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# Author's Accepted Manuscript

Gastrocnemius muscle-tendon interaction during walking in typically-developing adults and children, and in children with spastic cerebral palsy

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#### Abstract

Background: Our understanding of the interaction of muscle bellies and their tendons in individuals with muscle pathology is limited. Knowledge of these interactions may inform us of the effects of musculoskeletal pathologies on muscle-tendon dynamics and the subsequent neurological control strategies used in gait. Here, we investigate gastrocnemius muscle-tendon interaction in typically-developing (TD) adults and children, and in children with spastic cerebral palsy (SCP).

Methods: We recruited six TD adults (4 female; mean age: 34 yrs. (24 - 54)), eight TD children (5 female; mean age: 10 yrs. (6 - 12)) and eight independently ambulant children with SCP (5 female; mean age 9 yrs. (6 - 12); 3 unilaterally-affected). A combination of 3D motion capture and 2D real-time ultrasound imaging were used to compute the gastrocnemius musculo-tendinous unit (MTU) length and estimate muscle belly and tendon lengths during walking. For the TD subjects, the measurements were made for heel-toe walking and voluntary toe-walking.

Results: The gastrocnemius muscle bellies of children with SCP lengthened during single support (p = 0.003). In contrast, the muscle bellies of TD subjects did not demonstrate an increase in length over the period of single support under heel-toe or toe-walking conditions.

Conclusion: We observed lengthening of the gastrocnemius muscle bellies in children with SCP during single support, a phase of the gait cycle in which the muscle is reported consistently to be active. Repeated lengthening of muscle bellies while they are active may lead to muscle damage and have implications for the natural history of gait in this group.

#### 1. Introduction

The gastrocnemius muscle consists of two heads (lateral and medial) which originate above the knee and share a common distal tendon that inserts into the calcaneus. The other major muscle of the calf is the soleus. The soleus originates from the proximal fibula and proximal medial border of the tibia, and also inserts into the calcaneus. Much of the internal tendon of the soleus and the external tendon of the gastrocnemius is shared. Several studies have quantified the interaction between the gastrocnemius muscle bellies and tendon to better understand the role of the gastrocnemius muscle-tendon unit (MTU) in human locomotion. To date, these studies have been performed largely in typically developing (TD) adults. Using a combination of kinematic data and simultaneous 2D ultrasound imaging, several researchers have measured the length changes of the medial gastrocnemius (MG) MTU, tendon and muscle fascicles in real-time during walking (Fukunaga et al., 2001; Lichtwark and Wilson, 2006; Ishikawa et al., 2005). The results of these studies demonstrated the complementary actions of the gastrocnemius muscle belly and its external tendon. These studies consistently reported that during the single support phase of gait, the gastrocnemius muscle fascicles act almost isometrically as the tendon increases in length. In preswing, the tendon recoils and the muscle fascicles contract concentrically so that the muscle tendon unit shortens quickly. In late swing, the muscle fascicles lengthen. This behaviour of the MG muscle is thought to be highly efficient as the contractile elements of the muscle do not perform any substantial negative external mechanical work, with the external tendon storing and releasing energy (Fukunaga et al., 2001). For a detailed review of recent work in muscle-tendon interaction during walking see Cronin and Lichtwark (2013).

The length of MTUs may be calculated from joint angles derived from movement analysis studies and estimates of muscle moment arms. Studies of the length of the gastrocnemius MTU have been performed in TD children (Eames et al., 1997) and also in children with spastic cerebral palsy (SCP) (Orendurff et al., 2002; Wren et al., 2004). However, these computations do not separate the contribution of the muscle belly and the external tendon to MTU length. Although research in muscle-tendon interaction in clinical populations has been conducted (Cronin et al., 2010), to our knowledge, these have not been performed in children with SCP or in TD children.

In TD subjects, the plantarflexor moments required in walking are similar to the measured plantarflexor torques during maximum voluntary contraction (Fosang and Baker, 2006). In

children with SCP, muscles are much smaller than in TD subjects as a proportion of their body mass, and their lean muscle mass may be reduced further (Fry et al., 2007; Barber et al., 2011; Noble et al., 2014). Furthermore, the gastrocnemius muscle is known to be active during single support in these children (Davids et al., 1999; Patikas et al., 2006; Romkes and Brunner, 2007; van der Krogt et al., 2010). Together these factors suggest that individuals with SCP may have difficulty sustaining an isometric contraction of the plantarflexors under loads typical of walking. More forceful contractions may be produced in eccentric contractions than in concentric contractions at the same muscle length in subjects with SCP (Damiano et al., 2001).

