sectional area

Whilst the participant lay relaxed in the prone position, the length of the GM tendon was measured using B-mode ultrasonography from the insertion into the calcaneus, to the GM MTJ (Maganaris and Paul 2002). The length of the GM tendon was measured using an inextensible tape, a method that has been previously validated when assessing the length of the GM muscle (Barber et al. 2011b).

Images were obtained using B-mode ultrasonography to measure Achilles tendon CSA (Pang and Ying 2006) at the mid-point between the tendon insertion into the tuber calcanei and the soleus MTJ. This method has previously been used in the assessment of GM tendon properties (Maganaris and Paul 2002) and reported to be a reliable technique (ICC = 0.81) in the assessment of Achilles tendon CSA (Ying et al. 2003). With the probe in an axial-plane, a video recording of the Achilles tendon CSA was obtained and images were extracted from the video for analysis offline. The mean Achilles tendon CSA of two images was reported and measured using Image J software.

7.3.9 Tendon stress and strain

GM tendon stress was calculated by dividing GM tendon force by its CSA, and GM tendon strain was determined by calculating the ratio of tendon elongation to the resting length of the GM tendon (Maganaris and Paul 1999, 2002; Maganaris et al. 2006; Onambele et al. 2007; Reeves et al. 2003).

7.3.10 Tendon stiffness

The force-elongation relations of the GM tendon were fitted with a second order polynomial function, which was forced through zero. GM tendon stiffness was then calculated from the gradient of tangential lines along the force-elongation relationship, at 10% intervals of PF MVIC force (Onambele et al. 2007) and the standardised force of 645 N.

7.3.11 Young's modulus

At each 10% interval of PF MVIC force and the 645 N standardised force, Young's modulus was calculated by multiplying GM tendon stiffness to the ratio of its respective resting length and CSA.

7.3.12 Statistics

All statistical analyses were performed using SPSS software (Version 19, SPSS Inc., Chicago Illinois). A mixed-design ANOVA was used to distinguish differences in tendon displacement, stiffness, stress, strain and Young's modulus between groups (3 levels; paretic, non-paretic and individuals without neurological impairment) and within-subject (10 levels; 10% intervals of PF MVIC). To minimise type I error with the additional outcome measures (as could occur with repeated ANOVA tests), a MANOVA was used to compare the differences and interactions in the joint torque, force, architectural, and tendon displacement, stiffness, stress, strain and Young's modulus at 645 N, of the paretic vs. non-paretic limbs vs. the dominant limb of control individuals. If a significant interaction was found, independent post-hoc t-tests (with Bonferonni corrections) were used to run paired comparisons. Where assumptions of sphericity were violated, a Greenhouse-Geisser correction was used prior to identifying differences in the data. Statistical significance was accepted at the $P < 0.05$. Effect size is reported as partial eta squared ($p\varepsilon^2$) and data are presented as the mean (SD), unless stated otherwise.

7.4 Results

No group differences in participant age (P = 0.575), stature (P = 0.604) and body mass (P = 0.061) were identified when comparing individuals with SCP and those without neurological impairment.

7.4.1 Tendon length, CSA and moment arm

With the ankle secured at 0 deg, GM tendon length was not different between the paretic limb, non-paretic limb and individuals without neurological impairment. Similarly moment arm length estimated at 0 deg, was also found not to be different between groups (Table 7.1; P = 0.698 and P = 0.302, respectively). Achilles tendon CSA was different between groups (Table 7.1; P = 0.001, $\eta\rho^2$ = 0.378) and post hoc pairwise comparisons showed that the Achilles tendon CSA of individuals without neurological impairment was 23% and 38% larger than the non-paretic and paretic tendons (P = 0.042 and P = 0.001, respectively). Interestingly however, there was no difference between the paretic and non-paretic Achilles tendon CSA (P = 0.344).

Table 7.1. Structural properties and moment arm of the Achilles tendon during rest in the paretic and non-paretic limbs of individuals with SCP, and individuals without neurological impairment.

	Paretic limb	Non-paretic limb	Controls
Tendon length (mm)	235 (26.7)	229 (22.9)	238 (25.0)
Tendon CSA (mm^2)	42.2 (12.6)*	52 (11.7)[†]	67.6 (17.2)
Moment arm length (cm)	6.05 (1.69)	5.08 (0.98)	5.54 (1.56)

Controls refer to individuals without neurological impairment. *Difference between paretic and controls (P = 0.001). [†]Difference between non-paretic groups and controls (P = 0.042).

7.4.2 Tendon force-elongation relation

The GM MTJ of the Achilles tendon moved proximally as force increased throughout the ramped PF MVIC in a curvilinear fashion (Figure 7.1). For both GM tendon force and elongation variables, there was a group by force interaction effect (P = 0.002, $\eta\rho^2$ = 0.334 and P = 0.010, $\eta\rho^2$ = 0.239, respectively). T-tests revealed that there was no difference between the non-paretic tendon forces or elongation throughout the PF MVIC, compared to the tendon of individuals without neurological impairment (both P > 0.05). The paretic tendon force was different from 10-100% of MVIC when compared to the non-paretic tendon (P < 0.01) and individuals without neurological impairment (P < 0.001). When assessing force at PF MVIC (Table 7.2), individuals without neurological impairment (P < 0.001) and

non-paretic tendon force (P = 0.006) was over two-fold greater than the paretic GM tendon force.

The elongation properties of the GM tendon followed a similar pattern whereby the paretic tendon elongation was different from 20-100% intervals of MVIC when compared to the non-paretic tendon (P < 0.05) and individuals without neurological impairment (P < 0.01). The paretic GM tendon elongated approximately half the distance of the non-paretic tendon (P = 0.015) and tendon individuals without neurological impairment (P = 0.005) travelled at PF MVIC (Table 7.2).
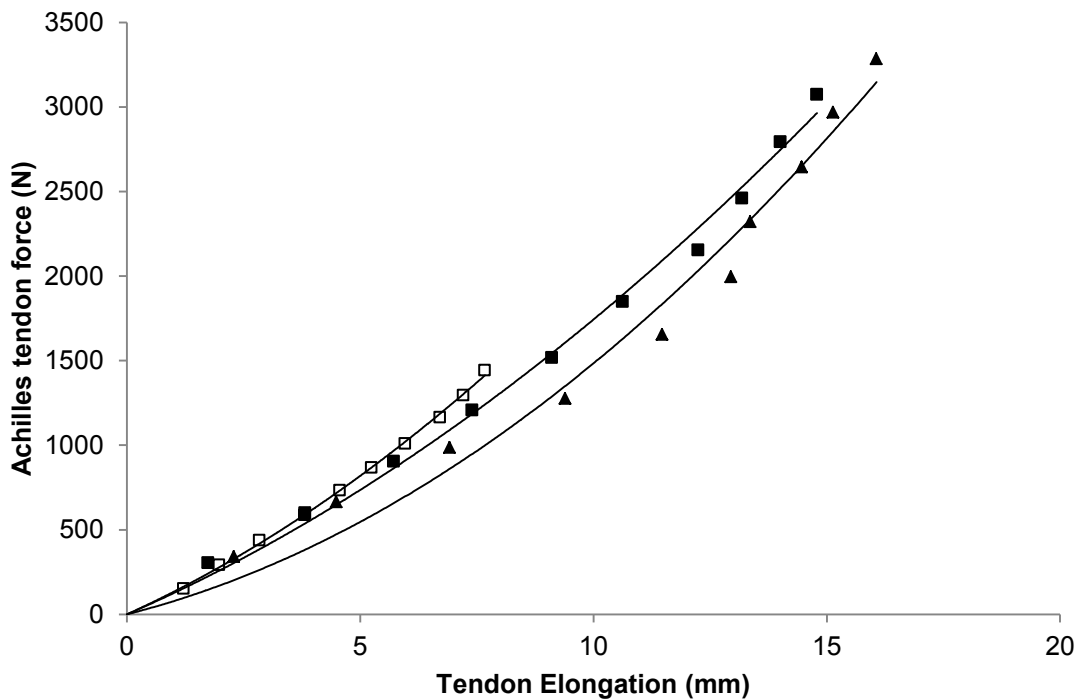
Figure 7.1. Achilles tendon force-elongation relationship in paretic (open squares) and non-paretic (closed squares) limbs and in individuals without neurological impairment (closed triangles). Error bars have been omitted for clarity.

7.4.3 Tendon stress-strain relation

Whereas tendon stress was similar between the paretic and non-paretic tendon and individuals without neurological impairment throughout the PF MVIC (P = 0.092), there was a tendon strain group-by-force interaction (P = 0.025, $\eta\rho^2$ = 0.202). Indeed, figure 7.2 shows that the paretic tendon strain between 20-100% of PF MVIC was lower than non-paretic tendon (P < 0.05) and individuals without neurological impairment (P < 0.05). Table 7.2 shows the strain placed upon the paretic tendon at PF MVIC was 20% and 30% less than the non-paretic limb (P = 0.029) and that of individuals without neurological impairment (P = 0.014),

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respectively. No differences in tendon strain were identified between the non-paretic limb and individuals without neurological impairment (P > 0.05).
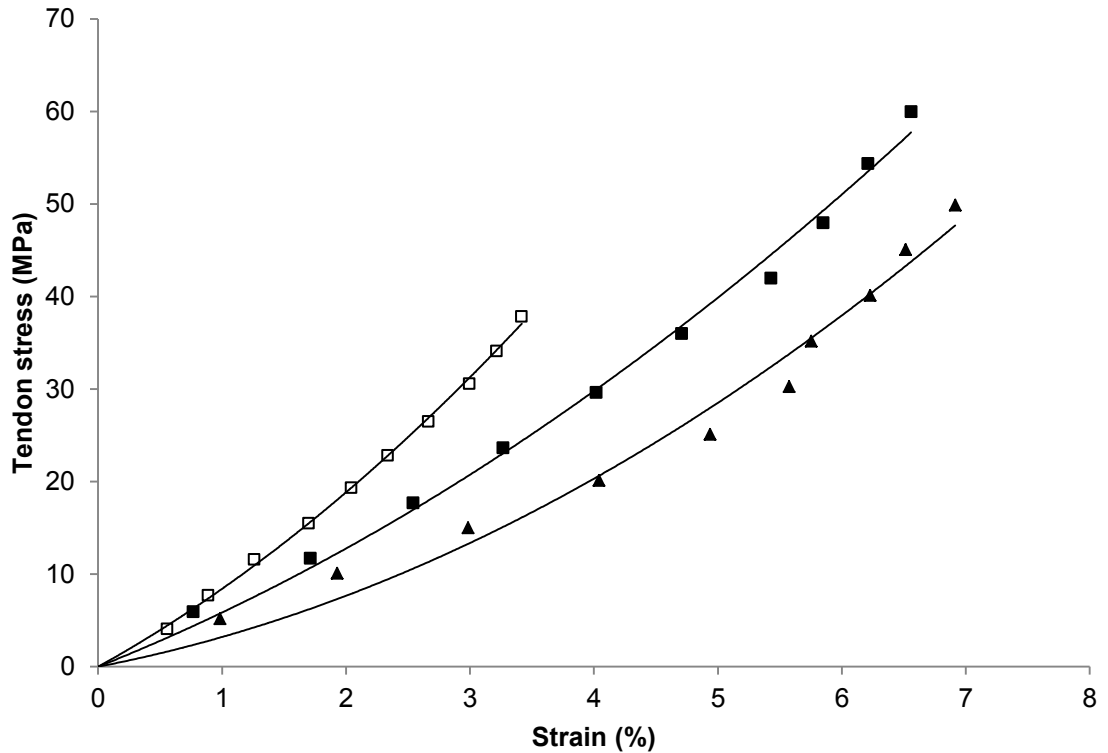
Figure 7.2. Achilles tendon stress-strain relationship in paretic (open squares) and non-paretic (closed squares) limbs and in individuals without neurological impairment (closed triangles). Error bars have been omitted for clarity.

