Morphological and mechanical properties of spastic muscle in children and young adults with spastic cerebral palsy

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Morphological and mechanical properties of spastic muscle in children and young adults with spastic cerebral palsy
Abstract

Individuals with spastic cerebral palsy (CP) commonly experience muscle weakness, reduced range of motion, and increased stiffness of affected joints, which together contribute to reduced functional capacity. There is increasing awareness that muscular, in addition to neural factors, contribute to these deficits. The purposes of this thesis were to (1) develop and validate new ultrasound-based methods for assessing morphological properties of the human medial gastrocnemius (MG) muscle in vivo, and (2) to investigate the morphological and passive and active mechanical properties of the MG muscle in children and young adults with spastic CP.

Validation of new methods for assessing muscle morphological properties in vivo. A freehand three-dimensional ultrasound (3DUS) approach for assessing MG muscle volume and length was developed and validated against equivalent measurements made using magnetic resonance imaging (MRI). Compared to MRI, the freehand 3DUS approach overestimated muscle volume by 1.1% and underestimated muscle belly length by 1.3%. The 3DUS approach was also found to be highly reliable. A clinical method for measurement of MG muscle and tendon length was also developed and shown to have high accuracy and reliability compared to freehand 3DUS (Appendix C).

MG muscle physiological cross-sectional area (PCSA) in spastic CP. Compared to typically developed age-matched peers, PCSA of the MG muscle was reduced by 22% in young children aged 2-5 years, and by 37% in young adults aged 15-21 years. Reductions in MG muscle PCSA in the CP groups were primarily explained by a lack of volumetric muscle growth, and contribute to the muscle weakness observed in spastic CP.

Passive and active MG mechanical properties in young adults with CP assessed using dynamometry. Passive ankle stiffness was 51% higher and passive MG fascicle strain was 47% lower in the spastic group CP compared to typically developed controls. These findings suggest
that the increased resistance to passive ankle dorsiflexion in spastic CP is related to the inability of MG muscle fascicles to elongate with increased passive force. Compared to the typically developed group, the spastic CP group also produced 56% less active ankle plantarflexion torque across the available range of ankle joint motion, and had greater levels of antagonist co-contraction and a longer Achilles tendon slack length. The increased Achilles tendon slack length may facilitate a greater storage and recovery of elastic energy and partially compensate for decreased force and work production by the muscles of the triceps surae during activities such as locomotion.

Overall findings from this thesis indicate that the morphological and mechanical properties of the MG muscle and Achilles tendon are altered in individuals with spastic CP, and together contribute to ankle plantarflexor muscle weakness, restricted ankle dorsiflexion range and increased ankle stiffness observed in CP. Treatments for improving function in spastic CP should be directed towards the muscular as well as neural system.
Acknowledgements

I thank greatly my supervisor Dr Glen Lichtwark. He captured my curiosity and opened the doors to biomechanics research. His guidance along the research path, and every stop on the way, was exceptional. I just tried to keep up. It has been a privilege to be able to count myself as part of his research group. I sincerely thank my supervisor A/Prof Rod Barrett, whose knowledge and carefulness have been a major component in the completion of this work. I am gracious for the freedom that he allowed me to complete this work, but also the assistance along the way. To both my supervisors, I hope our collaborations continue into the future.

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In addition, I thank A/Prof Rob Herbert for input all the way along my PhD journey. We have had numerous valuable discussions and he has provided a number of memorable occasions. I am grateful to A/Prof Ros Boyd for her assistance with subject recruitment and opportunities to present my research to the CP community in Brisbane. I also thank Mr Adam Bowern for his technical assistance whenever I needed it. I am grateful to Mr Andrew Hegarty from Queensland X-Ray for his MRI technical assistance and the staff of the Hugh Williamson Gait Analysis Laboratory, The Royal Children’s Hospital, Melbourne for their assistance with my study in young children. I thank Dr Leanne Johnston from the CP League Queensland for her assistance with subject recruitment.
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Lee Barber

September 2011
Peer reviewed publications


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Chapter 1. Introduction

1.1. Background

Spastic cerebral palsy (CP) arises as a result of a non-progressive brain lesion at or around the time of birth that causes permanent and progressive postural and movement disorders (Rosenbloom, 2007). Spastic CP has an incidence of 2.0–2.5 occurrences per 1000 live births in developed nations, making spastic CP the most common cause of physical disability in children (Odding et al., 2006). Individuals with spastic CP present clinically with increased joint stiffness, reduced joint range of motion and reduced voluntary strength of the affected limb/s. Although the key features of spastic CP are neural in origin, it has become apparent that the mechanisms underlying these impairments also involve a combination of muscular and tendinous factors (Poon and Hui-Chan, 2009).

Spastic muscle is characterised by an increased resistance to stretch, and often shortens to produce, what is commonly termed, muscle contractures. Studies to date have sought to describe the morphological adaptation of muscle in individuals with spastic CP. These studies have tended to focus on the medial gastrocnemius (MG) muscle in individuals aged 6-12 years and support the notion that muscles in the paretic lower limb of children with spastic CP have significant muscle tissue and fibre atrophy, reduced muscle volumes, length and cross-sectional area and variable fascicle lengths compared to the unaffected limb and compared to typically developed (TD) peers (Fry et al., 2007, Lieber et al., 2004, Malaiya et al., 2007, Mohagheghi et al., 2008). What we currently don’t know is what the mechanism of these muscle morphological adaptations is, at what age the adaptations occur, and how they progress over time. At present there is a need to investigate muscle properties in very young children with spastic CP to determine when the muscle morphological adaptations occur. Further, it is important to investigate muscle properties young adults with spastic CP, where fixed contractures have already developed, to determine how muscle
Introduction

morphological adaptations progress over time. The MG muscle was selected for investigation in this thesis as it is an important muscle during locomotion and lower limb activities of daily living. Also, in children with spastic CP the MG undergoes significant morphological alterations during development and is the focus of corrective orthopaedic and conservative management (Bandholm et al., 2009, Malaiya et al., 2007, Steele et al., 2010).

The active and passive mechanical properties of the muscle and tendon, such as the force-length relationship of muscle and the strain and stiffness of the muscle-tendon unit influence joint compliance and force production which in turn affect muscle function. Altered muscle-tendon unit mechanical properties have been reported in stroke survivors with muscle spasticity (Gao et al., 2009, Gao and Zhang, 2008, Zhao et al., 2009). However to date only Alhusaini et al., (2010), has investigated spastic muscle mechanical properties in individuals with spastic CP. Alhusaini et al., (2010) reported increased ankle stiffness in children with spastic CP compared to TD children. However it remains unclear what musculo-articular structures contribute to these differences in stiffness detected at the joint level. There is conflicting reports of the affects of neurological disorders on the Achilles tendon. Zhao et al. (2009) reported a longer and more compliant tendon in stroke patients whereas Hoang et al. (2009) found no difference in tendon slack lengths and strains between ambulatory individuals with multiple sclerosis and healthy controls. No study of tendon mechanical properties of lower limb muscles in individuals with spastic CP has been conducted.

Since the secondary effects of spastic CP disrupt posture and movement, conservative, pharmacological, neurosurgical and orthopaedic management strategies are directed toward the musculoskeletal system (Graham and Selber, 2003). Treatment of musculoskeletal abnormalities in spastic CP typically address one or more of spasticity, muscle stiffness, muscle contracture, muscle weakness, dynamic and static joint deformity, bony deformities, and/or abnormal motor control. Understanding the morphological and mechanical properties of the muscle-tendon unit is fundamental in determining the mechanisms underlying the development and progression of
muscle contracture, joint stiffness and muscle weakness and the corresponding loss of motor function in individuals with spastic CP. New knowledge of muscle morphology and mechanics in spastic CP has the potential to contribute to the development of more targeted and hence more effective treatments.

1.2. Statement of the problem

Affected muscles of individuals with spastic CP are altered secondary to the upper motor neuron lesion and may develop contracture which contributes to increased joint stiffness, reduced joint range of motion and reduced voluntary strength. These impairments progress with age resulting in the affected individual experiencing general difficulty in performing functional tasks. Orthopaedic surgery and/or neurosurgery may ultimately be required to maintain functional independence in some individuals with spastic CP. The mechanisms underlying the development and progression of altered muscle and tendon morphological and mechanical properties remain unclear. Further understanding will guide the development, specificity and monitoring of treatment modalities for spastic CP.

1.3. Significance of the problem

Cerebral palsy is the largest single cause of childhood disability with an incidence of 2.0–2.5 occurrences per 1000 live births (Odding et al., 2006). The economic burden of CP to the Australian economy is approximately $1.47 billion dollars per year, of which $300 million could be directly attributed to health care and support costs associated with disability (Access Economics, 2008). Understanding the mechanisms that contribute to disability and loss of function is of utmost importance in improving the quality of life in this population.
1.4. General purpose

The general purpose of this thesis was to investigate the morphological and mechanical properties of the MG muscle in children and young adults with spastic CP.

1.5. Specific purpose

The specific purposes of this thesis were to:

1. Develop and validate new methods for measuring morphological and mechanical properties of the MG muscle using ultrasound (Chapter 3, Appendix C);

2. Investigate muscle morphological properties of the MG muscle in young children and young adults with and without spastic CP (Chapter 4);

3. Investigate the passive mechanical properties of the MG muscle in young adults with and without spastic CP (Chapter 5);

4. Investigate the active mechanical properties of the MG muscle and Achilles tendon in young adults with and without spastic CP (Chapter 6).
1.6 Thesis Organisation

This thesis has seven chapters and four appendices.

Chapter 1 provides a general introduction to the thesis.

A review of the relevant literature is presented in Chapter 2, which provides a synthesis and critical appraisal of the literature to date encompassing the topics of spastic CP, morphological properties of spastic muscle, mechanical properties of spastic muscle and tendon and measurement of muscle morphological and mechanical properties.

Chapter 3 demonstrates the validity and reliability of a freehand three dimensional ultrasound (3DUS) method for measuring the volume and length of the human medial gastrocnemius (MG) muscle \textit{in vivo}. This approach is used in subsequent chapters to assess muscle volumes and lengths of the MG in young children and young adults with spastic CP and age matched TD individuals. This study addressed the first stated purpose of the thesis.

In Chapter 4 muscle volume, physiological cross sectional area (PCSA), length, and muscle fascicle length and pennation angle of the MG muscle in children with spastic CP who have received no previous surgical or pharmaceutical intervention are compared to TD children aged between 2-5 years. This study provides the first data regarding morphological properties of spastic muscle in this age group and addresses the second specific purpose of the thesis.

Chapter 5 describes a study that compared the \textit{in vivo} passive mechanical properties of the ankle joint and MG muscle fascicles in young ambulant adults with spastic CP and TD control participants. Fundamental differences in architectural and mechanical properties of the ankle joint and MG muscle fascicle are discussed in relation to increased ankle stiffness in the spastic CP group. This study addressed the second and third specific purpose of the thesis.
Chapter 6 presents the study comparing the *in vivo* MG muscle fascicle active torque-length and Achilles tendon properties in young adults with spastic CP with TD controls. Active MG torque-length and Achilles tendon properties are assessed under controlled conditions on a dynamometer. Factors contributing to muscle weakness in spastic CP and Achilles tendon adaptations to spasticity are discussed. This investigation provides the first *in vivo* MG muscle fascicle active torque-length and Achilles tendon strain and stiffness data for individuals with spastic CP and addresses the fourth specific purpose of the thesis.

Chapter 7 summarises the results of the thesis experimental chapters and discusses the integration of the findings of each experiment with broader literature. Methodological considerations from the experiments are raised, future directions proposed and clinical relevance highlighted.

Appendices A and B provide supporting information for Experiments 2, 3 and 4 (Chapters 4–6).

Appendix C is a validation and reliability study comparing a novel US-tape measure method and 3DUS for measuring MG muscle length in a TD and spastic CP participants. The potential clinical use of this tool for muscle length measurement is highlighted. This study further addressed the first stated purpose of the thesis.

Appendix D is an invited commentary on the original publication of Experiment 2 (Chapter 4).
## 1.7. Abbreviations

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<tr>
<td>2D</td>
<td>Two-Dimensional</td>
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<tr>
<td>3D</td>
<td>Three-Dimensional</td>
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<tr>
<td>3DUS</td>
<td>Three-Dimensional Ultrasound</td>
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<tr>
<td>CP</td>
<td>Cerebral Palsy</td>
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<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>MG</td>
<td>Medial Gastrocnemius</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>PCSA</td>
<td>Physiological Cross-Sectional Area</td>
</tr>
<tr>
<td>TD</td>
<td>Typically Developed</td>
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<tr>
<td>GMFCS</td>
<td>Gross Motor Function Classification System</td>
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1.8. Acknowledgement of published papers

Included in this thesis are published papers in Chapters 3-6 and Appendix C which are co-authored with other researchers. My contribution to each co-authored paper is outlined at the front of each relevant chapter. The bibliographic details for these papers are:

**Chapter 3:**

**Chapter 4:**

**Chapter 5:**

**Chapter 6:**
Appendix C:


Appropriate acknowledgements of those who contributed to the research but did not qualify as authors are included in each published paper.

Lee Barber
September 2011

Supervisor: A/Prof Rod Barrett
September 2011
Introduction
Chapter 2. Literature review

2.1. Spastic CP

CP is the most common cause of physical disability affecting children in the developing countries, with an incidence of 2.0–2.5 occurrences per 1000 live births (Odding et al., 2006). It is not a single entity but a heterogeneous collection of clinical syndromes characterised by abnormal motor patterns (Reddihough and Collins, 2003). The definition of CP stresses two features, firstly, it is the result of a lesion in the immature brain which is non-progressive and secondly, it is a disorder of posture and movement which is permanent but can progress with age (Rosenbloom, 2007). CP is subdivided according to the motor type and its topographical distribution. Spastic CP is the most common motor type of CP with a prevalence of 70% including the most common topographical syndromes - spastic hemiplegia (one side of the body affected), spastic diplegia (lower limbs affected) and spastic quadriplegia (all four limbs affected) (Reddihough, 2011).

2.1.1. Features of spastic CP

Spastic CP is considered an upper motor neuron syndrome characterised by positive and negative features. The positive features are a result of the loss of upper motor neuron inhibition on lower motor neurons and include spasticity, impaired motor unit firing and clonus. The negative features result from a loss of upper motor neuron excitation of lower motor neurons and include weakness, fatiguability, poor balance and sensory deficits.

Spasticity is the key positive feature causing motor impairment in spastic CP. Spasticity has been defined as a velocity dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks resulting from hyper-excitability of the stretch reflex (Lance, 1980) and is the resistance felt in a muscle when the associated joint is moved quickly.
Various measures have been used to assess spasticity in the clinical setting, including the Ashworth scale or Modified Ashworth Scale (MAS) (Bohannon and Smith, 1987) and the Tardieu Scale and Modified Tardieu (Haugh et al., 2006), and the research laboratory setting, using well-controlled quantitative measures based on motorised mechanical perturbations to the joint and electrophysiological approaches (Chung et al., 2004, Dietz et al., 1991). While the predominance of research into spastic CP has focused on the neural features of the condition, the basic mechanisms underlying the mechanical features and functional deficits that occur after the development of spasticity are not clearly understood.

2.1.2. Musculoskeletal pathology in spastic CP

The positive and negative features of the CP create a neural and mechanical interaction that produces a cascade of musculoskeletal pathology. The primary musculoskeletal pathology in spastic CP involves adaptations to the skeletal muscle. One aspect of the skeletal muscle adaptations in spastic CP is historically termed contracture and is defined as a fixed shortening of a muscle (often referred to as the muscle belly) in relation to the length of the accompanying long bone (Bache et al., 2003). Muscle contractures are commonly assumed by clinicians to be caused by a shortened muscle. However, numerous adaptations occur to the muscles morphological structure including reduced muscle volume, length and variability in fascicle length (Fry et al., 2007, Lieber et al., 2004, Malaiya et al., 2007, Mohagheghi et al., 2008). Changes also occur at the muscle fibre structure level including sarcomere re-arrangement, increased intramuscular connective tissue and fat content and extracellular matrix integrity (Friden and Lieber, 2003, Lieber and Friden, 2002, Lieber et al., 2004, Smith et al., 2011). The cause of the muscle adaptations in spastic CP is not confirmed. The muscle adaptations may result in a reduced joint range of motion, a decrease in mobility, may be painful and create additional musculoskeletal pathology such as bony torsion, joint instability and degenerative arthritis (Andersson and Mattsson, 2001, Graham and Selber, 2003) but it is currently unknown what functional limitations muscle morphological changes in spastic CP place on the muscle and tendon.
2.1.3. Gross motor development in spastic CP

Gross motor function in spastic CP is related to the site and severity of the cerebral lesion. A child with spastic CP will be recognised by delayed gross motor development and the presence of abnormal movement and posture patterns (Bache et al., 2003). Cross-sectional studies of motor behaviour in children with CP have demonstrated characteristic patterns of motor development according to the severity of the condition and can be predicted and reliably classified using the using the Gross Motor Function Measure (GMFM) and Gross Motor Function Classification System (GMFCS) (Palisano et al., 2000, Palisano et al., 2007, Palisano et al., 2008, Russell et al., 2000). The GMFM is a standardized observational instrument designed and validated to measure change in gross motor function over time in children with CP. There are two versions of the GMFM, the original 88 item measure (GMFM-88) and a 66 item measure (GMFM-66). Items span the spectrum of activities in lying and rolling up to walking, running and jumping skills. The expanded and revised GMFCS for CP is a five-level classification system of severity of motor function and is based on self-initiated movement, with emphasis on sitting, transfers, and mobility and includes five age bands (Palisano et al., 2007). The primary criterion for distinctions between levels is based on functional limitations, the need for hand-held mobility devices (such as walkers, crutches, or canes) or wheeled mobility, and to a much lesser extent, quality of movement. For example, the general descriptions are: GMFCS Level I - “Walks without Limitations” and GMFCS Level 5 – “Transported in a Manual Wheelchair” (Palisano et al., 2007).

Briefly, the typical trajectory for children with spastic CP is that after a period of delayed attainment of motor skills (4-6 years), gross motor function (e.g. gait) often plateaus at 8-12 years and can decline in adolescence (Beckung et al., 2007, Day et al., 2007a, Day et al., 2007b, Palisano et al., 2000). This gradual loss of functional capacity can ultimately necessitate increased assistance for activities of daily living and the use of walking aides and wheelchairs for mobility.
2.1.4. Management of spastic CP

Since the secondary effects of spastic CP disrupt posture and movement, conservative, pharmacological, neurosurgical and orthopaedic management strategies are directed toward the musculoskeletal system (Graham and Selber, 2003). Treatment of musculoskeletal abnormalities must address one or more of spasticity, muscle stiffness, muscle contracture, dynamic and static joint deformity, bony deformities, and abnormal motor control (Gage, 1991, Koman et al., 2004).

The treatment options that are used change with the age and developmental stage of the child with spastic CP (Figure 2.1). Children younger than 3 or 4 years rarely develop significant fixed deformities, joint contractures, or bony deformities; therefore, many respond to physiotherapy, oral pharmacological agents, neuromuscular blocking agents, and orthotics (Graham et al., 2000, Koman et al., 2004). However, the consensus is that spasticity associated with CP should be treated before children reach the age of 5 or 6 years, so that contracture progression is reduced or do not have the chance to develop (Graham et al., 2000). The spasticity management technique used for children with CP is determined primarily by the clinical findings. Spasticity management involves the use of a continuum of modalities throughout childhood and most children are managed with a combination of treatments (e.g. the use of a combination of physiotherapy and casting can increase the beneficial effects associated with intramuscular injections of botulinum toxin) (Glanzman et al., 2004, Love et al., 2010). Available treatment options include physiotherapy, device-assisted modalities, oral pharmacological intervention, and surgery.

Physiotherapy, occupational therapy, device-assisted modalities (e.g. electrical stimulation), orthotics, casting, or any combination of these methods are important early management options for children with spastic CP. These treatments are used to maintain or improve joint range of motion, facilitate or strengthen weak muscles, inhibit or weaken spastic agonist muscles, provide support, and improve or normalise motor development in affected muscles (Damiano and Abel, 1998, Damiano et al., 2010).
As the child with spastic CP grows older, the frequency of fixed contractures, joint subluxation, dislocation, and bony deformity increases, and the need for surgical intervention increases. Surgical management of the arms and legs in patients with spastic CP is designed to correct muscle contracture, decrease spasticity, reduce deformity, improve ambulation, improve function, facilitate care activities and improve health-related quality of life (Harryman, 1992, Van Heest et al., 1999). Improved outcomes to Achilles lengthening surgery, gastrocnemius recession and derotation osteotomy procedures is seen in patients older than 6-8 years (Koman et al., 2003). For children who undergo osseous or soft-tissue procedures before skeletal maturity, chemomodulation of spasticity, casting, or both might be necessary during rapid periods of growth to maintain the benefits of the surgical procedure (Graham and Selber, 2003). Skeletally mature children and adults can benefit from chemodenervation or neuromuscular blockade (botulinum toxin) to manage painful spasticity or to achieve specific functional or positional needs (Bache et al., 2003, Koman et al., 2004).

