

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

3 Barbara M. Kalkman¹, Lynn Bar-On², Francesco Cenni^{2,3}, Constantinos N. Maganaris¹, Alfie
4 Bass⁴, Gill Holmes⁴, Kaat Desloovere², Gabor J. Barton¹, Thomas D. O'Brien¹

5

6 **Affiliation**

7 ¹Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Byrom
8 Street, Liverpool L3 3AF, UK

9 ²Department of Rehabilitation Sciences, KU Leuven, Tervuursevest 101, 3001 Leuven, Belgium

10 ³Department of Mechanical Engineering, KU Leuven, Celestijnenlaan 300, 3001 Leuven, Belgium

11 ⁴Alder Hey Children's NHS Foundation Trust, Eaton Road, L12 2AP Liverpool, UK

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13 **Running Head:** Acute effect of stretching in cerebral palsy

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15 **Corresponding Author:**

16 Barbara M. Kalkman

17 Research Institute for Sport and Exercise Sciences

18 Liverpool John Moores University

19 Tom Reilly Building

20 Byrom Street

21 Liverpool L3 3AF

22 b.m.kalkman@2014.ljmu.ac.uk

23 +44 75 96 722045

24

1 **New Findings:**

2 **What is the central question of this study?**

3 Can the increased range of motion seen acutely after stretching in children with CP be explained by
4 changes in the stiffness of the medial gastrocnemius fascicles?

5 **What is the main finding and its importance?**

6 We show for the first time that passive muscle and tendon properties are not changed acutely after a
7 single bout of stretching in children with cerebral palsy and do therefore not contribute to the increase
8 in range of motion. This contradicts common belief and what happens in healthy adults.

9

10 **Abstract:**

11 Stretching is often used to increase/maintain joint range of motion (ROM) in children with cerebral palsy
12 (CP) but the effectiveness of these interventions is limited. Therefore, this study aimed to determine the
13 acute changes in muscle-tendon lengthening properties that contribute to increased ROM after a bout
14 of stretching in children with CP. Eleven children with spastic CP (age:12.1(3)y, 5/6 hemiplegia/diplegia,
15 7/4 GMFCS level I/II) participated in this study. Each child received 3 sets of 5x20s passive, manual static
16 dorsiflexion stretches separated by 30s rest, and 60s rest between sets. Pre- and immediately post-
17 stretching, ultrasound was used to measure medial gastrocnemius fascicle lengthening continuously
18 over the full ROM and an individual common ROM pre- to post-stretching. Simultaneously, 3D motion of
19 two marker clusters on the shank and the foot was captured to calculate ankle angle, and ankle joint
20 torque was calculated from manually applied torques and forces on a 6DoF load cell. After stretching,
21 ROM was increased (9.9° (12.0), p=0.005). Over a ROM common to both pre and post measurements,
22 there were no changes in fascicle lengthening or torque. The maximal ankle joint torque tolerated by

1 the participants increased (2.9(2.4) Nm, $p=0.003$) and at this highest passive torque maximal fascicle
2 length was 2.8(2.4) mm greater ($p=0.009$) when compared to before stretching. These results indicate
3 that the stiffness of the muscle fascicles in children with CP remain unaltered by an acute bout of
4 stretching.

5 **Introduction:**

6 Children with cerebral palsy (CP) typically present with a reduced range of motion (ROM) and increased
7 ankle joint stiffness compared to typically developing children. It has been shown that increased muscle
8 stiffness and decreased muscle length contribute to this reduced ROM and impaired function (Geertsen
9 *et al.*, 2015). Stretching therapies are commonly used as a non-invasive treatment in children with CP. In
10 clinical practice the assumption is that repeated bouts of stretching can increase muscle length, and
11 consequently decrease muscle stiffness and therefore delay the onset of muscle contractures and defer
12 or avoid surgery (Odéen, 1981; Herbert, 2004; Wiart *et al.*, 2008). However, despite an improved ankle
13 ROM (Theis *et al.*, 2015), the scientific evidence does not confirm these assumptions and a significant
14 gap exists between the clinical rationale for stretching and the supporting evidence (for systematic
15 reviews see: Pin *et al.*, 2006; Wiart *et al.*, 2008). Given that stretching interventions cause discomfort to
16 children and are demanding of them and their families (Hadden & Von Baeyer, 2002), stronger evidence
17 is required to support their application and optimise their effectiveness.