7.4.4 Tendon stiffness

Figure 7.1 demonstrates how the paretic tendon stiffness follows a similar path to non-paretic tendon and tendon of individuals without neurological impairment. However the length of the paretic stiffness line at each 10% interval was

considerably shorter than non-paretic tendon and tendon of individuals without neurological impairment, resulting in a group-by-force interaction effect (P = 0.002, $\eta\rho^2$ = 0.343). The non-paretic tendon stiffness did not differ from that of the paretic tendon (P > 0.05) or of individuals without neurological impairment (P > 0.05). Contrary to this, the stiffness of the paretic limb was different at each 10% interval to PF MVIC compared with the tendon of individuals without neurological impairment (P < 0.001). Furthermore, at MVIC, the tendon of individuals without neurological impairment was over three-fold stiffer than the paretic tendon (P = 0.001; table 7.2).

7.4.5 Tendon Young's modulus

An interaction effect between group and force was identified in terms of Young's modulus (P = 0.009, $\eta\rho^2$ = 0.264). Figure 7.2 illustrates how the length of the paretic tendon curve is considerably shorter compared to the non-paretic and tendon of individuals without neurological impairment, however a difference from 60-100% of PF MVIC was only exhibited between the Young's modulus of the paretic tendon and that of individuals without neurological impairment (P < 0.05). At PF MVIC, table 7.2 shows the tendon of individuals without neurological impairment was double the stiffness of the paretic tendon (P = 0.009). No difference was found throughout the PF MVIC between the paretic and non-paretic tendons (P > 0.05), and the non-paretic tendon compared to individuals without neurological impairment (P < 0.05).

Table 7.2. Elastic properties of the Achilles tendon at PF MVIC in the paretic and non-paretic limbs of individuals with SCP, and individuals without neurological impairment.

	Paretic limb	Non-paretic limb	Controls
Achilles tendon force (N)	1442 (499)[ab]	3075 (1680)	3285 (1040)
Tendon elongation (mm)	7.67 (6.00)[cd]	14.8 (6.49)	16.1 (6.58)
Tendon stiffness (N·mm^{-1})	696 (565)[e]	1312 (858)	2096 (1112)
Tendon stress (MPa)	37.8 (21.1)	60.0 (30.9)	49.9 (14.0)
Tendon strain (%)	3.42 (3.07)[fg]	6.56 (3.20)	6.92 (3.03)
Young's modulus (GPa)	3.76 (1.91)[h]	5.86 (3.49)	7.56 (3.94)

[a]Difference between paretic and non-paretic groups (P = 0.003). [b]Difference between paretic and control groups (P = 0.001). [c]Difference between paretic and non-paretic groups (P = 0.015). [d]Difference between paretic and control groups (P = 0.005). [e]Difference between paretic and control groups (P < 0.001). [f]Difference between paretic and non-paretic groups (P = 0.029). [g]Difference between paretic and control groups (P = 0.014). [h]Difference between paretic and control groups (P = 0.009).

7.4.6 Tendon properties at a standardised force of 645 N

To account for the deficit in force achieved by the paretic limb, a comparison was conducted at the PF MVIC tendon force achieved by the weakest participant across all limbs tested (Table 7.3). No difference between individuals without neurological impairment, the paretic tendon, non-paretic tendon were identified

when assessing absolute displacement (P = 0.131), stiffness (P = 0.525), strain (P = 0.236), or Young's modulus (P = 0.127). Nevertheless, tendon stress was different between groups (P = 0.001, $\eta\rho^2$ = 0.371), with the paretic tendon stress being 39% higher than that recorded in individuals without neurological impairment.

Table 7.3. Elastic properties of the Achilles tendon at a standardised MVIC force (645 N) in the paretic and non-paretic limbs of individuals with SCP, and individuals without neurological impairment.

	Paretic limb	Non-paretic limb	Controls
Tendon elongation (mm)	3.77 (1.88)	4.63 (1.65)	5.52 (2.31)
Tendon stiffness (N·mm^{-1})	392 (274)	291 (158)	360 (182)
Tendon stress (MPa)	16.6 (5.11)*	13.0 (2.78)	10.1 (2.42)
Tendon strain (%)	1.65 (0.97)	2.06 (0.81)	2.36 (1.05)
Young's modulus (GPa)	2.22 (1.55)	1.35 (0.76)	1.36 (0.86)

Controls refer to individuals without neurological impairment. *Difference between paretic and controls (P = 0.001).

7.5 Discussion

The purpose of the present study was to establish whether *in vivo* differences exist in the elastic properties of the paretic tendon of individuals with SCP during a ramped PF MVIC, compared to the contralateral non-paretic limb and the

dominant limb of age-matched controls without neurological impairment. The primary findings showed that the GM tendon standardised force elastic properties and Young's modulus (i.e. assessed at 645 N) are not different from the contralateral non-paretic limb or the limb of individuals without neurological impairment. Due to the competitively lower force generated by the paretic limbs compared to the control limbs, at PF MVIC, the paretic GM tendon was more compliant compared to individuals without neurological impairment. When the tendon force-elongation relation was made relative to the structural properties of the tendon CSA and length to calculate stress and strain, respectively, the Young's modulus at PF MVIC showed that the paretic tendon of individuals with SCP was up to two times stiffer than the tendon of age-matched controls without neurological impairment.

The elongation of the paretic GM tendon at PF MVIC travelled approximately half the distance of the non-paretic tendon and individuals without neurological impairment. At face value, such a difference in displacement would result in the paretic tendon being reported as having stiffer material properties (Onambele et al. 2006; Pearson and Onambele 2005) than the elastic structures of the dominant limb of age-matched controls. However as the tendon deforms relative to the force generated by the contractile component, it may be considered that the primary difference in the force-elongation and stress-strain relation in the present study is due to lower forces generated as a result of impaired neural signalling to the paretic muscle of individuals with SCP. Previously in Chapter 5 and 6 the paretic muscle of individuals with hemiplegic SCP have been shown to produce lower

torques than the non-paretic contralateral limb and the dominant limb of age-matched controls without neurological impairment primarily as a result of impaired activation of the contractile tissue (Elder et al. 2003; Stackhouse et al. 2005). In individuals without neurological impairment, lower torques from weaker individuals have been reported to follow a similar pattern when tendon force is calculated (O'Brien et al. 2010b). Therefore, any apparent difference in the material properties of the paretic GM tendon is due to greater strength of the non-paretic limb and control participants without neurological impairment.

As a result of weakness, many studies have assessed the effects of the morphological properties that are linked with muscle strength (Aagaard et al. 2001), however no such study has assessed the effects of the in-series elastic component. The present study identified no differences between resting moment arm and GM tendon length between the limbs of individuals with SCP and individuals without neurological impairment. These similarities in GM tendon length is consistent with findings construed from Mohaghegi et al. (2007), but are in contrast to the clinical definition of the paretic muscle, as it is often referred to as being 'shorter in length' (Graham and Selber 2003), thus suggesting that the tendon would be longer when accounting for the deficit in muscle length. The observed deviation in findings is likely to be due to the method employed to assess tendon length; Malaiya et al. (2007) did not account for ankle angle, whereas in the present study ankle and knee angle was secured at the participants anatomical zero and full extension, respectively. Tendon lengths have not been reported to be different between individuals with spinal cord injury when

joint angle was controlled for, but tendon CSA was also found to be 17% smaller when compared to age-matched controls without neurological impairment (Maganaris et al. 2006). This reduction in tendon CSA resulted in the tendon being more compliant than individuals without neurological impairment, which is similar to the findings in the present study at PF MVIC.

The *in vivo* tendon stress transmitted through the GM tendon at PF MVIC ranged from 38-60 MPa and was not different across the different limbs tested in the present study, which is similar to previous findings in healthy men without neurological impairment (32 MPa; Maganaris and Paul 2002). Although the data presented is comparable to results previously reported by others, the level of variability in the paretic limb is relatively high in comparison to the control groups across all variables presented, which is likely to be due to the range of impairment across individuals with SCP. Nevertheless, in cadavers of individuals without neurological impairment, the stresses recorded at Achilles tendon failure were shown to be velocity dependant, ranging between 71 and 86 MPa (Wren et al. 2001). Whether these *in vitro* findings in stress failure apply to the paretic tendon is debateable as the *in vivo* strain at PF MVIC was lower than that reported in the control limbs. Resultantly, the Young's modulus of the paretic tendon was lower when compared to the tendon of individuals without neurological impairment. This may suggest that the internal collagen and/or extracellular matrix composition of the tendon is altered, similar to the reported differences in the composition of the paretic muscle of individuals with SCP (Friden and Lieber 2003; Lieber et al. 2003; Lieber et al. 2004), however it is likely to be due to the large difference in tendon

force between groups. Maganaris et al. (2006) identified this as being a considerable factor when assessing the tendon properties individuals with spinal cord injury and presented Young's modulus calculated at a common force achieved by all participants.

As with previous reports examining tendon properties, the data is regularly presented at MVIC (Abellaneda et al. 2009; Kongsgaard et al. 2011; Kubo et al. 2001b; Onambele et al. 2007; Onambele et al. 2006; Pearson and Onaimbele 2006; Reeves et al. 2003). With the large between group differences in PF MVIC force in the present study, a standardised force (645 N) was used to examine the elastic properties of the tendon. The standardised force compared to the data obtained from PF MVIC reflects that the paretic tendon is smaller rather than more compliant, as evidenced by a similar tendon stiffness, Young's modulus and higher a stress in the paretic limb compared to the control groups. This finding is consistent with the previous reports addressing the properties of the passive and active properties of the paretic muscle (as discussed in chapter 3 and 4). This may be a result of chronic loading on the tendon complex, as differing mechanical properties in the tendon may not be due solely to its CSA, but also improved structural integrity of collagen fibril packing, cross-linking and/or the ratio between collagen type I and III (Bailey et al. 1998; Kjær 2004; Wang 2006; Reed and Iozzo 2002). Based on the findings from the present study, this would suggest that the material properties of the paretic tendon are similar to that of the non-paretic tendon and that of individuals without neurological impairment. This is consistent with SCP muscle histological (Ito et al. 1996) and passive stiffness data (Chapter

4), which reports no difference compared to age matched controls. Therefore any difference in paretic tendon elastic properties in the present study can be attributed to regular physical exercise and changes at PF MVIC are due to the force applied to the tendon and its respective size.

