Mechanical and material properties of the plantarflexor muscles and Achilles tendon in children with spastic cerebral palsy and typically developing children

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Abstract

Background: Children with spastic cerebral palsy (CP) experience secondary musculoskeletal adaptations, affecting the mechanical and material properties of muscles and tendons. CP-related changes in the spastic muscle are well documented whilst less is known about the tendon. From a clinical perspective, it is important to understand these alterations in tendon properties in order to tailor interventions or interpret clinical tests more appropriately. The purpose of the study was to compare the mechanical and material properties of the Achilles tendon in children with cerebral palsy to those of typically developing children.

Methods: Using a combination of ultrasonography and motion analysis we determined tendon mechanical properties in ten children with spastic cerebral palsy and ten aged-matched typically developing children. Specifically, we quantified muscle and tendon stiffness, tendon resting length, tendon strain, cross sectional area, Young’s Modulus and the strain rate dependence of tendon stiffness.

Findings: Children with CP had a greater muscle to tendon stiffness ratio compared to typically developing children. Despite a smaller tendon cross sectional area and greater resting tendon length, no group differences were observed in tendon stiffness or Young’s Modulus. The slope describing the stiffness strain-rate response was steeper in children with cerebral palsy.

Interpretation: The results highlight that the mechanical and material properties of the Achilles tendon in children with spastic CP should be considered when implementing interventions that affect function and when interpreting clinical tests of spasticity.
1. Introduction

Spastic cerebral palsy (CP) results from damage to the developing brain before, during or shortly after birth (Reddihough and Collins, 2003). During maturation, secondary musculoskeletal adaptations occur, which can affect the mechanical and material properties of muscles and tendons. Previous research has primarily focused on the atypical development of the muscle in children with spastic CP compared to their typically developing (TD) peers. There is consistent evidence of a shorter gastrocnemius muscle belly length (Malaiya et al., 2007; Wren et al., 2010) reduced muscle volume (Malaiya et al., 2007), increased connective tissue (Booth et al., 2001), and increased muscle and fascicle stiffness (Barber et al., 2011; Fridén and Lieber, 2003; Smith et al., 2011). The adaptations of the tendon in children with spastic CP are less well established. However, the tendon also plays an integral role in movement, alongside the muscle. The mechanical properties of the tendon govern the transfer of muscular forces to the bone, and the storage and return of elastic energy during functional activities (Lichtwark and Wilson, 2008). It is possible that the aforementioned CP-related changes in the mechanical properties of the muscle, result in secondary mechanical adaptations of the tendon, which have implications for functional movement. Thus, the overall goal of this study was to characterise the mechanical properties of the tendon in children with spastic CP, and compare them to TD children.

As the muscle and tendon are closely integrated in the production of movement, the mechanical properties of both structures in children with spastic CP should not be considered independent to one another. Importantly, the length and compliance of the Achilles tendon can affect the force-generating capacity of the muscles (Lichtwark et al., 2007; Lichtwark and Wilson, 2008). For example, certain tendon compliance may allow muscle fibres to operate close to an optimal length and at relatively low shortening velocities, thereby aiding force
production (Lichtwark et al., 2007; Lichtwark and Wilson, 2006). Understanding CP-related differences in the tendon’s mechanical properties alongside mechanical properties in the muscle would provide useful insights into the mechanisms underlying CP-specific characteristics of functional movement. Therefore, the first specific aim of this study was to compare plantarflexor muscle and Achilles tendon stiffness in children with spastic CP and TD children.

