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**Article:**

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<https://doi.org/10.1113/EP087053>

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This is the peer reviewed version of the following article: Kalkman BM, Bar-On L, Cenni F, et al. Muscle and tendon lengthening behaviour of the medial gastrocnemius during ankle joint rotation in children with cerebral palsy. *Exp Physiol*. 2018;103:1367–1376, which has been published in final form at <https://doi.org/10.1113/EP087053>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

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Experimental Physiology

<https://ep.msubmit.net>

**EP-RP-2018-087053R2**

**Title:** Muscle and tendon lengthening behaviour of the medial gastrocnemius during ankle joint rotation in children with cerebral palsy

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**Author Conflict:** No competing interests declared

**Running Title:** Muscle properties in children with cerebral palsy

**Abstract:** Children with cerebral palsy (CP) commonly present with reduced ankle range of motion (ROM) partly due to changes in mechanical properties of the muscle-tendon-unit (MTU). Detailed information about how muscle and tendon interact to contribute to joint rotation is currently lacking, but may provide essential information to explain the limited effectiveness of stretching interventions in children with CP. The purpose of this study was to quantify which structures contribute to MTU lengthening and thus receive the stretch during passive ankle joint rotation. Fifteen children with CP

(age:11.4{plus minus}3y) and 16 typically developing (TD) children (age:10.2{plus minus}3y) participated. Ultrasound was combined with motion tracking, joint torque and electromyography to record fascicle, muscle and tendon lengthening of the medial gastrocnemius during passive ankle joint rotations over the full and a common ROM. In children with CP, relative to MTU lengthening, muscle and fascicles lengthened less (CP: 50.4%, TD: 63% of MTU lengthening;  $p<0.04$ ) and tendon lengthened more (CP: 49.6%, TD: 37% of MTU lengthening,  $p<0.01$ ) regardless of the ROM studied. Differences between groups in the amount of lengthening of the underlying structures during a similar amount of joint rotation and MTU displacement indicate possible differences in tissue mechanical properties due to CP, which are not evident by assessment on a joint level. These factors should be considered when assessing and treating muscle function in children with CP, for example during stretching exercises as the muscle may not receive much of the applied lengthening stimulus.

**New Findings:** What is the central question of this study? Which structures of the medial gastrocnemius muscle tendon unit contribute to its lengthening during joint rotation and thus receive the stretching stimulus? What is the main finding and its importance? We show for the first time, that muscle and tendon lengthen differently between children with CP and TD children during a similar amount of muscle tendon unit lengthening or joint rotation. This indicates possible differences in mechanical muscle and tendon properties due to CP, which is not evident by assessment of muscle function on a joint level.

**Dual Publication:** No

**Funding:** Liverpool John Moores University (LJMU): Barbara Maria Kalkman, Scholarship; Fonds Wetenschappelijk Onderzoek (Flemish Research Foundation): Lynn Bar-On, 12R4215N

1 Muscle and tendon lengthening behaviour of the medial gastrocnemius  
2 during ankle joint rotation in children with cerebral palsy

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1 **Abstract**

2 Children with cerebral palsy (CP) commonly present with reduced ankle range of motion (ROM) partly  
3 due to changes in mechanical properties of the muscle-tendon-unit (MTU). Detailed information about  
4 how muscle and tendon interact to contribute to joint rotation is currently lacking, but may provide  
5 essential information to explain the limited effectiveness of stretching interventions in children with CP.  
6 The purpose of this study was to quantify which structures contribute to MTU lengthening and thus  
7 receive the stretch during passive ankle joint rotation. Fifteen children with CP (age:11.4±3y) and 16  
8 typically developing (TD) children (age:10.2±3y) participated. Ultrasound was combined with motion  
9 tracking, joint torque and electromyography to record fascicle, muscle and tendon lengthening of the  
10 medial gastrocnemius during passive ankle joint rotations over the full and a common ROM. In children  
11 with CP, relative to MTU lengthening, muscle and fascicles lengthened less (CP: 50.4%, TD: 63% of MTU  
12 lengthening;  $p<0.04$ ) and tendon lengthened more (CP: 49.6%, TD: 37% of MTU lengthening,  $p<0.01$ )  
13 regardless of the ROM studied. Differences between groups in the amount of lengthening of the  
14 underlying structures during a similar amount of joint rotation and MTU displacement indicate possible  
15 differences in tissue mechanical properties due to CP, which are not evident by assessment on a joint  
16 level. These factors should be considered when assessing and treating muscle function in children with  
17 CP, for example during stretching exercises as the muscle may not receive much of the applied  
18 lengthening stimulus.

19 **New Findings:**

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

21 Which structures of the medial gastrocnemius muscle tendon unit contribute to its lengthening during  
22 joint rotation and thus receive the stretching stimulus?