7.6 Conclusion

This is the first study to assess the stiffness properties of the paretic tendon in hemiplegic individuals with SCP, compared to the non-paretic limb and dominant limb of individuals without neurological impairment. At PF MVIC paretic tendon stiffness and Young's modulus was found to be more compliant compared to the non-paretic and limb of individuals without neurological impairment. However, these findings are predominantly due to differences in tendon force and strain, as when standardised stiffness and Young's modulus were assessed no difference was identified between groups. This data suggests that active individuals with SCP are likely to have a similar intrinsic tendon composition compared to the control limbs a result of regular physical exercise.

Chapter 8

General discussion

The primary aim of this thesis was to ascertain the neuromuscular and tendon determinants of strength and range of motion (ROM) in the paretic limb of physically active adults with spastic cerebral palsy (SCP). Based on the existing literature, it is evident that there is no information available that addresses the underpinning neuromuscular and tendon physiology concerning muscular weakness and decreased ROM in the paretic limbs in adult individuals with SCP (Chapter 2). Prior to the work conducted in the present thesis, the majority of the information addressing SCP was based on children and adolescents that have previously been described as a sedentary population (Longmuir and Bar-Or 2000). In the assessment of the muscle and tendon, it has been shown that the effects of disuse have a negative impact on the neural and structural properties of the contractile and elastic structures (de Boer et al. 2007; Maganaris et al. 2006; Narici and Cerretelli 1998; Narici and Maganaris 2007; Sargeant et al. 1977; de Boer et al. 2008). This may exacerbate the severity of the SCP impairment as a result of the influences sedentary lifestyles have on functional performance. In order to address this notion of elevated impairment as a result of a sedentary lifestyle, the present thesis aimed to address the following aims in active men with SCP:

1. To assess whether testing posture has an effect on the reliability of passive ROM and stiffness measurements of the muscle.

2. To determine the passive ROM and stiffness properties of the paretic muscle, and examine whether anatomical cross sectional area (ACSA) and/or muscle length have an influence of the passive properties.

3. To ascertain the degree of muscle weakness in men with SCP, and to assess whether muscle size, agonist activation and/or antagonist activation can account for the deficits in strength.

4. To identify whether there is a difference in paretic muscle specific force *in vivo.*

5. To assess the *in vivo* structural and mechanical properties of the paretic tendon in men with SCP.

Prior to assessing individuals with SCP, it was suggested in Chapter 2 that it was unknown whether different testing postures have an influence on the measure of passive muscle properties. In Chapter 3, the inter- and intra-test reliability was assessed in the prone (Morse et al. 2008a) and seated (Kay and Blazevich 2009) postures in the assessment of passive muscle properties in individuals without neurological impairment. The results showed that the prone testing posture was not a reliable method to use when assessing the passive properties of muscle stiffness. Although it was not measured, by process of elimination, it was estimated that heel displacement and segmental translation of the participant's body during the passive dorsiflexion trial lead to large variances between testing day 1 and 2. The agreement between passive muscle stiffness and MTJ displacement were also poor, suggesting that the values obtained between the different postures are not comparable. In contrast, the seated posture was found to be a reliable measure of the passive muscular properties when statistically assessed by, ICC, CV and LoA. Unlike the prone posture the seated position controlled for the segmental translation and heel displacement simply because of

the back support the participant was fastened into during the passive stretch procedure. The specific variables identified as being reliable were; gastrocnemius medialis (GM) myotendinous junction (MTJ) displacement at a standardised passive torque and end ROM, and GM stiffness at a standardised force only.

Following the first experimental study of the thesis (Chapter 3), the seated posture was utilised in all subsequent chapters to assess the passive muscle properties of the paretic limb in active adults with SCP (Chapter 4). Surprisingly and in contrary to the hypothesis, the results showed that the paretic muscle of active individuals with SCP was not different from individuals without neurological impairment in terms of absolute passive GM stiffness (Chapter 4), GM specific force (Chapter 6), and standardised GM tendon stiffness and Young's modulus (Chapter 7). These results were independent of any neural influences directly confounding the measures as EMG (during passive protocols), and agonist and antagonist activation (during maximal voluntary isometric contraction (MVIC) protocols) were accounted for. Subsequently no difference was established between the paretic, non-paretic limbs and that of controls without neurological impairment, providing additional *in vivo* data to the clinical definition of active ambulatory men with hemiplegic SCP. However, when the passive properties of the muscle were quantified relative to the structural dimensions of the paretic muscle, the *in vivo* elastic modulus of the GM muscle was reported to be 2-fold stiffer than that of individuals without neurological impairment, which is similar to the results reported in children with SCP where muscle tendon unit (MTU) stiffness is higher than the control group (Alhusaini et al. 2010; Barber et al. 2011a; Vaz et al. 2006), however this is generally at the expense of reduced passive ROM, which is not the case in

the present cohort of active men with SCP. As no difference was identified in the *in vivo* passive strain, the increased elastic modulus was due to the increased stress placed upon the muscle. The greater stress was due to the smaller ACSA of the paretic GM of individuals with SCP. Therefore these differences from previous reports must be due to the activity and stretching status of physically active men with SCP in the present cohort. Potential mechanisms for these observations in passive muscle behaviour may be due to the regular effects of stretching 'dampening' the stretch reflex which is typically activated particularly when the joint reaches end ROM as a protective feature of SCP (Sheean 2002). Additionally as the paretic fibres rest at a reduced sarcomere length (Friden and Lieber 2003), regular stretching may have altered the number of sarcomeres in series over a period of time permitting the increased ROM available to the present paretic limb without the negative effects of casting immobilisation as previously reported (Brouwer et al. 2000; Sargeant et al. 1977). Whilst at a cellular level, the differing isoforms of the titin protein within SCP muscle in may also facilitate the changes observed within the present study (Magid and Law 1985; Neagoe et al. 2003), however, further research is required to confirm whether these mechanisms do impact on the passive properties of the paretic muscle.

In the present thesis two measures of stiffness have been included; tendon and passive muscle. The stiffness of the tendon is recorded during MVIC, and represents a functional measure to proprioception, balance and fascicular shortening under contraction (Maganaris et al. 2006; Onambele et al. 2006; Pearson and Onambele 2005), the passive stiffness represents an *in vivo* measure of the possible limitations to the diagnostic characteristic of ROM.

Although these measures represent the structural components of the MTU; tendon stiffness represents the compliance of the Achilles tendon under loading, and passive stiffness represents the passive components of the muscle and aponeurosis under stretch. Based on these distinctions, it is not surprising that tendon compliance is not associated with the passive properties of the muscle (Kubo et al. 2001a).

In relation to the active properties of the muscle, Chapter 5 quantified the strength of the paretic muscle of individuals with SCP whilst measuring agonist activation, antagonist coactivation and size of the gastrocnemius, compared to individuals without neurological impairment. Indeed PF MVIC torque of the paretic limb was 52% less than the torque generated by age-matched controls, which is similar to the findings from previous studies (Barber et al. 2012; Elder et al. 2003; Stackhouse et al. 2005). More interestingly, the deficit in torque within the paretic limb was associated with impaired activation of the gastrocnemius and to a lesser degree, lower muscle ACSA. Furthermore when a regression analysis was conducted on all of the determinants previously associated with muscle strength (Chapter 2), agonist activation was reported to account for 57% of the variation in PF MVIC torque. This data showed that agonist activation is a key determinant of PF MVIC torque in the paretic limb, and so is muscle ACSA but to a relatively lesser degree. Indeed, these findings coincide with the primary mechanism effecting functional performance and the severity of the impairment in individuals with SCP due to the lesion to the motor cortex. As a result of this central impairment resulting in abnormal signalling down the neural tracts, secondary impairments, within the skeletal muscle are established (Ito et al. 1996; Koman et

al. 2004; Lieber et al. 2003; Malaiya et al. 2007; McNee et al. 2009; Mohagheghi et al. 2007). The muscle fibre types in the paretic muscle of individuals with spinal cord lesion and stroke have been shown to have an increased proportion of type II fibres within the paretic musculature (Grimby et al. 1976; Sjostrom et al. 1980). This would suggest that as a result of impaired signalling to the paretic musculature, some motor units are unable to be innervated resulting in this deviation in fibre type expression and resultant fibre atrophy (Edström 1970), which may account for some of the differences observed in gastrocnemius size. With the disuse of high-threshold motor units, this would theoretically reduce the amount of cross bridges formed during contraction; explaining the reduced PF MVIC torque in the paretic musculature.

The findings from Chapter 5 would suggest that during MVIC, a failure to match the torque of participants without neurological impairment is almost entirely attributable to GM ACSA and agonist activation as previously discussed. However, a limitation in the measurement of MVIC torque and ACSA is that they do not account for differences in muscle architecture, muscle size, neural activation, and moment arm length, unless calculated separately. Alternatively, specific force describes the force generating capacity of a muscle whilst accounting for all of the factors associated to influence force measurements (Chapter 6). The findings showed that the specific force of the GM was not different compared to individuals without neurological impairment. This data suggests that in active adults with SCP that there is no impairment in material properties of the muscle at fascicle level, which provides indirect evidence to support the theory that secondary impairments reported within the muscle may be a result of disuse because children with SCP

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tend to be sedentary (Longmuir and Bar-Or 2000). Though whether this theory is true requires further research and should aim to directly assess whether the claims made in the present thesis regarding physically active vs sedentary individuals with SCP are accurate, now that physically active adults with SCP have been compared to active adults without neurological impairment.

As discussed in chapter 6, there were a number of limitations within the calculation of specific force. Fascicle length was measured at a comparable length rather than the angle at which peak torque occurred (Reeves et al. 2004b), due to the fact that 0 deg ankle angle was used to control for the different resting angles between the participants. As presented in individuals without neurological impairment the plantarflexors were on the ascending limb of the force length relation, it can be assumed that specific force was underestimated in the investigation (Maganaris et al. 2001; Morse et al. 2007b; Reeves et al. 2004b). However, in order to be able to compare between individuals that have limited dorsiflexion ROM, a consistent joint angle was chosen for the measurement of specific force at 0 degrees. In addition to this, in individuals without neurological impairment the difference in Achilles moment arm length during MVIC has been shown to be approximately 1.5 cm longer than when measured during rest (Maganaris et al. 1998a), which would have overestimated Achilles tendon force and specific force.