We have assessed the gastrocnemius muscle-tendon interaction in TD adults and children, and in children with SCP. We hypothesised that the gastrocnemius muscle belly would lengthen over the period of single support in the children with SCP, and maintain an isometric length in TD subjects during a heel-toe gait. We also hypothesised that toe-walking would induce lengthening of the gastrocnemius muscle belly over the period of single support in TD subjects.

#### 2. Methods

#### 2.1 Subjects

The study was approved by the local research ethics committee. Written consent was obtained from the adult subjects and from the parents or guardians of the children in this study. Oral consent was obtained from all participants. Table 1 provides details on the subjects recruited for this study. Table 2 summarises the diagnoses, GMFCS levels and previous intervention histories of subjects with SCP.

## [Tables 1 and 2 about here]

Subjects with SCP were included in the study if they met the following inclusion criteria:

- Diagnosis of spastic cerebral palsy
- Age range: 7 12 years
- Independent ambulator
- Equinus or plantigrade foot position at initial contact

Subjects with SCP were excluded from the study if they had significant minimum knee flexion in single support (>20 degrees), significant foot deformities or previous surgical intervention within the last two years (e.g. muscle lengthening) or botulinum toxin or serial casting within the previous six months

#### 2.2. Experimental Procedure

Measurements of the gastrocnemius MTU, muscle belly and tendon length changes were made for all subjects for barefoot walking at each subject's self-selected walking speed. To make these measurements, we collected anthropometric data, knee and ankle joint kinematics and two-dimensional ultrasound images of the gastrocnemius musculotendinous junction (MTJ) from each subject. A motion analysis system (Vicon 612, Motion Systems, Oxford, UK) consisting of nine infrared source cameras (M-Series) operating at 120Hz was used to collect the 3D positions of 14mm retroreflective markers applied at specific locations on the subjects' limbs (Helen Hayes marker set). We used these data to inform the Plug-in-Gait™ (Vicon Motion Systems) model to calculate joint angles (flexion/extension) at the ankle and knee. The method we used to measure changes in gastrocnemius muscle belly and tendon length differed from those reported in published literature (Fukunaga et al., 2001) where rather than imaging the gastrocnemius muscle fibres, we imaged the gastrocnemius MTJ in real time as a subject walked using twodimensional ultrasound imaging (Figure 1). During walking, the MTJ moves as the muscle belly contracts and this movement was tracked using a 7.5 MHz linear array 2D ultrasound probe (UST-5710-7.5 Linear Array, Aloka, Japan). Four retro-reflective markers were attached to the probe using double-sided adhesive tape to track its 3D position in the analysis volume as shown in Figure 2. The ultrasound images were collected using Vicon<sup>TM</sup> Workstation<sup>™</sup> software by connecting the video output from the ultrasound scanner to a Broadway Pro<sup>TM</sup> frame grabber (Data Translation Inc., USA).

[Figures 1 and 2 about here]

Initially, a minimum of three kinematic data trials were collected for each subject without the simultaneous collection of ultrasound images of the gastrocnemius MTJ. Following this, the ultrasound probe was firmly attached to a subject's leg using adjustable elastic straps in a position that enabled the MTJ to be clearly seen. The probe was orientated so that the linear sensor array ran proximally-to-distally. Once the subject was familiar with walking with the ultrasound probe attached, joint kinematic data and ultrasound images of the MTJ were collected simultaneously. As a subject walked, an operator pushed the ultrasound machine at a distance of approximately 1.5m behind the subject. At least one successful walking trial was collected with the ultrasound probe attached from each subject. Measurements were made when the TD subjects walked in their natural heel-toe pattern and then when they walked on their toes. The children with SCP were independently ambulant and adopted their natural pattern of walking which was either toe-walking or plantigrade at initial contact.