Tendon stiffness is determined by both its dimensions and material properties. Regarding the former, a long tendon with a small cross-sectional area will be more compliant than a short tendon with a large cross-sectional area. Regarding the latter, the tendon’s material properties are independent of its dimensions and depend primarily on collagen fibre type, size and organisation (Silver et al., 2003). One way to differentiate between the dimensional and material properties of the tendon is to calculate Young’s modulus, which can be thought of as tendon stiffness normalised by its dimensions. It has previously been demonstrated that in TD children, tendon stiffness increases with maturation (Waugh et al., 2012), due to changes in both dimensions and material properties. The adaptations of the tendon occur in response to chronic mechanical loading, predominantly due to an increase in muscle size and force (Kubo et al. 2001; Wu et al. 2010). Within the context of CP, a lack of mechanical loading from the spastic muscle due to atrophy and weakness (Malaiya et al., 2007) may cause atypical growth of the tendon. The lack of mechanical loading and inability to fully weight bear in more severely affected CP children (GMFCS IV and V) could exacerbate this weakness and atypical tendon growth. The Achilles tendon has been shown to be longer than in TD children, and cross-sectional area has been reported to be smaller (Gao et al., 2011), presumably as an adaptation to the atypical shortening of the muscle belly (Barber et al., 2012; Wren et al., 2010). One might expect these dimensional changes to cause the tendon to
be more compliant in children with spastic CP. However, Barber et al. (2012) did not find any differences in tendon stiffness between children with CP and TD children, which could indicate concomitant alterations in tendon cross-sectional area and maturation of the tendon’s material properties, independent of dimensions. Understanding the CP-related changes in the dimensional and material properties to tendon stiffness is an important prerequisite to better understand movement efficiency and control in children with spastic CP. Therefore, the second specific aim was to compare dimensions and material properties of the Achilles tendon between children with spastic CP and TD children.

Another mechanical property of the tendon is its viscoelasticity. Tendons recoil elastically after stretch, but they also become stiffer at higher loading rates (Knudson, 2007, pp. 73; Pearson et al., 2007; Theis et al., 2012). Previously, we have shown that the slope of the strain-rate-stiffness relationship is lower in TD children than in adults (Theis et al., 2012). This phenomenon may partly explain previously observed differences in movement efficiency between adults and children (Ebbeling et al., 1992). From a clinical perspective, it is important to understand the strain-rate-tendon stiffness relationship in children with CP, as it could lead to more differentiated (and therefore more meaningful) assessments of spasticity. Thus, the third specific aim of the study was to determine the strain-rate response of the Achilles tendon in children with spastic CP and TD children.
2. Methodology

2.1 Participants

Ten children with clinically diagnosed diplegic or quadriplegic spastic CP participated in this study. The CP group had knee deformities of less than 10 degrees and ankle deformities of less than 15 degrees (5 males, 5 female; age 11.4 ± 3.0 y; 6 children were GMFCS III, 4 children GMFCS IV). Participants that were GMFCS level III were able to fully weight bear and walk with an assistive frame. Those participants at GMFCS level IV were predominate wheelchair users but could weight bear with assistance and walk short distances with an assistive frame. In addition, none of the CP participants received botulinum toxin type A (Botox) injections or serial casting in the past 6 months or had orthopaedic surgery of the lower extremities in the past 12 months. Physical therapy routines of the CP groups consisted of approximately 3-4 hours per week of dynamic movement activities and use of a standing frame.

Ten age-matched TD children (5 males, 5 female; age 12.0 ± 2.9 y) also participated in this study. Children and their guardians provided informed assent and consent, respectively. The study was approved by the institutional and relevant NHS Ethics Committees.

2.2 Protocol and Instrumentation

To measure ankle torque, participants were seated on the dynamometer chair. The right knee was straightened to full extension for the TD group, and for the CP group the knee was straightened as much as possible, which was on average 7.0 ± 2.0° from full extension across CP participants. The relative hip angle was set to 85° for both groups. The lateral malleolus of the right ankle was aligned with the rotational axis of the dynamometer arm. The dynamometer footplate was positioned perpendicularly to the tibia, and this was considered
to be 0°. Stabilisation straps were applied tightly over the foot, thigh and chest to minimise movement of the upper body or leg.