Despite limitations in strength and range of motion often being linked with the contractile tissue within the paretic MTU (Alhusaini et al. 2010; Elder et al. 2003; Stackhouse et al. 2005; Vaz et al. 2006), the tendon plays an integral part within

the MTU as it deforms and transmits the contractile forces generated by the muscle to the bone, to enable joint movement (Maganaris and Paul 2002). The rate at which the force is transferred to the bone is due to the stiffness of the tendon structures (Reeves et al. 2003; Morse et al. 2005a; Pearson and Onaimbele 2006). Yet research into the *in vivo* structural and mechanical properties of the tendon in individuals with SCP is scarce. The findings from Chapter 7 reported that the tendon of adults with SCP at MVIC was more compliant than the structure of individuals without neurological impairment, and when Young's modulus was examined the tendon of individuals without neurological impairment was double the stiffness of the paretic tendon when assessed at plantarflexion MVIC. However, these differences in the tendon elastic properties at MVIC were largely confounded by the difference in the strain and force generated by the paretic limb, compared to the non-paretic limb and that of individuals without neurological impairment. Therefore, when the properties of the tendon where assessed at a standardised force (645 N), the paretic tendon stiffness and Young's modulus was similar to that of individuals without neurological impairment. Based on the data from chapter 7, it can be assumed that the intrinsic properties of the tendon across all experimental groups are similar. Tendon stress at 645 N was also greater in the paretic limb compared to individuals without neurological impairment as a result of the smaller CSA of the paretic tendon. The mechanism for this variation in the paretic tendon CSA can be related to data from spinal cord injuries (Maganaris et al. 2006) and training (Kubo et al. 2001b) studies. Kubo et al. (2001b), demonstrated that through increases in muscle MVIC torque and size after a 12 week isometric knee extension programme, the tendon stiffness and Young's modulus properties increased as a

result of the training, whereas tendon CSA did not change. This relationship between activity and tendon stiffness is also evident when activity is reduced; for example, in men that have had spinal cord paralysis ranging from 1.5-24 years the tendon CSA decreased as a result of the sustained decrease MVIC torque and subsequent forces applied to the paretic tendon over a chronic period of time (Maganaris et al. 2006). This finding can be directly applied to the present SCP model where the mechanism for impaired paretic tendon CSA is as a result of weakness in the paretic musculature.

From the data presented in this thesis (Chapters 4-7), it is clear that the primary impairment impacting on plantarflexor MVIC torque (Chapter 5) and GM force (Chapter 6) and tendon properties (Chapter 7) in individuals with SCP results from impaired neural communication from the central nervous system to the skeletal muscle, and to a lesser extent a decline in the contractile ACSA. As a result of this decrease in PF MVIC torque and muscle ACSA, this subsequently impacts on the size of the tendon (as explained later in the discussion). Although the findings were consistent in PF MVIC torque across chapters 4, 5, 6 and 7, there was inherent variability within the measure with a difference of approximately 20% between the trials (Table 8.1). The lowest MVC was recorded in the assessment of activation (Chapter 5), whereby both SCP and individuals without neurological impairment produced their lowest PF MVIC values compared to the other chapters. It is likely that this variability is one of the limitations of the interpolated twitch technique, and can be attributed to 1) the participants being apprehensive of the percutaneous electrical stimulation, and 2) the twitch application is applied during the plateau of an MVIC, which in this instance was lower than the MVIC

acquired from the trials used in Chapter 4 and 6. The lower PF MVIC value achieved during the tendon investigation (chapter 7), can be attributed to the lower torque that was achieved during an incremental or graded contraction as opposed to the maximal exertions during chapter 4 and 6, as has been suggested previously (Onambele et al. 2007). It should be noted, that although this variability exists during different types of PF MVIC trials, limits of agreement (3.52 ± 10.9 Nm), CV (6.39%) and ICC (0.942) analyses demonstrated that the seated PF MVIC method was reliable between test days 1 and 2.

Table 8.1. Variability of PF MVIC torque data from chapters 4-6.

PF MVIC torque (Nm)	Paretic limb	Non-paretic limb	Controls
Chapter 4	100 (57.2)	152 (49.9)	187 (26.9)
Chapter 5	79.5 (46.6)	138 (42.9)	167 (33.3)
Chapter 6	102 (55.8)	153 (47.7)	190 (25.7)
Chapter 7	83.9 (50.5)	139 (51.7)	165 (25.1)

With regard to the GM passive properties and whilst accounting for the neural influences there appeared to be no limitations to passive ROM, as with the findings in chapter 5, 6 and 7, any difference in stiffness were attributable to differences in muscle size. Contrary to notions that secondary impairment within muscle tissue occurs as a result of the neural impairment in children with SCP, this thesis provides evidence to demonstrate that the material properties of the muscle

and tendon are likely to be similar to that of age-matched active individuals without neurological impairment. To confirm the assumptions in the material properties of the paretic MTU in physically active men with SCP, future studies should take biopsy samples from active and sedentary individuals with SCP to assess whether physical status does impair the intrinsic properties of the muscle and expression of proteins such as titin. Nevertheless, the results from the present thesis should be used as normative values for individuals that are recovering from injury or as a reference to refer to when examining progress after the completion of an exercise intervention.

As stated in chapter 1, the spectrum of impairment within SCP alone is vast, ranging from independent and ambulant individuals that have minimal impairment to severely impaired patients that require care and are non-ambulatory. It is likely that the information presented within the current thesis applies predominantly to those individuals with CP that are ambulant. However, through the assessment of MTU function, it may be possible to determine whether similarities between ambulant and non-ambulant individuals with SCP exist. To help confirm the concepts developed from the results of this thesis, future work should compare physically active and sedentary individuals with SCP to ascertain whether habitual exercise is beneficial to the passive and/or active properties of the MTU, and performance tasks. It is understood that with resistance training, increases in muscle strength have consistently been reported in the paretic limb of children with SCP and linked with improved functional performance and gait (Damiano and Abel 1998; Damiano et al. 1995a; Damiano et al. 1995b), which in part may be linked to increases in muscle size (McNee et al. 2009). However, these studies did not

provide information regarding neural, architectural and morphological adaptation to resistance exercise in individuals with SCP. Understanding whether or to what extent muscle activation and coactivation in the paretic SCP muscle adapts with training may provide further information into designing specific interventions to enhance muscular strength. In elderly individuals without neurological impairment, heavy resistance strength training has been showed to increase neural activation as measured by surface EMG (Aagaard et al. 2001). Although such findings are related to impaired neural signalling to the muscle as a result of disuse, whether this information applies to individuals with SCP has yet to be determined.

In individuals with hemiplegic SCP, resistance training could benefit functional performance and independence based on the fact that the avoidance strategy of selectively loading the non-paretic limb may induce a detraining influence on the paretic limb. As with any disuse and reloading study (Narici and Cerretelli 1998; Sargeant et al. 1977; Stevens et al. 2006), there is likely to be an observable increase in activation in individuals with SCP who are sedentary in nature. It is unlikely that in the present SCP population that this activation increase would be observed with resistance training, as they are habitually active and familiar with the plantarflexor movement in both limbs. Therefore the lower activation levels in the present SCP cohort reflect the damage to the motor cortex and the descending neural pathways inherent as a result of the impairment; however this remains to be confirmed experimentally.

Within the SCP research, the mode at which strength is typically assessed is completed by performing isometric contractions to assess the MVIC capabilities of individuals with SCP at a set angle (Damiano and Abel 1998; Damiano et al. 1995b). Although isometric contractions can provide reliable information relating to muscular strength, it does not provide any information on the dynamic properties of the contractile properties throughout the available ROM. As spasticity is defined as a velocity dependent resistance to stretch and is associated with impaired ROM and muscular weakness (Graham and Selber 2003; Sheean 2002; Sheean and McGuire 2009), isokinetic strength tests are able to examine the variability of these two variables simultaneously. Previous studies have only assessed the effects of strength training in relation to isokinetic strength, whereby the subsequent increases in isokinetic strength have been associated with gait, function (MacPhail and Kramer 1995; McCubbin and Shasby 1985; Sharp and Brouwer 1997). Similarly, the MTU of individuals without neurological impairment are suggested to exhibit viscous and elastic properties and have been suggested to adapt acutely to exercise or stretch interventions (Magnusson et al. 2008). Changes in tissue temperature and/or conditioning exercises have been suggested to change the viscoelastic properties of the muscle (Maganaris 2003; Morse et al. 2008a; Magnusson et al. 1997). Morse et al. (Morse et al. 2008a) reported that after five passive stretches (which consisted of the MTU being taken to end ROM and being held for 1 min), a 21% increase in MTJ displacement accounted for the entire 17% increase in ROM. Whether such information applies to individuals with SCP is debateable as the benefits of manual and passive stretching are conflicting (Pin et al. 2006). Although the information in stretching in SCP is limited and inconsistent,

improving strength through an increased functional range of motion in the paretic limb may improve motor function in the paretic limbs of individuals with SCP.

As a result of impaired foot positioning during the swing phase of gait (Dietz et al. 1981), increased tendon compliance at PF MVIC (Chapter 7), and muscular weakness (Chapter 5) it is possible that falls may occur during such day-to-day activities. The prevalence of injuries in elderly populations as a result of accidental falls is the fifth leading cause of mortality, with the aetiology resulting in weakness, balance deficits and gait deficiencies (Rubenstein 2006). Throughout the thesis it has been established that similar deficits exist in individuals with SCP. Weakness of individuals with SCP reside not only from inactivity, but in the paretic limbs the employment of avoidance strategies result in further weakening of the MTU (Chapter 5 and 7) and also the bone due to disuse oesteoperosis (Takata and Yasui 2001). Although it is evident that individuals with SCP are likely to be subject to an increased risk of injury, a fundamental determinant related to limiting falls and improving balance in elderly individuals is elastic properties of the tendon (Narici et al. 2005; Karamanidis et al. 2008; Onambélé et al. 2008; Onambele et al. 2006; Reeves 2006). Onambele et al. (2006) demonstrated that the stiffness of elastic component during single leg balancing trials was associated with single leg balance ability in elderly individuals. As elderly individuals have more compliant tendons at MVIC, the tendon must elongate further for any given force it is subjected to subsequently reducing the transmission time of the forces from the contractile tissue to the bone. Although it is likely that balance impairments in ambulant individuals with SCP are prevalent (Bax et al. 2005), further research is

required to determine whether this data actually applies to the participants studied within the present thesis.

Conclusion

In summary, the present thesis aimed to examine the neuromuscular determinants of strength and passive properties of the tendon and muscle in physically active men hemiplegic SCP. Prior to the investigation of the neuromuscular and tendon properties in individuals with SCP, an initial reliability investigation was required (as highlighted in Chapter 2) to discriminate between the reliability of the prone (Morse 2011; Morse et al. 2008a) and seated postures (Kay and Blazevich 2009) used to assess the passive properties of the plantarflexor musculature (Chapter 3). The elastic properties of the muscle were examined during passive stretch conditions at 22 Nm (the highest passive torque attained by all participants) and end ROM and the inter-postural agreement between the seated and prone postures were too low to make any comparison between the postures. Similarly, the intra-postural assessment of the prone posture was found to be unreliable. The seated posture, however, was found to be a highly reliable posture when assessing GM MTJ displacement, GM stiffness and ankle angle. Although the results at end ROM may be subject to psychological and neural confounding factors (McHugh et al. 1998; McNair and Stanley 1996; Toft et al. 1989) impacting on GM MTJ displacement and GM stiffness measures, presenting these variables should be done with an understanding of the increased level of error.

