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

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Running Head: Acute effect of stretching in cerebral palsy

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New Findings:

What is the central question of this study?

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

What is the main finding and its importance?

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

Abstract:

Stretching is often used to increase/maintain joint range of motion (ROM) in children with cerebral palsy (CP) but the effectiveness of these interventions is limited. Therefore, this study aimed to determine the acute changes in muscle-tendon lengthening properties that contribute to increased ROM after a bout of stretching in children with CP. Eleven children with spastic CP (age:12.1(3)y, 5/6 hemiplegia/diplegia, 7/4 GMFCS level I/II) participated in this study. Each child received 3 sets of 5x20s passive, manual static dorsiflexion stretches separated by 30s rest, and 60s rest between sets. Pre- and immediately post-stretching, ultrasound was used to measure medial gastrocnemius fascicle lengthening continuously over the full ROM and an individual common ROM pre- to post-stretching. Simultaneously, 3D motion of two marker clusters on the shank and the foot was captured to calculate ankle angle, and ankle joint torque was calculated from manually applied torques and forces on a 6DoF load cell. After stretching, ROM was increased (9.9° (12.0), p=0.005). Over a ROM common to both pre and post measurements, there were no changes in fascicle lengthening or torque. The maximal ankle joint torque tolerated by the participants increased (2.9(2.4) Nm, p=0.003) and at this highest passive torque maximal fascicle length was 2.8(2.4) mm greater (p=0.009) when compared to before stretching. These results indicate that the stiffness of the muscle fascicles in children with CP remain unaltered by an acute bout of stretching.

Introduction:

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

Altered muscle-tendon properties in CP may leads to a reduced ROM, but these alterations may also mediate the response to stretching interventions as seen in typically developing individuals. For example, previous studies show that in CP, muscles are shorter (Fry *et al.*, 2004; Malaiya *et al.*, 2007; Oberhofer *et al.*, 2010), tendon slack length is longer (Gao *et al.*, 2011; Barber *et al.*, 2012) and relative muscle to tendon stiffness is increased (Kalkman *et al.*, 2016). It is unknown how these altered properties mediate the acute response of a muscle to stretching in individuals with CP. In typically developing adults, acutely after 5 minutes of conditioning stretches, ROM was increased and joint stiffness decreased (Morse *et al.*, 2008). Based on an increase in muscle belly length in the absence of an increase in fascicle length and pennation angle changes, the authors attributed these changes to a reduction in the stiffness of the intramuscular connective tissue. The question then arises, do spastic muscles respond to stretch in a similar way, since changes have been shown in both the quality and amount of intramuscular connective tissue in children with CP (Smith *et al.*, 2011). It has been shown that ankle ROM in children with CP improved immediately after stretching and this was accounted for by an increase in length at maximal joint angles of all three structures that make up the muscle-tendon-unit (MTU) of the medial gastrocnemius, i.e. muscle belly, fascicles and tendon (Theis *et al.*, 2013). However, the gain of MTU lengthening after stretching reported in this study seems extremely large (18.5mm) for an increase in ankle ROM of only 9.8°. Also, no information was reported about any changes in joint torque and thus in the passive properties of the involved structures.

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

Methods:

Ethical approval

The study was approved by the NHS research ethics committee in the UK (Project No: 15/LO/0856) and the University Hospital's ethics committee in Leuven, Belgium (Project No: S S57384). The study was conducted in accordance with the Declaration of Helsinki. This study was not registered in a data base. Written parental consent was obtained from the parents, and written assent was given by children in accordance with local regulations.

Participants:

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

Experimental design:

Participants attended the hospital on one occasion. During this visit, participants underwent an acute bout of passive ankle dorsiflexion stretches applied by a physiotherapist. Stretches were applied to the leg that was most affected as defined by spasticity scores. Before and within 10 minutes after the stretching session, measurements of ankle angle, passive joint torque and medial gastrocnemius muscle fascicle lengthening during a passive stretch were taken. During these measurements, participants lay prone on a bed with the lower leg supported on an inclined cushion such that the knee was ~20° flexed. The leg was positioned in a custom-made orthosis, to control ankle movement in the sagittal plane (figure 1). The axis of rotation of the orthosis was aligned with the lateral malleolus. Each participant underwent 3 trials of passive ankle dorsiflexion movements taking 5 seconds to complete one movement, which resulted in an average velocity of 10deg/s. At least 10 seconds rest was taken between individual repetitions. The maximal ROM was defined as the point where either the participant indicated the threshold or the examiner felt the joint reach the end of the passive movement. Forces and torques at the ankle were measured at 200 Hz using a six degrees-of-freedom force sensor load-cell (ATI mini45: Industrial Automation) attached to the orthosis under the ball of the foot. 3D kinematics were collected with 3 cameras at 120 Hz from 2 clusters of 3 markers placed on the foot-plate of the orthosis and on the shank (Optitrack, US). Surface electromyography (sEMG), placement defined with ultrasound, was used to collect signals at 1600 Hz from the lateral gastrocnemius and soleus muscles (Zerowire, Cometa, Milan, IT). When, during joint rotation, the sEMG signal exceeded 10% of the maximum voluntary contraction value (collected with a hand-held dynamometer prior to the stretch trials), the corresponding trial was discarded. To measure muscle fascicle lengthening, a B-mode ultrasound probe (Telemed Echoblaster, Lithuania, 60 Hz) was securely fixed over the mid belly of the medial gastrocnemius muscle. Guidance regarding probe alignment was adhered to for minimising measurement errors (Bénard *et al.*, 2009; Bolsterlee *et al.*, 2016). Resting fascicle length was measured with the knee flexed at ~20° and the foot hanging off the edge of the bed.

Stretching intervention:

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

Data analysis:

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

Statistics

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

Results:

Eleven trials in 9 participants were excluded based on excessive RMS-EMG activity. This equates to 20% of the total number of trials. There were at least 2 trials per participant available for analysis. No differences were found pre- to post-stretching in the average RMS-EMG in any of the movement zones analysed for the lateral gastrocnemius (p=0.25) or the soleus (p=0.96, figure 4). Resting fascicle length (figure 3C) or resting ankle angle did not appear to change post-stretching (table 2). ROM increased significantly by 9.9° (12°) (p=0.01). This was accompanied by a 3.9(3.7) mm increase in MTU lengthening (p=0.01) and a 3.0(2.4) mm increase in fascicle lengthening (p=0.01) over the full ROM (table 2). There was an increase of 2.4(2.1) Nm in the maximal torque that was applied to the ankle after stretching (figure 3A). The change in pennation angle during muscle lengthening was not altered post-stretching (p=0.230), thus fascicle length resolved along the axis of the MTU increased by 3.1(2.6) mm after stretching (p=0.007). No changes were found in the amount of fascicle lengthening over a common ROM (p=0.301) pre- to post-stretching. Ankle stiffness calculated at 5 common torque values between 0 and 12 Nm were not different pre- to post-stretching (p=0.63). Fascicle lengthening vs change in ankle angle and torque are visualised in figure 2.

Discussion

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

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

This study was designed to assess any changes in muscle-tendon properties in response to the clinical practice of a therapist manually stretching the ankle to its end ROM. As such, we did not control, or set out to identify, what determines the maximum joint torque that can be applied or tolerated. However, there are a few possible explanations for this change after stretching that may be considered. The maximal ROM, when determined by the examiner is clinically defined as the "end-feel" of movement due to tissue stretch (Magee, 2014). The position at which this end-feel occurs will depend, among others, on pain tolerance, warm-up, or acquaintance between clinician and patient. These factors would all change after a bout of stretching and could contribute to the greater joint torque applied after stretch, as observed in this study. Additionally, we may hypothesise that the children experienced an increased stretch tolerance. It has been shown repeatedly in healthy adults that an increased tolerance to an uncomfortable stretch sensation is related to an increased ROM after stretching (Magnusson *et al.*, 1996; Folpp *et al.*, 2006; Konrad & Tilp, 2014). Future work should evaluate whether this has practical implications in the therapy of children with CP.