The management of individuals with spastic CP continues to evolve. The availability of validated and reliable outcome instruments that can be used to assess multiple outcomes of various therapeutic options, including the technical success of surgical procedures and functional status will facilitate the appropriate evaluation of the interventions currently used to manage children and adults with spastic CP.
Literature review

Figure 2.1. Musculoskeletal pathology and management in children with spastic CP.

Understanding the musculoskeletal pathology in spastic CP assists in the selection of appropriate treatment strategies. In the younger children from 2 to 6 years of age, spasticity management predominates. Between the ages of 7 and 11 years, the majority of children will undergo surgery to correct deformities and improve gait (Bache et al., 2003).

2.2 Morphological properties of spastic muscle

Muscle morphology refers to the anatomical size and shape of a muscle, and the macroscopic arrangement of the muscle fibres (Lieber and Friden, 2000). This section provides a critical appraisal and synthesis of the literature to date that have assessed muscle volume, fascicle length, pennation angle and muscle length in spastic CP. Most morphological studies have been cross-sectional and involved children with spastic CP in the ages of 6-12 years.

2.2.1 Muscle volume

Muscle volume is indicative of overall muscle growth, is reflective of the number of sarcomeres in series and in parallel, and is linearly related to muscle power. A recent systematic review (Barrett and Lichtwark, 2010) concluded that there was consistent evidence for a reduced calf muscle volume in the paretic limb in spastic CP compared to TD peers and the non-paretic limb in spastic
CP. In older children and young adults with spastic CP there is a 26-57% difference in calf muscle volumes relative to TD individuals (Table 2.1). The two studies that compared lower leg muscle volumes on the non-paretic side in individuals with spastic CP and TD muscles reported no significant difference (Elder et al., 2003, Malaiya et al., 2007).

Table 2.1. Characteristics of studies investigating lower leg muscle volume in children with spastic CP. To facilitate direct comparisons, percentage difference between the spastic CP and TD groups were calculated from muscle volume data normalised to body weight.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Motor function</th>
<th>Age (yrs)</th>
<th>Muscle</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaiya et al. (2007)</td>
<td>16 SCP</td>
<td>Ambulant</td>
<td>8 (4-12)</td>
<td>MG</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>15 TD</td>
<td></td>
<td>10 (4-13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fry et al. (2007)</td>
<td>7 SCP</td>
<td>Independently</td>
<td>8 (6-10)</td>
<td>MG</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>10 TD</td>
<td>ambulant</td>
<td>9 (6-12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oberhofer et al. (2009)</td>
<td>6 SCP (4H, 2D)</td>
<td>GMFCS I-II</td>
<td>H: 10 (1), D: 10 (2)</td>
<td>MG + LG</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5 TD</td>
<td></td>
<td>10 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortland (2009)</td>
<td>14 SCP (2H, 7D)</td>
<td>Independently</td>
<td>17 (14-22)</td>
<td>MG</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>14 TD</td>
<td>ambulant</td>
<td>Age, sex matched</td>
<td>LG</td>
<td>39</td>
</tr>
<tr>
<td>Elder et al. (2003)</td>
<td>28 SCP</td>
<td>Unknown</td>
<td>8 (2)</td>
<td>PF</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>14 TD</td>
<td></td>
<td>9 (2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Acronyms: Spastic CP (SCP), Medial gastrocnemius (MG), lateral gastrocnemius (LG), plantarflexors (PF), hemiplegia (H), diplegia (D). Mean (SD or range).

2.2.2 Muscle fascicle length

Muscle fascicle length, which represents the number of sarcomeres working in series, is the primary determinant of muscle excursion, with shorter muscle fascicles having a reduced range through which they can develop force and power, a reduced maximum shortening speed, and a
reduced length at which they develop passive forces (Lieber and Friden, 2000) (Figure 2.2). The findings for comparisons of fascicle length between the paretic side of individuals with spastic CP and their TD counterparts are inconsistent. Some studies report no differences in fascicle length for the MG (Lieber et al., 2004, Malaiya et al., 2007, Shortland et al., 2002) while others report a reduction in fascicle length in paretic muscle relative to TD muscle and also to the non-paretic MG in hemiplegia (Mohagheghi et al., 2007, Mohagheghi et al., 2008). Shortland et al. (2002) and Shortland et al. (2004) reported no differences in fascicle length for the MG muscle independent of the ankle joint angle (MDF, resting or 30° plantarflexion) or whether the data were normalised for leg length. Malaiya et al. (2007) found no differences in the normalised fascicle length of MG between the non-paretic side in individuals with spastic CP and TD individuals or between the paretic and non-paretic sides in individuals with spastic CP independent of the ankle angle tested (MDF and resting angle). However, the absolute fascicle length of MG was significantly shorter at the resting ankle angle in those with spastic CP. In contrast, Mohagheghi et al. (2008) reported significantly shorter fascicle lengths for MG in individuals with spastic CP when reported in absolute terms and when normalised to leg length, and for the proximal, mid, and distal aspects of the MG on the paretic compared with the non-paretic side (Mohagheghi et al., 2007).

Figure 2.2. Fascicle length and pennation angle of the MG.

The apparent disagreement in the reported results may stem from several methodological issues, such as recruitment of participants with differences in the type of the underlying brain injury (diplegia versus hemiplegia), differential levels of motor impairments involving the muscles
scanned and the suitability of the normalisation technique of fascicle length (Mohagheghi et al., 2008, Moreau et al., 2009). A proper resolution to the issue of whether muscle fascicles are shortened in individuals with spastic CP will require measurement of muscle fascicle length while controlling for muscle tension. It is currently not feasible to measure muscle tension directly in vivo; instead, this requires attempting to solve the force-sharing problem at the joints involved. Such an approach has been used to assess the passive properties of the gastrocnemius in multiple sclerosis by measuring changes in the passive ankle torque at different knee angles (Hoang et al., 2009, Hoang et al., 2005). It is important to establish if muscle fascicle length is altered in spastic CP because it could contribute to improved understanding of the mechanism of muscle contracture with possible implications for treatment.

2.2.3 Pennation angle

The pennation angle of a muscle is the angle formed by the individual muscle fascicles with the line of action of the muscle and determines the effectiveness of force transmission to the tendon (Figure 2.2). Comparisons of pennation angle on the paretic side in individuals with spastic CP and in TD individuals have produced somewhat inconsistent findings. The significant reductions in the pennation angle of the MG reported by Malaiya et al. (2007) and Shortland et al. (2002) were not supported by Gao et al. (2011) who demonstrated no difference. There were also no significant differences in fascicle angle between paretic and non-paretic sides in individuals with spastic CP or between the non-paretic side and TD individuals have been reported (Malaiya et al., 2007, Shortland et al., 2002). The observed differences in fascicle angle depended on ankle joint angle and have not been ascribed any major functional significance (Malaiya et al., 2007, Shortland et al., 2004).

2.2.4 Muscle length

Muscle length is the distance between the musculo-tendinous junctions proximally and distally and may also be termed the muscle belly (Figure 2.3). Reduced muscle length is suggested to be
indicative of muscle contracture (Fry et al., 2003). There is consistent evidence that MG muscle length is reduced on the paretic side in individuals with spastic CP compared with their TD counterparts in absolute and relative terms at all ankle joint angles (Fry et al., 2007, Fry et al., 2004, Malaiya et al., 2007, Oberhofer et al., 2009). With the exception of absolute length at the maximum dorsiflexion (MDF) angle, Malaiya et al. (2007) also reported that MG length was significantly reduced on the paretic compared with the non-paretic side in individuals with spastic CP. Some authors have suggested that this may represent a reduced fibre diameter and corresponding contraction of the aponeurosis to which the muscle fibres attach and may explain why MG muscle length in individuals with spastic CP is typically reduced in the absence of large reductions in muscle fascicle length (Malaiya et al., 2007, Shortland et al., 2002). However there is little to no evidence to support this hypothesis at present.

Figure 2.3. Muscle length of the MG from 3D volume rendering of a MRI of the lower limb. LG: lateral gastrocnemius, SOL: Soleus.
2.2.5 Muscle PCSA

The functional significance of muscle morphology and structure can be further understood by considering the PCSA of the muscle, which is proportional to the muscle’s maximal force generating capacity (Haxton, 1944, Powell et al., 1984). The PCSA is obtained from the ratio of muscle volume to optimal fascicle length (which is sometimes corrected by the cosine of the pennation angle), and represents the number of muscle fascicles working in parallel (Lieber and Friden, 2000) (Equation 1).

\[
PCSA = \frac{\text{Muscle Volume} \times \cos \theta}{\text{Fascicle Length}}
\]

Equation 1.

To date only one study (Malaiya et al., 2007) has measured muscle volume, fascicle length and pennation angle in the same participants with spastic CP. In the study by Malaiya, et al. (2007) the differences in PCSA (calculated at a common joint angle) for the MG between the spastic CP and TD groups aged 4-12 years were almost completely explained by differences in muscle volume.

The PCSA in normal muscle is primarily determined by the sum of the cross-sectional areas of all the muscle fibres within the muscle, and so a reduced PCSA in individuals with spastic CP could be explained by a reduced number of fibres and/or a decrease in the average fibre cross-sectional area (Malaiya et al., 2007). Only a limited number of muscle cells can be analysed in histological studies, and so it is difficult to know how muscle fibre number influences PCSA in individuals with spastic CP. However, there is evidence to suggest that muscle cells from patients with spasticity are severely atrophic. Spastic muscle fibres have been reported to be, on average, less than one-third the size of normal fibres of upper extremity muscles in patients with wrist flexion contractures (Friden and Lieber, 2003, Lieber et al., 2003). Reduced muscle volume in individuals with spastic CP may be explained by a reduced muscle fibre diameter and corresponding contraction of the aponeurosis to which the muscle fibres attach (Malaiya et al., 2007, Shortland et al., 2002). A further finding that distinguishes normal muscle and spastic muscle is the total area of
muscle fibre bundles accounted for by muscle fibres, which is only 40% in the latter compared with 95% in the former (Lieber et al., 2003). This finding is explained by a greater proportion of extracellular matrix material in the muscle fibres of individuals with spastic CP (Lieber et al., 2003) and is consistent with reports of collagen accumulation and thickened endomysium in individuals with spastic CP, findings that were also shown to be significantly correlated with clinical measures of function (Booth et al., 2001).

Investigations of spastic lower limb muscle morphological properties to date have included children with spastic CP with ages ranging from 4 to 12 years (Elder et al., 2003, Fry et al., 2007, Malaiya et al., 2007, Mohagheghi et al., 2007). Physical development is varied across these ages and treatment and management strategies may be quite mixed. It therefore remains unclear at what age changes in muscle volume and length start to occur in children and it is difficult to determine the time course of normal muscle development and how this might relate to the increasing muscle weakness and corresponding decline in motor function that occur with ageing in individuals with spastic CP. Studies on younger children who have received no previous surgical or pharmaceutical intervention are required to determine at what age’s changes in muscle volume and length start to occur, as this could have implications for the management of spastic CP in this age group.

2.3. Mechanical properties of spastic muscle and tendon

Isolated skeletal muscle studies indicate that the force-length relationship varies with the extent of myofilament overlap in the sarcomere and approximates a bell-shaped curve. Muscle force is maximal at intermediate (optimal) muscle lengths and declines at shorter and longer relative lengths (Gordon et al., 1966). However, the force exerted by muscle is not dependent solely on the active muscle contraction. In vivo muscle consists of a substantial amount of cytoskeletal components and connective tissue, including the tendon. When stretched beyond resting length these structures exert a passive force that combines with the active contribution due to the
contractile process (Edman, 1992, Kulig et al., 1984). These active and passive components contribute to the mechanical properties of the muscle (Figure 2.4.).

Figure 2.4. Representation of the force-length relationship of muscle. Total force = active force + passive force.

Other mechanical properties such as muscle and tendon stress, strain and stiffness have also been studied using isolated animal or human muscle material (Zajac, 1989). However measurements taken from isolated or preserved musculotendinous material may have altered properties (Smith et al., 1996) and be inappropriate when interpreting in vivo human function (Maganaris, 2001, Maganaris et al., 2001, Maganaris and Paul, 1999). Contraction and movement specific musculoskeletal geometry data as opposed to resting-state or cadaveric data provides further insight into individual and task specific muscle-tendon mechanical properties. As opposed to isolated fibre experiments, the operating length range of the muscle-tendon unit under in vivo condition is confined by the anatomical constraints of the skeleton and the morphological features of the muscle. A number of studies have demonstrated the feasibility of accurate in vivo estimates of human muscle active and passive mechanical properties using the combination of 2D ultrasound, MRI, EMG, electrical stimulation and dynamometry (Herzog et al., 1991a, Herzog et al., 1991b, Hoang et al., 2005, Hoang et al., 2007, Maganaris, 2001, Maganaris et al., 2001,
Maganaris and Paul, 1999). Hoang et al. (2005, 2007) outlined a reliable method that enabled measurement of passive force-length properties of human gastrocnemius in vivo and concluded that the relaxed human gastrocnemius muscle-tendon unit falls slack over about one-quarter of its in vivo length and that muscle fascicle strains are much greater than tendon strains. Maganaris (2001) and Maganaris (2003) calculated the active force-length curve in a number of intact human lower limb muscles and indicated that during maximal contractions the muscles operate in the ascending limb and plateau region of the typical bell-shaped curve obtained from experiments on isolated muscle. Furthermore, different results have been obtained between muscles and between subject groups (Gao and Zhang, 2008, Herzog et al., 1991a, Herzog et al., 1991b, Herzog and ter Keurs, 1988, Smeulders et al., 2004), possibly because of muscle structural differences, muscle function specificity and/or anatomical constrains of the skeletal system.

Experimental measurements of isolated tendons have revealed relatively consistent mechanical properties among different tendons (Zajac, 1989). Loading applied to an isolated tendon by a force transducer allows length and force to be measured. Tendon strain is the change in length of the tendon relative to the starting length and expresses the compliance of the tissue. Load and strain provide insight into the unique properties of the tendon relative to other tissues (e.g. muscle fascicles) and materials (e.g. wood, plastic). In vivo tendon properties are more difficult to estimate although Zajac (1989), using estimations of tendon strain and literature values, concluded that tendons strain approximately 3% during muscle contraction. More recently ultrasonography and MRI studies have reported strains of the Achilles tendon ranging from 5–10% during isometric contractions and dynamic movements (Flinn et al., 2003, Lichtwark and Wilson, 2005, Maganaris and Paul, 2002, Muramatsu et al., 2001). In addition, Hoang et al. (2007) demonstrated that the Achilles tendon contributed more than half of the total compliance of the MG muscle-tendon unit during passive lengthening. The length and compliance of the Achilles tendon can also uncouple the length changes of the fascicles from that of the muscle-tendon unit, and thereby influence the force-generating capacity of the muscle (Lichtwark and Wilson, 2007, Lichtwark and Wilson, 2008) For example, a more compliant tendon allows the muscle fibres to operate closer to optimal
length and at lower shortening speeds, thereby increasing force and work production of the muscle-tendon unit during locomotion. Lichtwark et al., 2007, Lichtwark and Wilson, 2006; however, this may also reduce muscle control and power output (Alexander, 2002).

2.3.1 Passive mechanical properties of spastic muscle

Resistance to stretch is caused by a combination of passive muscle stiffness, neurally mediated reflex stiffness and active muscle stiffness (Lieber et al., 2004). Sinkjaer and Magnussen (1994) and Mirbagheri et al. (2001) demonstrated, in the spastic leg of hemiparetic stroke and spinal cord injured patients respectively, that the increased passive muscle stiffness accounted for almost all of the increase in joint stiffness measured in the muscles of the spastic limb. Increased non-neurally mediated passive joint stiffness in subjects with upper motor neuron lesions has been attributed to shorter muscles (Sinkjaer and Magnussen, 1994, Zhao et al., 2009) and muscles with shorter fascicles (Gao et al., 2009). However conflicting findings have been reported in spastic CP, because some studies report reductions in resting fascicle length (Mohagheghi et al., 2008, Moreau et al., 2009), while others report no differences (Malaiya et al., 2007, Shortland et al., 2002).

Studies of whole muscle level architectural properties in spastic CP have primarily been confined to measurements of resting muscle, fascicle length and pennation angle. These measurements are typically made at the resting joint angle, where muscle tension is assumed to be negligible, and so do not reveal information about muscle stiffness. The stiffness of individual muscle cells has however been reported to be substantially increased in spastic wrist muscle (Friden and Lieber, 2003), which depending on the number of muscle cells in series and their corresponding lengths, could explain joint level mechanical properties in spastic CP. More recently increased stiffness has been attributed to changes in the cellular and extracellular properties of muscle in individuals with spastic CP (Smith et al., 2011, Smith et al., 2009).

In the only study to date investigating passive mechanical properties at the joint level in spastic CP, the ankle joint of children aged 4-9 years with spastic CP was reported to be more stiff than TD
peers (Alhusaini et al., 2010). While the increased joint stiffness reported by Alhusaini et al. (2010) is consistent with findings for other forms of muscle spasticity (Gao et al., 2009, Hoang et al., 2009), the stiffness characteristics at the individual muscle fascicle level in spastic CP remain unknown. Furthermore, contracture is considered progressive during child development, so the passive mechanical properties of muscle from children assessed by Alhusaini et al. (2010) may be different from young adults where underlying growth is complete and fixed muscle contracture have developed.

2.3.2 Active mechanical properties of spastic muscle

Lower limb force production is reduced in spastic CP (Damiano et al., 1995, Elder et al., 2003, Engsberg et al., 2000, Wiley and Damiano, 1998) and is directly related to functional performance in this population (Damiano et al., 2000). Spasticity reduces the ability to activate muscles maximally (Rose and McGill, 2005, Stackhouse et al., 2005) and causes greater amounts of antagonistic muscle activation (co-contraction) (Elder et al., 2003). However, research increasingly shows that spasticity is not the sole culprit in producing the motor dysfunction of spastic CP (Damiano et al., 2000). Muscle morphology and structure and the muscle force-length relationship may play a more significant role (Lieber and Friden, 2002, Moreau et al., 2010, Smith et al., 2011, Wiley and Damiano, 1998).

In individuals with spastic muscle Gao and Zhang (2008) reported a decreased width of the active force-length relation in stroke survivors, which would be expected to decrease the muscle force-generating capacity at short and long lengths respectively. However no such studies of the active force-fascicle length relation have been conducted to date in spastic CP.

2.3.3. Mechanical properties of the Achilles tendon in spastic muscle

The Achilles tendon interacts with the triceps surae muscles and plays an important role in force transmission and energy storage and return during functional activities. There are no studies of
tendon mechanical properties of lower limb muscles in individuals with spastic CP to date. However several studies in individuals with related neurological conditions have been conducted, albeit with conflicting findings. Zhao et al. (2009) reported a longer and more compliant Achilles tendon in stroke patients, whereas Hoang et al. (2009) found no difference in Achilles tendon slack length and peak strain in ambulatory individuals with multiple sclerosis, relative to controls. Studies of muscle mechanical and Achilles tendon properties in spastic CP are required to improve understanding of force production and motor dysfunction in the population.

2.4 Measurement of muscle morphological and mechanical properties

Advances in medical imaging techniques such as ultrasound and magnetic resonance imaging (MRI) have now made it possible to better quantify how muscle and tendon design might be coupled to function in vivo (Fry et al., 2004, Holzbaur et al., 2007). These technologies also present us with the opportunity to assess how muscles adapt to different stimuli that affect muscular control. MRI is considered to be the criterion standard for measuring muscle morphology in vivo (Elliott et al., 1997, Holzbaur et al., 2007, Mitsiopoulos et al., 1998, Scott et al., 1993). However, this technique is expensive, may not be available, takes a substantial amount of time for each scan (typically >2 min) and in some cases patients require sedation. B-mode ultrasound may be used for the same purpose and compared to other imaging techniques has a number of advantages. It is well-suited to detect objective changes in structure and organization in muscle tissue in a manner that makes low demands on the patient, the equipment is cheaper and more portable, large objects can be scanned with multiple sweeps, scans can be performed quickly, in almost any subject position and the muscle can be visualised in real time (Fry et al., 2007, Malaiya et al., 2007, Reeves et al., 2004a, Whittaker et al., 2007). Ultrasound has been the preferred modality for making muscle architectural measurements in the spastic CP literature to date (Fry et al., 2004, Malaiya et al., 2007, Mohagheghi et al., 2007, Mohagheghi et al., 2008, Moreau et al., 2009,
Aspects of muscle morphology and architecture, such as muscle cross sectional area, fascicle length and pennation angle, can be visualised in real time and measured from B-mode images (Friederich and Brand, 1990, Fry et al., 2004, Lichtwark et al., 2007, Reeves et al., 2004a). A number of these 2D characteristics have also been incorporated into regular geometric models to approximate muscle volume (Albracht et al., 2008, Esformes et al., 2002, Infantolino et al., 2007, Miyatani et al., 2004). A direct three-dimensional (3D) representation of a muscle is, however, more favourable when making morphological measurements as the variable shape of a muscle over its length will be taken into account.