As a direct result of the findings from chapter 3, the first experimental study on individuals with SCP aimed to assess the passive properties of the muscle (Chapter 4). Surprisingly it was identified that no impairment in ROM was observed, subsequently providing additional in vivo data to the clinical definition of individuals with SCP. When the smaller CSA of individuals with SCP was accounted for, the passive stress of the muscle was significantly higher than the contralateral non-paretic muscle and the muscle of individuals without neurological impairment, subsequently resulting in a stiffer elastic modulus in the paretic GM. Therefore, throughout the same ROM a smaller GM CSA in physically active adults with SCP have to dissipate larger relative torque compared to the control muscles, consequently causing the muscle to elongate to the same extent as the non-paretic muscle under stretch. In this particular physically active SCP population it is possible that their regular training may have facilitated these observed findings.

Having considered the difference in the paretic GM CSA compared to the control groups (chapter 4), chapter 5 aimed to address the effects muscle size, activation and coactivation have on PF MVIC torque across the paretic, non-paretic and control groups. The data from active adults with hemiplegic SCP showed that muscle weakness as a result of muscular and neural differences in the paretic limb. This was confirmed as PF MVIC torque was on average 47% weaker than the non-paretic limb and control group. The paretic gastrocnemius ACSA was smaller compared to the control group, though no difference was observed

between paretic and non-paretic limbs. The paretic limb demonstrated an inability to innervate the PF muscles and increased antagonist coactivation was also evident when compared to the non-paretic limb and control group. Although the differences between the paretic limb and control group were identified, the multiple regression on the paretic limb identified that muscle activation was a significant predictor of gastrocnemius PF MVIC torque. This demonstrates that the key determinant of weakness in individuals with SCP is their ability to activate the gastrocnemius muscle, regardless of the differences identified in paretic gastrocnemius ACSA and antagonist coactivation.

As a result of the identified neural and muscular differences in the paretic limb (chapter 5), the natural progression was to account for muscle size, neural, moment arm length and muscle architectural factors affect PF MVIC and calculate the intrinsic force producing capacity of the GM muscle. The findings from chapter 6 showed that the paretic GM of physically active individuals with SCP has a similar specific force generating capacity to the non-paretic muscle and the GM of control individuals. This study also demonstrates how the pennation angle and fascicle length of the paretic muscle at MVIC were different from the control group. Nevertheless, weakness observed in the paretic GM is not due to the intrinsic material properties of the muscle, but because of the smaller PCSA of the muscle.

As the majority of research examining SCP impairment is conducted on the active and passive neuromuscular properties *in vivo* (Chapter 4, 5 and 6), chapter 7 was the first study to provide data on the elastic properties of the tendon. The data

showed that at PF MVIC, the paretic tendon stiffness and Young's modulus was found to be more compliant compared to the non-paretic and limb of individuals without neurological impairment. However, these findings are predominantly due to differences in tendon force and strain, as when standardised stiffness and Young's modulus were assessed no difference was identified between groups. This data suggests that active individuals with SCP are likely to have a similar intrinsic tendon composition compared to the control limbs a result of regular physical exercise.

References

Aagaard P, Andersen JL, Dyhre-Poulsen P, Leffers AM, Wagner A, Peter Magnusson S, Halkjær-Kristensen J, Simonsen EB (2001) A mechanism for increased contractile strength of human pennate muscle in response to strength training: Changes in muscle architecture. Journal of Physiology 534 (2):613-623.

Abellaneda S, Guissard N, Duchateau J (2009) The relative lengthening of the myotendinous structures in the medial gastrocnemius during passive stretching differs among individuals. Journal of Applied Physiology 106 (1):169-177.

Ada L, Canning CG, Low SL (2003) Stroke patients have selective muscle weakness in shortened range. Brain 126 (3):724-731.

Adams GR, Hather BM, Dudley GA (1994) Effect of short-term unweighting on human skeletal muscle strength and size. Aviat Space Environ Med 65 (12):1116-1121.

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

Alfredson H, Pietilä T, Jonsson P, Lorentzon R (1998) Heavy-Load Eccentric Calf Muscle Training For the Treatment of Chronic Achilles Tendinosis. The American Journal of Sports Medicine 26 (3):360-366.

Alhusaini AAA, Crosbie J, Shepherd RB, Dean CM, Scheinberg A (2010) Mechanical properties of the plantarflexor musculotendinous unit during passive dorsiflexion in children with cerebral palsy compared with typically developing children. Developmental Medicine and Child Neurology 52 (6):E101-E106.

Alkner BA, Tesch PA (2004) Efficacy of a gravity-independent resistance exercise device as a countermeasure to muscle atrophy during 29-day bed rest. Acta Physiol Scand 181 (3):345-357.

Allen GM, Gandevia SC, McKenzie DK (1995) Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. Muscle and Nerve 18 (6):593-600.

Andersson C, Grooten W, Hellsten M, Kaping K, Mattsson E (2003) Adults with cerebral palsy: walking ability after progressive strength training. Developmental Medicine and Child Neurology 45 (4):220-228.

Atkinson G, Nevill AM (1998) Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Medicine 26 (4):217-238.

Ayalon M, Ben-Sira D, Hutzler Y, Gilad T (2000) Reliability of isokinetic strength measurements of the knee in children with cerebral palsy. Developmental Medicine and Child Neurology 42 (6):398-402.

Bailey AJ, Paul RG, Knott L (1998) Mechanisms of maturation and ageing of collagen. Mech Ageing Dev 106 (1-2):1-56.

Bampouras TM, Reeves ND, Baltzopoulos V, Maganaris CN (2006) Muscle activation assessment: Effects of method, stimulus number, and joint angle. Muscle and Nerve 34 (6):740-746.

Barber L, Barrett R, Lichtwark G (2011a) Passive muscle mechanical properties of the medial gastrocnemius in young adults with spastic cerebral palsy. J Biomech 44 (13):2496-2500.

Barber L, Barrett R, Lichtwark G (2011b) Validity and reliability of a simple ultrasound approach to measure medial gastrocnemius muscle length. Journal of Anatomy 218 (6):637-642.

Barber L, Barrett R, Lichtwark G (2012) Medial gastrocnemius muscle fascicle active torque-length and Achilles tendon properties in young adults with spastic cerebral palsy. J Biomech.

Bax M, Goldstein M, Rosenbaun P, Leviton A, Paneth N, Dan B, Jacobsson B, Damiano D (2005) Proposed definition and classification of cerebral palsy, April 2005. Developmental Medicine and Child Neurology 47 (8):571-576.

Behm DG, St-Pierre DMM, Perez D (1996) Muscle inactivation: Assessment of interpolated twitch technique. Journal of Applied Physiology 81 (5):2267-2273.

Binder-Macleod SA, McDermond LR (1992) Changes in the force-frequency relationship of the human quadriceps femoris muscle following electrically and voluntarily induced fatigue. Physical Therapy 72 (2):95-104.

Bishop D (1997) Reliability of a 1-h endurance performance test in trained female cyclists. Medicine and Science in Sports and Exercise 29 (4):554-559.

Bland JM, Altman DG (1986) Regression analysis. Lancet 1 (8486):908-909.

Blundell SW, Shepherd RB, Dean CM, Adams RD, Cahill BM (2003) Functional strength training in cerebral palsy: a pilot study of a group circuit training class for children aged 4-8 years. Clinical Rehabilitation 17 (1):48-57.

Booth CM, Cortina-Borja MJF, Theologis TN (2001) Collagen accumulation in muscles of children with cerebral palsy and correlation with severity of spasticity. Developmental Medicine and Child Neurology 43 (5):314-320.

Bottinelli R, Pellegrino MA, Canepari M, Rossi R, Reggiani C (1999) Specific contributions of various muscle fibre types to human muscle performance: An in vitro study. Journal of Electromyography and Kinesiology 9 (2):87-95.

Bressel E, McNair PJ (2002) The effect of prolonged static and cyclic stretching on ankle joint stiffness, torque relaxation, and gait in people with stroke. Physical Therapy 82 (9):880-887.

Brouwer B, Davidson LK, Olney SJ (2000) Serial casting in idiopathic toe-walkers and children with spastic cerebral palsy. Journal of Pediatric Orthopaedics 20 (2):221-225.

Brown JK, Rodda J, Walsh EG, Wright GW (1991) Neurophysiology of lower-limb function in hemiplegic children. Developmental Medicine and Child Neurology 33 (12):1037-1047.

Brown P (1994) Pathophysiology of spasticity. Journal of Neurology Neurosurgery and Psychiatry 57 (7):773-777.

Burgess KE, Graham-Smith P, Pearson SJ (2009) Effect of acute tensile loading on gender-specific tendon structural and mechanical properties. Journal of Orthopaedic Research 27 (4):510-515.

Burtner PA, Qualls C, Woollacott MH (1998) Muscle activation characteristics of stance balance control in children with spastic cerebral palsy. Gait Posture 8 (3):163-174.

Canning CG, Ada L, O'Dwyer N (1999) Slowness to develop force contributes to weakness after stroke. Archives of Physical Medicine and Rehabilitation 80 (1):66-70.

Cans C, De-la-Cruz J, Mermet MA (2008) Epidemiology of cerebral palsy. Paediatrics and Child Health 18 (9):393-398.

Cornbleet SL, Woolsey NB (1996) Assessment of hamstring muscle length in school-aged children using the sit-and-reach test and the inclinometer measure of hip joint angle. Physical Therapy 76 (8):850-855.

Curran-Everett D, Benos DJ (2004) Guidelines for reporting statistics in journals published by the American Physiological Society. American Journal of Physiology - Advances in Physiology Education 28:85-87.

Dadebo B, White J, George KP (2004) A survey of flexibility training and hamstring strains in professional football clubs in England. British Journal of Sports Medicine 38 (4):388-394.

Damholt V, Termansen NB (1978) Asymmetry of plantar flexion strength in the foot. Acta Orthop Scand 49 (2):215-219.

Damiano D, Abel M, Romness M, Oeffinger D, Tylkowski C, Gorton G, Bagley A, Nicholson D, Barnes D, Calmes J, Kryscio R, Rogers S (2006) Comparing functional profiles of children with hemiplegic and diplegic cerebral palsy in GMFCS Levels I and II: Are separate classifications needed? Developmental Medicine and Child Neurology 48 (10):797-803.

Damiano DL (1993) Reviewing muscle cocontraction: Is it a developmental, pathological, or motor control issue? Physical and Occupational Therapy in Pediatrics 12 (4):3-20.

Damiano DL, Abel MF (1998) Functional outcomes of strength training in spastic cerebral palsy. Archives of Physical Medicine and Rehabilitation 79 (2):119-125.

Damiano DL, Kelly LE, Vaughn CL, Westcott SL, Lowes LP (1995a) Effects of quadriceps femoris muscle strengthening on crouch gait in children with spastic diplegia. Physical Therapy 75 (8):658-671.

Damiano DL, Martellotta TL, Sullivan DJ, Granata KP, Abel MF (2000) Muscle force production and functional performance in spastic cerebral palsy: Relationship of cocontraction. Archives of Physical Medicine and Rehabilitation 81 (7):895-900.