The participants’ available range of motion (ROM) was determined by dorsi- and plantarflexing the foot at 10°·s⁻¹, until any discomfort was reported. This occurred between 6.3 ± 0.7° dorsiflexion and 20.3 ± 4.0° of plantarflexion for the CP group, and 19.0 ± 8.3° dorsiflexion and 23.1 ± 3.6° of plantarflexion for the TD group. The dynamometer system was then set to apply passive angular rotations to the right ankle joint at constant angular velocities of 1, 10 and 30°·s⁻¹ within the available ROM. Participants were instructed to relax the muscles of the lower limb. Three rotations were recorded at each angular velocity; the order of angular velocities was randomised. Electrical activity of the medial gastrocnemius (EMG) was monitored throughout the rotations (Trigno wireless system, Delsys Inc., Ltd., Boston, USA). Both torque and EMG signals were sampled at 1000 Hz. Torque data were filtered using a low-pass, fourth-order, zero-lag Butterworth filter with a cut-off frequency of 14 Hz.

Muscle and tendon elongation were measured as the displacement of the medial gastrocnemius muscle-tendon junction. The muscle-tendon junction (MTJ) was visualised using B-mode ultrasonography (Megas GPX, Esaote, Italy; 45 mm Linear array probe, 10 MHz transducer scanning). The 2D coordinates of the MTJ were then manually digitised (Peak Performance, Cambridge, UK). Muscle-tendon junction position data were then filtered using a low-pass fourth-order zero-lag Butterworth filter with a 3.25 Hz cut-off frequency.

2.3 Derivation of dependent variables
Motion analysis markers were placed on the calcaneus, medial and lateral epicondyles, and the handle of the ultrasound probe. These were tracked using 3D motion analysis. The coordinates of two markers from the handle of the ultrasound probe, combined with the coordinates of the muscle-tendon junction in the ultrasound image, the global 2D position of the muscle-tendon junction was calculated in the sagittal plane. Tendon length was defined as the linear distance from its insertion on the calcaneus to the medial gastrocnemius muscle-tendon junction. Medial gastrocnemius muscle length was defined as the distance between the medial femoral epicondyle and the global 2D coordinates of the muscle-tendon junction. Thus, both medial gastrocnemius and Achilles tendon were modelled as straight lines. The resting length of the Achilles tendon was calculated as the length at which there was a sustained increase in ankle torque above zero (Barber et al., 2012), and thus, where tendon slack had been taken up. Tendon resting length was expressed in absolute terms at this point and normalised by resting muscle-tendon unit length.

Tendon stiffness was calculated as the change in muscle-tendon force divided by the corresponding change in Achilles tendon length. Muscle-tendon force was calculated over the range, neutral (0 degrees) to maximum dorsiflexion, by dividing ankle torque by the Achilles tendon moment arm. Moment arm was calculated using the tendon excursion method as the mathematical derivative of tendon excursion with respect to the angular displacement of the corresponding joint. According to recommendations of Fath et al. (2010), a third-order polynomial was fitted to approximate the relationship between tendon elongation and angular displacement from 5° dorsiflexion to 5° plantarflexion, and differentiated at the neutral ankle position to obtain the moment arm. An estimate of “total” plantarflexor muscle stiffness was derived using a similar method to that described by Morse et al. (2008). For this purpose, the change in force was divided by changes in medial gastrocnemius muscle length.
Muscle and tendon stiffness for both groups was determined during dorsiflexion in the $10^\circ \cdot s^{-1}$ trial. Stiffness was calculated relative to each participant’s maximal force, subsequently referred to as stiffness$_{REL}$. Specifically, we determined the slope of the force-elongation curve, between 20-80% of each participant’s peak force. Tendon stiffness measured on three separate occasions and over the range of 20-80% gave a coefficient of variation of 6.0% for the CP group ($n = 6$) and 6.8% for the TD group ($n = 10$).

Tendon stiffness was also calculated in a common force region (subsequently referred to as stiffness$_{COM}$) to allow inferences about the passive mechanical properties of the tendon between groups. For this purpose, we used range, which corresponded to 20% and 80% of peak force from the second weakest participant (corresponding to an absolute force range of 30.2 – 120.0 N). For this analysis, we excluded the weakest participant, as 20% of their peak force was lower than the minimum force for some of the stronger participants.

The tendon’s Young’s Modulus was calculated by multiplying tendon stiffness$_{COM}$ by its resting length and dividing by tendon cross-sectional area. For this purpose, we used a modified silicon ultrasound gel pad (Aquaflex 2 × 9 cm, Parker Labs Inc., NJ, USA) to take three discrete ultrasound images of the Achilles tendon’s cross-sectional area, approximately 30 mm proximally to the tendon insertion (Magnusson et al., 2001).