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

- 1 We show for the first time, that muscle and tendon lengthen differently between children with CP and
- 2 TD children during a similar amount of muscle tendon unit lengthening or joint rotation. This indicates
- 3 possible differences in mechanical muscle and tendon properties due to CP, which is not evident by
- 4 assessment of muscle function on a joint level.
- 5

## 1 **Introduction**

2 Cerebral palsy (CP) is a non-progressive disorder caused by a brain lesion occurring in the early stages of  
3 development (Graham *et al.*, 2016). Children with spastic CP usually show increased ankle joint stiffness  
4 and reduced range of motion (ROM) compared to typically developing (TD) children (Alhusaini *et al.*,  
5 2010). It has been reported that muscles of children with CP undergo significant changes in their  
6 mechanical properties, which contribute to the reduced ROM (Mathewson & Lieber, 2015). In the  
7 management of CP, treatment is often aimed at maintaining or increasing ankle ROM. Stretching  
8 therapies are commonly used, assuming they can increase muscle length and/or reduce its stiffness  
9 (Zhao *et al.*, 2011; Theis *et al.*, 2015). However, we have recently demonstrated that improvements in  
10 ROM acutely after stretching are not caused by changes in muscle properties, but might be due to an  
11 increase in stretch tolerance (Kalkman *et al.*, 2018). This is corroborated by others who show that the  
12 effectiveness of long term stretching interventions to improve fascicle length and/or passive muscle  
13 stiffness is uncertain (Wiar *et al.*, 2008; Theis *et al.*, 2015; Craig *et al.*, 2016). To understand the reason  
14 for the limited effectiveness of these stretching therapies it is essential to know if the muscle fascicles  
15 are actually lengthening and receiving the stretch stimulus when rotating the joint, or whether other  
16 structures of the MTU take up the stretch.

17 Previous studies of medial gastrocnemius (MG) muscle architecture in individuals with CP using  
18 ultrasound consistently report shorter muscle bellies compared to TD subjects (Fry *et al.*, 2004; Barrett  
19 & Lichtwark, 2010). In addition, longer Achilles tendon has been reported in children with CP (Wren *et al.*,  
20 2010; Barber *et al.*, 2012), which could be a compensation for the shorter muscle belly length when  
21 MTU length is similar. Furthermore, some studies have reported smaller resting muscle fascicle lengths  
22 in children with CP than TD children (Mohagheghi *et al.*, 2008; Matthiasdottir *et al.*, 2014), but others  
23 have not detected differences (Shortland *et al.*, 2002; Mathewson *et al.*, 2014). Inconsistencies can be

1 attributed to different normalisation procedures, small sample sizes and the heterogeneity of symptoms  
2 associated with CP.

3 From investigations of muscle lengthening (defined as the displacement of the MG muscle-tendon-  
4 junction relative to the origin of the muscle), we know that when passively rotating the joint in children  
5 with CP, the MG muscle belly lengthens less compared to TD children (Matthiasdottir *et al.*, 2014).  
6 However, it is the passive stiffness of a muscle relative to its tendon that has implications for treating  
7 impaired joint function, because this determines how these two structures interact when lengthened by  
8 joint rotation. In TD adults it has been shown that when stretched, muscle fascicles undergo much  
9 smaller changes in length than the whole muscle-tendon unit (MTU) and both the tendon and  
10 intramuscular connective tissue contribute significantly to increased MTU length during joint rotation  
11 (Herbert & Moseley, 2002; Morse *et al.*, 2008). It is not known how muscle belly and tendon lengthen  
12 relative to each other during passive joint rotation in children with CP. Furthermore, due to the pennate  
13 nature of the medial gastrocnemius muscle, the lengthening of its muscle belly will depend on both the  
14 properties of the muscle fascicles and the connective tissue that ties them together. A relation that has  
15 not been studied before.

16 In children with CP, the amount of MG fascicle lengthening during passive joint rotation shows  
17 inconsistent results, with some studies indicating that there is no difference (Matthiasdottir *et al.*, 2014),  
18 while others found less fascicle lengthening (Barber *et al.*, 2011) in CP vs TD. This discrepancy could  
19 possibly be explained by differences in the ROM over which the results are compared between groups. A  
20 decreased ROM in children with CP could confound findings when comparisons are made over the full  
21 ROM. In fact, any comparison between CP and controls over absolute joint angles is inherently limited,  
22 because differences in the muscle's moment arm (Kalkman *et al.*, 2017) and passive joint torque  
23 (Alhusaini *et al.*, 2010) will influence the joint angle-tissue lengthening relationship.



1 Therefore, in contrast to previous investigations, we aim to explore muscle fascicle, muscle belly and  
2 tendon lengthening simultaneously during passive joint rotation in order to understand which tissue  
3 takes up the stretch. Furthermore, we innovatively compare the lengthening of these tissues over  
4 different ranges, accounting for the interaction between joint and underlying structures, and allowing  
5 for more robust conclusions.

6 Ultrasound has proved a valuable tool to improve understanding of *in vivo* behaviour of muscle and  
7 tendon during contraction and joint rotation. However, a calculation of the tissues' mechanical  
8 properties during passive joint rotation is more difficult as several assumptions are inferred. The passive  
9 torque measured at the ankle is a combination of different muscles and passive structures, and the  
10 contribution of each force-bearing structure to the net joint torque cannot be quantified *in vivo* nor can  
11 it be assumed to remain constant throughout the ROM. Nevertheless, the resulting passive elongations  
12 of muscle and tendon in response to stretch allow drawing conclusions about the relative contribution  
13 of the muscular and tendinous structures to ROM.

