Does long-term passive stretching alter muscle-tendon unit mechanics in children with spastic cerebral palsy?

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Abstract

Background: Cerebral Palsy causes motor impairments during development and many children may experience excessive neural and mechanical muscle stiffness. The clinical assumption is that excessive stiffness is thought to be one of the main reasons for functional impairments in cerebral palsy. As such, passive stretching is widely used to reduce stiffness, with a view to improving function. However, current research evidence on passive stretching in cerebral palsy is not adequate to support or refute the effectiveness of stretching as a management strategy to reduce stiffness and/or improve function. The purpose was to identify the effect of six weeks passive ankle stretching on muscle-tendon unit parameters in children with spastic cerebral palsy.

Methods: Thirteen children (8-14 y) with quadriplegic/diplegic cerebral palsy were randomly assigned to either an experimental group (n=7) or a control group (n=6). The experimental group underwent an additional six weeks of passive ankle dorsiflexion stretching for 15 minutes (per leg), four days per week, whilst the control group continued with their normal routine, which was similar for the two groups. Measures of muscle and tendon stiffness, strain and resting length were acquired pre and post intervention.

Findings: The experimental group demonstrated a 3° increase in maximum ankle dorsiflexion. This was accompanied by a 13% reduction in triceps surae muscle stiffness, with no change in tendon stiffness. Additionally, there was an increase in fascicle strain with no changes in resting length, suggesting muscle stiffness reductions were a result of alterations in intra/extra-muscular connective tissue.

Interpretation: The results demonstrate that stretching can reduce muscle stiffness by altering fascicle strain but not resting fascicle length.
1. Introduction

Children with spastic cerebral palsy (CP) experience significant alterations in soft tissue structures within the muscle-tendon unit (MTU), secondary to neurological impairment (Alhusani et al., 2010; Barber et al., 2011; Tardieu et al., 1988). Specifically, a shortening of the muscle through a loss of both in-series (Mohagheghi et al., 2008) and in parallel (Shortland et al., 2002) sarcomeres, along with an increase in the accumulation of connective tissue (Williams and Goldspink, 1973; 1984; Williams 1988) can all contribute to greater MTU stiffness, which is often assessed as an increased resistance to stretch. Tendon stiffness may also be reduced (Couppe et al., 2012; de Boer et al., 2007) and thus not have sufficient resistance to counteract any increase in muscle stiffness. The overall increase in the stiffness of the MTU is likely to be a contributing factor in the development of contractures (Lannin and Ada, 2011; Herbert and Balnave, 1993; Herbert and Crosbie, 1997; Farmer and James, 2001) and subsequent loss of function (Eek & Beckung, 2008).

One main role of therapeutic intervention in CP is to oppose contracture (Farmer and James, 2001; Lieber and Bodine-Fowler, 1993). To this end, passive stretching has been a common method of rehabilitation for a number of years (Damiano, 2009; National Institute for Health and Care Excellence, 2012). Previous animal research has suggested that long-term stretch to oppose contracture may also reduce MTU stiffness. Two mechanisms have been proposed to be responsible for such a reduction in MTU stiffness. Firstly, it could be the case that chronic stretching could cause an increase in the number of in series sarcomeres (Williams and Goldspink, 1973; Williams, 1988; Tabary et al., 1972; Heslinga et al., 1995), which in turn would lead to an increase in the length of the muscle fibre (Williams and Goldspink, 1971). Secondly, MTU stiffness could be reduced through a reduction of accumulated connective tissue within the muscle (Williams and Goldspink, 1973; 1984; Williams, 1988).
Previous research investigating stretching in human populations has demonstrated increases in joint range of motion (ROM) (McPherson et al., 1984; Miedaner and Renander, 1987), and decreased joint stiffness in response to long-term stretching (Kubo, et al., 2002; McPherson et al., 1984; Nakamura et al., 2012; O’Dwyer et al., 1994). Often, such decreases in MTU stiffness are interpreted to be reflective of changes in muscle stiffness. However, MTU stiffness is influenced by several structures surrounding the corresponding joint, including muscles, tendons, ligaments and muscle fascia (Wright, 1973). Consequently, it is important to understand the mechanisms underlying any changes in MTU stiffness, in response to passive stretching.