Lastly, relative tendon stiffness (stiffness$_{REL}$) was calculated from the slope of the force-elongation curve, corresponding to 20-80% of each participant’s peak force. Stiffness$_{REL}$ was plotted against angular velocities of 1, 10 and $30^\circ \cdot s^{-1}$. A linear regression line was fitted through the strain-rate-stiffness relationship to calculate the slope of the line.
2.4 Statistical analysis

To address the first specific aim of this study, we determined differences in muscle and tendon stiffness\textsubscript{REL} between groups using a mixed design repeated measures ANOVA. Here, we tested for a structure (muscle vs. tendon) × group (CP vs. TD) interaction. In case of significance, Bonferroni corrected \textit{t}-tests were performed to locate any between group differences and between structure differences.

Regarding the second specific aim, we performed a MANOVA on stiffness\textsubscript{COM}, tendon cross-sectional area, absolute resting tendon length and resting tendon length expressed as a percentage of muscle-tendon unit length, with Bonferroni corrected \textit{t}-tests. One further independent \textit{t}-test was performed on Young’s modulus. In addition to these statistical tests, we also determined the effect sizes (ES) (Cohen’s D) to describe group differences for all dependent variables.

With regards to the third specific aim of the study, a mixed design repeated measures ANOVA was performed to test for a main effect of strain-rate on tendon stiffness\textsubscript{REL}, and a group × strain-rate interaction. In case of main effect significance, two 1 × 3 repeated ANOVA’s were performed. Following significance, paired \textit{t}-tests with Bonferroni correction were performed to determine within-group differences in strain-rate. In case of a significant interaction, independent \textit{t}-tests were performed to compare tendon stiffness\textsubscript{REL} between groups at each strain-rate. One further independent \textit{t}-test was used to compare the slope of the strain-rate-stiffness\textsubscript{REL} relationship between groups.
3. Results

There was a significant group × structure interaction ($F_{1, 18} = 8.45, P = 0.02$). The main effect of structure on stiffness$_{REL}$ was non-significant ($F_{1, 18} = 2.29, P = 0.15$). Follow up independent samples $t$-tests revealed that muscle stiffness$_{REL}$ was significantly greater in CP compared to TD ($t_{18} = 2.86, P = 0.010, ES = -0.95$), with no difference in tendon stiffness$_{REL}$ ($t_{18} = 0.21, P = 0.83, ES = 0.16$). Follow up paired samples $t$-tests revealed muscle stiffness$_{REL}$ was significantly greater than tendon stiffness$_{REL}$ in CP ($t_{9} = 2.99, p = 0.041$), whilst this effect was non-significant in TD ($t_{9} = 1.03, P = 0.33$) (Figure 1).

The main effect of the MANOVA on tendon cross-sectional area, absolute and normalised resting tendon lengths and stiffness$_{COM}$ was significant (Hotelling’s $T^2 = 1.42, F_{4, 14} = 7.13, P < 0.01$). Post-hoc analyses revealed that tendon cross-sectional area was significantly smaller in CP compared to TD ($t_{18} = -5.16, P < 0.01$). In addition, normalised resting tendon length was significantly greater in CP compared to TD ($t_{18} = 2.72, P = 0.013, ES = -1.05$). In contrast, absolute resting tendon length and tendon stiffness$_{COM}$ were not significantly different between groups ($t_{18} = 0.13, P = 0.90; ES = -0.05; t_{17} = -1.05, P = 0.31, ES = 0.48$), respectively. In addition, there were no differences in Young’s modulus between groups ($t_{17} = 0.44, P = 0.67, ES = -0.21$) (Figure 2) (Table 1).
The strain-rate by group ANOVA on stiffness_{REL} revealed a significant main effect of strain rate ($F_{2, 29} = 24.35, P < 0.01$). The strain-rate by group interaction was also significant ($F_{2, 29} = 6.13, P = 0.02$). Independent samples t-tests revealed no significant difference between groups at any strain rate ($1^{\circ}\cdot s^{-1}$: $t_{18} = 0.16, P = 0.88$; $10^{\circ}\cdot s^{-1}$: $t_{18} = 0.34, P = 0.74$; $30^{\circ}\cdot s^{-1}$: $t_{18} = -1.25, P = 0.28$). The analysis of effect sizes revealed that the group differences at the low velocities were small (ES = 0.14 and 0.16 at $1^{\circ}\cdot s^{-1}$ and $10^{\circ}\cdot s^{-1}$, respectively) whilst the group effect was moderate at $30^{\circ}\cdot s^{-1}$ (ES = -0.29) (Figure 3).