#### 2.3 Estimation of Gastrocnemius MTU, muscle belly and tendon length

Joint kinematic and two-dimensional ultrasound image data was analysed and processed using a program written in Matlab® Version 7 (Mathworks, Inc. USA) which used the Motion Lab Systems C3DServer script (Shriners Motion Analysis Lab, USA) to read the kinematic data files. Since the ultrasound image data was collected at a different frequency (25Hz) to the marker trajectory data (120Hz), the program first matched the marker trajectory frames with the ultrasound image frames. Following this, the program displayed each frame of the ultrasound video file and the measurer (GK) manually marked the position of the MTJ (Figure 1). For further details on the repeatability of this method, see Supplementary Materials, Appendix B. Using the coordinates of the four markers attached to the ultrasound probe, a reference axis system was defined on the probe. This was used to determine the 3D coordinates of the gastrocnemius MTJ, using the method detailed by Fry et al. (2004). From this, the 3D position of the gastrocnemius MTJ was determined for each ultrasound frame. An estimate of gastrocnemius MTU length was derived from knee flexion/extension and ankle dorsi/plantarflexion according to the imaging-based model of Eames et al. (1997). Tendon lengths were estimated as the Euclidean distance between the MTJ and the

insertion point of the tendon in the calcaneus (determined using the 3D position of the heel marker). Muscle belly length was estimated by subtracting the tendon length from the MTU length.

#### 2.4 Statistical Analysis

A single representative trial was selected for each subject. To test the hypotheses, we calculated the change in absolute length of the gastrocnemius muscle belly over the period of single support, and conducted paired t-tests. As we applied multiple statistical tests (children with SCP, and TD children and adults heel-toe and toe-walking) the Bonferroni adjustment was applied to change the level of significance from p < 0.05 to p < 0.01.

We also computed the percentage changes in MTU, muscle belly and tendon length over the gait cycle from its length at initial contact. From this, a variable  $\Delta L_{SS}$ , the percentage change in length of the muscle belly over the period of single support (not necessarily the maximum change in length over this period) was derived. The independent t-test was applied to test for a difference in the mean value of  $\Delta L_{SS}$  between the following groups of subjects:

- Children with SCP and TD children who walked in their natural heel-toe pattern
- Children with SCP and TD children who voluntarily toe walked

In addition, a paired t-test was applied to test for a difference in the mean value of  $\Delta L_{SS}$  in adults and TD children, between heel-toe and toe-walking.

#### 3. Results

For heel-toe walking, the MTU, the muscle belly and tendon length results of the TD adults are consistent with those reported in published literature (Fukunaga et al., 2001; Lichtwark and Wilson, 2006; Ishikawa et al., 2005) where during loading the gastrocnemius MTU shortens, primarily due to changes in tendon length (Figure 3; normalised lengths are presented in Supplementary Materials, Appendix C). The muscle belly remains at a near constant length for much of single support shortening only towards the end of this phase. This results in a significant reduction in the change of length over the whole of single

support. During preswing there is shortening of both muscular and tendinous components and in swing, the muscle belly and tendon lengthen, restoring their original position at initial contact. Our results for heel-toe walking in TD children are similar to those for TD adults, however the change in absolute length of the muscle belly over the period of single support was not significant.

## [Figure 3 about here please]

For toe-walking TD adults and children, the muscle belly and tendon profiles differ from the profiles observed for heel-toe walking where just after initial contact, the muscle bellies for the TD subjects shorten concentrically as their tendons stretch. In single support, the muscle bellies lengthen gradually, however, a greater increase in the percentage change in length of the muscle belly from its reference length at initial contact is seen in the TD adults than in the TD children (See Supplementary Materials, Appendix D). However, the change in absolute length of the gastrocnemius muscle belly over the period of single support was not significant for the TD subjects. For the TD adults who toe-walked,  $\Delta L_{SS}$  was found to be significantly different to the near-isometric action of the muscle belly in heel-toe walking (p = 0.003). For the TD children, there was no significant difference in  $\Delta L_{SS}$  between heel-toe and toe-walking (p = 0.152). Our purpose in asking TD subjects to walk on their toes was to encourage these subjects to generate plantarflexor moments similar to the group of children with SCP. We have included representative knee and ankle joint kinematics and plantarflexor moment data from each subject group and for each walking pattern in Supplementary Materials, Appendices E and F.