18 Altered muscle-tendon properties in CP may leads to a reduced ROM, but these alterations may also
19 mediate the response to stretching interventions as seen in typically developing individuals. For
20 example, previous studies show that in CP, muscles are shorter (Fry *et al.*, 2004; Malaiya *et al.*, 2007;
21 Oberhofer *et al.*, 2010), tendon slack length is longer (Gao *et al.*, 2011; Barber *et al.*, 2012) and relative
22 muscle to tendon stiffness is increased (Kalkman *et al.*, 2016). It is unknown how these altered
23 properties mediate the acute response of a muscle to stretching in individuals with CP. In typically

1 developing adults, acutely after 5 minutes of conditioning stretches, ROM was increased and joint
2 stiffness decreased (Morse *et al.*, 2008). Based on an increase in muscle belly length in the absence of an
3 increase in fascicle length and pennation angle changes, the authors attributed these changes to a
4 reduction in the stiffness of the intramuscular connective tissue. The question then arises, do spastic
5 muscles respond to stretch in a similar way, since changes have been shown in both the quality and
6 amount of intramuscular connective tissue in children with CP (Smith *et al.*, 2011). It has been shown
7 that ankle ROM in children with CP improved immediately after stretching and this was accounted for by
8 an increase in length at maximal joint angles of all three structures that make up the muscle-tendon-unit
9 (MTU) of the medial gastrocnemius, i.e. muscle belly, fascicles and tendon (Theis *et al.*, 2013). However,
10 the gain of MTU lengthening after stretching reported in this study seems extremely large (18.5mm) for
11 an increase in ankle ROM of only 9.8°. Also, no information was reported about any changes in joint
12 torque and thus in the passive properties of the involved structures.

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

1 **Methods:**

2 *Ethical approval*

3 The study was approved by the NHS research ethics committee in the UK (Project No: 15/LO/0856) and
4 the University Hospital's ethics committee in Leuven, Belgium (Project No: S 557384). The study was
5 conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained from
6 the parents, and written assent was given by children in accordance with local regulations.

7 *Participants:*

8 Eleven children aged 8-16 years old, diagnosed with spastic CP were recruited for participation in this
9 study. Patient characteristics can be found in table 1. Children were excluded if they had Botulinum
10 Toxin-A injection to the lower limb muscles less than 6 months prior to testing, any lower limb neuro- or
11 orthopaedic surgery or a baclofen pump. Children were recruited through the gait lab of Alder Hey
12 Children's Hospital in Liverpool and the University Hospital in Leuven.

13 *Experimental design:*

14 Participants attended the hospital on one occasion. During this visit, participants underwent an acute
15 bout of passive ankle dorsiflexion stretches applied by a physiotherapist. Stretches were applied to the
16 leg that was most affected as defined by spasticity scores. Before and within 10 minutes after the
17 stretching session, measurements of ankle angle, passive joint torque and medial gastrocnemius muscle
18 fascicle lengthening during a passive stretch were taken. During these measurements, participants lay
19 prone on a bed with the lower leg supported on an inclined cushion such that the knee was ~20° flexed.
20 The leg was positioned in a custom-made orthosis, to control ankle movement in the sagittal plane
21 (figure 1). The axis of rotation of the orthosis was aligned with the lateral malleolus. Each participant
22 underwent 3 trials of passive ankle dorsiflexion movements taking 5 seconds to complete one