14 In order to explain the lack of change in muscle properties after stretching in children with CP (Kalkman  
15 *et al.*, 2018), the purpose of this study was to quantify which structures contribute to MTU lengthening  
16 and thus receive the stretch stimulus during passive ankle joint rotation. We hypothesized that the  
17 muscle belly and the fascicles would lengthen less in CP compared to TD children and that the tendon  
18 would lengthen more.

## 19 **Method**

### 20 *Ethical approval*

21 The study was approved by the National Health Service research ethics committee in the UK (project no  
22 15/LO/0856) and the University Hospital's ethics committee in Leuven, Belgium (project no. S S57384).  
23 The study was conducted in accordance with the *Declaration of Helsinki*. This study was not registered in

1 a database. Written parental consent was obtained and written assent was given by children in  
2 accordance with local regulations.

### 3 *Participants*

4 Fifteen Children with CP and sixteen TD children aged 6-16 years were recruited for participation  
5 through the gait lab of Alder Hey Children's NHS Foundation Trust in Liverpool, UK and the University  
6 Hospital in Pellenberg, Belgium. Patient characteristics can be found in table 1. Five of the TD children  
7 were assessed with the same protocol for a second time after a two-hour break to determine reliability  
8 of the full measurement protocol. Children with CP were excluded if they had Botulinum Toxin-A  
9 injection to the lower limb muscles 6 months prior to testing, a baclofen pump, any lower limb neuro- or  
10 orthopaedic surgery or less than 20 degrees of ankle movement in the sagittal plane (to ensure  
11 sufficient stretch in the medial gastrocnemius muscle). Patients had previously received on average 2.3  
12 Botulinum Toxin-A injections All TD children were free from neuromuscular or skeletal disorders.

### 13 *Experimental protocol*

14 Participants lay prone on a bed with the lower leg supported on an inclined cushion such that the knee  
15 was  $\sim 20^\circ$  flexed, the leg was positioned in a custom-made orthosis, to control ankle movement in the  
16 sagittal plane (Figure 1A; Part of the experimental protocol has been published previously (Kalkman *et*  
17 *al.*, 2018). The axis of rotation of the orthosis was aligned with the lateral malleolus. The foot was  
18 secured to a rigid footplate with the help of an adjustable insole that ensured heel contact with the  
19 footplate during ankle rotation. The leg tested was the most affected, defined by clinical spasticity  
20 scores (Tardieu *et al.*, 1954; Bohannon & Smith, 1987), and the left in TD. Each participant underwent  
21 two trials involving three passive movements by manually rotating the foot from maximal plantarflexion  
22 to maximal dorsiflexion aiming for a maximum angular velocity of  $15 \pm 5^\circ/\text{sec}$ , which is slow enough to  
23 not elicit a stretch reflex (Bar-On *et al.*, 2013) and at least 10 seconds rest in between individual

1 repetitions (Bar-On *et al.*, 2013). The reliability of data measured using the same equipment has been  
2 previously reported (Schless *et al.*, 2015). Forces and torques around the ankle were measured at 200Hz  
3 using a six degrees-of-freedom force sensor load-cell (ATI mini45: Industrial Automation) attached to the  
4 orthosis under the ball of the foot. The point of attachment of the load-cell to the orthosis could be  
5 adjusted according to foot length. 3D kinematics were collected with 3 cameras at 120Hz from 2 clusters  
6 of 3 markers placed on the foot-plate of the orthosis and on the shank and a single marker placed on the  
7 most superficial part of the posterior calcaneal tuberosity (Optitrack, US). Surface electromyography  
8 (sEMG), placed on the middle of the muscle belly as defined with ultrasound, collected signals at 1600Hz  
9 from the lateral gastrocnemius and soleus muscles during all trials and from the MG during the trials  
10 measuring muscle belly lengthening (Zerowire, Cometa, Milan, IT). Raw EMG signals were filtered with a  
11 sixth-order zero-phase Butterworth bandpass filter from 20 to 500 Hz. The root mean square envelope  
12 of the sEMG (RMS-EMG) was extracted by applying a low-pass 30 Hz sixth-order zero phase Butterworth  
13 filter on the squared signal. When, during joint rotation the RMS-EMG signal exceeded 10% of the  
14 maximum voluntary contraction value (collected prior to the stretch trials), the corresponding trial was  
15 discarded (Haberfehlner *et al.*, 2015).

## 16 *Ultrasound*

17 A B-mode ultrasound scanner (Telemed Echoblaster, Lithuania) with a 59mm linear transducer rigidly  
18 fitted with a cluster of 4 markers was used to identify the location of the medial femoral condyle in a  
19 local reference frame defined by the shank cluster.

20 To define myotendinous junction (MTJ) displacement, the probe with cluster was securely fixed over the  
21 MG MTJ using a custom-made holder. The long axis of the probe was aligned with the line of action of  
22 the muscle to minimize out of plane movement. The MTJ was tracked at 30Hz in the local reference  
23 frame on the shank during the first three passive movements.