In support of the notion that passive stretching could potentially decrease muscle stiffness, we have recently demonstrated that the spastic medial gastrocnemius muscle and fascicles in children with diplegic CP elongate following acute stretching (Theis et al., 2013). This finding provides us with a theoretical basis for the hypothesis that any stretch-induced increases in ROM and reductions in MTU stiffness could be accompanied by decreases in muscle stiffness. In addition, we have also shown the muscle to be stiffer than the tendon in CP. Therefore, although muscle and fascicle elongation does take place, the degree of stretch taken up by the muscle may not be sufficient to induce expected changes (e.g. increase in fascicle length, reduction in the accumulated connective tissue, etc.) in the mechanical properties of the MTU.

Our previous results also demonstrated that the Achilles tendon elongates following acute bouts of stretching (Theis et al., 2013). Since the tendon is a passive structure, applying any form of load over a long period (e.g., resistance training or stretching) could theoretically
result in an increase in tendon stiffness (Reeves et al., 2006). However, current evidence suggests that in contrast to resistance training, long-term, moderate duration stretching does not provide a sufficient stimulus to elicit any significant alterations in tendon stiffness in healthy individuals (Kubo et al., 2002; Kay et al., 2009). It is not clear whether tendons of spastic muscles respond in a similar fashion to long term stretching.

Understanding the effect of stretching on the spastic muscle, fascicles and its tendon will have important implications for the practice of stretching in spastic CP. Therefore, the purpose of this study was to investigate the effects of six weeks of passive stretching on ankle joint, triceps surae and Achilles tendon mechanical properties in children with spastic CP. We hypothesised that any reductions in joint stiffness would be accompanied by alterations in the mechanical properties of the muscle and fascicles, but not the tendon. An addition of sarcomeres in response to stretching would be expected to increase resting muscle fascicle length, whilst reductions in accumulated connective tissue would be expected to alter muscle and fascicle strain, but not resting length.
2. Methodology

2.1 Participants
Thirteen children with spastic CP (seven male, six female; mean age 10.3 (3.0 y)) participated in this study. Seven children had diplegic CP, and six children had quadriplegic CP. It was important to maintain a homogenous group so only children with both lower limbs affected were included in this study. Participants were recruited from a local school for children with disabilities. Children were identified by a physiotherapist as diplegic or quadriplegic, and had a clinical diagnosis of spasticity in the lower limbs. Children were randomly assigned to an experimental (n=7, diplegic CP = 4; quadriplegic CP = 3) or control group (n=6, diplegic CP = 3; quadriplegic CP = 3). Six participants were classified as GMFCS level III and seven were classified as GMFCS level IV as assessed by a physiotherapist. None of these children had received any form of lower limb surgery, 24 months prior to participation in the study, and none had received Botulinum toxin injections to the legs 4 months prior to participation. All children were wheelchair users, but were ambulant with the use of a walking aid. The study was approved by the institutional as well as the relevant local NHS Ethics Committees. The study was conducted in accordance with the Declaration of Helsinki. Written parental consent was obtained in addition to written assent from the children.

2.2 Experimental design
The experimental group completed a six week stretching programme in addition to their normal physical activity routine. The control group did not receive the additional stretching programme but continued with their normal routine. Normal routines of both groups were very similar in activity and volume, as all participants were recruited from the same centre. These routines consisted of approximately 3-4 hours per week of dynamic movement.
activities and use of a standing frame. Before and after the six week intervention period, all participants underwent two testing sessions. Pre-intervention data were collected 24-48 hours prior to the first stretching session. Post-intervention data were collected 48-72 hours after the final stretching session. During the pre- and post-testing sessions, we obtained data for the mechanical properties of the muscle, tendon and fascicles of the right leg. Six participants (three control group and three experimental group) were also tested again in a follow-up session, three weeks after the cessation of the stretching intervention.

2.2.1 Stretching programme design. Each participant in the experimental group received an ankle dorsiflexion stretch to both the right and left legs, applied by a clinician. Stretches were performed four days per week for six weeks. The stretch was performed for a total of 15 minutes on each leg, in 60 s repetitions followed by a 30 s rest period. These stretch durations and frequencies were chosen based on durations frequently used in clinical practice (Wiart et al., 2008).