For the children with SCP, the gastrocnemius MTU lengthened at initial contact and continued to do so in single support with a peak percentage change in length of 3% (See Supplementary Materials, Appendix D). This lengthening can be, in part, attributed to the lengthening of the muscle bellies with an additional contribution made from the lengthening tendon. The change in absolute length of the gastrocnemius muscle belly over the period of single support was significant in the children with SCP (p = 0.003). Additionally,  $\Delta L_{SS}$  for the children with SCP and the TD children who walked in their natural heel-toe pattern were found to be significantly different (p = 0.001). The lengthening of the muscle

belly in the children with SCP was also much greater than the lengthening seen in the toe-walking TD subjects.  $\Delta L_{SS}$  was found to be significantly different (p = 0.008) between the children with SCP and the TD children who toe walked voluntarily.

#### 4. Discussion

We have measured gastrocnemius muscle-tendon dynamics in TD children and adults, and in children with SCP. The results of this study supported our first hypothesis that the gastrocnemius muscle belly would lengthen during single support in children with SCP in contrast to their typically peers. In contradiction of our second hypothesis, we observed near isometric or shortening behaviour of the gastrocnemius muscle belly during the single support period of the gait cycle in TD adults and children when these subjects walked in their natural heel-toe or toe-walking gaits. The change in length of the MTU in single support, in both TD adults and children adopting their natural heel toe-walking pattern, is accommodated by the external tendon. This may allow the musculo-tendinous unit to behave near its energetic optimum as first described by Fukunaga et al. (2001). In contrast, in children with SCP, the gastrocnemius muscle bellies elongated during single support by a mean of more than 2% of the muscle belly length.

#### 4.1. Clinical Implications

Large eccentric actions of the gastrocnemius muscle belly during single support may be due to an incapacity of these muscles to maintain an isometric contraction in the presence of external forces (plantarflexor moments; Supplementary Materials, Appendix F). The forces produced by the plantarflexors may be compromised in children with SCP in whom the distal muscle volume is greatly reduced (Fry et al., 2007; Noble et al., 2014; Barber et al., 2011) and the non-myofibrillar content increased (Noble et al., 2014). This insufficiency may be exacerbated by an inability to activate the available muscular resources (Stackhouse et al., 2005; Rose and McGill, 2005), and by abnormal muscle activation patterns in walking (Berger et al., 1982). Muscle weakness could also arise from activation at a non-optimal sarcomere length secondary to a shortened muscle position during toe-walking.

There is little data to support significant alterations in tendon compliance in the calf of individuals with SCP (Barber et al., 2011). We cannot directly infer from our data the forces

crossing the individual components of the triceps surae, but increased stiffness of the tendinous components would be consistent with the reduced excursion of tendon that we observed during the stance phase of children with SCP. However, the compliance of the portion of the MTU from the distal end of the gastrocnemius to the calcaneum may be influenced by the action of the soleus muscle belly with its broad insertion into the external tendon of the gastrocnemius.

With recovery intervals of an appropriate duration, eccentric exercise training may be an effective method of building muscle (for review see Roig et al., 2009). However, under conditions of repeated eccentric contraction, without recovery, significant damage may occur. Rader and Faulkner (2006) found that the muscles of older mice that were exposed to lengthening contraction protocol, suffered permanent reductions in muscle mass. Furthermore, satellite cells are responsible for muscle fibre repair and replacement after damage and a recent study by Smith et al. (2013) has demonstrated a reduction in the number of satellite cells in the hamstrings of children with cerebral palsy. In fact, Dayanidhi et al. (2015) showed that the number of satellite cells in the gracilis muscle of children with cerebral palsy (expressed as number per 100 myofibers) was reduced by 70% when compared to their age-matched peers. So, it is possible that in children with SCP, muscles damaged by eccentric lengthening may not have the opportunity or resources to recover fully, leading to irreversible and deleterious changes in muscle quality and further weakness.

The eccentric activation of the gastrocnemius and any subsequent damage to these muscles may have implications for the natural history of gait and mobility in children with SCP. Analyses of induced acceleration suggest that the capacity for the gastrocnemius to provide vertical support to the body in normal walking is significant but that in moderate and severe crouch the capacity of the gastrocnemius to accelerate the body upwards is reduced (Steele et al., 2013). In contrast, in crouch gait, the knee extensors have a greater capacity to accelerate the body upwards. It follows that subjects who cannot generate sufficient plantarflexion moments may flex their limbs to increase the relative capacity of the knee and hip extensors to maintain support of the body (Steele et al. 2013).