Freehand 3D ultrasound (3DUS) involves combining B-mode ultrasound scanning and 3D motion analysis to provide a direct *in vivo* measurement of tissue structure. A stack of B-mode images is created by recording consecutive ultrasound scans while simultaneously tracking the position and orientation of the ultrasound transducer using 3D motion analysis. Coordinate transformations are used to map the individual 2D B-mode images into space and a 3D rendering of the tissue of interest can be constructed for morphological measurement purposes. Freehand 3DUS systems have been used to make direct volume and length measures of the small lower leg muscles of TD children and children with spastic diplegic CP (Fry et al., 2007, Fry et al., 2004, Malaiya et al., 2007). This has enabled the researchers to effectively evaluate the muscle morphological differences between the two populations, quantify the musculotendinous deformity and assess changes due to surgery and over time. Muscle belly lengths measures have also been made in adults using freehand 3DUS and measurement on one subject has shown repeatable results (Fry et al., 2003).

Delcker et al. (1999) reports that good muscle volume accuracy can be achieved in measurements of small-sized, cadaveric muscles. However, these results cannot necessarily be applied to *in vivo* measurements of large human leg muscles because such scans require multiple ultrasound sweeps to capture the whole muscle volume as a result of the limited field of view of standard ultrasound transducers (40 – 60 mm). Recently, Weller et al. (Weller et al., 2007), showed that a freehand
3DUS system, using multiple sweeps, provided excellent precision and accuracy in the
measurement of volume of isolated dog muscles when compared with measurements based on
computed tomography and a water displacement method. The system used by Weller et al. (2007),
also provided repeatable measures of muscle volume measurement in live dogs.

Despite the continued use of 3DUS systems for the determination of muscle morphological factors
in humans, no validation or reliability study has been reported in humans, in vivo. In addition, the
accuracy and repeatability of large muscle volume and muscle belly length measures, requiring
multiple ultrasound sweeps, in different joint positions has not been assessed.

Freehand 3DUS and B-mode ultrasound appears to be well suited to assessing muscle properties
underlying muscle weakness and contracture in spastic CP, as well as associated changes resulting
from maturity and treatment interventions in the clinical environment. Ultrasound also offers the
potential to study muscle and tendon properties during dynamic tasks, thereby providing insight
into how muscles generate power during functional activity (Lichtwark et al., 2007, Lichtwark and
Wilson, 2006).
2.5 Summary

- CP is the most common cause of physical disability in children. Although the key feature of spastic CP is spasticity and is neural in origin, significant alterations in the morphology and mechanical properties of the muscle occur.

- Investigations of the morphology of spastic muscle have focused on children aged 6-12 years, but how the muscle morphological changes develop in younger children and progress across the age span is unknown.

- A single study has investigated the passive mechanical properties of the ankle in individuals with spastic CP and has reported increased stiffness relative to TD individuals. Therefore, further investigations of the passive properties of muscle in spastic CP are required.

- There have been no investigations of the active mechanical properties or tendon properties in individuals with spastic CP. Therefore, further investigations of the active properties of muscle and tendon in spastic CP are required.

- The development of ultrasound technologies offers potential for measuring the morphological and mechanical properties of spastic muscle in all ages.
Chapter 3. Validation of a freehand 3D ultrasound system for morphological measures of the medial gastrocnemius muscle

Acknowledgement of co-authorship:

I have made a substantial contribution in the conception and design of this study, analysis and interpretation of the research data, and the drafting and critical revising of the final manuscript.
3.1 Introduction

Muscle volume and muscle length are important morphological properties of muscle. Both are related to the physiological cross-sectional area (PCSA) of a muscle and provide an indication of its force producing capacity (Fukunaga et al., 2001, Reeves et al., 2004b). Direct muscle volume and muscle length measures can be used to examine muscle contracture and observe changes due to surgery or specific training interventions (Fry et al., 2007, Kawakami et al., 2008). Furthermore, accurate estimates of muscle volume and length are important in musculoskeletal modelling where variability in cadaveric muscles has made extrapolation to living, healthy individuals’ problematic (Fukunaga et al., 1997).

Magnetic resonance imaging (MRI) is considered to be the “gold standard” modality for direct measurement of muscle volume and length in vivo (Holzbaur et al., 2007, Mitsiopoulos et al., 1998). However, this technique is expensive, may not be available, takes a substantial amount of time for each scan (typically >2 min), in some cases patients require sedation and in some clinical groups MRI is contraindicated due to metallic implants such as cardiac pacemakers. In contrast, two-dimensional (2D) B-mode ultrasound is suited to detect aspects of muscle morphology, such as muscle cross sectional area, fascicle length and pennation angle in a safe, objective and relatively inexpensive manner (Lichtwark et al., 2007, Maganaris, 2003, Whittaker et al., 2007). Such measures have been incorporated into regular geometric models to approximate muscle volume (Albracht et al., 2008, Miyatani et al., 2004). A direct three-dimensional (3D) representation of a muscle is, however, more favourable when making morphological measurements as the variable shape of a muscle over its length will be taken into account.

Freehand 3DUS involves combining 2D ultrasound scanning and 3D motion analysis to provide a direct in vivo measurement of tissue structure. A stack of 2D B-mode images is created by recording consecutive ultrasound scans while simultaneously tracking the position and orientation of the ultrasound transducer using 3D motion analysis. Coordinate transformations are used to map
the individual 2D B-mode images into space and a 3D rendering of the tissue of interest can be constructed for morphological measurement purposes. Freehand 3DUS systems have been used to make direct volume and length measures of the small lower leg muscles of TD children and children with spastic diplegic CP (Fry et al., 2007, Malaiya et al., 2007). This has enabled the researchers to effectively evaluate the muscle morphological differences between the two populations and assess changes due to surgery and over time. Muscle belly lengths measures have also been made in adults using freehand 3DUS and measurement on one subject has shown repeatable results (Fry et al., 2003).

Delcker et al. (1999) reports that good muscle volume accuracy can be achieved in measurements of small-sized, cadaveric muscles. However, these results cannot necessarily be applied to in vivo measurements of large human leg muscles because such scans require multiple ultrasound sweeps to capture the whole muscle volume as a result of the limited field of view of standard ultrasound transducers (40 – 60 mm). Recently, Weller et al., (2007), showed that a freehand 3D ultrasonography system, using multiple sweeps, provided excellent precision and accuracy in the measurement of volume of isolated dog muscles when compared with measurements based on computed tomography and a water displacement method. The system used by Weller et al. (2007), also provided repeatable measures of muscle volume measurement in live dogs.

Despite the continued use of 3DUS systems for the determination of muscle morphological factors in humans, no validation or reliability study has been reported in humans, in vivo. In addition, the accuracy and repeatability of large muscle volume and muscle belly length measures, requiring multiple ultrasound sweeps, in different joint positions has not been assessed. We hypothesise that 3DUS will be a valid and reliable method to determine muscle volume and length when compared to MRI. Therefore the purpose of this study is to (1) validate and (2) assess the reliability of the measurement of MG muscle volume and muscle belly length in vivo using multiple sweeps freehand 3DUS system compared to MRI at a range of ankle joint angles.
3.2 Methods

3.2.1 Subjects

Five male and five female subjects (age $26 \pm 5$ years, height $174 \pm 8$ cm) volunteered to participate in the study. All subjects were healthy university staff or students and provided informed consent in accordance with institutional guidelines (GU Ref No: PES/31/07/HREC). Potential subjects were excluded from the study if they had any history of lower leg injury or surgery, were pregnant or had metal implants.

3.2.2 Experimental design

Freehand 3DUS scans of the right lower leg were performed on the relaxed muscle to assess muscle volume and muscle belly length of the MG. MRI scans of the right lower leg were performed the following day to assess MG muscle volume and muscle belly length. Both the freehand 3DUS scans and the MRI scans were performed at three ankle angles, $15^\circ$ dorsiflexion (DF), $0^\circ$ dorsiflexion (N) and $-15^\circ$ dorsiflexion (PF), with a constant knee angle of $25^\circ$ of knee flexion for each subject. Three freehand 3DUS scans were performed and analysed separately at each ankle joint angle to assess repeatability of measures at each angle.

3.2.3 3DUS set-up and calibration

B-mode ultrasound images were recorded at 25 Hz using a PC-based ultrasound scanner with a 128-element beamformer and a 10.0 MHz linear transducer with 60 mm field of view (HL9.0/60/128Z, Telemed Echo Blaster 128 Ext-1Z system, Lithuania). Position and orientation of the transducer were recorded by tracking three reflective markers rigidly attached to the transducer (Figure 3.1) using an optical motion analysis system recording at 100 Hz (8-camera MX13, Vicon Motion Systems Ltd., Oxford, UK).
Figure 3.1. 3DUS transducer setup. A Perspex frame with three retro-reflective markers was rigidly attached to the ultrasound transducer using casting material.

A three volt square wave was produced during recording of ultrasound data, which triggered synchronous collection of the motion analysis data. A 66.7 ms time delay was measured and the data adjusted accordingly. Stradwin software (v3.5, Mechanical Engineering, Cambridge University, UK) was used to integrate the ultrasound images with the transducer kinematic data for frame manipulation, 3D visualisation and reconstruction. As 3D reconstruction was not performed in real-time, customised Matlab (7.6.0 R2008a, The MathWorks, Massachusetts, USA) scripts were used to calculate the 3D position and orientation of the ultrasound transducer and to convert recorded ultrasound video and kinematic data files to Stradwin data file formats to be loaded into Stradwin.

Prior to scanning, the system was spatially calibrated following the single-wall phantom calibration protocol provided in the Stradwin software (Prager et al., 1998). Briefly, this involves scanning a planar surface (the floor of a flat-bottomed water bath which is clearly definable) and performing a least-square fit to estimate the best three translation and three rotations of the line data which fit a plane, which is then used as the spatial calibration. The three translation and rotation offsets were
added to the sensor measurements to calculate the 3D position of the B-mode scans during reconstruction. To ensure consistent acoustic performance and minimise the variability of sound velocity the temperature of the water was maintained between 26-28°C during the single-wall phantom calibration of the freehand 3D ultrasound and subsequent data collection in the water bath. This water temperature also helped to maintain patient comfort during the testing.

3.2.4 3DUS measurements

To eliminate tissue compression and enhance visualisation, the right MG was scanned using the ultrasound transducer while the subjects were kneeling in a water bath. Water at a temperature between 26-28°C covered the entire lower leg. The foot was rigidly stabilised at each ankle joint angle using solid blocks (Figure 3.2.). Knee angle was maintained at 25° of knee flexion by having the leg supported at the end of the water bath and the torso resting on an adjustable bench. Knee and ankle angles were measured using a plastic goniometer.

Figure 3.2. Subject position for 3DUS scanning in the water bath (cut-away). The subjects were kneeling and water covered the entire lower leg. The foot was rigidly stabilised at DF, N and PF using solid blocks. Knee angle was maintained at 25° of knee flexion by having the leg supported
at the end of the water bath and the torso resting on an adjustable bench. Ultrasound images were acquired by performing two or three overlapping parallel sweeps over the length of the MG muscle (highlighted 3DUS scan area). A stack of 2D B-mode ultrasound images was acquired by manually moving the ultrasound transducer over the length of the MG muscle in a transverse orientation at a steady speed. Due to the size of the adult MG muscle and the limited size of the field of view of the ultrasound transducer, two or three overlapping parallel sweeps were necessary to cover the muscle. Ultrasound settings such as power, gain, image depth, and focal depth were optimised to allow ease of identification of the collagenous tissue that defines the outer border of the muscle.

All post-scanning processing was performed in Stradwin. Because multiple sweeps of the MG were required, dividing planes were placed between overlapping ultrasound images (Figure 3.3A). Segmentation was performed manually by outlining the perimeter of the MG in each 2D ultrasound image (Weller et al., 2007). Once segmentation was complete, surface interpolation through the segmentation contours created a rendered 3D image of the muscle belly (Figure 3.3B).

The proximal insertion of the MG was difficult to visualise in the B-mode images so muscle volume (ml) and muscle belly length (mm) measures were made proximally from the most superficial aspect of the medial femoral condyle to the distal musculotendinous junction. To assess the reliability of the segmentation method used for the determination of the MG volume and length, ten randomly selected freehand 3DUS scans were re-analysed. One operator (LB) performed all of the ultrasound image processing.
3.2.5. Phantom volume validation

The accuracy of the freehand 3DUS using single and multiple sweeps was also assessed using twenty water-filled latex condom phantoms containing various volumes of water (26 – 296 ml). The water-filled condoms were imaged and the volumes estimated using the methods defined above. Each reconstructed volume was compared to the known volume of water within the condom. Water volume was calculated using the measured water mass (g) / 0.9978 g.cm$^{-3}$ (the density of water at 22º C).
3.2.6. MRI set-up and measurements

Subjects lay supine on the MRI gantry. Knee and ankle angles were reproduced from measurements in the water bath and maintained using foam bolsters and adjustable straps. Axial MRI scans were recorded, such that the right MG of each subject was scanned from the proximal insertion on the femur to the distal musculotendinous junction where the gastrocnemius connects to the Achilles tendon. All subjects were scanned using a General Electric Signa HDx 1.5 Tesla MRI scanner (Milwaukee, WI, USA.). Adequate anatomical coverage was achieved using a 12 Channel Body Array Coil (GE Healthcare). Images were acquired in the axial plane using a standard 2D spin echo pulse sequence – 400 ms repetition time; 12 ms time to echo; 25 kHz receiver bandwidth; 320 x 288 image matrix (with zip 512 interpolation); 23 x 17.3 cm field of view and 5 mm slice thickness, with varied interslice gap (3 – 5 mm) to allow 40 slices for each subject's anatomical coverage.

The muscle boundaries of the MG were manually segmented in all corresponding axial plane images using a piecewise linear boundary provided by the software program 3D Slicer (Version 2.6-opt, Harvard University, Boston, USA). Between 25 and 35 contour curves were segmented for each muscle (Figure 3.3C) and surface rendering performed for measurement of volume and muscle belly length (Figure 3.3D). Measurements were made proximally from the most superficial aspect of the medial femoral condyle to the distal musculotendinous junction. To assess the reliability of the segmentation method used for the determination of the MG volume and length, ten randomly selected MRI scans were re-analysed. One individual (LB) performed all of the MRI image processing.

3.2.7. Statistical analysis

The limits of agreement method (Bland and Altman, 1986) was used to assess the agreement between (1) the freehand 3DUS and MRI-based measurement of muscle volume and muscle belly length for the MG at three ankle joint angles (DF, N, PF) and, (2) the freehand 3DUS-based...
measurement of volume and the known water volume of the condom phantoms. Intra-session reliability of muscle volume and muscle belly length measurements made using freehand 3DUS over three trials was assessed using the intra-class correlation coefficient (ICC). To assess the reliability of the segmentation method used for the determination of the MG volume and length, ten randomly selected freehand 3DUS scans and ten randomly selected MRI scans were re-analysed and the respective ICC was calculated.

3.3 Results

3.3.1. Validity
The mean muscle volume (± SD) assessed in the study was 274 ± 75 ml (Figure 3.4) and the mean muscle belly length (± SD) was 247 ± 20 mm (Figure 3.5). There was a tendency for the 3DUS to overestimate the muscle volume by 1.90 ml (1.1%) and to underestimate muscle belly length by 3.0 mm (1.3%) across all joint angles (Table 3.1). The 95% confidence intervals (CI) for the level of agreement between 3DUS and MRI were 18 ml for muscle volume and 10 mm for muscle belly length (Figures 3.4, 3.5). 3DUS underestimated condom phantom volumes by 0.9 ± 1.7 ml, (95% CI = 3.4 ml), which corresponded to a percentage difference of 0.7 ± 2.6%.

3.3.2. Reliability and Repeatability
The ICCs for repeated freehand 3DUS measures of muscle volume and muscle belly length were greater than 0.99 and 0.98 respectively (Table 3.2). The mean muscle volumes (± SD) for each trial of the segmentation process reliability were 276 ± 76 and 274 ± 77 ml (ICC = 0.99) for freehand 3DUS, and, 273 ± 81 and 271 ± 81 ml (ICC = 0.99) for MRI. The mean muscle belly lengths (± SD) for the freehand 3DUS trials were 243 ± 16 and 245 ± 15 mm (ICC = 0.97), and MRI trials 251 ± 21 and 252 ± 22 mm (ICC = 0.99).
Figure 3.4. Scatter plots and Bland–Altman plots of MG volume measured by freehand 3DUS and MRI. Scatter plots, MRI versus mean 3DUS, and Bland–Altman plots, difference (3DUS-MRI) versus average of values measured by MRI and 3DUS, of the MG volume in three ankle positions (15°, 0° and -15° dorsiflexion). The diagonal line in the scatter plots corresponds to the line of perfect agreement. The horizontal lines on the Bland–Altman plots represent the mean difference and the upper and lower 95% limits of agreement.
Figure 3.5. Scatter plots and Bland–Altman plots of MG muscle belly length measured by freehand 3DUS and MRI. Scatter plots, MRI versus mean 3DUS, and Bland–Altman plots, difference (3DUS-MRI) versus average of values measured by MRI and 3DUS, of the MG muscle belly length in three ankle positions. The diagonal line in the scatter plots corresponds to the line of perfect agreement. The horizontal lines on the Bland–Altman plots represent the mean difference and the upper and lower 95% limits of agreement.
Table 3.1. Comparison of muscle volume and muscle belly length measurements of the MG between freehand 3DUS and MRI. Data are presented as mean ± 1 SD.

<table>
<thead>
<tr>
<th>Muscle volume</th>
<th>Ankle joint position</th>
<th>MRI (ml)</th>
<th>3DUS (ml)</th>
<th>Mean difference (ml)</th>
<th>Mean difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>273 ± 83</td>
<td>278 ± 80</td>
<td>-4.8 ± 9.1</td>
<td>-2.1 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>271 ± 80</td>
<td>274 ± 77</td>
<td>-3.1 ± 8.9</td>
<td>-1.6 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>PF</td>
<td>274 ± 81</td>
<td>272 ± 77</td>
<td>2.2 ± 8.9</td>
<td>0.4 ± 3.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>273 ± 79</td>
<td>275 ± 75</td>
<td>-1.9 ± 9.1</td>
<td>-1.1 ± 3.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscle belly length</th>
<th>Ankle joint position</th>
<th>MRI (mm)</th>
<th>3DUS (mm)</th>
<th>Mean difference (mm)</th>
<th>Mean difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>255 ± 20</td>
<td>255 ± 20</td>
<td>-0.6 ± 5.0</td>
<td>-0.1 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>251 ± 21</td>
<td>247 ± 20</td>
<td>4.2 ± 5.1</td>
<td>1.7 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>PF</td>
<td>245 ± 19</td>
<td>240 ± 18</td>
<td>5.4 ± 4.7</td>
<td>2.2 ± 1.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>250 ± 20</td>
<td>247 ± 20</td>
<td>3.0 ± 5.4</td>
<td>1.3 ± 2.2</td>
</tr>
</tbody>
</table>
Table 3.2. Reliability of intra-session repeated measures of muscle volume and muscle belly length by freehand 3DUS system assessed using the Intra-class correlation coefficient (ICC). Experimental data are presented as mean ± 1 SD.

<table>
<thead>
<tr>
<th>Muscle volume (ml)</th>
<th>Ankle joint position</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td></td>
<td>278 ± 83</td>
<td>277 ± 81</td>
<td>278 ± 76</td>
<td>0.998</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>274 ± 76</td>
<td>271 ± 79</td>
<td>276 ± 76</td>
<td>0.997</td>
</tr>
<tr>
<td>PF</td>
<td></td>
<td>273 ± 77</td>
<td>270 ± 79</td>
<td>274 ± 77</td>
<td>0.998</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscle belly length (mm)</th>
<th>Ankle joint position</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td></td>
<td>255 ± 20</td>
<td>255 ± 20</td>
<td>256 ± 20</td>
<td>0.991</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>245 ± 17</td>
<td>248 ± 22</td>
<td>248 ± 20</td>
<td>0.988</td>
</tr>
<tr>
<td>PF</td>
<td></td>
<td>240 ± 20</td>
<td>239 ± 18</td>
<td>240 ± 18</td>
<td>0.988</td>
</tr>
</tbody>
</table>

### 3.4. Discussion

This study has demonstrated good agreement between multiple sweeps freehand 3DUS and MRI measures across a range of MG volumes and varying ankle joint angles. The mean percentage difference between the two methods was minimal with freehand 3DUS overestimating the volume measured using MRI by 1.1%. In further support, only 0.7% variability was calculated when comparing the freehand 3DUS system to known volume water-filled condom phantoms in measurements over a range of volumes from 26 – 296 ml. The results of this validation study support those of previous studies on smaller muscles. Weller et al. (2007) found that freehand 3DUS underestimated the in vitro dog muscle volume by 3.33 ml compared to CT volume measures and overestimated by 1.38 ml compared to the water displacement method. Delcker et al. (1999) reported a 10% difference between freehand 3DUS and the water displacement method in cadaveric human hand muscles. The current study had a tendency for the 3DUS to overestimate the muscle volume by 1.90 ml. Comparing the accuracy of our study to the previous validation studies
is problematic considering the dissimilarities in the methods used; however the multiple sweeps 3DUS method is valid for measuring large *in vivo* muscle volumes.