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

16 *Stretching intervention:*

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

1 *Data analysis:*

2 Data analysis was carried out using custom-made software (Matlab R2015 and Python 2.7.11). Kinematic
3 and kinetic data were filtered using a 2nd order low pass Butterworth filter with a cut-off frequency of 6
4 Hz and averaged over the 3 stretches for each individual. Anatomical calibration of the shank and foot
5 reference frames were applied to obtain the ankle angle (Leardini *et al.*, 2007). The calculation of net
6 ankle joint moment is described in figure 1 (Bar-On *et al.*, 2013; Schless *et al.*, 2015). A modified semi-
7 automated tracking software (Cronin *et al.*, 2011; Gillett *et al.*, 2013) was used to track the fascicles and
8 aponeuroses. Fascicle length was calculated by extrapolating the fascicle as a straight line to the
9 intersection point with the aponeuroses. Pennation angle (α) was measured as the angle between the
10 fascicle and the deep aponeurosis. Next, to determine separate contribution of fascicles and the tendon-
11 aponeurosis to total MTU lengthening, fascicle length resolved along the axis of the MTU was calculated
12 ($l_{fas_resolved} = l_{fas} * \cos \alpha$). Changes in fascicle lengthening were analysed over the full ROM (to
13 maximal dorsiflexion angles) and over a ROM common to all subjects from -25° to -5° (with negative
14 angles reflecting plantarflexion). To define ankle stiffness, a second-order polynomial was fitted for each
15 individual through the 3 repetitions of the passive torque-angle curve, the slope of this polynomial was
16 defined at 5 equally distributed torque values between 0 and 12 Nm that could be achieved by all
17 participants. Raw EMG signals were filtered with a 6th order zero-phase Butterworth bandpass filter
18 from 20 to 500 Hz. The root mean square envelope of the sEMG (RMS-EMG) was extracted by applying a
19 low-pass 30 Hz 6th order zero-phase Butterworth filter on the squared signal. To assess any change in
20 RMS-EMG post stretching the RMS-EMG signal was quantified over three equal zones of the ROM. The
21 zones were defined as the time windows corresponding to 10-36.6% ROM, 36.6-63.3% ROM and 63.3-
22 90% ROM. Average RMS-EMG per position zone was defined as the area underneath the RMS-EMG
23 curve divided by the duration of the corresponding time zone. All RMS-EMG values are expressed
24 relative to the maximum voluntary contraction value (collected prior to the stretch trials).

1 *Statistics*

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

10 **Results:**

11 Eleven trials in 9 participants were excluded based on excessive RMS-EMG activity. This equates to 20%
12 of the total number of trials. There were at least 2 trials per participant available for analysis. No
13 differences were found pre- to post-stretching in the average RMS-EMG in any of the movement zones
14 analysed for the lateral gastrocnemius ($p=0.25$) or the soleus ($p=0.96$, figure 4). Resting fascicle length
15 (figure 3C) or resting ankle angle did not appear to change post-stretching (table 2). ROM increased
16 significantly by 9.9° (12°) ($p=0.01$). This was accompanied by a $3.9(3.7)$ mm increase in MTU lengthening
17 ($p=0.01$) and a $3.0(2.4)$ mm increase in fascicle lengthening ($p=0.01$) over the full ROM (table 2). There
18 was an increase of $2.4(2.1)$ Nm in the maximal torque that was applied to the ankle after stretching
19 (figure 3A). The change in pennation angle during muscle lengthening was not altered post-stretching
20 ($p=0.230$), thus fascicle length resolved along the axis of the MTU increased by $3.1(2.6)$ mm after
21 stretching ($p=0.007$). No changes were found in the amount of fascicle lengthening over a common ROM
22 ($p=0.301$) pre- to post-stretching. Ankle stiffness calculated at 5 common torque values between 0 and

1 12 Nm were not different pre- to post-stretching ($p=0.63$). Fascicle lengthening vs change in ankle angle
2 and torque are visualised in figure 2.