Toe-walking gives rise to increased mean plantarflexor moments in stance when compared to heel-toe walking (Supplementary Materials, Appendix F). Intervention such as muscle-

tendon lengthening surgery can change a toe-walking pattern to a heel-toe pattern reducing the persistent stance phase loading of the plantarflexors (Rose, S.A. et al., 1993). This may serve to reduce the degree of eccentric lengthening of the gastrocnemius. Increasing muscle strength may also reduce eccentric lengthening. Mcnee et al. (2009) observed increases in plantarflexor volume following plantarflexor strengthening.

#### 4.2. Limitations

We have measured the excursion of the gastrocnemius MTJ, and not directly the excursion of the gastrocnemius muscle fascicles. It is possible that some of the excursion of the MTJ could be explained by changes in pennation of the gastrocnemius muscle fascicles. However, Lichtwark and co-workers (Lichtwark et al., 2007) found no significant changes in pennation angle during single support in TD subjects. Additionally, we hypothesized length changes throughout single support. However, inspection of Figure 3 suggests that some lengthening of the gastrocnemius muscle belly amongst TD subjects may take place in the first portion of single support, perhaps corresponding to the large plantarflexor moments in this portion of the gait cycle.

The impact of previous surgical or non-surgical interventions on muscle-tendon interaction, which our subjects with SCP (Table 2) have had may have influenced the relative contributions of muscle and tendon. In any form of research involving children with SCP, it is difficult to find participants that have not been touched by some significant intervention. If children without intervention had been recruited, they would have represented a very able sub-group, the results from which would not have been generalizable.

Securing an ultrasound probe to the leg of each of our subjects may have had an effect on a subject's gait. To assess this, a comparison of the average MTU length changes calculated using knee and ankle joint kinematics collected from all subjects with and without the attachment of the ultrasound probe were made. The results are presented in the Supplementary Material, Appendix G. No statistically significant difference was found between the maximum and minimum MTU length changes for each subject group. However, to improve subject experience and the data collection aspects of the study, future work will involve using a portable lightweight ultrasound machine that a subject can carry on their back.

Another limitation is the 2D nature of the model used to estimate the gastrocnemius MTU length which estimates the length changes in the sagittal plane. If the ankle should move out of the sagittal plane, e.g. in the case of dynamic hindfoot varus or valgus motion, this may introduce an error in the MTU length measurement. Future work will involve using a 3D ultrasound imaging system to track the 3D position of the MTJ.

#### 5. Conclusion

We analysed the gastrocnemius muscle-tendon interaction in TD adults and children and in children with SCP. In the heel-toe and toe-walking TD subjects, isometric or shortening behaviour of the gastrocnemius muscle belly was observed. However, children with SCP demonstrated the greatest eccentric lengthening of the muscle belly during stance. Persistent eccentric lengthening of the muscle belly in children with SCP may cause permanent muscle damage with negative consequences for the natural history of gait in these children.

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#### 7. Conflict of Interest

None of the authors have conflicts of interest to declare.

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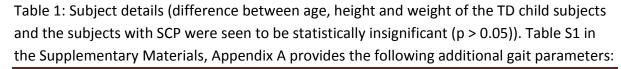
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V.C.C.C.C.

self-selected walking speed, knee and ankle angles at initial contact and maximum dorsiflexion angles during single support for all subject groups

Table 2: Diagnosis, GMFCS level, previous intervention history and selected side (limb) for analysis of subjects with SCP

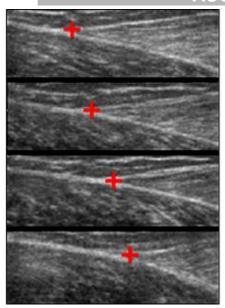
Figure Legends

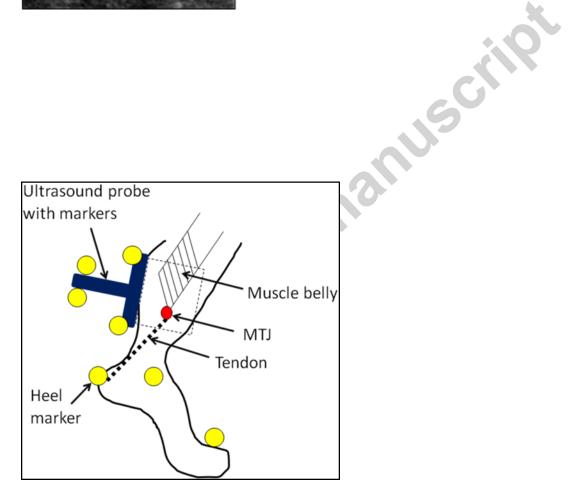
Figure 1: Two-dimensional ultrasound images showing the position of the gastrocnemius musculotendinous junction (MTJ) in multiple frames of the gait cycle in a typically-developing subject.