Our muscle belly length measurements compare favourably with the values made from adult cadavers (Wickiewicz et al., 1983) and are consistent with the expected relationship between length and joint angle (Fry et al., 2003). Agreement of measurement of muscle belly length between MRI and freehand 3DUS at each ankle joint angle was also good. The freehand 3DUS system underestimated the muscle belly length by 1.3% as compared to the MRI measures. Measurements at DF are almost identical but at N and PF there appears to be an underestimation of muscle belly length by the freehand 3DUS. This may be due to numerous confounding factors including background muscle activation, variable connective tissue image quality due to tissue stretch and/or depth, difficulty in imaging the most prominent posterior aspect of the medial femoral condyle with B-mode ultrasound and subject position (supine versus prone) effecting passive forces acting on the muscle bulk despite the same joint configuration. Furthermore, MRI length measurements may be inaccurate due to the axial slice widths, which in this study were 5 mm. This limits the accuracy of the length measures using the MRI technique to distance between axial slice planes. This may also account for the lack of difference in length measurement that was observed between the DF and N positions.

The intra-session reliability of the freehand 3DUS system to measure muscle volume and muscle belly length was very high with all ICCs greater than 0.997 and 0.988, respectively. A 0.9% underestimation in volume and 0.6% overestimation difference in muscle belly length between re-analysed scans indicated that the manual segmentation process is also a major source of measurement error. To note, repeat MRI scans were not performed but a repeated segmentation process produced a difference of 0.7% for volume and -0.5% for muscle belly length indicating one source of error in our current ‘gold standard’. Unlike techniques for examining bone, there is currently no image processing technique that is able to automatically threshold and segment muscles from MRI to minimize the manual processing error. Considering the variability implicated
with the segmentation process of both imaging methods, much of the calculated differences in muscle volume and muscle belly length may be explained simply by manual segmentation error. It is encouraging that only small percentage differences in accuracy and repeatability between the two techniques were found making its potential application to deriving PCSA and estimating the force generating capacity of muscle acceptable.

Ultrasound images are subject to distortion due to tissue compression from the transducer and, in general, have poor resolution of deep muscles (Fry et al., 2004, Infantolino et al., 2007). In this study, the focus was on superficial muscle, and to enhance image quality and eliminate transducer pressure, a water bath was used. The use of a water bath also assisted the multiple sweeps scanning procedure by allowing sufficiently overlapping parallel sweeps and the maintenance of an orthogonal transducer orientation to the skin surface. Lack of overlap resulted in gaps in the 3D-rendered muscle and, hence, inaccuracies in the measurement of the muscle volume and muscle belly length. Usually echogenic gel is the coupling medium used between the skin and the ultrasound transducer during scanning. Our experience using gel for single sweep scanning was positive but for multiple sweeps scanning was mixed in regard to recorded image quality and tissue deformation differences between sweeps which ultimately affected the post-processing procedures. While water baths can be specifically designed for examining peripheral musculature of the upper and lower limb, other coupling medium may be required to be developed to make this technology more applicable to clinical settings. To ensure quality ultrasound images for 3D reconstruction, muscles must have clearly identifiable borders. The latter can pose a problem if muscles have been affected by pathology, are partly fused to other muscles, or insert on poorly defined aponeuroses. Further investigations of the validity of 3DUS for volume and length measurements in other specific muscles and using difference subject populations may be required.

Concluding Remarks

This study has demonstrated that accurate and repeatable measurement of relatively large muscle volume and muscle belly length in vivo is possible using multiple sweeps freehand 3DUS imaging
over a large range of ankle joint angles. Errors in length and volume measurement of less than 2% can be considered negligible when using morphology measures to make estimates of muscle force or a muscle's length range, however for smaller muscles, the percentage errors are likely to increase as was the case in the study by Delcker and colleagues (1999). Ultrasound, compared to other imaging techniques, can be performed quickly and in almost any subject position, can scan large objects using multiple sweeps, and is relatively cheaper and more portable. Also other measures of in vivo muscle morphology can be obtained during the brief time for data collection such as anatomical cross-sectional area, muscle-tendon length, fibre length and pennation angle allowing an individual subject estimation of PCSA within the setting of a biomechanics laboratory. The freehand 3DUS system lends itself to in vivo muscle morphological measurements for monitoring changes due to age, function, pathology, surgery and training and researchers may consider the use of freehand 3DUS interchangeably with MRI.
Chapter 4. Medial gastrocnemius muscle volume and fascicle length in children aged 2-5 years with cerebral palsy

Acknowledgement of co-authorship:

I have made a substantial contribution in the conception and design of this study, analysis and interpretation of the research data, and the drafting and critical revising of the final manuscript.
4.1. Introduction

Spastic CP is a group of non-progressive motor impairment syndromes that occur secondary to lesions of the brain in the early stages of development (Bax et al., 2005). Spasticity is the key feature of spastic CP, and is neural in origin. However it is clear that spastic muscle also undergoes significant morphological and structural alterations during development. Involved muscles often shorten to create muscle contractures, which contribute to increased joint stiffness, reduced joint range of motion and may contribute to reduced voluntary strength. These impairments progress over time and limb movement and general functional ability often declines (Bache et al., 2003, Graham and Selber, 2003, Wren et al., 2004). As the secondary effects of spastic CP disrupt posture and movement, most conservative, drug and surgical management strategies attempt to remediate musculoskeletal dysfunction.

A recent systematic review (Barrett and Lichtwark, 2010) concluded that there was consistent evidence for a reduced muscle volume across a range of muscles in the paretic limb in spastic CP compared with both the non-paretic limb and TD peers (Elder et al., 2003, Fry et al., 2007, Fry et al., 2004, Lampe et al., 2006, Lieber et al., 2004, Malaiya et al., 2007, McNee et al., 2009). The reduced muscle volume in spastic CP is likely to contribute to muscle weakness and loss of motor function reported in spastic CP. A further conclusion from the review was consistent evidence for a reduced gastrocnemius muscle length in spastic CP, which may be explained by a reduced muscle fibre diameter and corresponding contraction of the aponeurosis to which the muscle fibres attach (Malaiya et al., 2007, Shortland et al., 2002). In contrast to findings for muscle length, there is inconsistent evidence for reduced muscle fascicle length in spastic CP. Some studies report no differences in fascicle length for the MG (Lieber et al., 2004, Malaiya et al., 2007, Shortland et al., 2002) while others report a reduction in fascicle length in paretic muscle relative to TD muscle and also to the non-paretic gastrocnemius muscle in hemiplegia (Mohagheghi et al., 2008, Moreau et al., 2009). The apparent disagreement in the reported results may stem from several methodological issues, such as recruitment of participants with differences in the type of the
underlying brain injury (diplegia versus hemiplegia), differential levels of motor impairments
involving the muscles scanned and the suitability of the normalization technique of fascicle length
(Mohagheghi et al., 2008, Moreau et al., 2009). It is important to establish if muscle fascicle length
is altered in spastic CP because muscle fascicle length is an important determinant of muscle
excursion, with shorter muscle fascicles having a reduced range through which they can develop
force and power, a reduced maximum shortening speed and a reduced length at which they develop
passive forces (Lieber and Friden, 2000).

The functional significance of muscle morphology and structure can be further understood by
considering the physiological cross-sectional area (PCSA) of the muscle, which is proportional to
the muscle’s maximal force generating capacity. The PCSA is obtained from the ratio of muscle
volume to optimal fascicle length (which is sometimes corrected by the cosine of the pennation
angle), and represents the number of muscle fascicles working in parallel (Lieber and Friden,
2000). To date only one study (Malaiya et al., 2007) has measured muscle volume, fascicle length
and pennation angle in the same participants with spastic CP. In the study by Malaiya, et al.
(Malaiya et al., 2007) the differences in PCSA (calculated at a common joint angle) for the MG
between the spastic CP and TD groups aged 4-12 years were almost completely explained by
differences in muscle volume. However, the extent to which this may be true for younger children
with spastic CP is unknown.

There is presently little information about the time course of structural changes to muscle in spastic
CP. Investigations of spastic lower limb muscle morphological properties to date have included
children with spastic CP with ages ranging from 4 to 12 years (Elder et al., 2003, Fry et al., 2007,
Malaiya et al., 2007, Mohagheghi et al., 2007). Physical development is varied across these ages
and treatment and management strategies may be quite mixed. It therefore remains unclear at what
age changes in muscle volume and length start to occur in children and how this progresses with
time. Studies on younger children who have received no previous surgical or pharmaceutical
intervention are required to determine at what age’s changes in muscle volume and length start to occur, as this could have implications for the management of spastic CP in this age group.

The purpose of the present study was therefore to compare muscle volume, PCSA, length, and muscle fascicle length and pennation angle of the MG muscle in children with hemiplegic and diplegic spastic CP who have received no previous surgical or pharmaceutical intervention and TD children aged between 2-5 years using ultrasound at three ankle joint angles. As there is no data regarding morphological properties of spastic muscle in this age group, our null hypothesis was that there would be no difference between the spastic CP and TD groups in the variables measured.

### 4.2. Methods

#### 4.2.1. Participants

Twenty TD children (11 boys, 9 girls) and fifteen children with spastic CP, (11 boys, 4 girls) aged 2-5 years participated in the study. The spastic CP group were recruited from patients referred to The Royal Children’s Hospital and to Monash Medical Centre, Melbourne, Australia for botulinum toxin injections (Ethical approval - Human Research Ethics Committee, Royal Children’s Hospital: HREC RCH:27062, HREC: 07083C). The spastic CP participants were hemiplegic (n = 5) or diplegic (n = 10) and were either level I (n = 10) or II (n = 5) on the Gross Motor Function Classification System (GMFCS) for Cerebral Palsy (Palisano et al., 2007) with clinically diagnosed gastrocnemius spasticity. All participants had a minimum of 0° ankle dorsiflexion range of motion with the knee extended. Children were excluded if they had had previous Botox treatment to the calf muscles or previous calf surgery. The TD children were recruited from the general population. All children were able to walk independently and potential participants were excluded if there was a history of previous lower leg injury or other developmental disorder affecting the lower limb (Ethical approval – Human Research Ethics Committee, Griffith University: GU Ref No:...
PES/29/07/HREC). All parents of participants provided informed consent to the research and to publication of the results in accordance with institutional guidelines.

4.2.2. Experimental protocol

Height, weight, leg length and fibula length were initially measured for each participant. Ultrasound scans of the lower leg were performed on the relaxed MG muscle at MDF, neutral (N) and maximum plantarflexion (MPF) with the knee fully extended (0° of flexion). For ultrasound measurements participants lay prone on a plinth, with the foot positioned off the end of the plinth to facilitate full range of ankle movement. MDF and MPF ankle angles were achieved using sufficient force to reach the end range of motion of the ankle joint in the sagittal plane. Ankle joint angles were measured using a standardised protocol with a plastic goniometer to the nearest degree. N ankle angle was defined as 90° between the line of the fibula and the base of the lateral foot. The force to achieve each ankle angle and maintain foot stability was applied by hand for ultrasound scanning. Only the most affected leg of the spastic CP participants and the right leg of the TD participants were scanned. Ultrasound scans were performed by two operators (LB and TH-I) and all data was processed and analysed by the one investigator (LB).

4.2.3. Ultrasound measures

A PC-based B-mode ultrasound scanner with a 128-element beamformer and a 10.0 MHz linear transducer with 60 mm field of view (HL9.0/60/128Z, Echo Blaster 128 Ext-1Z system, Telemed, Vilnius, Lithuania) was used to record ultrasound images at 25 Hz.

Two dimensional, B-mode ultrasound (2DUS) of MG muscle fascicles were examined following the recommendations of Benard et al. (Bénard et al., 2009). The probe was aligned to the midline of the muscle so that the long axis of the US transducer was aligned with the line of action of the fascicles in the mid-belly of the muscle. Muscle fascicle length was defined as the straight line distance between the upper muscular fascia and the lower muscular fascia parallel to the lines of
collagenous tissue visible on the image. This measurement was consistently made in the middle of image where the full length of the fascicle could be visualised. The pennation angle was defined as the angle made between the upper fascia (the line of action of the tendon) and the direction of the muscle fascicles and has been described previously (Lichtwark et al., 2007). This method for obtaining representative ultrasound images of the MG fascicle and pennation angle has been shown to minimize measurement errors (Bénard et al., 2008, Bénard et al., 2009).

Freehand three-dimensional ultrasound (3DUS) was used to measure muscle volume and length as has been described and validated previously (Experiment 1) and an ultrasound gel couplant was used at the transducer-soft tissue interface to minimise the acoustic impedance mismatch between air and skin. 3DUS involves combining 2D ultrasound images of the transverse plane of the muscle with 3D motion data to reconstruct the muscle volume in 3D. Reconstructions and volume and length measurements were performed using Stradwin software (v3.5, Mechanical Engineering, Cambridge University, UK) (Experiment 1).

PCSA was calculated using equation 1. Fascicle length and pennation angle (θ) were obtained from measurements made at the neutral ankle angle.

\[
PCSA = \frac{\text{Muscle Volume} \times \cos \theta}{\text{Fascicle Length}}
\]

Equation 1

4.2.4. Statistical analysis

Demographic, anthropometric and PCSA data from the most affected side of the spastic CP group and the right leg of the TD were compared using a one-way analysis of variance (ANOVA) (SPSS Statistics 18.0.0). Pearson product moment correlation coefficients were computed for the relation between the muscle volume, muscle length, fascicle length, pennation angle and age in months. Correlation coefficients were also computed for the relationship between muscle length, fascicle length, pennation angle and ankle angle. The selection of covariates for the dependent variables
followed the procedure outlined in Mohagheghi et al. (Mohagheghi et al., 2008). Pearson product-moment correlation coefficients were computed for the relations between muscle volume and body mass, and muscle length and fascicle length and fibula length and ankle angle. If these correlations were significant and the slopes of the regression lines were not significantly different between groups, then the relevant anthropometric variable was used as a covariate in the subsequent group comparisons. A 2 x 3 full factorial general linear model (GLM) with relevant covariates was used to assess the effect of between group factors (spastic CP versus TD) and within group factors (MDF, N, MPF ankle joint angle) and their interactions on the dependent measures (muscle volume, muscle length, fascicle length and pennation angle). Pairwise comparisons were used to assess group differences in each measure at the three ankle joint angles. Values are presented as the mean ± one standard deviation (SD) and the alpha level for assessing statistical significance was set at 0.05.

4.3. Results

4.3.1. Participant characteristics

Demographic, anthropometric, and ankle angle data for the spastic CP and TD groups are presented in Table 4.1. Fibula length was significantly greater for the TD group (p = 0.01).
Table 4.1. Demographic, anthropometric, and ankle angle data for the spastic CP and TD groups.

<table>
<thead>
<tr>
<th></th>
<th>Spastic CP (n = 15)</th>
<th>TD (n = 20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>45 (15)</td>
<td>48 (14)</td>
<td>0.64</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>16 (3)</td>
<td>16 (3)</td>
<td>0.40</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>99 (9)</td>
<td>101 (9)</td>
<td>0.61</td>
</tr>
<tr>
<td>Leg length (cm)</td>
<td>47 (8)</td>
<td>48 (7)</td>
<td>0.92</td>
</tr>
<tr>
<td>Fibula length (cm)</td>
<td>18 (3)</td>
<td>20 (2)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Maximum dorsiflexion angle (°)</td>
<td>8 (7)</td>
<td>26 (6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Maximum plantarflexion angle (°)</td>
<td>-53 (6)</td>
<td>-54 (5)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Data are mean (1 SD). Asterisk (*) indicates significance group difference (p < 0.05). Positive ankle joint angles indicate dorsiflexion.

Group had a significant main effect on ankle angle with the spastic CP group more plantarflexed, -15 (2)°, than the TD group, -9 (2)° (F (1, 33) = 5.19, p = 0.03). A significant group by ankle angle interaction was also detected (F (1, 33) = 5.90, p = 0.02). Pairwise comparisons revealed that the spastic CP group had significantly reduced ankle angle at MDF (p < 0.001) but not at MPF.

4.3.2. Correlation analysis

MG muscle volume was significantly correlated with age in months for the spastic CP and TD groups, r = 0.65 and 0.63 respectively (both p < 0.01) and muscle length was significantly correlated with age for both groups at all ankle angles (r = 0.72-0.81, p < 0.01) (Appendix A, Figure A.1). MG muscle volume was significantly correlated with body mass for both groups at all ankle angles tested (r = 0.67-0.74, p < 0.01) and muscle length was significantly correlated with fibula length for both groups at all ankle angles tested (r = 0.79-0.88, p < 0.001) (Appendix A, Figure A.2). The slopes of the regression lines were not significantly different between groups. Body mass and fibula length were used as covariates for subsequent group comparisons of muscle volume and length respectively. MG fascicle length was not significantly correlated with fibula
length in the spastic CP group ($r = 0.17-0.56$, $p > 0.05$) or the TD group respectively ($r = 0.08-0.43$, $p > 0.05$). Pearson’s $r$-values, 95% confidence intervals and $p$-values for all correlation analyses are presented in the Appendix A, Table A.1.

4.3.3. Analysis of variance

Normalised MG muscle volume was significantly less in the spastic CP group, 25 (2) ml, than the TD group, 33 (2) ml, ($F (1, 32) = 8.13$, $p = 0.008$) (Figure 4.3A). PCSA was significantly less in the spastic CP group, 5.3 (0.5) cm$^2$, than the TD group, 7.3 (0.5) cm$^2$, ($F (1, 33) = 7.31$, $p = 0.01$).

No significant main group effects were detected for MG muscle length ($p = 0.28$), fascicle length ($p = 0.44$) or pennation angle ($p = 0.11$) (Figure 4.3B-D). However significant group by ankle angle interactions were detected for MG fascicle length ($F (1, 33) = 26.42$, $p < 0.001$) (Figure 4.3C) and pennation angle ($F (1, 33) = 18.28$, $p < 0.001$) (Figure 4.3D). Pairwise comparisons revealed that fascicle length was significantly longer at MPF ($p < 0.001$) and pennation angle was significantly smaller at MPF ($p = 0.002$) for the spastic CP group compared to the TD group.
Figure 4.3. Muscle volume, muscle length, fascicle length and pennation angle versus ankle angle for the spastic CP and TD groups (A-D). Spastic CP ( ■ ) and TD ( ○ ) groups. Data are mean ( ± SEM). Means for muscle volume are statistically adjusted for body weight and means for muscle length are statistically adjusted for fibula length. Positive ankle angle indicates dorsiflexion. Asterisk (*) indicates significance group difference (p < 0.01).

4.4. Discussion

The main finding of this study was that muscle volume was significantly reduced by 22% in the spastic CP compared to the TD group in children aged 2-5 years. Smaller muscle volume has been consistently reported in studies of independently ambulatory children aged 4-12 years with spastic CP (Table 4.2) (Elder et al., 2003, Fry et al., 2007, Malaiya et al., 2007, Shortland, 2009). We therefore confirm reductions in muscle volume of the MG in children with spastic CP compared to TD children at an earlier age than previously reported. The magnitude of the observed muscle
volume reduction in spastic CP relative to TD muscle reported here is at the low end of the 26-57% differences for studies of calf muscle volumes of older children and young adults reported elsewhere (Elder et al., 2003, Fry et al., 2007, Malaiya et al., 2007, Oberhofer et al., 2009, Shortland, 2009) (Table 4.2). While this appears to indicate a general lack of volumetric growth during development in spastic CP, the differences in condition severity and lack of surgical and pharmacological treatments in the present group compared to older age categories studied previously makes it difficult to compare study results. One possibility, for example, is that the spastic CP participants in the present study had milder spastic CP and hence experienced smaller reductions in muscle volume compared to TD muscle relative to volume reductions reported other studies. Significant positive correlations were however found between muscle volume and age, and muscle length and age in both groups, which is suggestive of muscle growth across the age span assessed in both groups in our cross-sectional study (Appendix A, Figure A.1, Table A.1). However longitudinal studies will be required to better understand the natural history of muscle growth during development in spastic CP and the effects of common treatment interventions.