1 Then, because MTJ and fascicles of the MG could not be visualized simultaneously, the US probe was  
2 fixed over the MG muscle belly to measure fascicle lengthening at 60Hz during the second three passive  
3 movements. Guidance regarding probe alignment was adhered to for minimising measurement errors  
4 (Bénard *et al.*, 2009).

#### 5 *Data analysis*

6 Data analysis was carried out using custom-made software (Matlab R2015a, Python 2.7.11). Anatomical  
7 calibration of the shank and foot reference frames was applied to obtain ankle angle (Leardini *et al.*,  
8 2007). During movement, displacement of the MTJ was manually tracked (Figure 1B) and muscle and  
9 tendon lengths were defined as the linear distances between the medial femoral condyle and the MTJ;  
10 and between the MTJ and the marker on the calcaneus, respectively. The MTU length was defined as  
11 the summation of muscle and tendon length. A modified semi-automated tracking software (Cronin *et*  
12 *al.*, 2011; Gillett *et al.*, 2013) was used to track fascicle length ( $l_{fas}$ ). Both aponeuroses and a fascicle were  
13 manually defined in the first frame of the video. Thereafter, the software automatically tracked and  
14 calculated fascicle length by extrapolating the defined fascicle to the intersection point with the defined  
15 aponeuroses during the movement. Pennation angle ( $\alpha$ ) was measured as the angle between the  
16 fascicle and the deep aponeurosis. Next, fascicle length resolved along the axis of the MTU was  
17 calculated trigonometrically:  $l_{fas\_resolved} = l_{fas} / \cos \alpha$ . The net ankle joint torque was calculated from  
18 the exerted torques and forces on the load-cell, measured external moment arms, and the predicted  
19 torque caused by gravity on the foot and orthotic (Bar-On *et al.*, 2013). All kinematic and kinetic  
20 variables were filtered using a 2<sup>nd</sup> order Butterworth filter with a cut-off frequency of 6Hz. Starting  
21 length (length at maximal plantar flexion angle) was subtracted from absolute muscle, tendon and  
22 fascicle length to compare lengthening of these structures over the full ROM and over a common ROM  
23 that could be achieved by all participants (-5° to -25°, with negative angles reflecting plantarflexion).  
24 Furthermore, all lengthening parameters were assessed over a common joint torque from 0Nm (defined

1 as slack length) to 3Nm and over a common amount of MTU lengthening (20mm). Muscle belly, fascicle  
2 and tendon lengthening was additionally expressed as a percentage of MTU lengthening. The  
3 parameters described above were calculated for the individual data curves. For visualization purposes,  
4 average curves were obtained by normalizing the trajectories of all variables to the stretch cycle and  
5 subsequently averaged over trials. These average curves are shown in Figure 2.

## 6 *Statistics*

7 All parameters were checked to be normally distributed using the Shapiro-Wilk test and by inspection of  
8 the q-q plots. All data were found to be normally distributed. The between session reliability in the TD  
9 children of lengthening parameters was analysed using intra-correlation coefficients (ICC, 3,k) and the  
10 standard error of measurement (SEM), calculated from one-way ANOVA. A 2-sample independent t-test  
11 was used to compare lengthening parameters between CP and TD groups. Relations between muscle  
12 and tendon lengthening, ROM and age were made using Pearsons  $r^2$ -values. All statistical analyses were  
13 performed in Matlab (Mathworks, R2015). The  $\alpha$ -level was set at 0.05. Effect sizes were expressed as  
14 Hedge's  $g$ , values of  $\approx 0.1$ ,  $\approx 0.2$  and  $\geq 0.3$  may be roughly considered small, medium and large effects  
15 (Hentschke & Stüttgen, 2011).

## 16 **Results**

17 Intra-correlation coefficients of the inter-session reliability ranged from 0.70-0.90. The full results of the  
18 reliability analysis are shown in table 2. At the starting position of the passive movement (individual  
19 maximal plantarflexion), joint angle was not different between TD and CP groups (mean (SD); CP:  $-38.3^\circ$   
20 (7.2), TD:  $-36.6^\circ$  (9.4),  $p=0.59$ , CI [-7.85 4.53]). At this angle, torque (CP: -1.5 (0.9) Nm, TD: -1.8 (0.5) Nm,  
21  $p=0.25$ , CI [-0.22 0.81]), absolute muscle (CP: 164.1 (28.8) mm, TD: 174.7 (30.9) mm,  $p=0.4$ , CI [-32.47  
22 13.4]), tendon (CP: 166.9 (29.6) mm, TD: 159.6 (24.7) mm,  $p=0.54$ , CI [-13.74 25.85]) and fascicle lengths

1 (CP: 25.0 (6.6) mm, TD: 28.4 (4.1) mm,  $p=0.08$ , CI [-7.34 0.42]) were not significantly different between  
2 children with CP and TD children.