The stretches were performed with the children seated in their wheelchair. To gain the initial stretch position, the leg was lifted and the knee was slowly guided into extension. To initiate a stretch of the triceps surae muscles, the clinician’s hand was cupped around the heel, with the palm of the hand flat against the foot. The knee was supported in an extended position and the ankle was slowly dorsiflexed, with pressure from the hand. Once in a maximal stretch position, the muscle was held for a period of 60 seconds.

2.2.2 Pre-, post- and follow-up data acquisition. During the pre-, post- and follow-up testing sessions, stiffness and strain of the medial gastrocnemius muscle and fascicles, and the Achilles tendon were measured. For this purpose, participants were seated on an
isokinetic dynamometer (Cybex Norm, Lumex, Ronkonkoma, NY, USA). The relative hip angle was set to 85 deg and the knee was straightened as much as possible, which was determined to be 7 (2) deg from full extension. The lateral malleolus of the right ankle was aligned with the centre of rotation of the dynamometer arm. Stabilisation straps were firmly tightened over the foot, thigh and chest. Three infrared LED motion capture cameras (Motion Analysis, Santa Rosa, USA) were positioned on one side of the dynamometer. Reflective markers were placed on the head of the first metatarsal, the medial malleolus, the calcaneus, the medial femoral epicondyle. In addition, two markers were placed on the handle of the ultrasound probe. All kinematic data were filtered using a low-pass, fourth-order, zero-lag Butterworth filter with a cut-off frequency of 5 Hz.

We initially determined each participant’s ROM, by manually dorsi- and plantarflexing the foot, until any discomfort was reported. A rotation of the right ankle joint at 10 deg·s\(^{-1}\) was then applied through the ROM. This angular velocity was chosen to avoid any stretch reflex activity in the spastic muscle (Thilmann et al., 1991). Participants were instructed to relax the muscles of the lower limb as much as possible during this time, while three continuous rotations were recorded. Muscle activity from the medial gastrocnemius and tibialis anterior muscles were monitored throughout the passive rotation using EMG to ensure muscle activity was not evoked by the rotations. EMG data of the tibialis anterior and the medial gastrocnemius muscles (EMGworks, Delsys Inc., Ltd., Boston, USA) as well as torque data from the dynamometer were collected 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. Data for all participants at each time point (pre-, post- and follow-up) were analysed after the final follow-up testing session.
Average joint stiffness was calculated as the slope of the torque-angle curve, between 20% and 80% of each participant’s peak torque. This interval was chosen so as to avoid the “toe region” at the lower end of the stiffness curve, and also to minimise the contribution of passive elastic structures such as ligaments, connective tissue and skin at the upper end of the stiffness curve (Abellaneda et al., 2009). This interval provided reliable stiffness data across trials in children with CP (coefficient of variation = 5.7%).

2.3 Data analysis

2.3.1 Muscle and Tendon Stiffness. Muscle and tendon stiffness were calculated from the slope of the torque-elongation curves, in the same range as joint stiffness, which corresponded to 20% and 80% of each participant’s peak torque. Achilles tendon stiffness was calculated as the change in passive ankle torque divided by the corresponding change in Achilles tendon length. An estimate of “total triceps surae muscle stiffness” was also derived from the data by dividing passive ankle torque and elongation of the medial gastrocnemius muscle. We deemed this as an appropriate measure of the total stiffness, which is experienced by the individual and can influence the control of movement. However, it is important to note that “muscle stiffness” measured in this way is a theoretical construct and does not solely represent stiffness of the medial gastrocnemius muscle. Rather, stiffness is attributable to all of the triceps surae muscle-tendon unit and other passive elastic structures – the contribution of which cannot be measured in vivo (Morse, et al., 2008). Since the medial gastrocnemius contributes substantially to plantarflexor force production with an extended knee angle (Cresswell et al., 1995), we considered it functionally important, and therefore, a more appropriate choice than the soleus to represent any pre to post intervention changes in this study.
2.3.2 Muscle and tendon length. For the calculation of muscle and tendon stiffness, elongation of the medial gastrocnemius muscle and Achilles tendon were measured, respectively. This was done by tracking the displacement of the gastrocnemius muscle-tendon junction, using B-mode ultrasonography (Megas GPX, Esaote, Italy; 45 mm Linear array probe, 10 MHz transducer scanning), captured at 25 Hz. A layer of water-based gel (Henley’s Medical, Hertfordshire, UK) applied between the ultrasound probe and skin enhanced acoustic transmission. The probe was placed perpendicularly to the skin surface above the muscle-tendon junction and orientated to reveal a line running between the aponeuroses of the medial gastrocnemius and soleus muscles. The probe was then fixed in position using a custom made holder. A 2 mm wide strip of echoabsorptive tape placed on the skin in contact with the probe provided a reference to which any probe movement could be identified. The 2D coordinates of the muscle-tendon junction were obtained by manual digitisation (Peak Performance, Cambridge, UK). Digitised muscle-tendon junction position data for both methods were filtered using a low-pass fourth-order zero-lag Butterworth filter with a 3.25 Hz cut-off frequency.