Although the participants in the spastic CP group had relatively high motor function (i.e. GMFCS I-II), they may have lower physical activity levels, and therefore experience reduced stimulus for muscle growth than the TD group (Eliakim et al., 2001, McNee et al., 2009). Genetic alterations have been identified in spastic CP muscle (Smith et al., 2009), which give rise to competing pathways for fibre hypertrophy and an increase in anabolic growth factors. We are unable to clarify the physiological mechanism of the reduced muscle volume in spastic CP from the present study, but this would be expected to be related to a reduced number of muscle fibres in parallel (hypoplasia) and/or decreased muscle fibre cross-sectional area (fibre atrophy) (Lieber et al., 2004, Shortland et al., 2002).
Table 4.2. Characteristics of studies investigating lower leg muscle volume in children with spastic CP. To facilitate direct comparisons, percentage difference between the spastic CP and TD groups were calculated from muscle volume data normalised to body weight.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Motor function</th>
<th>Age (yrs)</th>
<th>Muscle</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>15 SCP</td>
<td>GMFCS I-II</td>
<td>4 (1)</td>
<td>MG</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>20 TD</td>
<td></td>
<td>4 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaiya et al. (2007)</td>
<td>16 SCP</td>
<td>Ambulant</td>
<td>8 (4-12)</td>
<td>MG</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>15 TD</td>
<td></td>
<td>10 (4-13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elder et al. (2003)</td>
<td>28 SCP</td>
<td>Unknown</td>
<td>8 (2)</td>
<td>PF</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>14 TD</td>
<td></td>
<td>9 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fry et al. (2007)</td>
<td>7 SCP</td>
<td>Independently</td>
<td>8 (6-10)</td>
<td>MG</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>10 TD</td>
<td>ambulant</td>
<td>9 (6-12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oberhofer et al. (2009)</td>
<td>6 SCP (4H, 2D)</td>
<td>GMFCS I-II</td>
<td>H: 10 (1), D: 10 (2)</td>
<td>MG +</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5 TD</td>
<td></td>
<td>10 (1)</td>
<td>LG</td>
<td></td>
</tr>
<tr>
<td>Shortland (2009)</td>
<td>14 SCP (2H, 7D)</td>
<td>Independently</td>
<td>17 (14-22)</td>
<td>MG</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>14 TD</td>
<td>ambulant</td>
<td>Age, sex matched</td>
<td>LG</td>
<td>39</td>
</tr>
</tbody>
</table>

Acronyms: Spastic CP (SCP), medial gastrocnemius (MG), lateral gastrocnemius (LG), plantarflexors (PF), hemiplegia (H), diplegia (D). Mean (SD or range).

The significantly reduced muscle volume in the spastic CP compared to TD group detected in the present study occurred with relatively small differences in fascicle length and pennation angle between the groups. These findings are consistent with those of Malaiya et al. (Malaiya et al., 2007), who also reported MG muscle volume reductions in 4-12 year old children with spastic CP in the absence of changes in fascicle length at resting ankle joint angle. Our findings indicate that reduced muscle volume is the major determinant of the reduction in PCSA, and hence reduced muscle force production capacity in young children with spastic CP.
The practical significance of these findings is that lack of volumetric muscle growth in young children with spastic CP and the associated muscle weakness are likely to contribute to functional limitations observed in young children with spastic CP (Bache et al., 2003, Graham and Selber, 2003). Given that muscle weakness tends to progress over time, and can be a barrier to mobility and independent living in later stages of development (Shortland, 2009), an important goal of treatment and management of spastic CP in young children may be to facilitate muscle growth through use of suitable exercise interventions (McNee et al., 2009). A possible explanation for the apparent lack of clinical awareness of reduced muscle growth in young children with spastic CP is that the 22% reduction in MG muscle volume detected in the present study corresponds to 8 ml, which may be below the detection threshold of routine clinical examination and therefore easily overlooked. These findings also suggest that treatments with the potential to compromise muscle growth (e.g. botulinum toxin) may need to be carefully considered when used in young children with spastic CP.

No group differences were found for muscle length, fascicle length or pennation angle at the neutral or MDF angles. However the spastic CP group had a reduced MDF angle. Assuming the moment arms are similar between both groups and that the MDF represents the end-range of motion (where passive forces are high), these results suggest that the observed restriction in ankle joint dorsiflexion range in the spastic CP group is likely to be related to greater passive stiffness of the MG and other plantarflexor muscles. However at present it remains unclear to what extent the hypothesised increase in muscle-tendon stiffness may be attributed to the muscle fascicles, tendon or both (Hoang et al., 2007, Lieber et al., 2004). For example it is possible that the spastic CP group has a different number of sarcomeres in series and/or that for a given fascicle length the sarcomeres are at a different length as previously reported for spastic wrist muscles (Lieber and Friden, 2002). This might explain the inability of fascicles to stretch further and hence reduce the observed dorsiflexion range in spastic CP. Combining measures of fascicle and sarcomere length in future studies may therefore prove a fruitful way of better understanding the mechanisms of joint restriction in spastic CP.
There are a number of limitations that need to be considered when interpreting the results of this study. Firstly, the study was limited to the MG muscle and so it remains unclear how muscle morphology and structure in other muscles are altered in young children with spastic CP. Secondly, although participants were instructed in the present study to relax their muscles during the testing procedure, we did not measure muscle activation and so cannot fully discount the possibility that there were differences in muscle activation between groups. Thirdly, fascicle length and pennation angle are highly dependent on muscle tension, therefore a consideration for future studies will be to control for muscle tension when comparing passive muscle properties between different groups (Hoang et al., 2007). Finally, a high degree of inherent heterogeneity in the spastic CP group can make it difficult to detect group differences in muscle properties. The extent of the brain lesion causing spastic CP, the degree of motor impairment and concomitant level of function would be expected to influence muscle properties. Also, children with hemiplegic and diplegic spastic CP have been included in this study. Although lower limb muscle fascicle lengths and volumes have been reported to be similar in relatively high functioning children with hemiplegic CP (Malaiya et al., 2007, Mohagheghi et al., 2007) and children with diplegic CP (Fry et al., 2007, Mohagheghi et al., 2008, Shortland et al., 2002), these groups have a dissimilar pathogenesis (Krageloh-Mann and Horber, 2007) and the muscle and tendon adaptations may follow distinct trajectories. In future, it will therefore be useful to examine the relation between muscle structural properties and motor function in spastic CP.

**Concluding remarks**

MG muscle PCSA was reduced in spastic CP compared to TD children aged 2-5 years, which was primarily explained by a 22% reduction in muscle volume in the spastic CP group. This reduction in PCSA is likely to contribute to muscle weakness in young children with spastic CP and provides preliminary evidence in support of the need for early intervention to minimise muscle volume loss in spastic CP. While no significant differences were found between muscle and fascicle lengths across the range of available dorsiflexion range of motion, the restricted ankle joint dorsiflexion
range in the spastic CP group is suggestive of group differences in the mechanical properties of the passive structures of the ankle complex including muscle fascicles and/or tendon.
Chapter 5. Passive muscle mechanical properties of the medial gastrocnemius in young adults with spastic cerebral palsy

Acknowledgement of co-authorship:

I have made a substantial contribution in the conception and design of this study, analysis and interpretation of the research data, and the drafting and critical revising of the final manuscript.
5.1. Introduction

Spastic CP is a group of non-progressive motor impairment syndromes that occur secondary to lesions of the brain in the early stages of development (Bax et al., 2005). Spasticity is the key feature of spastic CP, and is neural in origin. However it is clear that spastic muscle also undergoes significant morphological and structural alterations in spastic CP which contribute to muscle weakness, restricted joint range of motion and increased passive joint stiffness (Gracies, 2005) and corresponding reductions in function (Eek and Beckung, 2008). The key to further understanding the mechanisms responsible for these joint level alterations is to examine architectural and mechanical properties of joint structures such as muscles and tendons (Lieber and Friden, 2001).

Perhaps the most consistent finding from muscle level studies in spastic CP to date is that muscles in spastic CP are smaller compared to TD peers when expressed in terms of volume, cross-sectional area or muscle thickness (Barrett and Lichtwark, 2010). This finding, together with reports of collagen accumulation (Booth et al., 2001) and altered neural activation in spastic CP (Poon and Hui-Chan, 2009), would be expected to contribute to the reported muscle weakness in spastic CP (Wiley and Damiano, 1998). However it remains less clear what musculo-articular structures are responsible for restricted range of motion and increased stiffness at the joint level in spastic CP; commonly referred to as contracture. Increased non-neurally mediated joint stiffness in subjects with upper motor neuron lesions has been attributed to shorter muscles (Zhao et al., 2009) and muscles with shorter fascicles (Gao et al., 2009). However conflicting findings have been reported in spastic CP, because some studies report reductions in resting fascicle length (Mohagheghi et al., 2008, Moreau et al., 2009), while others report no differences (Malaiya et al., 2007, Shortland et al., 2002). It has also been suggested that reductions in muscle belly length reported in the absence of reductions in fascicle length in spastic CP could at least be partially explained by muscle fibre atrophy (Malaiya et al., 2007). Furthermore, although there is evidence for longer and more compliant tendons post-stroke (Zhao et al., 2009), no studies of tendon mechanical properties have been conducted in spastic CP.
Studies of whole muscle level architectural properties in spastic CP as mentioned above have primarily been confined to measurements of resting muscle belly and fascicle length/angle. These measurements are typically made at the resting joint angle, where muscle tension is assumed to be negligible, and so do not reveal information about muscle stiffness. The stiffness of individual muscle cells has however been reported to be substantially increased in spastic wrist muscle (Friden and Lieber, 2003), which depending on the number of muscle cells in series and their corresponding lengths, could explain joint level mechanical properties in spastic CP. In the only study to date investigating passive mechanical properties at the joint level in spastic CP, the ankle joint of children aged 4-9 years with spastic CP was reported to be more stiff than TD peers (Alhusaini et al., 2010). While the increased joint stiffness reported by Alhusaini et al. (2010) is consistent with findings for other forms of muscle spasticity (Gao et al., 2009, Hoang et al., 2009), the stiffness characteristics at the individual muscle fascicle level in spastic CP remain unknown. Furthermore, contracture is considered progressive during child development, so the passive mechanical properties of muscle from children assessed by Alhusaini et al. (2010) may be different from young adults where underlying growth is complete and fixed muscle contracture have developed.

The purpose of this study was to compare the *in vivo* passive mechanical properties of the ankle joint and MG muscle in young adults with spastic CP and TD control subjects. We hypothesized that passive ankle stiffness would be greater in spastic CP and be accompanied by lower MG muscle fascicle strains. The MG muscle was chosen for analysis because it is commonly affected by contracture in neurological conditions such as spastic CP (Malaiya et al., 2007) and because of its functional importance in locomotion (Steele et al., 2010).
5.2. Methods

5.2.1. Subjects

Nine young adults with spastic CP (6 males, 3 females, aged 17(2) years, range 15-21 years) and ten TD young adults (5 males, 5 females, aged mean (1SD) 18(2) years, range 15-20 years) participated in the study. The spastic CP group were recruited from the Queensland Cerebral Palsy League and the TD participants were recruited from the general population. The spastic CP participants were either hemiplegic (n=7) or diplegic (n=2). All were level I on the Gross Motor Function Classification System for Cerebral Palsy (Palisano et al., 2007) and their modified Ashworth scale was 1(1) (range 0-2) indicating relatively high motor function. The inclusion criteria for the spastic CP group were having clinically diagnosed gastrocnemius spasticity and no heel to ground contact during stance phase of gait assessed using qualitative video analysis. The exclusion criteria for the spastic CP group were having orthopaedic surgery within the last 2 years, botulinum toxin injection to the lower extremities within the last 12 months and tactile hypersensitivity in the lower extremities. Potential TD participants were excluded if there was a history of previous foot, ankle or lower leg injury or previous lower limb surgery. Ethics approval was obtained from the Institutional Human Research Ethics Committee and all relevant ethics guidelines were followed.

5.2.2. Experimental protocol

Height, weight, leg length, fibula length and MG muscle volume were initially measured for each participant. The passive mechanical properties of the ankle joint and MG muscle were subsequently assessed on a Biodex System 4 (Biodex Medical Systems Inc, New York). All measurements were made on the MG muscle of the most affected side of the participants in the spastic CP group and the MG muscle of the right side of the TD group. Participants were positioned prone on an adjustable plinth with the foot fixed to a footplate using a customised belt retention system to minimise heel lift. The knee was maintained at $0^\circ$ extension or maximum extension (whichever was greater). The limits of ankle joint range of motion, MDF and maximum
plantarflexion (MPF) were determined by applying a manual force to the foot plate and recording the corresponding ankle angle. Each participant then underwent three separate trials involving three cycles of full ankle range of motion at 20°.sec\(^{-1}\). Ankle torque, ankle angle and electromyography (EMG) data were recorded at 1000 Hz using a custom LabView program (National Instruments, Austin, Texas) and USB data acquisition device (NI:USB-6259 BNC, National Instruments, Austin, Texas). A synchronisation pulse triggered simultaneous ultrasound, 3D motion capture, dynamometer and EMG data collection.

5.2.3. Ultrasound

A PC-based B-mode ultrasound scanner with a 128-element beamformer and a 6-10 MHz linear transducer with 60mm field of view (Echo Blaster 128 Ext-1Z system, Telemed, Vilnius, Lithuania) was used to record ultrasound images at 25 Hz. Freehand three-dimensional ultrasound (3DUS) was used to measure muscle volume as has been described and validated previously (Experiment 1). Reconstructions and volume measurements were performed using Stradwin software (v3.5, Mechanical Engineering, Cambridge University, UK) (Experiment 1).

MG muscle fascicle length changes were recorded using two-dimensional, B-mode ultrasound. The ultrasound probe was fixed to the muscle using elastic wrap so that the long axis of the US transducer was aligned with the line of action of the fascicles in the mid-belly of the muscle. Muscle fascicle length was defined as the straight line distance between the upper muscular fascia and the lower muscular fascia parallel to the lines of collagenous tissue visible on the image. This method for aligning the transducer has been shown to minimize measurement errors in fascicle length and pennation angle (Bénard et al., 2008, Bénard et al., 2009).

Fascicle length changes during passive lengthening were subsequently estimated using a semi-automated computerised block-matching method that has been previously used to measure gastrocnemius-Achilles muscle-tendon junction displacement (Zhao et al., 2009). Briefly, the
fascicle endpoints were selected at the point of intersection with the superficial and deep aponeurosis of the MG. Five tracking pixel blocks 4.6mm x 1.8mm (39 × 15 pixels) situated along both the superficial and deep aponeuroses were used to search for a best match in an 8.1 mm × 5.3 mm surrounding region in the next frame. The average displacement of the five regions was regarded as the estimated displacement of the superficial and deep aponeuroses and each end of the selected fascicle. Interpolation was used to estimate sub-pixel displacements, and a Kahlman filter was used to smooth the estimated displacements between successive image frames. Fascicle tracking points on each frame were also checked visually and any observed errors manually corrected.

5.2.4. 3D motion capture

Reflective markers were placed on the foot, shank and thigh (3 per segment). Marker trajectories were recorded at 100 Hz using a 10-camera 3D motion analysis system (MX13, Vicon Motion Systems, Oxford, UK) and used to calculate ankle and knee joint angles.

5.2.5. EMG

An eight channel Bagnoli surface EMG system (Delsys Inc., Boston, MA) was used to record whether any unwanted lower leg muscle activity was present during the passive movements of ankle joint. Single differential parallel-bar surface electrodes were placed over the muscle bellies of the MG, lateral gastrocnemius, soleus and tibialis anterior muscle. EMG signals were amplified (× 1000) and band-pass filtered (20-450 Hz). All EMG signals were full wave rectified and low pass filtered (6 Hz), and trials were discarded if the maximum value of the envelope was greater than five percent of the activity associated with a pre-recorded maximum voluntary isometric contraction performed at MDF.
5.2.6. Passive ankle joint and MG muscle fascicle mechanical properties

Passive ankle joint and MG muscle fascicle mechanical properties were determined during lengthening by averaging individual participant data across cycles and then fitting a third order polynomial to the torque-angle data. To account for the torque generated by gravity acting on both the foot and the footplate, a geometric fit of the gravitational moment was performed on the torque signal (Appendix B). The gravitational moment was modelled using a generalised sinusoid equation (Andersen et al., 2010) in the region where passive moments were lowest. Because a range of joint angles where there is zero passive force does not exist in the ankle joint (Clarke et al., 2010), the fit was constrained to bias the effect of weight. The gravitational fit was constrained using experimental measures of the relationship between joint position and torque with a 1kg mass, similar to that of the foot (Clauser et al., 1969), located within the centre of the foot restraints.

Slack angle was defined as the angle at which the ankle torque exceeded 0 Nm of plantarflexor torque, and change in ankle angle was defined as the difference between MDF angle and slack angle length. Peak ankle torque was defined as the maximum ankle torque during the passive trial. Ankle stiffness was calculated from the slope of the torque-angle curve between 30-100% of maximal ankle torque. MG physiological cross sectional area (PCSA) was in turn computed from the ratio of MG muscle volume to MG fascicle slack length, where slack length was defined as the fascicle length when torque exceeded 0 Nm of plantarflexor torque. Change in MG fascicle length was defined as the difference between MG fascicle length at MDF and MG fascicle slack length. MG fascicle strain was calculated from the change in fascicle length (maximal fascicle length-slack length) as a percentage relative to the slack length.

5.2.7. Statistical analysis

A between group general linear model (SPSS Statistics 18.0.0) was used to test differences in outcome measures between the spastic CP and TD groups. Values are presented as the mean ± standard error of measurement (SEM) and the alpha level for assessing statistical significance was set at 0.05. Standardised effect sizes are reported as Cohen’s $d$. 
5.3. Results

5.3.1. Participant characteristics

The spastic CP group were significantly shorter, had a significantly lower MDF angle, MG volume and PCSA than the TD group (Table 5.1). There were no significant group differences in age, mass, leg length, fibula length or knee angle (Table 5.1).

5.3.2. Joint and fascicle level mechanical properties

No trials were excluded on the basis of excessive EMG activity and no significant differences in normalised EMG amplitude were detected between groups for any muscle. Compared to TD, the mean ankle torque-angle curve was shifted to the left (Fig. 2A), and the mean ankle stiffness was higher across the measured torque range in the spastic CP group (Fig. 2B). In addition, the mean change in fascicle length was lower in the spastic CP group (Fig. 2C), which contributed to a higher slope of the ankle torque versus fascicle length curve in the spastic CP group (Fig. 2D).
Table 5.1. Demographic, anthropometric, knee and ankle angle and MG muscle size data for the spastic CP and TD groups.

<table>
<thead>
<tr>
<th></th>
<th>Spastic CP</th>
<th>TD</th>
<th>F (1,17)</th>
<th>p</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>17 (1)</td>
<td>18 (1)</td>
<td>0.1</td>
<td>0.72</td>
<td>-0.3</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>57 (3)</td>
<td>61 (2)</td>
<td>1.3</td>
<td>0.26</td>
<td>-0.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 (2)</td>
<td>171 (2)</td>
<td>5.3</td>
<td>0.03*</td>
<td>-1.2</td>
</tr>
<tr>
<td>Leg length (cm)</td>
<td>86 (3)</td>
<td>90 (2)</td>
<td>1.9</td>
<td>0.19</td>
<td>-0.5</td>
</tr>
<tr>
<td>Fibula length (cm)</td>
<td>36 (1)</td>
<td>38 (1)</td>
<td>1.6</td>
<td>0.22</td>
<td>-0.7</td>
</tr>
<tr>
<td>Knee angle (°)</td>
<td>10 (1)</td>
<td>8 (1)</td>
<td>3.3</td>
<td>0.09</td>
<td>0.6</td>
</tr>
<tr>
<td>Maximum dorsiflexion angle (°)</td>
<td>6 (1)</td>
<td>21 (1)</td>
<td>50.9</td>
<td>&lt; 0.01*</td>
<td>-5.0</td>
</tr>
<tr>
<td>MG muscle volume (ml)</td>
<td>134 (20)</td>
<td>223 (21)</td>
<td>9.9</td>
<td>&lt; 0.01*</td>
<td>-1.5</td>
</tr>
<tr>
<td>MG PCSA (cm²)</td>
<td>34 (3)</td>
<td>53 (5)</td>
<td>11.7</td>
<td>&lt; 0.01*</td>
<td>-1.6</td>
</tr>
</tbody>
</table>

Data are mean (1 SEM). Asterisk (*) indicates significance group difference (p < 0.05). ES = Effect size (Cohen’s d).
Figure 5.2. Ankle torque versus ankle angle, ankle stiffness versus ankle torque, MG fascicle length versus ankle angle and ankle torque versus MG fascicle length for the spastic CP (SCP) and TD groups (A-D). Data are mean (1 SEM). Positive ankle joint angles indicate dorsiflexion.
The spastic CP group had significantly greater ankle stiffness and significantly smaller slack angle, change in ankle angle, change in MG fascicle length and MG fascicle strain relative to the TD group (Table 5.2). There were no significant group differences in peak ankle torque or MG fascicle slack length (Table 5.2).