3 Movements were performed with an average maximal angular velocity of 12.7 (4.2) °/s. No movements  
4 were excluded due to inaccurate movement velocity. In the children with CP, eleven trials were  
5 excluded due to an elevated EMG signal. This equates to 10% of the total number of trials. A minimum  
6 of 2 trials per participant was available for analysis. The full ROM was 13° smaller towards dorsiflexion in  
7 the CP group. Absolute muscle and fascicle lengthening over full ROM were on average 9mm smaller in  
8 CP. Absolute tendon lengthening was similar between groups. Over the common ROM that could be  
9 achieved by all participants (-25° to -5°) absolute muscle and fascicle lengthening was on average 3mm  
10 smaller in CP and absolute tendon lengthening did not differ between groups. At -5°, being the most  
11 dorsiflexed position all participants could achieve, joint torques were significantly larger in children with  
12 CP (2.34 (1.77) Nm) than TD children (0.49 (0.94) Nm). Over a common joint torque (0 Nm to 3 Nm),  
13 absolute muscle and fascicle lengthening was on average 3.2 mm smaller in CP, and absolute tendon  
14 lengthening did not differ between groups. When analysed over a common range of MTU lengthening  
15 (20mm), absolute muscle and fascicle lengthening were on average 2.5mm smaller in CP and absolute  
16 tendon lengthening was on average 2.6 mm larger in CP. Finally, when expressed as a percentage of  
17 MTU lengthening, relative muscle lengthening was smaller and relative tendon lengthening larger in  
18 children with CP over all the studied ROMs (Table 3, Figure 2).

19 Pennation angle was not different between groups regardless the range over which it was studied  
20 ( $p>0.05$ ). Fascicle lengthening resolved along the axis of the MTU was 8.2 (3.2) mm and 11.5 (2.0) mm  
21 respectively for CP and TD children over the common ROM ( $p<0.01$ ). Over the full ROM this was 16.3  
22 (6.3) mm and 26.5 (7.0) mm for CP and TD children ( $p<0.01$ ).

1 Muscle lengthening increased significantly with age in TD children while in children with CP, tendon  
2 lengthening increased with age (Figure 3). Significant correlations were found between muscle and  
3 tendon lengthening with ROM in children with CP (Figure 4).

#### 4 **Discussion**

5 Regardless of whether groups were compared according to common joint angle, joint torque, or relative  
6 to MTU lengthening, muscle and fascicle lengthening were always smaller in children with CP than TD  
7 children (Table 3). This confirms previous findings of smaller muscle and fascicle lengthening during  
8 passive ankle dorsiflexion in children with CP (Barber *et al.*, 2011; Matthiassdottir *et al.*, 2014). By  
9 simultaneously studying the relative contributions of the muscle and tendon to MTU lengthening, we  
10 also found that in TD children the muscle lengthens more than the tendon (63:37%) while in children  
11 with CP they lengthen equally (50:50%). This indicates greater stiffness of the muscle relative to tendon  
12 in children with CP than TD. Our data cannot distinguish whether this difference is caused by a stiffer  
13 MG muscle (Friden & Lieber, 2003), or by a longer tendon (Wren *et al.*, 2010; Barber *et al.*, 2012),  
14 making it more compliant in children with CP. However, the joint moments required to lengthen the  
15 muscle-tendon unit are greater in children with CP (Alhusaini *et al.*, 2010) and others have reported  
16 Achilles tendon stiffness not to be different between CP and TD (Theis *et al.*, 2016). Therefore, it is  
17 reasonable to conclude that an increased muscle stiffness contributes more to a difference in the  
18 relative lengthening between muscle and tendon as observed in this study. However, regardless of  
19 whether the explanation lies in the muscle, tendon or a combination, this reduced stretch at the muscle  
20 relative to the tendon might explain the lack of change in muscle properties both acutely (Kalkman *et*  
21 *al.*, 2018) and after long term (Theis *et al.*, 2015) stretching interventions.

22 Previous studies on muscle properties in children with CP analysed fascicle lengthening only over the full  
23 (Barber *et al.*, 2011) or a common ROM (Matthiassdottir *et al.*, 2014). However, due to differences in

1 Achilles tendon moment arm (Kalkman *et al.*, 2017) and joint stiffness (Alhusaini *et al.*, 2010) between  
2 TD and CP participants, comparison of lengthening parameters between groups only in terms of joint  
3 angles should be interpreted with caution. When data is analysed over a common ROM, it should  
4 additionally be noted that this common ROM could be at a different position relative to the full ROM in  
5 individual children and that children with CP develop torque earlier in their ROM. In this study the  
6 torque at the limit of the common dorsiflexion range ( $-5^{\circ}$ ) was higher in children with CP compared to  
7 TD children. To circumvent these issues, we compared our data in two additional ways. Firstly, over a  
8 common torque range to assure a similar stretching stimulus to the MTU. However, joint torque is also  
9 affected by differences in Achilles tendon moment arm, co-contraction and intrinsic joint stiffness.  
10 Therefore, lengthening values were additionally compared over a common MTU lengthening.  
11 Nevertheless, irrespective of the method used, we always found that relative to MTU lengthening,  
12 muscle lengthening is smaller and tendon lengthening larger in children with CP. This consistency  
13 confirms that the above changes in the mechanical behaviour of the MTU of children with CP are  
14 substantial and independent of the range used. However, when assessing the effect of an intervention  
15 or comparing different subgroups of children with CP, the differences may be less pronounced and the  
16 method of analysis will likely be important. This is a vital consideration when decomposing the causes of  
17 a reduced ROM in the clinical decision-making process.