3 **Discussion**

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

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

18 This study was designed to assess any changes in muscle-tendon properties in response to the clinical
19 practice of a therapist manually stretching the ankle to its end ROM. As such, we did not control, or set
20 out to identify, what determines the maximum joint torque that can be applied or tolerated. However,
21 there are a few possible explanations for this change after stretching that may be considered. The
22 maximal ROM, when determined by the examiner is clinically defined as the “end-feel” of movement
23 due to tissue stretch (Magee, 2014). The position at which this end-feel occurs will depend, among
24 others, on pain tolerance, warm-up, or acquaintance between clinician and patient. These factors would

1 all change after a bout of stretching and could contribute to the greater joint torque applied after
2 stretch, as observed in this study. Additionally, we may hypothesise that the children experienced an
3 increased stretch tolerance. It has been shown repeatedly in healthy adults that an increased tolerance
4 to an uncomfortable stretch sensation is related to an increased ROM after stretching (Magnusson *et al.*,
5 1996; Folpp *et al.*, 2006; Konrad & Tilp, 2014). Future work should evaluate whether this has practical
6 implications in the therapy of children with CP.

7 The greater ROM achieved after the bout of stretching in this study resulted in a 3.9 mm increase in
8 MTU lengthening. Eighty percent of this increase in maximal MTU length was accounted for by resolved
9 fascicle lengthening, which was calculated as the lengthening of the fascicles along the axis of the MTU.
10 The remaining 20% thus should be due to stretching of the in series elastic component, which includes
11 the Achilles tendon distal to the muscle belly and/or the connective tissue within the muscle. These
12 results contradict earlier findings of Theis *et al.* (2013), who showed muscle and tendon to contribute
13 equally to the increase in MTU lengthening seen after an acute bout of stretching in children with CP.
14 However, the gain in MTU lengthening of 18.5mm reported in this study seems extremely large for a
15 change in ankle angle of only 9.8°. Such a displacement of the MTU would imply moment arm values of
16 11cm which are much larger than those previously reported in children (Waugh *et al.*, 2011; Kalkman *et*
17 *al.*, 2017) or adults (Maganaris *et al.*, 2000).

18 Long-term stretching interventions are based on the assumption that they affect muscle fascicle length
19 and stiffness by changing in series sarcomere number or alter the mechanical tissue properties. An
20 advantage of the addition of sarcomeres in series would be to change the active excursion range of the
21 muscle. Such plasticity of muscle fibres to stretch has been shown in several animal studies (Tabary *et*
22 *al.*, 1972; Williams & Goldspink, 1973) where prolonged positioning of muscles at increased length over
23 several weeks resulted in increased fibre length and in-series sarcomere number (Williams & Goldspink,
24 1973). However, it is not known whether this finding applies to spastic human muscle, in particular

1 when sarcomeres are already over lengthened (Mathewson *et al.*, 2014). Nonetheless, for any
2 remodelling of the muscle to take place, the fascicles must experience sufficient stretching stimulus. In
3 a previous study we have shown that when rotating the ankle joint, this stretching stimulus to the
4 muscle fascicles is smaller in children with CP than their typically developing peers (Kalkman *et al.*,
5 2016). Similarly, it has been showed that when stretching over the full ROM, muscle and tendon
6 lengthen less than in TD children (Hösl *et al.*, 2015). This may explain the lack of consistent and
7 substantial functional improvements seen after long term stretching interventions in these patients.
8 Here on the other hand, we show that after 20 minutes of stretching, the stretching stimulus to the
9 muscle fascicles can be acutely increased, thereby giving the potential for remodelling of the muscle to
10 occur. Future research should assess whether the increase in ROM seen after long term stretching
11 interventions in children with CP is due to an increase in stretch tolerance, as is shown here acutely, or if
12 indeed any remodelling of the muscle takes place.