Damiano DL, Quinlivan J, Owen BF, Shaffrey M, Abel MF (2001) Spasticity versus strength in cerebral palsy: relationships among involuntary resistance, voluntary torque, and motor function. European Journal of Neurology 8:40-49.

Damiano DL, Vaughan CL, Abel MF (1995b) Muscle response to heavy resistance exercise in children with spastic cerebral palsy. Dev Med Child Neurol 37 (8):731-739.

de Boer MD, Maganaris CN, Seynnes OR, Rennie MJ, Narici MV (2007) Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. Journal of Physiology 583 (3):1079-1091.

de Boer MD, Seynnes OR, di Prampero PE, Pišot R, Mekjavić IB, Biolo G, Narici MV (2008) Effect of 5 weeks horizontal bed rest on human muscle thickness and architecture of weight bearing and non-weight bearing muscles. European Journal of Applied Physiology 104 (2):401-407.

Del Santo Jr M, Marches F, Ng M, Hinton RJ (2000) Age-associated changes in decorin in rat mandibular condylar cartilage. Arch Oral Biol 45 (6):485-493.

Dietz V, Quintern J, Berger W (1981) Electrophysiological studies of gait in spasticity and rigidity: evidence that altered mechanical properties of muscle contribute to hypertonia. Brain 104 (SEP):431-449.

Dodd KJ, Taylor NF, Graham HK (2003) A randomized clinical trial of strength training in young people with cerebral palsy. Developmental Medicine and Child Neurology 45 (10):652-657.

Dressler MR, Butler DL, Wenstrup R, Awad HA, Smith F, Boivin GP (2002) A potential mechanism for age-related declines in patellar tendon biomechanics. Journal of Orthopaedic Research 20 (6):1315-1322.

Edström L (1970) Selective changes in the sizes of red and white muscle fibres in upper motor lesions and Parkinsonism. J Neurol Sci 11 (6):537-550.

Elder GCB, Stewart G, Cook K, Weir D, Marshall A, Leahey L (2003) Contributing factors to muscle weakness in children with cerebral palsy. Developmental Medicine and Child Neurology 45 (8):542-550.

Esformes JI, Narici MV, Maganaris CN (2002) Measurement of human muscle volume using ultrasonography. European Journal of Applied Physiology 87 (1):90-92.

Essex C (2003) Hyperbaric oxygen and cerebral palsy: No proven benefit and potentially harmful. Developmental Medicine and Child Neurology 45 (3):213-215.

Falk B, Usselman C, Dotan R, Brunton L, Klentrou P, Shaw J, Gabriel D (2009) Child-adult differences in muscle strength and activation pattern during isometric elbow flexion and extension. Applied Physiology, Nutrition and Metabolism 34 (4):609-615.

Friden J, Lieber RL (2003) Spastic muscle cells are shorter and stiffer than normal cells. Muscle & Nerve 27 (2):157-164.

Fukunaga T, Roy RR, Shellock FG, Hodgson JA, Edgerton VR (1996) Specific tension of human plantar flexors and dorsiflexors. Journal of Applied Physiology 80 (1):158-165.

Gage JR, Deluca PA, Renshaw TS (1995) Gait analysis: Principles and applications: Emphasis on its use in cerebral palsy. Journal of Bone and Joint Surgery - Series A 77 (10):1607-1623.

Gajdosik RL, Vander Linden DW, McNair PJ, Riggin TJ, Albertson JS, Mattick DJ, Wegley JC (2005) Viscoelastic properties of short calf muscle-tendon units of older women: Effects of slow and fast passive dorsiflexion stretches in vivo. European Journal of Applied Physiology 95 (2-3):131-139.

Gajdosik RL, Vander Linden DW, Williams AK (1999) Influence of age on length and passive elastic stiffness characteristics of the calf muscle-tendon unit of women. Physical Therapy 79 (9):827-838.

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81 (4):1725-1789.

García-Morales P, Buschang PH, Throckmorton GS, English JD (2003) Maximum bite force, muscle efficiency and mechanical advantage in children with vertical growth patterns. Eur J Orthod 25 (3):265-272.

Gemperline JJ, Allen S, Walk D, Rymer WZ (1995) Characteristics of motor unit discharge in subjects with hemiparesis. Muscle and Nerve 18 (10):1101-1114.

Gillette PD, Fell RD (1996) Passive tension in rat hindlimb during suspension unloading and recovery: Muscle/joint contributions. Journal of Applied Physiology 81 (2):724-730.

Given JD, Dewald JPA, Rymer WZ (1995) Joint dependent passive stiffness in paretic and contralateral limbs of spastic patients with hemiparetic stroke. Journal of Neurology Neurosurgery and Psychiatry 59 (3):271-279.

Gleim GW, Stachenfeld NS, Nicholas JA (1990) The influence of flexibility on the economy of walking and jogging. Journal of Orthopaedic Research 8 (6):814-823.

Graham HK, Selber P (2003) Musculoskeletal aspects of cerebral palsy. Journal of Bone and Joint Surgery - Series B 85 (2):157-166.

Grimby G, Broberg C, Krotkiewska I, Krotkiewski M (1976) Muscle fiber composition in patients with traumatic cord lesion. Scand J Rehabil Med 8 (1):37-42.

Guette M, Gondin J, Martin A (2005) Time-of-day effect on the torque and neuromuscular properties of dominant and non-dominant quadriceps femoris. Chronobiol Int 22 (3):541-558.

Häkkinen K, Kallinen M, Izquierdo M, Jokelainen K, Lassila H, Mälkiä E, Kraemer WJ, Newton RU, Alen M (1998) Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. Journal of Applied Physiology 84 (4):1341-1349.

Halbertsma JPK, Van Bolhuis AI, Göeken LNH (1996) Sport stretching: Effect on passive muscle stiffness of short hamstrings. Archives of Physical Medicine and Rehabilitation 77 (7):688-692.

Hanna SE, Rosenbaum PL, Bartlett DJ, Palisano RJ, Walter SD, Avery L, Russell DJ (2009) Stability and decline in gross motor function among children and youth with cerebral palsy aged 2 to 21 years. Developmental Medicine and Child Neurology 51 (4):295-302.

Harlaar J, Becher JG, Snijders CJ, Lankhorst GJ (2000) Passive stiffness characteristics of ankle plantar flexors in hemiplegia. Clinical Biomechanics 15 (4):261-270.

Harridge SDR, Kryger A, Stensgaard A (1999) Knee extensor strength, activation, and size in very elderly people following strength training. Muscle and Nerve 22 (7):831-839.

Hassani A, Patikas D, Bassa E, Hatzikotoulas K, Kellis E, Kotzamanidis C (2006) Agonist and antagonist muscle activation during maximal and submaximal isokinetic fatigue tests of the knee extensors. Journal of Electromyography and Kinesiology 16 (6):661-668.

Herbert RD, Gandevia SC (1999) Twitch interpolation in human muscles: Mechanisms and implications for measurement of voluntary activation. J Neurophysiol 82 (5):2271-2283.

Higbie EJ, Cureton KJ, Warren GL, Prior BM (1996) Effects of concentric and eccentric training on muscle strength, cross-sectional area, and neural activation. Journal of Applied Physiology 81 (5):2173-2181.

Hsu A-L, Tang P-F, Jan M-H (2002) Test-retest reliability of isokinetic muscle strength of the lower extremities in patients with stroke. Archives of Physical Medicine and Rehabilitation 83 (8):1130-1137.

Hunter SK, Thompson MW, Ruell PA, Harmer AR, Thom JM, Gwinn TH, Adams RD (1999) Human skeletal sarcoplasmic reticulum Ca2+ uptake and muscle function with aging and strength training. Journal of Applied Physiology 86 (6):1858-1865.

Ikeda AJ, Abel MF, Granata KP, Damiano DL (1998) Quantification of cocontraction in spastic cerebral palsy. Electromyogr Clin Neurophysiol 38 (8):497-504.

Impellizzeri FM, Bizzini M, Rampinini E, Cereda F, Maffiuletti NA (2008) Reliability of isokinetic strength imbalance ratios measured using the Cybex NORM dynamometer. Clinical Physiology and Functional Imaging 28 (2):113-119.

Ito J, Araki A, Tanaka H, Tasaki T, Cho K, Yamazaki R (1996) Muscle histopathology in spastic cerebral palsy. Brain & Development 18 (4):299-303.

Jones DA, Rutherford OM, Parker DF (1989) Physiological changes in skeletal muscle as a result of strength training. Q J Exp Physiol 74 (3):233-256.

Karamanidis K, Arampatzis A, Mademli L (2008) Age-related deficit in dynamic stability control after forward falls is affected by muscle strength and tendon stiffness. Journal of Electromyography and Kinesiology 18 (6):980-989.

Kawakami Y, Muraoka Y, Kubo K, Suzuki Y, Fukunaga T (2000) Changes in muscle size and architecture following 20 days of bed rest. Journal of gravitational physiology : a journal of the International Society for Gravitational Physiology 7 (3):53-59.

Kay AD, Blazevich AJ (2009) Moderate-duration static stretch reduces active and passive plantar flexor moment but not Achilles tendon stiffness or active muscle length. Journal of Applied Physiology 106 (4):1249-1256.

Kippers V, Parker AW (1987) Toe-touch test. A measure of its validity. Physical Therapy 67 (11):1680-1684.

Kjær M (2004) Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. Physiol Rev 84 (2):649-698.

Klein CS, Rice CL, Marsh GD (2001) Normalized force, activation, and coactivation in the arm muscles of young and old men. Journal of Applied Physiology 91 (3):1341-1349.

Klinge K, Magnusson SP, Simonsen EB, Aagaard P, Klausen K, Kjaer M (1997) The effect of strength and flexibility training on skeletal muscle electromyographic activity, stiffness, and viscoelastic stress relaxation response. American Journal of Sports Medicine 25 (5):710-716.

Koman LA, Smith BP, Shilt JS (2004) Cerebral palsy. Lancet 363 (9421):1619-1631.

Kongsgaard M, Nielsen CH, Hegnsvad S, Aagaard P, Magnusson SP (2011) Mechanical properties of the human Achilles tendon, in vivo. Clinical Biomechanics 26 (7):772-777.

Kuban KCK, Leviton A (1994) Cerebral palsy. N Engl J Med 330 (3):188-195.

Kubo K, Akima H, Kouzaki M, Ito M, Kawakami Y, Kanehisa H, Fukunaga T (2000) Changes in the elastic properties of tendon structures following 20 days bed-rest in humans. European Journal of Applied Physiology 83 (6):463-468.

Kubo K, Kanehisa H, Fukunaga T (2001a) Is passive stiffness in human muscles related to the elasticity of tendon structures? European Journal of Applied Physiology 85 (3-4):226-232.

Kubo K, Kanehisa H, Ito M, Fukunaga T (2001b) Effects of isometric training on the elasticity of human tendon structures in vivo. Journal of Applied Physiology 91 (1):26-32.

Labeit S, Kolmerer B (1995) Titins: Giant proteins in charge of muscle ultrastructure and elasticity. Science 270 (5234):293-296.

Lance JW (1981) Disordered muscle tone and movement. Clin Exp Neurol 18:27-35.