18 This study, and others before us (Morse *et al.*, 2008), observed a discrepancy between the amount of  
19 fascicle and muscle belly lengthening during a passive stretch. This decoupling of the elongation of the  
20 fascicles from that of the whole muscle can be explained by deformation of intra-muscular connective  
21 tissue (endomysium and perimysium), extra-muscular connective tissue (epimysium) and the  
22 aponeurosis (Lieber *et al.*, 2017). Additional analysis of the current data to explore fascicle:muscle  
23 lengthening showed that over a common ROM, muscle belly lengthening could be entirely explained by  
24 the resolved fascicle lengthening in both groups. This may imply that the increased resistance to stretch



1 of the muscle in children with CP results from similar changes in the lengthening characteristics of both  
2 the fascicles and passive connective tissue. When studied over the full ROM, average muscle belly  
3 lengthening in the CP group was 1.4mm larger than the resolved fascicle lengthening, while in the TD  
4 group muscle belly lengthening was equal to resolved fascicle lengthening. This could suggest that  
5 structures other than the fascicles, such as the perimysium and tissue between the fibres, deform to  
6 provide the additional lengthening required to achieve maximal dorsiflexion angles in children with CP,  
7 while in TD children this is not the case. Consistent with this interpretation, both intramuscular  
8 connective tissue (Malaiya *et al.*, 2007) and the expression of extracellular matrix production-related  
9 genes were found to be dramatically increased in spastic muscles and correlated with muscle  
10 mechanical properties, such as stiffness (Smith *et al.*, 2012).

11 It has been shown that muscle contractures already start developing at an early age in children with CP  
12 (Willerslev-Olsen *et al.*, 2013) and that growth is an important factor contributing to the development of  
13 contractures (Švehlík *et al.*, 2013). Therefore, it is important to capture the critical age at which  
14 treatment is most effective and consider the changes that occur in muscle-tendon properties with  
15 maturation. It has been reported that gastrocnemius muscle belly length increases with age in TD  
16 children (Bénard *et al.*, 2011; Weide *et al.*, 2015). However, muscle lengthening from 0 to 4Nm  
17 dorsiflexion torque, was not found to increase with age (Bénard *et al.*, 2011; Weide *et al.*, 2015). This is  
18 not supported by our data, since we found a tendency for muscle lengthening to increase with age in TD  
19 children (Figure 3). The inconsistency might be caused by differences in age range and methodology of  
20 applying the dorsiflexion torque. Interestingly, the increase in muscle lengthening with age was absent  
21 in children with CP, for whom tendon lengthening increased with age. This may indicate that muscle  
22 stiffness increases with age in children with CP, which is consistent with the progression of disease  
23 (Graham *et al.*, 2016). This is consistent with previous results showing impaired muscle growth and  
24 increased stiffness with age in children with CP (Willerslev-Olsen *et al.*, 2018) Additionally, it indicates a

1 possibility that the Achilles tendon acts as a compensation mechanism to partly preserve ROM, despite a  
2 shorter and stiffer muscle as children with CP grow.

3 The relative contribution of fascicle, muscle and tendon lengthening to ROM may be important in  
4 determining the best treatment. We show that both muscle and tendon lengthening are related to ROM  
5 in children with CP (Figure 4). A lack of this relationship in TD children shows that the MG does not play  
6 an essential role in determining their ROM. Stretching is often used to increase ROM in children with CP  
7 and is assumed to increase muscle length. However, the smaller muscle belly lengthening, caused by a  
8 change in the relative stiffness between muscle and tendon will lead to a smaller physiological stimulus,  
9 which may possibly explain the lack of effectiveness of stretching therapies (Wuart *et al.*, 2008). Altering  
10 the relative stiffness before starting stretching therapies, either by making the muscle less stiff, or by  
11 increasing the stiffness of the tendon might make stretching exercises more effective. The large  
12 variability amongst participants in the current study and those reported in literature suggests that  
13 patient- and muscle-specific information may be required to facilitate individualized treatment  
14 programs.

15 This study has some limitations. Currently, it is not possible to measure muscle and tendon stiffness  
16 during passive rotation in an intact joint, because there are no *in vivo* techniques to quantify forces in  
17 the muscle-tendon unit. However, the results of our study show less MG MTJ displacement in children  
18 with CP compared with TD children. Therefore, it is likely that in children with CP, the reduced  
19 contribution of the MG muscular component to MTU lengthening can, at least partly, be explained by an  
20 increased stiffness in the MG muscle. The SEM values of all parameters were lower than the average  
21 difference between groups, nonetheless, reliability of the calculated parameters was lower than  
22 expected based on Cenni *et al.*, 2018. Future studies could reduce possible sources of error by applying  
23 motorized instead of manual movements and automatic tracking algorithms for feature identification. In  
24 the current study, fascicle and tendon length were represented as straight lines, thus neglecting possible