Table 5.2. Ankle joint level and MG fascicle level measures for the spastic CP and TD groups.

<table>
<thead>
<tr>
<th></th>
<th>Spastic CP</th>
<th>TD</th>
<th>F (1,17)</th>
<th>p</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ankle joint level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slack angle (deg)</td>
<td>-8.4 (2)</td>
<td>-1.0 (2)</td>
<td>10.1</td>
<td>&lt; 0.01*</td>
<td>-1.5</td>
</tr>
<tr>
<td>Peak ankle torque (Nm)</td>
<td>10.1 (1)</td>
<td>11.4 (1)</td>
<td>0.7</td>
<td>0.4</td>
<td>-1.3</td>
</tr>
<tr>
<td>Ankle stiffness (Nm.deg⁻¹)</td>
<td>0.65 (0.1)</td>
<td>0.43 (0.04)</td>
<td>4.9</td>
<td>0.04*</td>
<td>1.0</td>
</tr>
<tr>
<td>Change in ankle angle (deg)</td>
<td>14.9 (1)</td>
<td>21.7 (1)</td>
<td>14.0</td>
<td>&lt; 0.01*</td>
<td>-1.7</td>
</tr>
<tr>
<td><strong>MG fascicle level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MG fascicle slack length (mm)</td>
<td>38.6 (3)</td>
<td>42.3 (2)</td>
<td>0.9</td>
<td>0.35</td>
<td>-0.4</td>
</tr>
<tr>
<td>Change in MG fascicle length (mm)</td>
<td>4.0 (1)</td>
<td>9.0 (1)</td>
<td>31.1</td>
<td>&lt; 0.01*</td>
<td>-2.6</td>
</tr>
<tr>
<td>MG fascicle strain (%)</td>
<td>10.8 (1.6)</td>
<td>20.3 (1.4)</td>
<td>19.4</td>
<td>&lt; 0.01*</td>
<td>-2.0</td>
</tr>
</tbody>
</table>

Data are mean (1 SEM). Asterisk (*) indicates significance group difference (p < 0.05). Negative ankle joint angles indicate plantarflexion. ES = Effect size (Cohen’s d).

**5.4. Discussion**

The present study has revealed fundamental differences in architectural and mechanical properties of the ankle joint and MG muscle fascicles between young adults with spastic CP and TD age-matched controls. At the joint level we found that during passive ankle dorsiflexion, the musculo-articular structures developed torque earlier in the angle range of motion, the ankle joint was stiffer, and maximum ankle dorsiflexion angle was lower in the spastic CP group. These findings are similar to those of previous studies of ambulant children with hemiplegic and diplegic spastic
CP that reported reduced ankle dorsiflexion range in affected limbs (Shortland, 2009, Svehlik et al., 2010) and increased passive ankle plantarflexion stiffness (Alhusaini et al., 2010). Although the 51% group difference in ankle stiffness in the present study was smaller than the 242% difference in ankle stiffness reported by Alhusaini et al. (2010), this may be partly explained by a higher level of function (lower average GMFCS) of our spastic CP participants. In addition Alhusaini et al. (2010) calculated ankle stiffness across the same ankle range of motion in the TD and spastic CP groups and subsequently a different segment of the torque-angle curve making direct comparison with findings from the present study difficult.

The unique contribution of the present study is that we demonstrated pronounced differences in the passive mechanical behaviour of the MG muscle fascicles, which underwent, on average 47% less strain in the spastic CP group in the absence of group differences in peak ankle torque. This suggests that the increased resistance to stretch during passive ankle dorsiflexion in individuals with spastic CP may be attributed at least in part to the inability of the muscle fascicles to elongate with added force. Consistent with previous reports of reduced muscle size in spastic CP (Experiment 2) (Malaiya et al., 2007, Shortland, 2009), we also found that the PCSA of the MG muscle was 37% lower in the spastic CP group. The finding that MG fascicles in spastic CP have reduced strain in conjunction with the MG muscle being 37% smaller in PCSA suggests differences in structural and/or material properties of the MG in spastic CP relative to TD, and is consistent with the finding that muscle cells are shorter and stiffer in the wrist muscles of individuals with spastic CP (Friden and Lieber, 2003). Although we did not investigate the specific mechanisms underlying the reduced fascicle strain reported here, an increase in weakly bound cross-bridges (Wang et al., 1991) and/or titin stiffness (Horowits, 1999) are believed to be the main factors that limit passive muscle extensibility. It is also possible that the spastic CP group has a different number of sarcomeres in series and/or that for a given fascicle length the sarcomeres are at longer lengths than in normal muscle as was reported for spastic wrist muscles (Lieber and Friden, 2002).
At present it is difficult to identify which structures cause the greater ankle stiffness in spastic CP. The steeper slope of the torque-fascicle length curve for the spastic CP group (Fig 1D) is suggestive of increased stiffness of the MG muscle fascicles in our spastic CP subjects. However the forces experienced by the MG are only a proportion of that transmitted through the Achilles tendon, with Achilles force being shared with the lateral gastrocnemius and the soleus (Albracht et al., 2008). It therefore remains possible that the observed group differences ankle stiffness could be attributed to other posterior leg muscles (e.g. soleus) and/or group differences in Achilles tendon moment arms. It was notable however from subjective analysis of our ultrasound data that the proximal soleus muscle lengthened in a similar manner to MG, and may therefore experience similar strains. Other explanations also include the possibility of group differences in the stiffness of the connective tissue which acts in parallel to the fibres (Gajdosik, 2001) and force transmission amongst joint structures (Huijing, 2009).

There are a number of methodological factors that warrant consideration when interpreting the findings of this study. Firstly, passive properties of the MG are influenced by knee and ankle joint angles (Brindle et al., 2008, Hoang et al., 2005) and we cannot fully exclude the possibility of small errors in our ankle torque measurements made on the dynamometer due to misalignment of the axes of the ankle and dynamometer and/or motion of the knee during testing. Secondly, MG muscle fascicles in the present study were represented as straight lines connecting the fascicle endpoints, thus neglecting possible effects of fascicle curvature of fascicle length. However muscle curvature effects have been reported to be small in passive compared to active contractions (Muramatsu et al., 2002). The possible underestimation of fascicle length in the present study would therefore be expected to be small in comparison to the large differences observed in the fascicle lengths between the spastic CP and TD groups. Finally, there was a tendency for greater variability in our passive measures in the spastic CP compared to TD group, which is likely to reflect the relative heterogeneity of the spastic CP group in terms of severity of the CP and previous treatment.
Concluding remarks

The spastic CP participants had a lower ankle range of motion and greater ankle stiffness compared to the TD individuals. The MG muscles were significantly smaller in the spastic CP group and the MG fascicles exhibited significantly less strain. Reduced MDF range of motion and increased passive ankle joint stiffness in individuals with spastic CP may be related to the inability of MG muscle fascicles to elongate with increased passive force.
Chapter 6. Medial gastrocnemius muscle fascicle active torque-length and Achilles tendon properties in young adults with spastic cerebral palsy

Acknowledgement of co-authorship:

I have made a substantial contribution in the conception and design of this study, analysis and interpretation of the research data, and the drafting and critical revising of the final manuscript.
6.1. Introduction

Individuals with spastic CP typically experience lower extremity muscle weakness, increased joint stiffness and reduced joint range of motion (Elder et al., 2003), which together contribute to reduced motor function in spastic CP (Damiano et al., 2000, Engsberg et al., 2000). Muscle weakness in particular, which can progress during development, has been suggested to be a primary impairment that can limit mobility in spastic CP (Damiano et al., 2000). The mechanisms responsible for muscle weakness in spastic CP are complex and multifaceted, with a range of neural and muscular factors being implicated. For example, individuals with spastic CP have been reported to have a reduced capacity to maximally activate their muscles (Stackhouse et al., 2005) and exhibit greater levels of antagonistic co-contraction (Rose and McGill, 2005). There is also strong and consistent evidence that the size of certain lower extremity anti-gravity muscles, such as the gastrocnemius, is reduced in spastic CP 3, (Barrett and Lichtwark, 2010). However in order to more fully characterise the factors responsible for muscle weakness in spastic CP it is necessary to also consider the role of intrinsic muscle properties such as the active and passive force-length relations, as well as the mechanical properties of the tendon to which the muscles attach.

The tension developed by a muscle is length dependent, with active and passive muscle forces contributing to total muscle tension. The active force generated by a muscle is a function of the number of cross-bridges formed, which depends on the extent of myo-filamentary overlap. Muscle force is maximal at intermediate (optimal) muscle lengths and declines at shorter and longer relative lengths (Gordon et al., 1966). Passive forces are believed to arise due to weakly bound cross-bridges (Wang et al., 1991) and/or titin stiffness (Horowits, 1999), and are generated at longer muscle lengths (Herzog et al., 1991b). In Experiment 3 it was reported that MG muscle fascicles (fibre bundles) undergo 47% less strain in spastic CP compared to controls at approximately equivalent passive ankle torques, suggesting that muscle fascicles have an increased resistance to passive stretch in spastic CP. Further, Gao and Zhang (2008) reported a decreased width of the active force-length relation in stroke survivors with muscle contracture, which would
be expected to decrease the muscle force-generating capacity at short and long lengths respectively. However no such studies of the active force-fascicle length relation have been conducted to date in spastic CP.

The Achilles tendon interacts with the *triceps surae* muscles and plays an important role in force transmission and energy storage and return during functional activities. Importantly, the length and compliance of the Achilles tendon can uncouple the length changes of the fascicles from that of the muscle-tendon unit, and thereby influence the force-generating capacity of the muscle (Lichtwark and Wilson, 2007, Lichtwark and Wilson, 2008). For example, a more compliant tendon allows the muscle fibres to operate closer to optimal length and at lower shortening speeds, thereby increasing force and work production of the muscle-tendon unit during locomotion (Lichtwark et al., 2007, Lichtwark and Wilson, 2006). There are no studies of tendon mechanical properties of lower limb muscles in individuals with spastic CP to date. However several studies in individuals with related neurological conditions have been conducted, albeit with conflicting findings. Zhao et al. (2009) reported a longer and more compliant Achilles tendon in stroke patients, whereas Hoang et al. (2009) found no difference in Achilles tendon slack length and peak strain in ambulatory individuals with multiple sclerosis, relative to controls. Studies of muscle mechanical and Achilles tendon properties in spastic CP are required to improve understanding of force production and motor dysfunction in the population.

The purpose of this study was to compare the *in vivo* MG muscle fascicle active torque-length and Achilles tendon properties in young adults with spastic CP with TD controls. We chose to study musculo-tendinous properties of the MG in young adults because we wanted to understand differences at full musculoskeletal maturity where potential confounding effects of growth are minimised. In this age group the degree of musculoskeletal deformity (e.g. contracture) in spastic CP may also be stable. We hypothesized that ankle plantar flexor strength would be reduced in spastic CP due to a reduced muscle physiological cross sectional area (PCSA). In the absence of previous studies of active MG fascicle torque-length and tendon properties in spastic CP, our null
hypotheses were that there would be no difference in the ankle torque-fascicle length relation and no difference in the tendon slack length and stiffness between the spastic CP and TD groups.

6.2. Methods

6.2.1. Participants

Nine young adults with spastic CP (6 males, 3 females, aged 17(2) years, range 15-21 years) and ten TD young adults (5 males, 5 females, aged mean (1SD) 18(2) years, range 15-20 years) participated in the study. A full description of participant characteristics, recruitment and inclusion criteria is provided in Experiment 3. The spastic CP group were significantly shorter and had a significantly lower MDF angle and MG PCSA than the TD group as reported previously in Experiment 3. There were no significant group differences in age, mass, leg length or fibula length. Ethics approval was obtained from the Institutional Human Research Ethics Committee and all relevant ethics guidelines were followed.

6.2.2. Data collection and analysis procedures

Active ankle torque, MG fascicle length and Achilles tendon properties were assessed under controlled conditions on a dynamometer (Biodex System 4, Biodex Medical Systems Inc, New York). Full details of passive fascicle force-length properties and ultrasound measurements of PCSA using three-dimensional ultrasound have been described in Experiment 1 and 3. All measurements were made on the most affected leg in the spastic CP group and the right leg of the TD group. Participants were positioned prone on an adjustable plinth with the foot fixed to a footplate using a customised belt retention system to minimise heel lift. The lateral malleolus was aligned with the axis of rotation of the dynamometer and the knee was maintained at 0° extension or maximum extension (whichever was greater). Reflective marker triads were placed on the foot, shank, thigh and ultrasound probe. Marker trajectories were recorded at 100 Hz using a 3D motion analysis system (10-camera MX13, Vicon Motion Systems, Oxford, UK) and were used to
calculate ankle and knee joint angles as well as position and orientation of the ultrasound probe. All ultrasound images were recorded 25 Hz using a PC-based B-mode ultrasound scanner with a 128-element beamformer and a 6-10 MHz linear transducer with 60mm field of view (Echo Blaster 128 Ext-1Z system, Telemed, Vilnius, Lithuania), and electromyographic (EMG) data were recorded from differential parallel-bar surface electrodes placed over the muscle bellies of the MG, lateral gastrocnemius, soleus and tibialis anterior muscle using an eight channel EMG system (Bagnoli, Delsys Inc., Boston, MA). Dynamometer and EMG data were recorded at 1000 Hz using a USB data acquisition device (NI:USB-6259 BNC, National Instruments, Austin, Texas) with custom LabView software (National Instruments, Austin, Texas) and band-pass filtered (20-450 Hz) prior to subsequent analysis. A synchronisation pulse generated by the ultrasound scanner was used to trigger simultaneous collection of the 3D motion analysis, dynamometer and EMG data.

6.2.3. Active MG force-length properties

The ultrasound probe was fixed to the skin over the MG muscle for measurement of fascicle length and pennation angle as previously described by Bénard et al. (2009) Passive ankle joint torque was initially assessed for each participant throughout the full ankle range of motion at 20°.sec⁻¹ as described previously in Experiment 3. Participants subsequently performed maximum voluntary isometric contractions (MVIC) of the plantar and dorsiflexor muscle groups at five ankle angles corresponding to 0, 25, 50, 75 100% of the range between maximum plantarflexion and MDF. The duration of each MVIC was four seconds with a rest period of two minutes between trials. Three trials were performed at each condition. The measured ankle plantarflexion torque-angle curves were corrected for gravity as described previously in Experiment 3, and were subsequently adjusted to remove the passive torques at the same fascicle length and to account for co-contraction of the tibialis anterior muscle (Morse et al., 2007). Tibialis anterior co-contraction was defined as the ratio of mean root mean square EMG amplitude in the active plantar flexor versus dorsiflexor MVICs at each angle (expressed as a percentage) (Morse et al., 2007). Fascicle lengths under all
conditions were estimated using a semi-automated computerised block-matching method as used in Experiment 3.

6.2.4. Achilles tendon properties

The ultrasound probe with attached reflective markers was positioned over the myotendinous junction of the MG to measure Achilles tendon length. Achilles tendon length was defined as the distance from the MTJ to the point of insertion onto the calcaneous within a three-dimensional global coordinate system (Lichtwark and Wilson, 2005). The ankle joint was initially passively rotated throughout range at $20^\circ.s^{-1}$ and Achilles tendon slack length was determined as the length of the Achilles tendon when the passive plantar flexion torque first exceeded zero during passive dorsiflexion rotations. The stiffness of the Achilles tendon was subsequently computed by dividing the ankle torque produced during the MVIC at MDF by the change in Achilles tendon length from the slack length to the length during MVIC at MDF (Lichtwark and Wilson, 2005). All torque and Achilles tendon length measurements were averaged from three trials.

6.2.5. Statistical analysis

A mixed general linear model (GLM) was used to assess the effect of one between group factor (spastic CP versus TD) and one within group (repeated) factors (ankle angle: 0, 25, 50, 75, 100%) and group related interactions on the dependent measures (absolute and normalised active ankle torque, co-contraction ratio, MG fascicle length). A between group GLM was also used to assess the effect of group on Achilles tendon properties (slack length and stiffness). All statistical analyses were performed using SPSS (IBM SPSS Statistics for Windows, Release 19.0.0, Chicago, Illinois). Data are presented as the mean (1SD) and the alpha level for assessing statistical significance was set at 0.05. Standardised effect sizes are reported as Cohen’s $d$. 
6.3. Results

Group had a significant main effect on active ankle torque (Figure 1A). The absolute active ankle plantarflexion torque was significantly lower (F(1,17)=26.28, p<0.01, ES=-2.36) for the spastic CP group (27.1 (4.8) Nm) compared to the TD group (60.8 (4.5) Nm). There was no significant group difference when the active ankle plantarflexion torque was normalised by MG PCSA (Figure 1B) and no significant group difference in active MG fascicle length at MDF (Figures 1C and D). The tibialis anterior co-contraction ratio during the plantarflexion MVICs was significantly larger (F(1,17)=8.23, p=0.02, ES=1.66) in the spastic CP group (13 (1) %) compared to the TD group (9 (1) %). Achilles tendon slack length was significantly longer in the spastic CP than the TD group (F(1,17)=10.68, p<0.01, ES=1.53) (Figure 2A). There were no differences in the stiffness of the Achilles tendon when computed in absolute (Figure 2A) or normalised terms (Figure 2B).
Figure 6.1. Active absolute and normalised ankle torque versus angle and ankle torque versus MG fascicle length. A. Ankle torque versus ankle angle, B. Normalised ankle torque versus ankle angle, C. Ankle torque versus MG fascicle length and D. Normalised ankle torque versus MG fascicle length for the spastic CP (SCP) and TD groups. Data are mean (1SEM). Positive ankle joint angles indicate dorsiflexion. Active ankle torques have been adjusted for gravity, co-contraction and passive torque.
Figure 6.2. Active ankle torque versus Achilles tendon length and normalised ankle torque versus Achilles tendon strain. A. Ankle torque versus Achilles tendon length and B. Normalised ankle torque versus Achilles tendon strain for the spastic CP (SCP) and TD groups. Data are mean (1SEM).

6.4. Discussion

Consistent with our hypothesis maximum isometric ankle plantarflexion torque was significantly lower in the spastic CP group. The mean strength of the spastic CP group across the available range of ankle joint range of motion was approximately half that of the TD group after group differences in co-contraction, which were significantly higher in the spastic CP group, had been accounted for. Our findings are in agreement with previous studies that report muscle weakness in spastic CP (Elder et al., 2003, Ross and Engsberg, 2002, Wiley and Damiano, 1998) which is likely to be a primary factor underlying motor impairment in this population (Damiano et al., 2000).

When the maximum isometric ankle plantarflexion torques were normalised by the PCSA of the MG muscle, which was 33% lower in the spastic CP group (Experiment 3), the spastic CP group remained 22% weaker than the TD control group, although this group difference was no longer statistically significant. This result indicates that reduced muscle PCSA is an important factor contributing to muscle weakness in spastic CP as previously reported (Experiment 2 and 3)(Malaiya et al., 2007, Shortland, 2009). Other factors which might be expected to explain the
observed 22% difference in maximal isometric ankle plantar flexor torque production normalised to MG PCSA include the increase in co-contraction and decreased central nervous system motor unit recruitment and discharge rates (Rose and McGill, 2005, Stackhouse et al., 2005) in the spastic CP group.

The active torque-fascicle length relations showed that the spastic CP and TD groups were operating on the ascending limb of the torque-fascicle length relation throughout the available range of motion of the ankle joint. The TD group was able to produce a 28% higher normalised active ankle torque at 9% longer MG fascicle lengths at MDF. Although these differences were not statistically significant, the functional significance of this finding may be that individuals with spastic CP may not be able to operate at MG fascicle lengths that are favourable for MG force production. The shorter fascicle lengths observed under active conditions are consistent with previous findings that the maximum MG fascicle length (at MDF) is also reduced under passive conditions in the same participants (Experiment 3). Unlike Gao et al. (2008) who reported a decreased width of the active force-length relation in stroke survivors, we were unable to assess the width of the torque-fascicle length relations because the fascicles in both groups were restricted to the ascending limb of the torque-MG fascicle length relation. However it seems that the range of fascicles lengths over which force can be produced are similar between the two groups.