The tendon strain-rates corresponding to angular velocities 1, 10 and $30^{\circ}\cdot s^{-1}$ were $0.12 \pm 0.02$, $0.43 \pm 0.04$, $0.81 \pm 0.03$ cm·s$^{-1}$ for the CP group and $0.14 \pm 0.03$, $0.46 \pm 0.10$, $0.88 \pm 0.09$ cm·s$^{-1}$ for the TD group, respectively. The group by strain-rate interaction also expressed itself in different strain-rate effects between groups. Post-hoc paired samples t-tests revealed that in CP, Achilles tendon stiffness_{REL} was significantly greater at $30^{\circ}\cdot s^{-1}$, than at $10^{\circ}\cdot s^{-1}$ and $1^{\circ}\cdot s^{-1}$ ($P = 0.007$). In TD, Achilles tendon stiffness_{REL} was greater at $10^{\circ}\cdot s^{-1}$ than at $1^{\circ}\cdot s^{-1}$ ($P = 0.001$), but not different between $10^{\circ}\cdot s^{-1}$ and $30^{\circ}\cdot s^{-1}$ ($P = 0.62$) (Figure 4.4). Finally, the slope of the strain-rate stiffness_{REL} curve was significantly steeper in CP (Slope = $21.5 \pm 18.7$ Ns·mm$^{-2}$, $R^2 = 0.85 \pm 0.11$) compared to TD (slope = $8.6 \pm 3.7$ Ns·mm$^{-2}$, $R^2 = 0.94 \pm 0.07$) ($t_{18} = 2.27, P = 0.04$).
4. Discussion

The first specific aim of this study was to compare plantarflexor muscle and Achilles tendon stiffness in children with spastic CP and TD children. For this purpose, we calculated muscle and tendon stiffness relative to each participant’s force at maximal dorsiflexion. The result that plantarflexor muscle stiffness was significantly greater in children with CP compared to TD children is consistent with previous findings (Barber et al., 2011; Friden and Lieber, 2003; Smith et al., 2011). Potential mechanisms underlying these spasticity-related differences in muscle stiffness include a reduced number of in-series sarcomeres (Smith et al., 2011) and remodelling of intra- and extra-muscular connective tissue (Booth et al., 2001).

More interestingly, we also found that children with CP had greater muscle compared to tendon stiffness, whereas in TD children the stiffness between the two structures was not different. This difference in the muscle to tendon stiffness ratio may have important implications for movement control. For example, in a healthy system, tendon stiffness is tuned to optimise muscle fibre shortening velocity and minimise muscle activation (Lichtwark and Wilson, 2008). In children with spastic CP, a greater muscle to tendon stiffness ratio may partly explain the high mechanical energy cost and greater mean energy expenditure experienced during walking (Rose et al., 1990; Olney et al., 1987).