1 effect of curvature. The influence of curvature has been reported to be small for passive fascicle length  
2 measurements in the MG (Muramatsu *et al.*, 2002). Neglecting tendon curvature leads to an  
3 overestimation of tendon lengthening in both groups especially at more plantarflexed ankle angles  
4 where the tendon is below slack length. Since we expect slack length to be at more plantarflexed angles  
5 in children with CP, an overestimation of tendon lengthening would be more likely in the TD children.  
6 Thus, controlling for tendon curvature would only amplify the between-group difference in tendon  
7 lengthening reported here. In addition, the methodology did not allow to visualise muscle belly and  
8 fascicle lengthening in the same trial. However, judged on the angle-time curves of the individual  
9 stretches, repetitions were considered repeatable between the two conditions. Also, in the TD children  
10 the leg tested was not randomized, but instead, always the left leg was assessed, which in most  
11 individuals would be the non-dominant leg. Since it is not known how limb dominance affects muscle  
12 lengthening, the lack of randomization for leg tested could have influenced our results. Furthermore,  
13 the effect of a single stretch on MTU properties has been shown to be negligible (Kalkman *et al.*, 2018).  
14 It is possible that previous treatments, i.e. Botulinum Toxin-A injections, received by the participants  
15 may have influenced the results of this study. Some studies show microstructural changes on the tissue  
16 level in animals (Pingel *et al.*, 2017) or observe an increase in muscle stiffness in *in silico* experiments  
17 (Wang *et al.*, 2018), while others report no changes in muscle stiffness (Alhusaini *et al.*, 2011) after  
18 Botulinum Toxin-A injections. Unfortunately, it is practically impossible to recruit a representative group  
19 of children with CP who have not had any interventions during their life. However, this does not  
20 confound the validity of the present results for the limited effect of the stretching intervention studied,  
21 as these interventions are applied regardless of Botulinum Toxin-A history. Finally, the exclusion of  
22 movements that showed muscle activation higher than a threshold helped minimize the effects of  
23 reflex-activity on the feature displacement. EMG activity during the analysed stretches was found to be

1 around 5% of RMS-MVC. However, small effects of background EMG activity below this threshold  
2 cannot be fully excluded.

3 In summary, this study demonstrates that when passively rotating the ankle joint to stretch the calf  
4 muscles, the tendon lengthens less than the muscle in TD children, while in children with CP, the muscle  
5 lengthens as much as the tendon. This suggests altered material properties of the muscle and tendon in  
6 children with CP. This should be considered when assessing and treating muscle function at joint level in  
7 children with CP, for example during stretching exercises.

## 8 **Additional information**

### 9 *Competing interests*

10 None declared.

### 11 *Author contributions*

12 Conception and design of the research: B.M.K., L.B., T.D.OB., C.N.M., K.D., G.H. and G.J.B. Data  
13 acquisition: B.M.K., L.B. and F.C. Data analysis: B.M.K. and L.B. Interpretation of the results: B.M.K., L.B.,  
14 C.N.M., T.D.OB., A.B. and G.H. Drafting the manuscript: B.M.K. and L.B. Editing and revision of the  
15 manuscript: B.M.K., L.B., F.C., K.D., A.B., G.H., G.J.B., C.N.M. and T.D.OB. All authors have read and  
16 approved the final version of this manuscript and agree to be accountable for all aspects of the work in  
17 ensuring that questions related to the accuracy or integrity of any part of the work are appropriately  
18 investigated and resolved. All persons designated as authors qualify for authorship, and all those who  
19 qualify for authorship are listed.

### 20 *Funding*

21 This study was funded by a joint scholarship between Alder Hey Children's Hospital and Liverpool John  
22 Moores University and by grant 12R4215N from the Flemish Research Foundation (FWO), Belgium.

1 *Acknowledgements*

2 We thank Erwin Aertbeliën from the department of mechanical engineering, KU Leuven, for his help  
3 with the calculations of net joint torque.

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5

## Figure Legends

**Figure 1. A.** Experimental design 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-plate were tracked with motion analysis and used to calculate the foot-plate angle in 3D. A single marker was placed on the most distal part of the calcaneus and additionally tracked in 3D using motion analysis. The ultrasound probe was placed above the medial gastrocnemius muscle-tendon junction (MTJ), or on the muscle belly, and the position and orientation of the image was tracked using motion analysis by means of a cluster of markers attached to the probe. **B.** Close-up of the foot attached with an insole to the foot plate of the orthotic. **C.** The MTJ was identified as the most distal insertion of the muscle into the tendon. **D.** Fascicle length was defined as the straight line distance between the upper and lower aponeurosis along the lines of collagenous tissue and pennation angle ( $\alpha$ ) was defined as the angle between the fascicle and the deep aponeurosis. With the exception of the ultrasound measurements, the same experimental setup was used in Kalkman et al. 2018.

**Figure 2** Muscle/tendon length (**A**) and fascicle length (**B**) versus ankle angle with the common ROM indicated in shaded grey; Muscle/tendon length (**C**) and fascicle length (**D**) versus muscle-tendon-unit (MTU) length; muscle/tendon length (**E**) and fascicle length (**F**) versus ankle torque. 95% Confidence Intervals are shown at 4 representative time points.

**Figure 3** Correlations between age and muscle (**A, B**), tendon (**C, D**) and fascicle (**E, F**) lengthening across the range of motion (ROM) for children with cerebral palsy (CP) and typically developing (TD) children. A regression line is shown for significant relationships.