The Achilles tendon slack length was significantly longer in the spastic CP group, on average by 10%. In the absence of other differences in muscle and tendon properties, an increased Achilles tendon slack length would be expected to increase the compliance of the tendon, because the tendon would extend further for the same applied force. One possible advantage of greater tendon compliance would be a greater capacity to store and recover strain energy. Such an adaptation may be favourable in spastic CP and allow the tendon to contribute more mechanical work and thereby compensate for decreased force production by the muscle. In the absence of other adaptations, the longer Achilles tendon slack length in SCP would result in a rightward shift of the muscle-tendon unit’s isometric force-length relationship, as the muscle would achieve peak force at a longer
length (Lieber et al., 1992). Muscles that have a high ratio of slack length to moment arm, such as the gastrocnemius, are most sensitive to changes in slack length (Hoy et al., 1990). However group differences in Achilles tendon slack length in the present study were not accompanied by significant differences in peak isometric fascicle lengths, suggesting that the interaction between the muscle fascicles and tendon under isometric conditions is complex and possibly influenced by other unknown factors.

It was notable that although the spastic CP group produced 53% less torque at MDF, the Achilles tendon underwent 45% less stretch relative to the slack length, and so there was no significant group difference in Achilles tendon stiffness. The Achilles tendon strain of 4.9% at maximum isometric force in the TD group was within the range reported elsewhere (Maganaris and Paul, 2002, Muramatsu et al., 2001). However, the strain at maximum isometric force for the spastic CP group was only 2.7%. This value was unexpectedly low because muscles with smaller cross-sectional areas tend to attach to tendons with smaller cross-sectional areas, so that the strain at maximum isometric force is relatively invariant (Zajac, 1989). Possible explanations for these findings are that the tendon cross-sectional area is maintained in the presence of smaller muscles in spastic CP, that the material properties (i.e. Elastic modulus) of the Achilles tendon is altered in CP or that the inability to maximally activate muscles limited the strain of the tendon. In order to better understand how the material properties of the tendon are affected in spastic CP it will be necessary to measure the cross sectional area of the Achilles tendon in future studies. Studies investigating the Achilles tendon mechanical properties in individuals with spasticity have revealed a longer and more compliant tendon in stroke patients (Zhao et al., 2009) and no difference in slack length and peak strain in individuals with multiple sclerosis (Hoang et al., 2009), relative to controls. Our findings in young adults with spastic CP highlights that muscle-tendon adaptations in different forms of spasticity are not the same, and may be dependent on the type and site of the upper motor neuron lesion and the time the lesion has been present.
There are several important limitations to our study. Firstly, our measurements of muscle properties were restricted to the MG muscle, which is just one of several synergistic plantar flexor muscles including the lateral gastrocnemius and the soleus that are responsible for producing the measured ankle torque. We therefore cannot be sure to what extent the MG was responsible for the measured ankle torque. A further assumption was that the observed MG muscle fascicle and Achilles tendon length changes were not caused by differences in the moment arm of the triceps surae complex between the two groups. Furthermore, our estimates of tendon slack length and stiffness were for the free tendon (external to the muscle belly), and so we do not know the mechanical properties of the aponeurotic tendon (internal to the muscle belly) at this time. Our measurements were made in individuals with mild spastic CP so it remains unknown how active muscle fascicle and tendon properties are affected in more severe spastic CP. To fully understand the influence of muscle contracture on function, it will be important to examine how these mechanical properties of muscle and tendon change during childhood development and what impact this has on the dynamic interaction between the muscle fascicles and tendon during functional tasks such as walking.

Concluding remarks
In addition to having smaller MG muscles and increased co-contraction of tibialis anterior, decreased ankle strength in spastic CP may be related to an inability to exert force at longer MG muscle fascicle lengths. We found little difference in torque-MG fascicle length relationship, which infers no difference in the contractile properties of the muscle. While tendon length was longer in the CP group there was no difference in the absolute stiffness of the tendons, suggesting adaptations to the tendon morphology or material properties to allow for sufficient storage and recovery of elastic energy during tasks like walking. This may partially compensate for the decreased force production by the muscles of the triceps surae.
Chapter 7. General discussion
7.1. Summary of experimental findings

The general purpose of this thesis was to investigate the morphological and mechanical properties of the MG muscle in children and young adults with spastic CP. The following section of the general discussion provides summary of the findings for each experiment that comprises the study.

Experiment 1 (Chapter 3). Validation of a freehand 3DUS for morphological measures of the MG muscle.

The freehand 3DUS system overestimated MG muscle volume by 1.1% and underestimated MG muscle belly length by 1.3%. The intra-class correlation coefficients for repeated freehand 3DUS system measures of muscle volume and muscle belly length were greater than 0.99 and 0.98 respectively. The intra-class correlation coefficients for the segmentation process of the freehand 3DUS system and MRI for muscle volume were both greater than 0.99 and muscle belly length were 0.97 and 0.99, respectively. It was concluded that the freehand 3DUS was a valid and reliable method for the measurement of human muscle volume and muscle belly length in vivo.

Experiment 2 (Chapter 4). Medial gastrocnemius muscle volume and fascicle length in children aged 2-5 years with cerebral palsy

MG muscle volume was 22% lower in the spastic CP group compared to the typically developing group, which in the absence of significant group differences in neutral fascicle length, gave rise to an approximately equivalent reduction in PCSA for the spastic CP group. Significant positive correlations were found between muscle volume and age, and muscle length and age within both groups. Maximum ankle dorsiflexion angle was also reduced in spastic CP compared to the typically developing group. It was concluded that the observed reduction in muscle PCSA in the spastic CP group would be expected to contribute to the clinically observed muscle weakness in spastic CP and suggests the need for early intervention to minimise loss of muscle PCSA in spastic CP.
Experiment 3 (Chapter 5). Passive muscle mechanical properties of the medial gastrocnemius in young adults with spastic cerebral palsy

The PCSA was found to be 37% lower in the spastic CP group. Mean ankle stiffness was found to be 51% higher and mean MG fascicle strain 47% lower in the spastic CP group. It was concluded that the increased resistance to passive ankle dorsiflexion in spastic CP was related to the inability of MG muscle fascicles to elongate with increased force.

Experiment 4 (Chapter 6). Medial gastrocnemius muscle fascicle active torque-length and Achilles tendon properties in young adults with spastic cerebral palsy

Compared to the TD group, the spastic CP group had 56% lower active ankle plantarflexion torque across the available range of ankle joint motion. When the ankle plantarflexion torques were normalised by the PCSA of the MG muscle the torque difference between groups was 22%. The spastic CP group had greater levels of antagonistic co-contraction which may, in part, explain the reduced torque in the spastic CP group. In addition, the Achilles tendon slack length was, on average, 10% longer in the spastic CP group. It was concluded that the increased Achilles tendon slack length may facilitate a greater storage and recovery of elastic energy and partially compensate for decreased force and work production by the muscles of the triceps surae during activities such as locomotion.
7.2. Synthesis of experimental findings

New insight into MG muscle morphology in individuals with spastic CP

There is consistent evidence that the morphology of distal lower limb muscles is altered in older children with spastic CP compared to TD peers (Fry et al., 2007, Lieber et al., 2004, Malaiya et al., 2007, Mohagheghi et al., 2008). Experiment 2 described volumetric deficits in the MG of young children aged 2–5 years with spastic CP of 22% and hence confirms earlier muscle morphological alterations than has previously been reported. The purpose of Experiment 2 was not to clarify the physiological mechanism of the reduced muscle volume in spastic CP, but this would be expected to be related to a reduced number of muscle fibres in parallel (hypoplasia) and/or decreased muscle fibre cross-sectional area (fibre atrophy) (Lieber et al., 2004, Shortland et al., 2002). There may be a number of possible explanations for the reduced muscle volume. Firstly, the young children with spastic CP in this study had relatively high motor function (GMFCS level I or II) but these children may still have lower physical activity levels compared to age matched TD children. Therefore, young children with spastic CP may experience reduced stimulus for muscle growth. Unfortunately, an evidence-based answer to the question of whether physical activity is essential for normal muscle growth to occur in TD or spastic CP children is not currently available. Although regular exercise can increase muscle volume in TD children (Eliakim et al., 2001) and regular resistance training can increase muscle volume in children with spastic CP (Damiano et al., 1995), the net gains caused by physical activity over the changes expected due to growth alone tend to be small during the years preceding puberty (Teran-Garcia et al., 2008). Secondly, genetic alterations have been identified in spastic CP muscle that give rise to competing pathways for fibre hypertrophy and an increase in anabolic growth factors (Smith et al., 2009). The transcriptional control of muscle in individuals with spastic CP was reported to be qualitatively different and was different to any of the other "altered use" muscle transcriptional models such as Duchenne muscular dystrophy and immobilization-induced muscle atrophy. Finally, it is possible that both of these explanations may be implicated in the reduced muscle volume in the spastic CP group.
The reduced muscle volume in the young children with spastic CP (Experiment 2) occurred in the absence of significant group differences in fascicle length and pennation angle which gave rise to an equivalent reduction in PCSA which is directly proportional to maximal muscle force-generating capacity in normal muscle (Fukunaga et al., 1992, Haxton, 1944). In conjunction with smaller muscles in the paretic limb, children with spastic CP have a reduced capacity to maximally activate their muscles (Stackhouse et al., 2005) and exhibit greater levels of antagonistic co-contraction (Rose and McGill, 2005) which all compromises muscle force production. The practical significance of these findings is that lack of volumetric muscle growth in young children with spastic CP and the associated muscle weakness are likely to contribute to the functional limitations observed in children with spastic CP and may be an important factor to the delayed acquisition of motor milestones observed in this group. For example, the muscles of the plantarflexor muscle group are essential for the maintenance of standing balance and to support and propel the body forward during gait, and deficits in muscle size may demand the adoption of an altered gait pattern. The finding of reduced muscle volume at a young age provides preliminary evidence in support of the need for early interventions, such as exercise, to minimise muscle volume loss in children with spastic CP.

The magnitude of the observed muscle volume reduction in the young children with spastic CP relative to TD muscle reported in Experiment 2 is at the lower end of the range of differences (26–57%) in calf muscle volumes in older children and young adults reported elsewhere (Elder et al., 2003, Fry et al., 2007, Malaiya et al., 2007, Oberhofer et al., 2009, Shortland, 2009). In addition, the volumetric deficits in the MG of high functioning (GMFCS I) young adults with spastic CP of 37% determined in Experiment 3 may suggest a lack of volumetric growth during development with a plateau of volume loss after puberty although the differences in condition severity and treatment history make it difficult to directly compare study results. The measurement of muscle volume, using freehand 3DUS, in the gait laboratory or clinical setting, may be useful as a means of monitoring longitudinal changes in muscle morphology which may predict the functional status of the individual with spastic CP and guide the selection of treatments.
Fundamental differences in the active and passive mechanical properties of the ankle joint, MG muscle fascicle and Achilles tendon in individuals with spastic CP.

Clinically, individuals with spastic CP may present with restricted joint range of motion, increased passive joint stiffness, and muscle weakness. Reduced ankle dorsiflexion range of motion in both young children and young adults with spastic CP was reported in Experiments 2 and 3 and is consistent with current literature investigating ambulatory children with spastic CP (Shortland, 2009, Svehlik et al., 2010). In addition, Experiment 3 found that during passive ankle dorsiflexion the spastic CP group had reduced ankle slack angle, suggesting the musculo-articular structures developed torque earlier in ankle range of motion, and greater ankle joint stiffness compared to the TD group. The findings of Experiments 2 and 3 are in accordance with the only study to date investigating passive mechanical properties at the joint level in spastic CP (Alhusaini et al., 2010) and also clinical observations. Investigation of the MG muscle fascicles in individuals with spastic CP (Experiment 3) found that increased ankle joint stiffness in individuals with spastic CP may be attributed at least in part to the reduced MG muscle fascicle strain. The reduced strain experienced by spastic CP muscle has recently been shown to be due to increased muscle fibre bundle stiffness with corresponding increase in collagen content of the muscle extracellular matrix and an increase of in vivo sarcomere length (Smith et al., 2011).

During MVICs throughout the available range of ankle range of motion the mean ankle torque of the young adult spastic CP group was 56% lower than the TD group (Experiment 4). The mechanisms responsible for muscle weakness in spastic CP are complex and multifaceted, with a range of neural and muscular factors being implicated. Reduced strength in individuals with spastic CP has been reported to be attributed to greater levels of antagonistic muscle co-contraction (Rose and McGill, 2005), reduced muscle PCSA (Elder et al., 2003), and reduced capacity to maximally activate muscles (Stackhouse et al., 2005).
Firstly, the percentage co-contraction of the antagonist *tibialis anterior* muscle during plantarflexor MVIC was significantly higher in the group with spastic CP, and was accounted for in the ankle torque values presented in Experiment 4. It is believed that the co-contraction would have had minimal contribution to plantarflexion weakness as the muscle volume (and associated PCSA) of the dorsiflexor muscles was much smaller than the plantarflexor muscles (Fukunaga et al., 1992).

Also, during plantarflexion MVIC at MDF, the *tibialis anterior* muscle was in a shortened position, thus placing the muscle fascicles at a short length and reducing the muscles ability to generate force. Secondly, reduced MG PCSA appears to be an important factor in the reduced ankle plantarflexor torque (Experiment 4) however as the other plantarflexor muscles, such as the soleus, are involved in ankle torque production, morphological alterations of these muscle, with comparable reductions in PCSA, must also be implicated. Thirdly, the spastic CP group had 9% shorter fascicle lengths during peak active ankle torque production (at MDF) which may be of functional significance as individuals with spastic CP may not be able to operate at MG fascicle lengths that are favourable for MG force production. Furthermore, Lieber and Friden (2002) and Smith et al. (2011) propose that the sarcomere lengths are longer in muscles of the paretic limb of individuals with spastic CP and thus fascicles composed of relatively longer sarcomeres may have functionally reduced operational lengths to maintain sarcomeres closer to optimal lengths for maximum force production. The further difference in torque production in the spastic CP group may be explained by decreased central nervous system motor unit recruitment and discharge rates (Rose and McGill, 2005, Stackhouse et al., 2005) and/or a selective loss of these motor units (Rose et al., 1994, Rose and McGill, 1998).

In the first study to investigate tendon mechanics in individuals with spastic CP it was found that the Achilles tendon slack length was 10% longer in the spastic CP than the TD group (Experiment 4). While the muscle-tendon unit of the gastrocnemius is of approximately the same length in both groups, the gastrocnemius muscle becomes stiffer and shorter in individuals with spastic CP compared to TD individuals (Fry et al., 2007, Malaiya et al., 2007). A concomitant increase in Achilles tendon length would therefore be expected in individuals with spastic CP.
The Achilles tendon active strain was significantly different in the spastic CP group compared to the TD group, 2.7% versus 4.9% respectively (Experiment 4). This may suggest that during functional tasks the Achilles tendon is stiffer in the spastic CP group. However, in the spastic CP group the Achilles tendon lengthened 45% less with 53% lower torque during MDF MVIC. This resulted in an estimate of the Achilles tendon stiffness from the slope of the Achilles tendon length-torque relationship that is the same in both groups (Experiment 4). A simplified linear estimate may not indicate the correct Achilles tendon length-torque relationship and more precise measurement of the Achilles tendon length change may be required to determine in vivo active tendon stiffness.

7.3. Clinical relevance

The finding of reduced muscle volume in young children with spastic CP has significant clinical implications. Smaller muscles are weaker and may be a barrier to gross motor development, mobility and function. Therefore an important objective for early treatment may be to facilitate muscle growth through use of suitable exercise interventions (McNee et al., 2009). These findings also suggest that treatments with the potential to compromise muscle growth (e.g. botulinum toxin) may need to be carefully considered when used in young children with spastic CP.

The greater resistance to elongation force in the muscle fascicles of individuals with spastic CP appears to be associated with clinically observed reduced joint range and increased joint stiffness. Increased fascicle stiffness, as well as increased extracellular matrix stiffness (Smith et al., 2011), should be targets for future treatments as opposed to treatments only targeting lengthening of the affected muscle.
7.4. Methodological considerations

Generally, there is a high degree of inherent heterogeneity in ambulant individuals with spastic CP. The extent of the brain lesion causing spastic CP, unilateral or bilateral involvement (hemiplegia or diplegia), the degree of motor impairment, and the concomitant level of function would be expected to influence these muscle and tendon properties. Throughout this thesis the measurements were made in individuals with relatively mild spastic hemiplegic and diplegic CP so it remains unknown how muscle morphological and muscle-tendon mechanical properties are affected in more severe spastic CP and also how muscle morphological and muscle-tendon mechanical properties vary between topographically classified groups.

When using freehand 3DUS for accurate *in vivo* morphological measurement a number of important methodological practices should be highlighted. Firstly, the single-wall phantom calibration of the freehand 3D ultrasound system (Chapter 3) should use the same water temperature for the water bath as is used for subsequent measurement of the *in vivo* tissue. Water temperature maintained between 26-28°C in the water baths in Chapters 3, 5 and 6. Using different water bath temperatures for the freehand 3D ultrasound calibration and measurement and/or using water bath temperatures outside the range of 25-30°C will affect the axial distance calculations of the ultrasound system and the scaling of the ultrasound image visualisation (Angelsen, 2000, Lubbers and Graaff, 1998, Telemed, 2008a, Telemed, 2008b). Incorrect axial distance calculations of the ultrasound system will result in distorted 3D renderings of the *in vivo* tissue of interest (Gee et al., 2008). Secondly, a gel couplant that transmits sound at a similar speed to biological tissue may also be used for freehand 3D ultrasound in vivo tissue measurement. During pilot studies a gel couplant was found to be appropriate, and more practical, for scanning smaller tissue volumes requiring a single freehand 3D ultrasound sweep (e.g. a young child’s MG muscle). A gel couplant was found to be inappropriate when scanning larger tissues that require two or three overlapping parallel sweeps (e.g. an adult MG muscle). During multiple sweeps of the transducer uneven tissue compression, due to transducer contact with the skin, resulted in distortion of the 3D rendering of
the tissue along the line of adjacent sweeps and inaccurate volume and length measurements. Importantly, the single-wall phantom water bath calibration of the freehand 3D ultrasound system, with water temperatures within the range of 25-30°C, is still valid for use with a gel couplant as the sound transmission speed is consistent between the gel and water.

The experiments of this thesis were limited to the MG muscle therefore it remains unclear how the morphology of other muscles are altered in individuals with spastic CP. The MG muscle is just one of several synergistic plantar flexor muscles, including the lateral gastrocnemius and the soleus, that are responsible for ankle torques. In the absence of information concerning force sharing ratios amongst synergistic ankle plantarflexor muscles in SCP and TD individuals, we assumed a constant value for the proportion of the Achilles tendon force transmitted via the MG (Albracht et al., 2008). In addition, measurement of passive length-tension relations of the MG is challenging as the muscle must be isolated from other structures such as synergistic muscles and ligaments and the behaviour of the muscle is influenced by motion about both the knee and ankle (Brindle et al., 2008, Herbert et al., 2002, Hoang et al., 2005). We also acknowledge that there is potentially force transfer between the triceps surae group as presented recently (Huijing, 2007, Huijing, 2009, Smeulders and Kreulen, 2007). We therefore cannot be sure to what extent the MG was responsible for the measured active and passive ankle joint torques. A further assumption was that the observed MG muscle fascicle and Achilles tendon length changes were not caused by differences in the moment arm of the triceps sure complex between the two groups (Lieber and Friden, 2002). The Achilles tendon moment arms were also assumed to be the same in each group (An et al., 1984), which may have led to an overestimate of the Achilles tendon moment arm and hence underestimate of Achilles tendon forces and stiffness in the spastic CP group. It will be important for future studies in active and passive mechanical properties of spastic muscle to address such issues of muscle force sharing, individual moment arm measurement and influences of knee position and force transfer on the muscle of interest.
Experiment 4 was the first study of the mechanical properties of the Achilles tendon in spastic CP. The estimates of tendon slack length and stiffness were for the free tendon (external to the muscle belly), and so we do not know the mechanical properties of the aponeurotic tendon (internal to the muscle belly) at this time. In order to better understand how the material properties of the tendon are affected in spastic CP it will also be necessary to measure the cross sectional area of the Achilles tendon in future studies.

During analysis of the MG muscle fascicles in Experiments 2-4 were represented as straight lines connecting the fascicle endpoints, thus neglecting possible effects of fascicle curvature or fascicle length. However muscle curvature effects have been reported to be small in passive compared to active contractions (Muramatsu et al., 2002). The possible underestimation of fascicle length in the present study would therefore be expected to be small in comparison to the large differences observed in the fascicle lengths between the spastic CP and TD groups.

### 7.5. Future research

Further investigations of the *in vivo* morphological and mechanical properties of spastic muscle need to consider the contribution of the other muscles of the *triceps surae*, such as the soleus, to reduced joint range of motion and stiffness. Methods such as those used by Hoang et al. (2005) are required to help isolate individual muscle relationships to total joint mechanics. In addition, the effect of fascicle stiffness of the muscles of the *triceps surae* on the stretch reflex excitability is unknown therefore studies may draw attention to the role of active stiffness during functional activities in individuals with spastic CP.