We also showed that tendon stiffness, when expressed relative to each participant’s force generating capacity, was not different between TD and CP. This finding is consistent with Barber et al., (2012), who demonstrated no differences in Achilles tendon stiffness, measured in absolute terms, in children with CP compared to TD children. To understand differences in the mechanical properties of the tendon, however, it is necessary to also quantify tendon stiffness over a common force range. Further, normalising tendon stiffness by its dimensions allows us to tease out eventual contributions of dimensions and material properties to tendon
stiffness in CP. Therefore, the second specific aim was to compare dimensions and material properties of the Achilles tendon between children with spastic CP and TD children. Our results demonstrate that absolute Achilles tendon length was not significantly different between children with CP and TD children. This result is in contrast to the findings by Barber et al. (2012) and Gao et al. (2011), and is likely to be explained by between subject differences in limb lengths. Support for this explanation is provided by our results that when expressed as a percentage of muscle-tendon unit length, the Achilles tendon was significantly longer in CP compared to TD children. The result that children with spastic CP had a smaller tendon cross-sectional area is consistent with the findings of Gao et al., (2011). A possible explanation for this finding is that tendon dimensions in children adapt in line with bone growth, which potentially develops slower in children with CP, due to a lack of weight-bearing activity (Samson-Fang and Stevenson, 1998). As a result of the relatively longer tendons and the smaller cross sectional area, we expected the tendon in CP to be more compliant than in TD children. However, we did not find a significant difference with an effect size of 0.48. It appears that the dimensional differences were not strong enough to elicit significant changes in tendon stiffness. Additionally, eventual dimension-related decreases in tendon stiffness were partially compensated for by a reverse change in Young’s Modulus (ES = -0.25), suggesting a different mechanism for alterations in the tendon’s material properties. The fact that Young’s modulus was not different between the groups suggests that the integrity of the tendon’s material properties is unaffected in children with spastic CP. This suggests that in spite of being weaker, the muscle provides the tendon with a sufficient loading stimulus so that the integrity is unaffected. However, it could also be the case that concomitant alterations in the microstructure of the tendon resulted in no overall observed changes in the material properties of the tendon in CP compared to TD children. These results are in line with the fact that a large portion of CP children in this study were able to fully
weight bear (GMFCS III). Future studies should investigate more severely affected children (GMFCS IV and V) who are unable to fully weight bear, to see if differences in tendon structure are observed due to a lack of mechanical loading.

The third specific aim of the study was to describe the strain-rate response of the Achilles tendon in children with spastic CP compared to TD children. Both groups showed an increase in absolute tendon stiffness with increasing strain-rate. This is consistent with previous studies of adults in both the patellar tendon (Pearson et al, 2007) and the Achilles tendon (Theis et al., 2012). In addition, we found that the slope of the strain-response was steeper in the CP group, indicating that at higher strain-rates (i.e., \(30^\circ \cdot s^{-1}\)), tendon stiffness was greater in children with spastic CP compared to TD children. This result is indicative of alterations in the tendon’s material properties, which could alter the viscoelastic response of the tendon. These results have important implications for the clinical test of spasticity. The fact that at \(30^\circ \cdot s^{-1}\) tendon stiffness is markedly higher in children with CP compared to TD children needs to be taken into consideration when performing clinical tests, to ensure that spasticity is not over-estimated.

It is important to recognise the limitations of the derivation of “muscle stiffness”. Firstly, torque measured at the ankle is not only attributable to the triceps surae muscle-tendon unit, but also to other passive structures. Second, our calculation of stiffness does not account for potential different contributions of the gastrocnemii and soleus to the total torque. It further assumes that all plantaflexor muscles lengthened uniformly during the passive dorsiflexion. In children with spastic CP, Barber et al. (2011) reported that soleus elongation was similar to that of the medial gastrocnemius; however, we cannot rule out the possibility of some systematic error in the calculation of muscle stiffness. Lastly, modelling the muscle as a
straight line does not take the actual individual muscle paths into consideration. From these limitations, it becomes clear that our measure of muscle stiffness is, to a certain extent, a theoretical construct. However, we believe that within the context of this study our measure of “global muscle stiffness” provides important insights into the interactions between CP-related differences in muscle and tendon properties.

In conclusion, our findings provide us with a more differentiated understanding of the tendon’s mechanical properties in children with spastic CP, which need to be taken into consideration when interpreting and treating movement abnormalities in this population. The fact that strain-rate-induced increases in tendon stiffness are more pronounced in children with CP compared to TD children have important consequences for the interpretability of current clinical tests of spasticity.

**Conflict of interest**

None of the authors have any commercial or other interests that create conflict of interest for the work presented here.
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Waugh