13 A number of assumptions in the present study should be acknowledged. Muscle fascicles were treated
14 as straight lines, thus neglecting possible effect of curvature. However, the influence of curvature has
15 been reported to be small for passive fascicle length changes in the medial gastrocnemius (Muramatsu
16 *et al.*, 2002). Ankle angle was measured in the sagittal plane as the angle between the shank and the
17 footplate that supported the foot. To minimise errors, a custom-made insole assured that the foot was
18 rigid to the footplate during the whole ROM. Not including a control group to check whether any
19 changes are actually due to the intervention, is a limitation in this study. However, we do not expect
20 muscle properties to change over the short time period that was assessed in this study. Therefore, we
21 do not believe the study design has confounded our conclusions. Furthermore, In a separate analysis,
22 four typically developing children were assessed for repeatability by performing the same protocol
23 before and after an hour break (Cenni *et al.*, 2016), no systematic changes were found in these children
24 and the study design was found to be reliable for applications that do not require sum-mm accuracy. It

1 was not possible to collect EMG recordings of the medial gastrocnemius muscle because we could not fit
2 an ultrasound probe and EMG electrodes on the small surface of a child's muscle. As an alternative, we
3 measured EMG of the lateral gastrocnemius and the soleus to assure joint rotations were passive. Also,
4 we need to acknowledge that even though EMG remained below 5% of the MVC values, we cannot
5 ascertain that muscles were fully passive. Also, we measured only the properties of one muscle of the
6 triceps surae group, however because we performed the stretching intervention with relatively more
7 knee extension the influence of the soleus muscle to the increased ROM is considered small. Finally, this
8 study was performed with a relatively small number of participants. Also, we had no information about
9 stretching interventions children were exposed to in their regular care. Validation of our results is
10 needed in a larger cohort of children with CP.

11 **Conclusions**

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

16 **Additional information**

17 *Competing interests*

18 No conflicts of interest, financial or otherwise, are declared by the authors.

19 *Author contributions*

20 BK, LB, TOB, CM, KD GH and GB contributed to conception and design of the research; BK, LB and FC to
21 data acquisition; BK and LB to data analysis; BK, LB, CM, TOB, AB and GH to the interpretation of the
22 results; BK drafted the manuscript; BK, LB, FC, KD, AB, GH, GB, CM and TOB edited and revised the
23 manuscript. All authors have read and approved the final version of this manuscript and agree to be

1 accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of
2 any part of the work are appropriately investigated and resolved. All persons designated as authors
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3 **Figure Legends**

4 **Figure 1: A)** Experimental set up showing leg placement in a custom-made orthosis. A hand-held force
5 sensor load-cell was used to measure net joint torque at the foot plate during passive stretch. Two
6 clusters of reflective markers on the shank and foot were tracked with motion analysis and used to
7 calculate the foot-plate angle in 3D. The ultrasound probe was placed on the muscle belly. **B)** Free body
8 diagram of the foot and foot plate. d_y and d_z correspond to the moment arm distances from the point of
9 force application of the load-cell to the lateral malleolus. F_z , F_y and M_x are the forces and moment
10 exerted on the load cell along the z, y and about the x direction respectively. M_{orthosis} is the predicted
11 moment caused by gravity on the orthosis. The joint moment is given by: $M_{\text{ankle}} = -F_z d_z - F_y d_y - M_x -$
12 M_{orthosis} (Bar-On *et al.*, 2013; Schless *et al.*, 2015)

13 **Figure 2:** Data shown are mean ($\pm 95\%$ CI calculated on normalized data (Cousineau, 2005)) values pre-
14 (blue) and post- (red) stretching of **(A)** fascicle length versus ankle angle, **(B)** joint torque versus ankle
15 angle, **(C)** fascicle length versus joint torque.

16 **Figure 3:** Individual data for **(A)** maximal torque applied around the ankle during a stretch, **(B)** fascicle
17 length at end-range dorsiflexion angle and **(C)** resting fascicle length. Data are shown pre and post
18 intervention. Individual data points (*) and group mean values (-).

19 **Figure 4: (A)** Average RMS-EMG response of the lateral gastrocnemius and soleus combined pre (blue)
20 and post (red) intervention. **(B)** Individual RMS-EMG signals versus ankle angle of the lateral
21 gastrocnemius and soleus pre (blue) and post (red) intervention.

22

Table 1. Participant characteristics

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

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

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

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Table 2. Mean (SD) lengthening values during passive ankle rotation pre- and post-stretching.

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

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