The time-course of changes to muscle and tendon morphological and mechanical properties in individuals with spastic CP during development is unknown. Longitudinal studies are required to track changes related to growth and the effects of spasticity. It would also be important to
determine the mechanisms for differences in muscle growth, using for example muscle biopsies, to measure muscle fibre number and size.

With the current methods to determine muscle-tendon morphological and mechanical properties, the relationship of these properties with functional capacity of individuals with spastic CP can be investigated. Using regression analysis and predictive forward dynamic simulations specific muscle-tendon properties may be highlighted as objectives for future treatment. Furthermore, the effects of treatments, such as botulinum toxin and recession surgery on muscle volume and muscle fascicle stiffness respectively, can be examined. The influence of treatments on muscle-tendon morphological and mechanical properties can also be monitored over time leading to individualised management strategies.

### 7.6. Conclusions

The valid and reliable measurement of relatively large muscle volume and muscle length *in vivo* is possible using multiple sweeps freehand 3DUS imaging over a large range of ankle joint angles. Ultrasound, compared to other imaging techniques, can be performed quickly and in almost any subject position, can scan large objects using multiple sweeps, and is relatively cheaper and more portable. Also other measures of *in vivo* muscle morphology can be obtained during the brief time for data collection such as anatomical cross-sectional area, muscle–tendon length, fibre length and pennation angle allowing an individual subject estimation of PCSA within the setting of a biomechanics laboratory.

Freehand 3DUS and B-mode ultrasound were used effectively to determine MG PCSA in individuals with spastic CP and TD from a young age to adults. In young children with spastic CP aged 2 to 5 years MG muscle PCSA was reduced by 27% and in young adults MG muscle PCSA was reduced by 37% compared with TD children. The reduced PCSA was primarily explained by an equivalent reduction in muscle volume in the groups with spastic CP. In relationship to other
literature this may suggest a lack of volumetric growth during development with a plateau of volume loss after puberty. This is yet to be proven therefore longitudinal studies will be required to gain better understanding of the natural history of muscle growth during development. The freehand 3DUS system lends itself to \textit{in vivo} muscle morphological measurements for monitoring changes due to age, function, pathology, surgery and training.

The muscle-tendon mechanical properties are altered individuals with spastic CP. In young children, the restricted ankle joint dorsiflexion range in the group with spastic CP is suggestive of group differences in the mechanical properties of the passive structures of the ankle complex, including muscle fascicles and/or tendons. More thorough investigations in young adults with spastic CP established that reduced MDF range of motion and increased passive ankle joint stiffness in individuals with spastic CP may be related to the inability of MG muscle fascicles to elongate with increased passive force. During MVIC of the ankle plantarflexor muscles decreased ankle strength in spastic CP was attributed to reduced MG PSCA and to a less extent increased co-contraction of tibialis anterior. In addition, individuals with spastic CP may not be able to operate at MG fascicle lengths that are favourable for force production. While Achilles tendon length was longer in the CP group there was no difference in the absolute stiffness of the tendons. This adaptation may allow for sufficient storage and recovery of elastic energy during tasks like walking and may partially compensate for the decreased force production by the muscles of the \textit{triceps surae}. It will be important to examine how the mechanical properties of muscle and tendon change during childhood development and what impact this has on the dynamic interaction between the muscle fascicles and tendon following lower limb surgery and during functional tasks such as walking.
Appendix A. Supporting information for Experiment 2

(Chapter 4)

Figure A.1. Scatter plots and corresponding regression lines of representative data at neutral ankle angle for muscle volume, belly length, fascicle length and pennation angle at neutral ankle angle versus age (A-D). Spastic CP (■) and TD (○) groups. Similar relationships were found at all ankle angles tested.
Figure A.2. Scatter plots and corresponding regression lines of representative data at neutral ankle angle for muscle volume versus weight, belly length versus fibula length, fascicle length versus fibula length and pennation angle versus fibula length at the neutral ankle angle (A-D). Spastic CP (■) and TD (○) groups. Similar relationships were found at all ankle angles tested.
Table A.1. Pearson’s correlation coefficients for defined variables (x versus y) in the spastic CP and TD group at the three ankle joint angles.

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<th>Variables</th>
<th>Spastic CP (n = 15)</th>
<th></th>
<th>TD (n = 20)</th>
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<tr>
<td></td>
<td>x</td>
<td>y</td>
<td>MPF</td>
<td>N</td>
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<td>Age</td>
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<td>Muscle volume</td>
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<td></td>
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Acronyms: Maximum plantarflexion (MPF), neutral (N), maximum dorsiflexion (MDF).

r (95% confidence intervals). Asterisk (*) indicates significant positive correlation (p < 0.05).
Appendix B. Supporting information for Experiment 3 (Chapter 5)

Figure B.1. Example ankle torque versus ankle angle data demonstrating the method for determining the gravity corrected torque (red solid line) from the raw torque signal (blue solid line). To account for the torque generated by gravity (green solid line) acting on both the foot and the footplate, a geometric fit of the gravitational moment was performed on the torque signal. The gravitational moment was modelled using a generalised sinusoid equation and this fit was performed in the region where passive moments were lowest (shaded area). This region was defined as 5 degrees either side of the joint angle where the slope of the angle versus torque...
relationship was minimum. Because a range of joint angles where there is zero passive force does not exist in the ankle joint, the fit had to be constrained to better reflect the effect of weight only. The gravitational fit was constrained using experimental measures of the relationship between joint position and torque with a 1 kg mass (black solid line), similar to that of the foot, located within the centre of the foot restraints. Solutions were constrained to achieve a joint moment within 20% of the maximum and minimum values of the torque measured across the range of motion using the 1 kg mass simulation.
Appendix C. Validity and reliability of a simple ultrasound approach to measure medial gastrocnemius muscle length

Acknowledgement of co-authorship:

I have made a substantial contribution in the conception and design of this study, analysis and interpretation of the research data, and the drafting and critical revising of the final manuscript.
**Abstract**

Fixed shortening of a muscle, or contracture, often develops in individuals with an upper motor neuron disorder. A clinical measure of muscle length would therefore be useful for identifying the presence of muscle contracture, tracking changes over time and evaluating the effect of interventions. This study compared a novel ultrasound-tape length method with a previously validated freehand 3D ultrasound method for measuring muscle length. The ultrasound-tape method intrasession reliability was also assessed. Resting MG muscle length was measured at three ankle joint angles in 15 typically developed (TD) adults and 9 adults with cerebral palsy (CP) using the two methods. The ultrasound-tape method on average overestimated the muscle length in the TD group by less than 0.1% (95% CI, 6%) and underestimated by 0.1% (95% CI, 6%) in the CP group compared to the 3D ultrasound method. Intrasession reliability of the ultrasound-tape method was high with intraclass correlation coefficients greater than 0.99. The ultrasound-tape method has sufficient accuracy to detect clinically relevant differences and changes in MG muscle length and may therefore be a useful clinical tool for assessing muscle length changes associated with contracture.
C.1. Introduction

Individuals with upper motor neuron lesions, such as those associated with spastic cerebral palsy (CP), stroke, traumatic brain injury and multiple sclerosis, and spinal cord injuries often present with contracture of affected muscles and associated motor impairment (Sheean, 2002). Contracture is defined as the fixed shortening of a muscle (often referred to as the muscle belly) in relation to the length of the accompanying long bone (Bache et al., 2003). Clinicians and researchers typically use joint range of motion as an indirect measure of muscle contracture (Fry et al., 2003, McDowell et al., 2000). However joint range of motion measures cannot differentiate the contributions of the multiple muscles that span the joint or the contributions of the muscle and tendon of individual muscles to the contracture. A clinical measure of individual muscle length would therefore be useful for identifying the presence of contracture, tracking muscle length changes over time and evaluating the effect of treatment interventions for spasticity and contracture such as physiotherapy, immobilisation (e.g. splinting), botulinum toxin injections or orthopaedic surgery.

Magnetic resonance imaging (MRI) is the modality of choice for direct measurement of muscle length *in vivo* (Mitsiopoulos et al., 1998, Oberhofer et al., 2009). However, this technique is expensive, may not be available, takes a substantial amount of time for each scan (typically >2 min) and in some cases patients require sedation. B-mode ultrasound (US) is commonly used to visualise muscle and tendon morphology and obtain quantitative information concerning muscle properties including muscle anatomical cross sectional area, muscle thickness, fascicle length and fascicle angle (Campbell and Wood, 2002, Maganaris, 2003, Ohata et al., 2008, Whittaker et al., 2007). Determination of *in vivo* muscle length is not possible with B-mode US as the field of view of the images is typically insufficient to visualise the whole muscle. Freehand 3D ultrasound (3DUS), which combines B-mode US with measures of 3D position, is an alternative method to measure muscle and tendon length and muscle volume (Experiment 1) (Fry et al., 2007, Malaiya et
al., 2007). However this approach requires 3D motion capture and time consuming analysis to manually segment each B-mode US image (Weller et al., 2007).

The purpose of this study was to determine the validity and reliability of muscle length measures obtained using a novel US and tape measure (US-tape) method in a typically developed and clinical sample consisting of individuals with CP. Muscle length measures of the MG obtained using the US-tape method were compared to measures obtained using 3DUS. The MG muscle was chosen for analysis because it is commonly affected by contracture in neurological conditions such as spastic CP (Malaiya et al., 2007) and stroke (Gao and Zhang, 2008) and because of its functional importance in locomotion (Liu et al., 2008, Steele et al., 2010).

C.2. Materials and Methods

C.2.1. Participants

Fifteen typically developed (TD) (5 females, 10 males, mean (SD) age 19 (3) years, mass 66 (8) kg, height 173 (7) cm) and nine individuals with spastic CP (3 females, 6 males, age 17 (2) years, mass 57 (10) kg, height 164 (7) cm), volunteered to participate in the study. The CP participants were either hemiplegic (n = 7) or diplegic (n = 2) and all were level I on the Gross Motor Function Classification System for Cerebral Palsy (Palisano et al., 2008) and were recruited through the Cerebral Palsy League Queensland. TD participants were healthy university staff or students. All participants provided written informed consent in accordance with institutional guidelines (CPLQ-2008/09-1023, GU Ref No: PES/21/08/HREC).

C.2.2. Experimental design

Three US-tape scans and three 3DUS scans were performed on the relaxed MG muscle of the right leg of the TD participants and on the most affected leg of the CP participants at each ankle angle - 30, 60 and 100% of the total ankle range of motion (ROM) from maximum plantarflexion (0% ROM) to MDF (100% ROM). The knee was maintained at 0° extension using a seat belt around the
thigh and the ankle range of motion was assessed and ankle angles passively positioned using an isokinetic dynamometer (Biodex System 4, Biodex Medical Systems Inc, New York).

C.2.3. Ultrasound measures

A PC-based B-mode US scanner with a 128-element beamformer and a 10.0 MHz linear transducer with 60 mm field of view (HL9.0/60/128Z, Telemed Echo Blaster 128 Ext-1Z system, Lithuania) was used for both the US-tape and 3DUS measures. US settings such as power, gain, image depth, and focal depth were optimised to allow ease of identification of the structures under investigation (Experiment 1).

The US-tape method involved the attachment of a metal tape measure to the US transducer at one end, and positioned over the Achilles tendon insertion on the calcaneus at the other end (Figure C.1). The proximal attachment of the MG was difficult to visualise with US so the most superficial aspect of the medial condyle of the femur was used as a standard proximal landmark for the US-tape method (Fig 1A). The tape distance from the calcaneus to the edge of the US transducer scan plane was recorded. A medial to lateral sweep of the transducer head was made over the medial femoral condyle whilst the tape was kept taught (at the same length) and the scan recorded. Post-processing of the scan was performed to identify the most superficial point of the condyle and the US depth and US distance of the condyle to the edge of the scan (Fig 1B). The most distal point of the muscle tendon junction (MTJ) was also scanned using a medial to lateral sweep of the transducer whilst the tape was kept taught (Fig 1C). The tape distance from the calcaneus to the edge of the US transducer scan plane was recorded. Post processing was performed and the US depth and US distance of the most distal MTJ point relative to the edge of the scan measured (Fig 1D). MTU length and tendon length were calculated using Pythagoras’ Theorem based on measures of: (a) Tape distance plus US distance and (b) US depth of the condyle/MTJ. Muscle length was calculated by subtracting tendon length from MTU length.
Figure C.1. US-tape method for measuring MG muscle belly length. (A) MTU length was measured from the calcaneus to the superficial aspect of the medial femoral condyle, posteriorly (tape distance + US distance). (B) Identification of the most superficial aspect of the condyle and the US depth and distance of the condyle to the edge of the scan. (C) Tendon length was measured from the calcaneus to the most distal aspect of the MTJ of the MG (tape distance + US distance). (D) Identification of the most distal point of the MTJ and the US depth and distance of the MTJ to the edge of the scan. Muscle belly length was calculated from the difference between MTU length and tendon length.

Freehand 3DUS was used to generate 3D reconstructions of the MG muscle as previously described in Experiment 1. The same anatomical landmarks were used for segmentation of the muscle volume as were used for the US-tape method - the most superficial aspect of the medial condyle of the femur proximally to the most distal point of the MTJ distally. Measurement of the muscle length was obtained from the 3D rendering using the Stradwin software measurement tools.
C.2.4. Statistical analysis

The level of agreement between the US-tape method and the freehand 3DUS-based measurement of MG muscle length was reported as the mean difference and corresponding 95% confidence interval (CI) (Bland and Altman, 1986) for the TD and CP groups each ankle joint angle assessed. Intra-session reliability of muscle length measurements made using the US-tape over three trials was assessed using the intra-class correlation coefficient, ICC (3,1).

C.3. Results

C.3.1 Validity

MG muscle length estimates using the US-tape method and freehand 3DUS, mean difference (mm), mean percentage difference and corresponding 95% CIs for the TD and CP groups at each ankle joint angle are presented in Table C.1. Mean muscle length (SD) averaged across the three ankle joint angles was 237 (12) mm in the TD group and 201(9) mm in the CP group. The US-tape method overestimated MG muscle length by 0.2 mm (<0.1%) in the TD group and by 0.3 mm (0.1%) in the CP group across all ankle joint angles. The 95% CIs for the mean difference between the two methods across all joint angles was 15 mm (6%) for the TD group and 13 mm (6%) for the CP group (Figures C.2 and C.3).
Table C.1. MG muscle length measured using the US-tape and freehand 3DUS methods for the TD and CP groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ankle joint angle (%)</th>
<th>Mean ankle angle (°)</th>
<th>Muscle length (mm)</th>
<th>Mean difference (mm)</th>
<th>95% CI</th>
<th>Mean difference (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>100</td>
<td>23 (4)</td>
<td>249 (26)</td>
<td>249 (27)</td>
<td>-0.1 (3)</td>
<td>12</td>
<td>0.0 (1)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-2 (3)</td>
<td>236 (24)</td>
<td>237 (27)</td>
<td>-0.9 (3)</td>
<td>14</td>
<td>-0.3 (1)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-21 (4)</td>
<td>225 (24)</td>
<td>225 (24)</td>
<td>0.3 (4)</td>
<td>17</td>
<td>0.2 (2)</td>
</tr>
<tr>
<td>CP</td>
<td>100</td>
<td>5 (5)</td>
<td>211 (39)</td>
<td>211 (39)</td>
<td>0.7 (4)</td>
<td>14</td>
<td>0.3 (1)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-13 (2)</td>
<td>202 (41)</td>
<td>202 (40)</td>
<td>0.2 (3)</td>
<td>12</td>
<td>0.1 (1)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-26 (3)</td>
<td>192 (35)</td>
<td>192 (36)</td>
<td>0.1 (4)</td>
<td>13</td>
<td>-0.1 (1)</td>
</tr>
</tbody>
</table>

ROM, range of motion; LoA, limit of agreement; CI, confidence intervals. Mean percentage difference is the ratio of the difference and the mean of the 3DUS and US-tape measures. Data are presented as mean (SD). ROM: Range Of Motion. CI: Confidence Interval. Positive ankle angle indicates dorsiflexion. 100% ROM = MDF.
Figure C.2. Scatter plots and Bland–Altman plots showing correspondence between 3DUS and US-tape measures of the MG muscle length in three ankle positions for TD individuals (100%, 60%, 30% of range of motion). The diagonal line (small dash) in the scatter plots corresponds to the line of perfect agreement. The horizontal lines on the Bland–Altman plots represent perfect agreement (small dash), the mean difference between the 3DUS and US-tape measurements (solid) and the upper and lower 95% confidence intervals (large dash).
Figure C.3. Scatter plots and Bland–Altman plots showing correspondence between 3DUS and US-tape measures of the MG muscle length in three ankle positions) for individuals with CP (100%, 60%, 30% of range of motion. The diagonal line (small dash) in the scatter plots corresponds to the line of perfect agreement. The horizontal lines on the Bland–Altman plots represent perfect agreement (small dash), the mean difference between the 3DUS and US-tape measurements (solid) and the upper and lower 95% confidence intervals (large dash).
C.3.2. Reliability

The ICCs for repeated intra-session US-tape measures of MG muscle length at each ankle angle for the TD and CP groups were greater than 0.99 (Table C.2).

Table C.2. Reliability of intra-session repeated measures of MG muscle length by the US-tape method assessed using the Intra-class Correlation Coefficient (ICC).

<table>
<thead>
<tr>
<th>Group</th>
<th>Ankle joint angle</th>
<th>MG muscle length (mm)</th>
<th>ICC (3,1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%) ROM</td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>TD</td>
<td>100</td>
<td>248(28)</td>
<td>249(27)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>235(27)</td>
<td>236(27)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>224(25)</td>
<td>225(25)</td>
</tr>
<tr>
<td>CP</td>
<td>100</td>
<td>211(41)</td>
<td>212(41)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>203(42)</td>
<td>202(42)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>192(36)</td>
<td>192(36)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD).

C.4. Discussion

This study demonstrated good agreement between freehand 3DUS and the US-tape measures of MG muscle length in the TD and CP groups across a range of ankle joint angles. Compared to our previously validated 3DUS measure (Experiment 1), the US-tape method overestimated MG muscle length by less than 0.1% in the TD group and underestimated MG muscle length by 0.1% in the CP group. Furthermore, the overall 95% CIs were 6% for the TD and CP groups respectively, suggesting that the error associated with our US-tape method is no more than ±3%. Importantly, the relative magnitude of error was unaffected by the ankle joint angle at which the measurements were made and the ICCs for intra-session reliability were all greater than 0.99.
To put our findings in a clinical perspective it is necessary to compare the accuracy of our US-tape method with the expected differences or changes in muscle length that the method may be used to detect in a given patient population. Unfortunately at present there is general paucity of information available on muscle length adaptation following upper motor neuron lesions, with published studies on this topic generally confined to CP (see Barrett and Lichtwark (2010) for review). CP studies to date report reductions in MG muscle length compared to TD, and in the paretic versus non-paretic limbs, of 8-31% (Fry et al., 2004, Malaiya et al., 2007). Further, MG muscle length changes following orthopaedic surgery in children with CP have been reported to be 5-12% (Fry et al., 2007). Although the present study was performed using young adults and not children, the ±3% confidence interval of the US-tape method reported here appears to be within acceptable limits for determining the presence of MG contracture relative to TD or the less affected limb, as well as assessing the effect of certain specific orthopaedic interventions reported in studies of CP to date. We are currently unaware of any studies that have measured the natural history of muscle length changes over time in any patient group following upper motor neuron lesion, and so it is currently difficult to assess the suitability of the US-tape method for this purpose. Further studies will be therefore required to determine the suitability of the US-tape method to assess the progression of muscle contracture over time and following conservative and pharmaceutical treatment interventions in specific patient groups with contracture for which data on the magnitude of muscle length adaptations are not currently available. It would also be of value in future to assess whether the method described here is suitable for assessing muscle length adaptations in other muscles affected by contracture (e.g. upper limb muscles).

Compared to other imaging modalities, we believe the US-tape measurement is a relatively simple and cost-effective technique for measuring muscle contracture in the clinical environment. Measuring muscle length using MRI (Eames et al., 1997) or 3DUS (Experiment 1) is time consuming (due to post-processing), expensive and may require patient sedation, and so is normally restricted to research rather than clinical investigations. In contrast we were able to make the required US recordings and perform the necessary post-processing to obtain MG muscle length estimates at each ankle joint angle within a few minutes for each participant using our US-tape
method. This method is easy to carry out and requires minimal ultrasonographic experience to localise the anatomical landmarks, scan and record the regions of interest.

Concluding remarks
This study demonstrated accurate measurement of MG muscle length using the US-tape method over a large range of ankle joint angles in young adults who are TD or have spastic CP. The ease, speed and accuracy of the US-tape method lends itself to clinical use for measurement of MG muscle lengths in individuals presenting with contracture such as those with stroke, multiple sclerosis or spastic CP.
Appendix D. Invited commentary on original publication Barber et al., (2011).


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References


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