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

Long-term stretching interventions are based on the assumption that they affect muscle fascicle length and stiffness by changing in series sarcomere number or alter the mechanical tissue properties. An advantage of the addition of sarcomeres in series would be to change the active excursion range of the muscle. Such plasticity of muscle fibres to stretch has been shown in several animal studies (Tabary *et al.*, 1972; Williams & Goldspink, 1973) where prolonged positioning of muscles at increased length over several weeks resulted in increased fibre length and in-series sarcomere number (Williams & Goldspink, 1973). However, it is not known whether this finding applies to spastic human muscle, in particular when sarcomeres are already over lengthened (Mathewson *et al.*, 2014). Nonetheless, for any remodelling of the muscle to take place, the fascicles must experience sufficient stretching stimulus. In a previous study we have shown that when rotating the ankle joint, this stretching stimulus to the muscle fascicles is smaller in children with CP than their typically developing peers (Kalkman *et al.*, 2016). Similarly, it has been showed that when stretching over the full ROM, muscle and tendon lengthen less than in TD children (Hösl *et al.*, 2015). This may explain the lack of consistent and substantial functional improvements seen after long term stretching interventions in these patients. Here on the other hand, we show that after 20 minutes of stretching, the stretching stimulus to the muscle fascicles can be acutely increased, thereby giving the potential for remodelling of the muscle to occur. Future research should assess whether the increase in ROM seen after long term stretching interventions in children with CP is due to an increase in stretch tolerance, as is shown here acutely, or if indeed any remodelling of the muscle takes place.

A number of assumptions in the present study should be acknowledged. Muscle fascicles were treated as straight lines, thus neglecting possible effect of curvature. However, the influence of curvature has been reported to be small for passive fascicle length changes in the medial gastrocnemius (Muramatsu *et al.*, 2002). Ankle angle was measured in the sagittal plane as the angle between the shank and the footplate that supported the foot. To minimise errors, a custom-made insole assured that the foot was rigid to the footplate during the whole ROM. Not including a control group to check whether any changes are actually due to the intervention, is a limitation in this study. However, we do not expect muscle properties to change over the short time period that was assessed in this study. Therefore, we do not believe the study design has confounded our conclusions. Furthermore, In a separate analysis, four typically developing children were assessed for repeatability by performing the same protocol before and after an hour break (Cenni *et al.*, 2016), no systematic changes were found in these children and the study design was found to be reliable for applications that do not require sum-mm accuracy. It was not possible to collect EMG recordings of the medial gastrocnemius muscle because we could not fit an ultrasound probe and EMG electrodes on the small surface of a child's muscle. As an alternative, we measured EMG of the lateral gastrocnemius and the soleus to assure joint rotations were passive. Also, we need to acknowledge that even though EMG remained below 5% of the MVC values, we cannot ascertain that muscles were fully passive. Also, we measured only the properties of one muscle of the triceps surae group, however because we performed the stretching intervention with relatively more knee extension the influence of the soleus muscle to the increased ROM is considered small. Finally, this study was performed with a relatively small number of participants. Also, we had no information about stretching interventions children were exposed to in their regular care. Validation of our results is needed in a larger cohort of children with CP.

Conclusions

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

Additional information

Competing interests

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

Author contributions

BK, LB, TOB, CM, KD GH and GB contributed to conception and design of the research; BK, LB and FC to data acquisition; BK and LB to data analysis; BK, LB, CM, TOB, AB and GH to the interpretation of the results; BK drafted the manuscript; BK, LB, FC, KD, AB, GH, GB, CM and TOB edited and revised the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Figure Legends