**Figure 4** Correlations between muscle **(A, B)**, and tendon **(C, D)** lengthening and range of motion (ROM) in children with cerebral palsy (CP) and typically developing (TD) children. A regression line is shown for significant relationships.

**Table 1. Participant characteristics**

<i>Participant characteristics</i>	<b>CP (n=15)</b>	<b>TD (n=16)</b>
Age (years, months)	11y 5m (3y)	10y 4m (3y)
Male/female (n)	10/5	7/9
Height (cm)	142 (20.3)	138.1 (19.1)
Mass (kg)	36 (18)	35 (15)
Tibia length (mm)	339.7 (54.3)	329.4 (52.7)
GMFCS (I-IV) (n)	9 I, 6 II	n/a
Diagnosis (n)	8 Diplegia, 7 Hemiplegia	n/a
*Modified Ashworth Score(Bohannon and Smith, 1987) (n=7) and Average Modified Tardieu(Tardieu et al., 1954) (n=8)	MAS: 1.5 (n=6); 3 (n=1) Tardieu: 2 (n=5); 3 (n=3)	n/a
Botulinum toxin-A injections >6 months prior to the study date. Mean (range)	2.3 (0-11)	n/a

Data are mean (SD) unless otherwise stated. CP: cerebral palsy; TD: typically developing; GMFCS: gross motor functional classification system (Palisano et al., 1997); n/a: not applicable.

\*Tardieu scores from children recruited at centre 2. MAS from children recruited at centre 1.

**Table 2. Mean (SD) of lengthening values in a subgroup (n=5) of TD children for repeatability analysis.**

	Session 1	Session 2	ICC	SEM
<u>Over common ROM (-25° to -5°)</u>				
Fascicle	10.3 (1.9)	8.9 (1.4)	0.753	2.0
Muscle	10.4 (3.6)	9.2 (2.7)	0.822	1.7
Tendon	7.0 (2.6)	5.3 (3.3)	0.663	2.1
<u>Over common MTU</u>				
Fascicle	6.9 (2.5)	6.5 (4.1)	0.74	1.6
Muscle	10.9 (1.8)	10.1 (2.4)	0.739	2.3
Tendon	9.0 (1.8)	9.9 (2.4)	0.908	0.9
<u>Over common torque</u>				
Fascicle	20.9 (5.2)	19.9 (4.4)	0.926	1.8
Muscle	22.9 (2.8)	22.6 (3.5)	0.737	3.1
Tendon	15.8 (4.6)	14.5 (5.5)	0.799	2.8

**Table 3. Mean (SD) lengthening values in children with cerebral palsy (CP) and typically developing (TD) children during passive ankle rotation.**

	Absolute lengthening (mm)			% of MTU lengthening	
	CP	TD	ES Hedge's g	CP	TD
<u>Over the full ROM</u>				<u>Over the full ROM</u>	
ROM (°)	48.0 (12.8)	60.6 (11.0) *	-1.03		
Fascicle	15.9 (6.2)	26.0 (4.3) **	-1.27	40.7 (10.7)	58.1 (14.3) **
Muscle	18.2 (5.4)	26.5 (7.0) **	-1.87	48.1 (9.2)	62.4 (9.2) **
Tendon	20.7 (8.1)	16.8 (6.7)	0.51	52.5 (8.8)	37.6 (9.2) **
<u>Over common ROM (-25° to -5°)</u>				<u>Over common ROM (-25° to -5°)</u>	
Fascicle	7.9 (3.2)	11.1 (2.1) **	-1.32	50.6 (20.4)	59.3 (14.6)
Muscle	8.5 (2.3)	11.4 (2.8) **	-1.20	53.9 (9.0)	64.9 (9.9) **
Tendon	7.6 (3.1)	6.45 (2.3)	0.32	46.1 (9.0)	35.1 (9.9) **
<u>From maximum 0 to 3Nm</u>				<u>From maximum 0 to 3Nm</u>	
ROM (°)	14.2 (3.2)	17.4 (5.6)	-0.81		
Fascicle	4.1 (1.6)	7.6 (3.2)**	-1.29	37.5 (9.6)	56.3 (14.9) **
Muscle	3.5 (1.9)	5.7 (2.5) *	-0.91	50.4 (9.3)	63.4 (8.5) **
Tendon	3.8 (2.9)	2.8 (1.3)	0.38	49.6 (9.3)	36.6 (8.5) **
<u>Over a common MTU range (0-20mm)</u>				<u>Over a common MTU range (0-20mm)</u>	
ROM (°)	17.7 (5.5)	18.7 (5.7)	-0.33		
Fascicle	5.7 (2.1)	7.6 (3.9) *	-0.52	25.4 (12.3)	39.1 (19.5) *
Muscle	9.9 (2.3)	12.1 (2.5) **	-1.08	49.3 (11.5)	61.4 (12.9) **
Tendon	10.1 (2.2)	7.9 (2.5) **	1.08	50.5 (11.2)	38.6 (12.8) **

ROM: range of motion; PF: plantar flexion; MTU: muscle-tendon unit; CI: confidence interval; SEM: inter-session standard error of measurement in TD children; ES: effect size.

\* Significant difference between CP and TD at  $p < 0.05$  (\*\*  $p < 0.01$ )