Lexell J, Downham D, Sjostrom M (1984) Distribution of different fibre types in human skeletal muscles. A statistical and computational study of the fibre type arrangement in M. vastus lateralis of young, healthy males. J Neurol Sci 65 (3):353-365.

Lexell J, Downham D, Sjostrom M (1986) Distribution of different fibre types in human skeletal muscles. Fibre type arrangement in m. vastus lateralis from three groups of healthy men between 15 and 83 years. J Neurol Sci 72 (2-3):211-222.

Lieber RL, Runesson E, Einarsson F, Friden J (2003) Inferior mechanical properties of spastic muscle bundles due to hypertrophic but compromised extracellular matrix material. Muscle & Nerve 28 (4):464-471.

Lieber RL, Steinman S, Barash IA, Chambers H (2004) Structural and functional changes in spastic skeletal muscle. Muscle and Nerve 29 (5):615-627.

Lippold OC (1952) The relation between integrated action potentials in a human muscle and its isometric tension. The Journal of physiology 117 (4):492-499.

Longmuir PE, Bar-Or O (2000) Factors influencing the physical activity levels of youths with physical and sensory disabilities. Adapted Physical Activity Quarterly 17 (1):40-53.

Macaluso A, Nimmo MA, Foster JE, Cockburn M, McMillan NC, De Vito G (2002) Contractile muscle volume and agonist-antagonist coactivation account for differences in torque between young and older women. Muscle and Nerve 25 (6):858-863.

MacPhail HEA, Kramer JF (1995) Effect of isokinetic strength-training on functional ability and walking efficiency in adolescents with cerebral palsy. Developmental Medicine and Child Neurology 37 (9):763-775.

Maganaris CN (2003) Tendon conditioning: Artefact or property? Proceedings of the Royal Society B: Biological Sciences 270 (SUPPL. 1):S39-S42.

Maganaris CN, Baltzopoulos V, Ball D, Sargeant AJ (2001) In vivo specific tension of human skeletal muscle. Journal of Applied Physiology 90 (3):865-872.

Maganaris CN, Baltzopoulos V, Sargeant AJ (1998a) Changes in Achilles tendon moment arm from rest to maximum isometric plantarflexion: In vivo observations in man. Journal of Physiology 510 (3):977-985.

Maganaris CN, Baltzopoulos V, Sargeant AJ (1998b) Differences in human antagonistic ankle dorsiflexor coactivation between legs; can they explain the moment deficit in the weaker plantarflexor leg? Exp Physiol 83 (6):843-855.

Maganaris CN, Baltzopoulos V, Sargeant AJ (2000) In vivo measurement-based estimations of the human Achilles tendon moment arm. European Journal of Applied Physiology 83 (4-5):363-369.

Maganaris CN, Paul JP (1999) In vivo human tendon mechanical properties. Journal of Physiology 521 (1):307-313.

Maganaris CN, Paul JP (2002) Tensile properties of the in vivo human gastrocnemius tendon. J Biomech 35 (12):1639-1646.

Maganaris CN, Reeves ND, Rittweger J, Sargeant AJ, Jones DA, Gerrits K, De Haan A (2006) Adaptive response of human tendon to paralysis. Muscle and Nerve 33 (1):85-92.

Magid A, Law DJ (1985) Myofibrils bear most of the resting tension in frog skeletal muscle. Science 230 (4731):1280-1282.

Magnusson SP (1998) Passive properties of human skeletal muscle during stretch maneuvers. Scandinavian Journal of Medicine and Science in Sports 8 (2):65-77.

Magnusson SP, Narici MV, Maganaris CN, Kjaer M (2008) Human tendon behaviour and adaptation, in vivo. The Journal of physiology 586 (1):71-81.

Magnusson SP, Simonsen EB, Aagaard P, Boesen J, Johannsen F, Kjaer M (1997) Determinants of musculoskeletal flexibility: Viscoelastic properties, cross-sectional area, EMG and stretch tolerance. Scandinavian Journal of Medicine and Science in Sports 7 (4):195-202.

Magnusson SP, Simonsen EB, Aagaard P, Kjaer M (1996a) Biomechanical responses to repeated stretches in human hamstring muscle in vivo. American Journal of Sports Medicine 24 (5):622-628.

Magnusson SP, Simonsen EB, Aagaard P, Sorensen H, Kjaer M (1996b) A mechanism for altered flexibility in human skeletal muscle. Journal of Physiology-London 497 (1):291-298.

Malaiya R, McNee AE, Fry NR, Eve LC, Gough M, Shortland AP (2007) The morphology of the medial gastrocnemius in typically developing children and children with spastic hemiplegic cerebral palsy. Journal of Electromyography and Kinesiology 17 (6):657-663.

Marbini A, Ferrari A, Cioni G, Bellanova MF, Fusco C, Gemignani F (2002) Immunohistochemical study of muscle biopsy in children with cerebral palsy. Brain & Development 24 (2):63-66.

McCubbin JA, Shasby GB (1985) Effects of Isokinetic Exercise on Adolescents With Cerebral Palsy. Adapted Physical Activity Quarterly 2 (1):56-64.

McHugh MP, Kremenic IJ, Fox MB, Gleim GW (1998) The role of mechanical and neural restraints to joint range of motion during passive stretch. Medicine and Science in Sports and Exercise 30 (6):928-932.

McNair PJ, Stanley SN (1996) Effect of passive stretching and jogging on the series elastic muscle stiffness and range of motion of the ankle joint. British Journal of Sports Medicine 30 (4):313-317.

McNee AE, Gough M, Morrissey MC, Shortland AP (2009) Increases in muscle volume after plantarflexor strength training in children with spastic cerebral palsy. Developmental Medicine and Child Neurology 51 (6):429-435.

Merletti R, Farina D, Gazzoni M (2003) The linear electrode array: A useful tool with many applications. Journal of Electromyography and Kinesiology 13 (1):37-47.

Merritt JL, McLean TJ, Erickson RP, Offord KP (1986) Measurement of trunk flexibility in normal subjects: Reproducibility of three clinical methods. Mayo Clin Proc 61 (3):192-197.

Mohagheghi AA, Khan T, Meadows TH, Giannikas K, Baltzopoulos V, Maganaris CN (2007) Differences in gastrocnemius muscle architecture between the paretic and non-paretic legs in children with hemiplegic cerebral palsy. Clinical Biomechanics 22 (6):718-724.

Moran HM, Hall MA, Barr A, Ansell BM (1979) Spinal mobility in the adolescent. Rheumatol Rehabil 18 (3):181-185.

Moreau NG, Teefey SA, Damiano DL (2009) In vivo muscle architecture and size of the rectus femoris and vastus lateralis in children and adolescents with cerebral palsy. Developmental Medicine and Child Neurology 51 (10):800-806.

Morrissey D, Roskilly A, Twycross-Lewis R, Isinkaye T, Screen H, Woledge R, Bader D (2011) The effect of eccentric and concentric calf muscle training on Achilles tendon stiffness. Clinical Rehabilitation 25 (3):238-247.

Morse CI (2011) Gender differences in the passive stiffness of the human gastrocnemius muscle during stretch. European Journal of Applied Physiology 111 (9):2149-2154.

Morse CI, Degens H, Jones DA (2007a) The validity of estimating quadriceps volume from single MRI cross-sections in young men. European Journal of Applied Physiology 100 (3):267-274.

Morse CI, Degens H, Seynnes OR, Maganaris CN, Jones DA (2008a) The acute effect of stretching on the passive stiffness of the human gastrocnemius muscle tendon unit. Journal of Physiology 586 (1):97-106.

Morse CI, Thom JM, Birch KM, Narici MV (2005a) Tendon elongation influences the amplitude of interpolated doublets in the assessment of activation in elderly men. Journal of Applied Physiology 98 (1):221-226.

Morse CI, Thom JM, Davis MG, Fox KR, Birch KM, Narici MV (2004) Reduced plantarflexor specific torque in the elderly is associated with a lower activation capacity. European Journal of Applied Physiology 92 (1-2):219-226.

Morse CI, Thom JM, Mian OS, Birch KM, Narici MV (2007b) Gastrocnemius specific force is increased in elderly males following a 12-month physical training programme. European Journal of Applied Physiology 100 (5):563-570.

Morse CI, Thom JM, Mian OS, Muirhead A, Birch KM, Narici MV (2005b) Muscle strength, volume and activation following 12-month resistance training in 70-year-old males. European Journal of Applied Physiology 95 (2-3):197-204.

Morse CI, Thom JM, Reeves ND, Birch KM, Narici MV (2005c) In vivo physiological cross-sectional area and specific force are reduced in the gastrocnemius of elderly men. Journal of Applied Physiology 99 (3):1050-1055.

Morse CI, Tolfrey K, Thom JM, Vassilopoulos V, Maganaris CN, Narici MV (2008b) Gastrocnemius muscle specific force in boys and men. Journal of Applied Physiology 104 (2):469-474.

Moseley AM, Crosbie J, Adams R (2001) Normative data for passive ankle plantarflexion-dorsiflexion flexibility. Clinical Biomechanics 16 (6):514-521.

Muramatsu T, Muraoka T, Takeshita D, Kawakami Y, Hirano Y, Fukunaga T (2001) Mechanical properties of tendon and aponeurosis of human gastrocnemius muscle in vivo. Journal of Applied Physiology 90 (5):1671-1678.

Narici M, Cerretelli P (1998) Changes in human muscle architecture in disuse-atrophy evaluated by ultrasound imaging. Journal of gravitational physiology : a journal of the International Society for Gravitational Physiology 5 (1):P73-74.

Narici MV, Binzoni T, Hiltbrand E, Fasel J, Terrier F, Cerretelli P (1996) In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. Journal of Physiology 496 (1):287-297.

Narici MV, Landoni L, Minetti AE (1992) Assessment of human knee extensor muscles stress from in vivo physiological cross-sectional area and strength measurements. Eur J Appl Physiol 65 (5):438-444.

Narici MV, Maganaris C, Reeves N (2005) Myotendinous alterations and effects of resistive loading in old age. Scandinavian Journal of Medicine & Science in Sports 15 (6):392-401.

Narici MV, Maganaris CN (2007) Plasticity of the muscle-tendon complex with disuse and aging. Exerc Sport Sci Rev 35 (3):126-134.

Neagoe C, Opitz CA, Makarenko I, Linke WA (2003) Gigantic variety: Expression patterns of titin isoforms in striated muscles and consequences for myofibrillar passive stiffness. J Muscle Res Cell Motil 24 (2-3):175-189.

Newham DJ, Hsiao SF (2001) Knee muscle isometric strength, voluntary activation and antagonist co-contraction in the first six months after stroke. Disabil Rehabil 23 (9):379-386.

Nielsen JB, Crone C, Hultborn H (2007) The spinal pathophysiology of spasticity - From a basic science point of view. Acta Physiologica 189 (2):171-180.

O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN (2010a) In vivo measurements of muscle specific tension in adults and children. Exp Physiol 95 (1):202-210.

O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN (2010b) Mechanical properties of the patellar tendon in adults and children. J Biomech 43 (6):1190-1195.