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

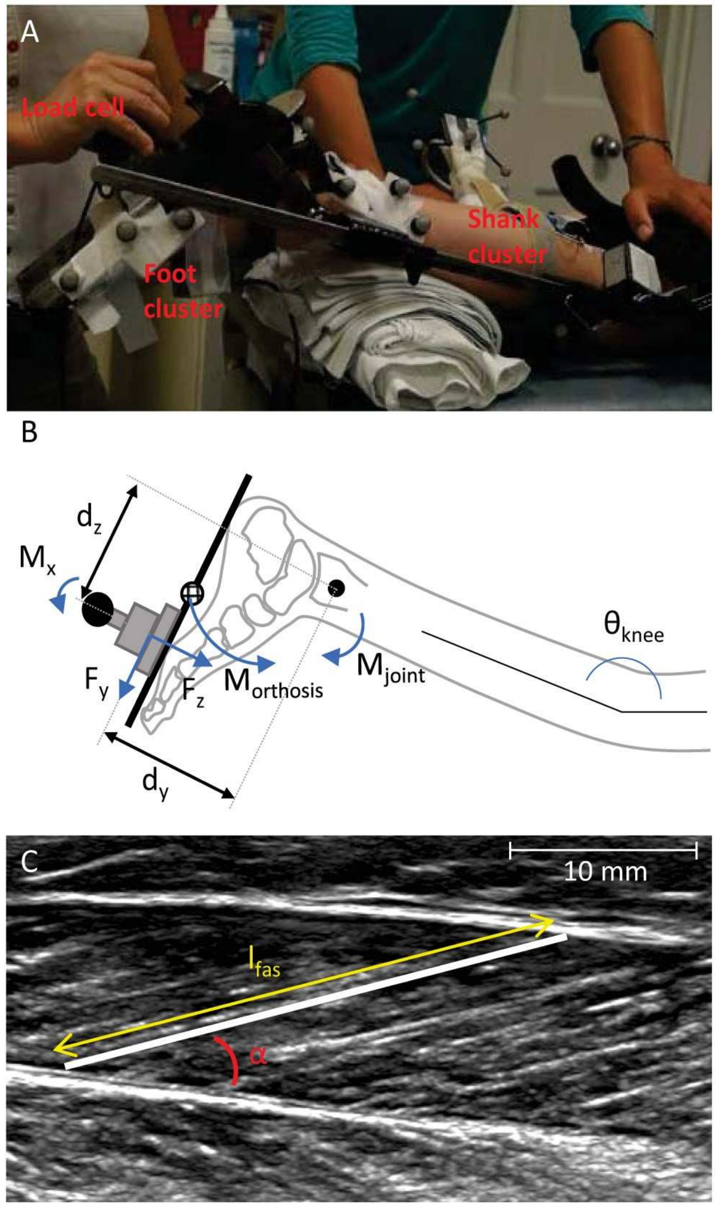

Figure 2: Data shown are mean (±95% CI calculated on normalized data (Cousineau, 2005)) values pre- (blue) and post- (red) stretching of **(A)** fascicle length versus ankle angle, **(B)** joint torque versus ankle angle, **(C)** fascicle length versus joint torque.