Figure 2: Placement of markers on the ultrasound probe and on the distal lower segments of the subjects.

Figures 3: (a), b), (c) Mean absolute gastrocnemius MTU, muscle belly and tendon length measured over one gait cycle in heel-toe walking TD adults and children and in children with SCP who were obligatory toe-walkers (d), (e), (f) Mean absolute gastrocnemius MTU, muscle belly and tendon length measured over one gait cycle in toe – walking TD adults and children and in children with SCP who were obligatory toe-walkers. Error bars indicate +/-one standard error of the measurement. OTO – opposite foot toe-off; OIC – opposite foot initial contact.

VCC6.6/FG,





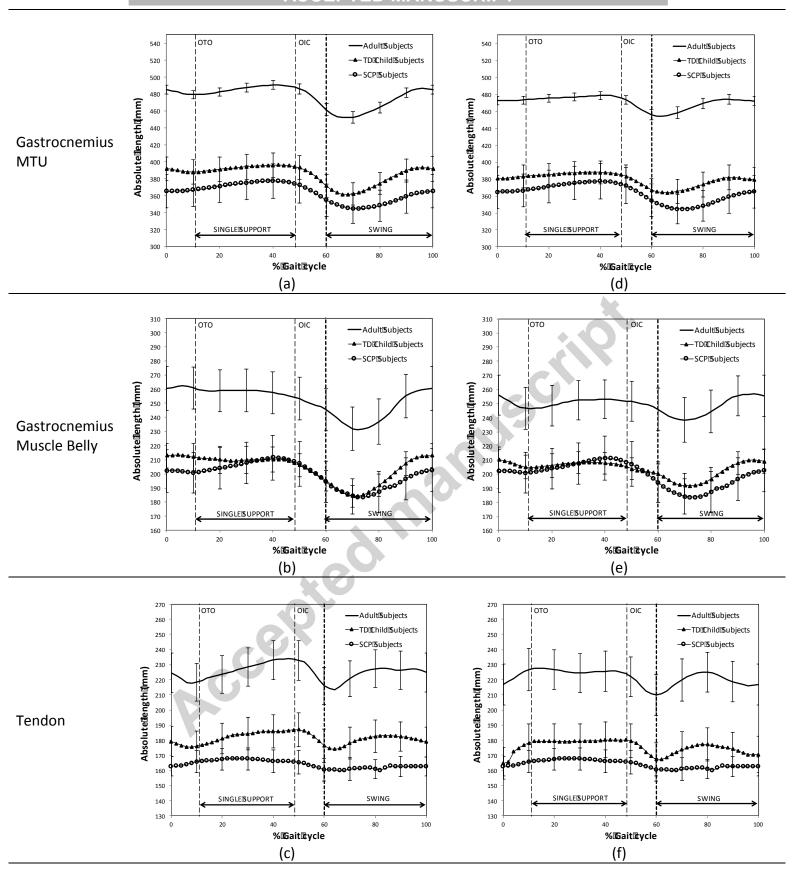


Figure 3

	TD Adults	TD Child Subjects	Subjects with SCP
No. of subjects	6	8	8
Gender	2 male, 4 female	3 male, 5 female	3 male, 5 female
Age (years)	34 ± 10	10 ± 2	9 ± 2
Height (cm)	170 ± 5	141 ± 13	136 ± 16
Weight (kg)	68 ± 7	36 ± 9	35 ± 15

<ol> <li>Subjects w</li> </ol>	ith SCP	No. of subjects
Diagnosis	Unilateral SCP	3
	Bilateral SCP	5
GMFCS Level	Level 1	3
	Level 2	5
	Serial casting	5
Previous intervention history	Botulinum toxin treatment	1
	Botulinum toxin treatment followed by serial casting	2
	Gastrocnemius slide surgery and digitorum longus lengthening	1
	No previous intervention history	1
Selected side	Unilateral SCP – Left limb	3
(limb) for	Bilateral SCP – Right limb	1
analysis	Bilateral SCP – Left limb	4