O'Dwyer NJ, Ada L, Neilson PD (1996) Spasticity and muscle contracture following stroke. Brain 119:1737-1749.

Odding E, Roebroeck ME, Stam HJ (2006) The epidemiology of cerebral palsy: Incidence, impairments and risk factors. Disabil Rehabil 28 (4):183-191.

Onambele GL, Burgess K, Pearson SJ (2007) Gender-specific in vivo measurement of the structural and mechanical properties of the human patellar tendon. Journal of Orthopaedic Research 25 (12):1635-1642.

Onambélé GL, Maganaris CN, Mian OS, Tam E, Rejc E, McEwan IM, Narici MV (2008) Neuromuscular and balance responses to flywheel inertial versus weight training in older persons. J Biomech 41 (15):3133-3138.

Onambele GL, Narici MV, Maganaris CN (2006) Calf muscle-tendon properties and postural balance in old age. Journal of Applied Physiology 100 (6):2048-2056.

Paasuke M, Ereline J, Gapeyeva H (2000) Twitch contraction properties of plantar flexor muscles in pre- and post-pubertal boys and men. European Journal of Applied Physiology 82 (5-6):459-464.

Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B (1997) Development and reliability of a system to classify gross motor function in children with cerebral palsy. Developmental Medicine and Child Neurology 39 (4):214-223.

Pang BSF, Ying M (2006) Sonographic measurement of Achilles tendons in asymptomatic subjects: Variation with age, body height, and dominance of ankle. J Ultrasound Med 25 (10):1291-1296.

Parry DAD, Barnes GRG, Craig AS (1978) A Comparison of the Size Distribution of Collagen Fibrils in Connective Tissues as a Function of Age and a Possible Relation between Fibril Size Distribution and Mechanical Properties. Proceedings of the Royal Society of London Series B Biological Sciences 203 (1152):305-321.

Pearson SJ, Onaimbele GNL (2006) Influence of time of day on tendon compliance and estimations of voluntary activation levels. Muscle and Nerve 33 (6):792-800.

Pearson SJ, Onambele GNL (2005) Acute changes in knee-extensors torque, fiber pennation, and tendon characteristics. Chronobiol Int 22 (6):1013-1027.

Pin T, Dyke P, Chan M (2006) The effectiveness of passive stretching in children with cerebral palsy. Developmental Medicine and Child Neurology 48 (10):855-862.

Portney LG, Watkins MP (1997) Power analysis and determination of sample size. In. Appleton and Lange, Norwalk, pp 651-667

Rassier DE, MacIntosh BR, Herzog W (1999) Length dependence of active force production in skeletal muscle. Journal of Applied Physiology 86 (5):1445-1457.

Reed CC, Iozzo RV (2002) The role of decorin in collagen fibrillogenesis and skin homeostasis. Glycoconj J 19 (4-5):249-255.

Reeves ND (2006) Adaptation of the tendon to mechanical usage. Journal of Musculoskeletal and Neuronal Interactions 6 (2):174-180.

Reeves ND, Maganaris CN, Ferretti G, Narici MV (2002) Influence of simulated microgravity on human skeletal muscle architecture and function. Journal of Gravitational Physiology 9 (1):153-154.

Reeves ND, Maganaris CN, Ferretti G, Narici MV (2005) Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. Journal of Applied Physiology 98 (6):2278-2286.

Reeves ND, Maganaris CN, Narici MV (2003) Effect of strength training on human patella tendon mechanical properties of older individuals. Journal of Physiology 548 (3):971-981.

Reeves ND, Maganaris CN, Narici MV (2004a) Ultrasonographic assessment of human skeletal muscle size. European Journal of Applied Physiology 91 (1):116-118.

Reeves ND, Narici MV, Maganaris CN (2004b) Effect of resistance training on skeletal muscle-specific force in elderly humans. Journal of Applied Physiology 96 (3):885-892.

Rose KJ, Burns J, Ryan MM, Ouvrier RA, North KN (2008) Reliability of quantifying foot and ankle muscle strength in very young children. Muscle and Nerve 37 (5):626-631.

Rubenstein LZ (2006) Falls in older people: epidemiology, risk factors and strategies for prevention. Age Ageing 35 (suppl 2):ii37-ii41.

Ruegg DG, Kakebeeke TH, Gabriel JP, Bennefeld M (2003) Conduction velocity of nerve and muscle fiber action potentials after a space mission or a bed rest. Clin Neurophysiol 114 (1):86-93.

Saitou K, Masuda T, Michikami D, Kojima R, Okada M (2000) Innervation zones of the upper and lower limb muscles estimated by using multichannel surface EMG. J Hum Ergol (Tokyo) 29 (1-2):35-52.

Sanger TD, Delgado MR, Gaebler-Spira D, Hallett M, Mink JW, Task Force Childhood Motor D (2003) Classification and definition of disorders causing hypertonia in childhood. Pediatrics 111 (1).

Sargeant AJ, Davies CTM, Edwards RHT, Maunder C, Young A (1977) Functional and structural changes after disuse of human muscle. Clin Sci Mol Med 52 (4):337-342.

Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K, Lømo T (1989) Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. J Muscle Res Cell Motil 10 (3):197-205.

Sharp SA, Brouwer BJ (1997) Isokinetic strength training of the hemiparetic knee: Effects on function and spasticity. Archives of Physical Medicine and Rehabilitation 78 (11):1231-1236.

Sheean G (2002) The pathophysiology of spasticity. European Journal of Neurology, Supplement 9 (1):3-9.

Sheean G, McGuire JR (2009) Spastic Hypertonia and Movement Disorders: Pathophysiology, Clinical Presentation, and Quantification. PM&R 1 (9):827-833.

Sjostrom M, Fugl-Meyer AR, Nordin G, Wahlby L (1980) Post-stroke hemiplegia; Crural muscle strength and structure. Scand J Rehabil Med 12 (Suppl. 7):53-67.

Smith RKW, Gerard M, Dowling B, Dart AJ, Birch HL, Goodship AE (2002) Correlation of cartilage oligomeric matrix protein (COMP) levels in equine tendon with mechanical properties: a proposed role for COMP in determining function-specific mechanical characteristics of locomotor tendons. Equine Vet J 34 (S34):241-244.

Spoor CW, Van Leeuwen JL, Meskers CGM, Titulaer AF, Huson A (1990) Estimation of instantaneous moment arms of lower-leg muscles. J Biomech 23 (12):1247-1259.

Stackhouse SK, Binder-Macleod SA, Lee SCK (2005) Voluntary muscle activation, contractile properties, and fatigability in children with and without cerebral palsy. Muscle and Nerve 31 (5):594-601.

Stackhouse SK, Dean JC, Lee SCK, Binder-MacLeod SA (2000) Measurement of central activation failure of the quadriceps femoris in healthy adults. Muscle and Nerve 23 (11):1706-1712.

Stevens JE, Pathare NC, Tillman SM, Scarborough MT, Gibbs CP, Shah P, Jayaraman A, Walter GA, Vandenborne K (2006) Relative contributions of muscle activation and muscle size to plantarflexor torque during rehabilitation after immobilization. Journal of Orthopaedic Research 24 (8):1729-1736.

Stokes M (1985) Reliability and repeatability of methods of measuring muscle in physiotherapy. Physiotherapy Practice 1 (2):71-76.

Suter E, Herzog W (2001) Effect of number of stimuli and timing of twitch application on variability in interpolated twitch torque. Journal of Applied Physiology 90 (3):1036-1040.

Takata S, Yasui N (2001) Disuse osteoporosis. J Med Invest 48 (3/4):147-156.

Taylor N, Sand PL, Jebsen RH (1973) Evaluation of hand function in children. Archives of Physical Medicine and Rehabilitation 54 (3):129-135.

Taylor NF, Dodd KJ, Graham HK (2004) Test-Retest Reliability of Hand-Held Dynamometric Strength Testing in Young People with Cerebral Palsy. Archives of Physical Medicine and Rehabilitation 85 (1):77-80.

Thom JM, Thompson MW, Ruell PA, Bryant GJ, Fonda JS, Harmer AR, De Janse Jonge XAK, Hunter SK (2001) Effect of 10-day cast immobilization on sarcoplasmic reticulum calcium regulation in humans. Acta Physiol Scand 172 (2):141-147.

Toft E, Espersen GT, Kalund S, Sinkjaer T, Hornemann BC (1989) Passive tension of the ankle before and after stretching. American Journal of Sports Medicine 17 (4):489-494.

Urbanchek MG, Picken EB, Kalliainen LK, Kuzon W.M, Jr. (2001) Specific force deficit in skeletal muscles of old rats is partially explained by the existence of denervated muscle fibers. Journals of Gerontology - Series A Biological Sciences and Medical Sciences 56 (5):B191-B197.

van Schaik CS, Hicks AL, McCartney N (1994) An Evaluation of the Length-Tension Relationship in Elderly Human Ankle Dorsiflexors. J Gerontol 49 (3):B121-B127.

Vaz DV, Mancini MC, Fonseca ST, Vieira DSR, de Melo Pertence AE (2006) Muscle stiffness and strength and their relation to hand function in children with hemiplegic cerebral palsy. Developmental Medicine and Child Neurology 48 (9):728-733.

Verschuren O, Ketelaar M, Takken T, van Brussel M, Helders P, Gorter JW (2008) Reliability of hand-held dynamometry and functional strength tests for the lower extremity in children with Cerebral Palsy. Disabil Rehabil 30 (18):1358-1366.

Walshe FMR (1935) The disorders of motor function following an ablation of part of the "leg area" of the cortex in man. Brain 58 (1):81-85.

Wang JHC (2006) Mechanobiology of tendon. J Biomech 39 (9):1563-1582.

Waugh CM, Blazevich AJ, Fath F, Korff T (2012) Age-related changes in mechanical properties of the Achilles tendon. Journal of Anatomy 220 (2):144-155.

Wiley ME, Damiano DL (1998) Lower-extremity strength profiles in spastic cerebral palsy. Developmental Medicine and Child Neurology 40 (2):100-107.

Williams PE, Goldspink G (1978) Changes in sarcomere length and physiological properties in immobilized muscle. Journal of Anatomy 127 (3):459-468.

Wren TAL, Yerby SA, Beaupré GS, Carter DR (2001) Mechanical properties of the human achilles tendon. Clinical Biomechanics 16 (3):245-251.

Ying M, Yeung E, Li B, Li W, Lui M, Tsoi CW (2003) Sonographic evaluation of the size of Achilles tendon: The effect of exercise and dominance of the ankle. Ultrasound Med Biol 29 (5):637-642.

Zipp P (1982) Recommendations for the standardization of lead positions in surface electromyography. Eur J Appl Physiol 50 (1):41-54.

Appendices

Appendix 1

Hussain AW, Onambele GL, Williams AG, Morse CI (2013) Passive stiffness of the gastrocnemius muscle in athletes with spastic hemiplegic cerebral palsy. European Journal of Applied Physiology

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Appendix 2

Hussain AW, Onambele GL, Willaims AG, Morse CI (2013) Muscle size, activation and coactivation in adults with cerebral palsy. Muscle & Nerve

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