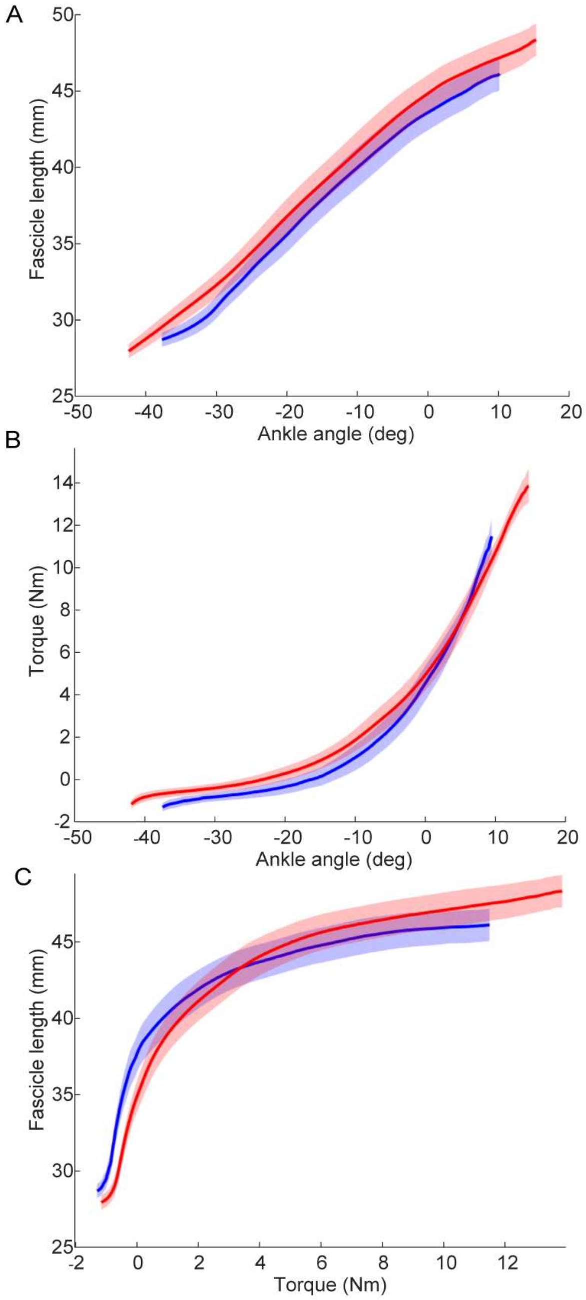

Figure 3: Individual data for **(A)** maximal torque applied around the ankle during a stretch, **(B)** fascicle length at end-range dorsiflexion angle and **(C)** resting fascicle length. Data are shown pre and post intervention. Individual data points (*) and group mean values (-).

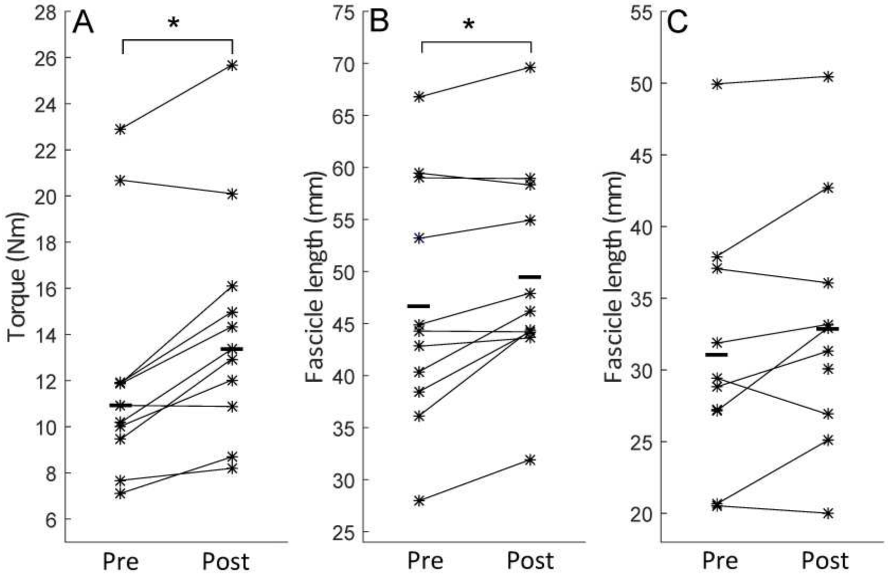

Figure 4: **(A)** Average RMS-EMG response of the lateral gastrocnemius and soleus combined pre (blue) and post (red) intervention. **(B)** Individual RMS-EMG signals versus ankle angle of the lateral gastrocnemius and soleus pre (blue) and post (red) intervention.

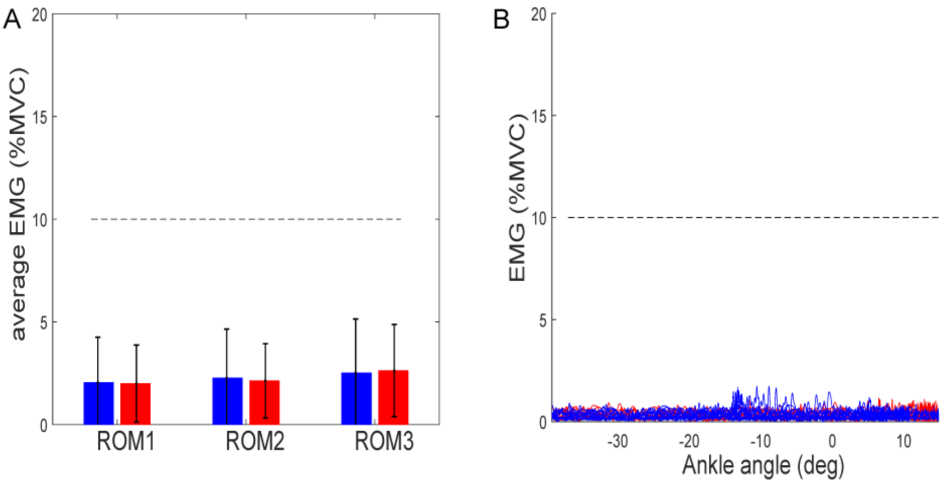

Table 1. Participant characteristics

Participant characteristics	**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

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