The lengths of the medial gastrocnemius muscle and Achilles tendon were combined with the ultrasound and motion analysis data as described previously (Theis et al., 2013). Specifically, the position of the muscle-tendon junction was calculated by combining coordinates of the handle of the ultrasound probe, with muscle-tendon junction coordinates obtained from the ultrasound images; to give the position of the muscle-tendon junction with respect to the global coordinate system of motion analysis. Medial gastrocnemius muscle length changes were calculated as the distance from the medial epicondyle marker to the global muscle-tendon junction marker, and the Achilles tendon changes, as the distance from the muscle-tendon junction to the calcanei, using custom written analysis software (Matlab v7.14,
MathWorks, Cambridge, UK). Medial gastrocnemius muscle and Achilles tendon resting lengths were defined as the corresponding lengths at 100% plantarflexion. The individualised ankle angle was chosen to account for the varying degrees of plantarflexion between participants. These resting lengths were used for the subsequent calculation of muscle and tendon strain, which was calculated by dividing elongation at maximum dorsiflexion, by resting length.

2.3.3 Fascicle strain. Medial gastrocnemius muscle fascicle strain was calculated by dividing change in fascicle length by fascicle resting length, which was quantified using open source digital measurement software (Image J, NIH, USA). These fascicle measurements were made at the mid-belly of the muscle. Three optimal and identifiable fascicles were selected and measured from deep to superficial aponeurosis. An average of the three fascicles was used for the subsequent analysis of resting fascicle length (coefficient of variation = 4.7%), taken with the ankle at 100% plantarflexion. For each participant, fascicle strain was calculated at maximum dorsiflexion (coefficient of variation = 6.8%).

Mechanical properties of the medial gastrocnemius and Achilles tendon were compared pre- to post-intervention, for both the experimental group and the control group. Muscle and tendon stiffness in six participants (three control and three experimental) was also obtained again during the follow-up session, three weeks after the cessation of the intervention period.

2.4 Statistical analysis
To assess the effect of stretching on joint, muscle and tendon mechanics, we performed mixed design ANOVAs with repeated measures on each dependent variable (maximum dorsiflexion angle, joint stiffness, muscle stiffness, muscle strain, fascicle strain, tendon
stiffness, tendon strain). Specifically, we tested for group (experimental vs. control) × time (pre- vs. post-intervention) interactions for each variable. In case of a significant interaction effect, follow-up t-tests with Bonferroni correction were used to detect pre- to post-differences for each group.
3. Results

Compliance to the stretching intervention in the experimental group was 99.4%, with just one participant from the experimental group missing one session. The first ANOVA revealed a significant group × time interaction effect on maximum dorsiflexion angle ($F_{1, 11} = 38.90, p < 0.001$). These results show that maximum dorsiflexion angle increased from pre- to post-intervention in the experimental group, but not in the control group (Figure 1).

Figure 1.

3.1 Joint stiffness

Results for joint stiffness indicated a significant group × time interaction effect ($F_{1, 11} = 43.00, p < 0.001$). Joint stiffness decreased significantly post-intervention in the experimental group but not in the control group (Figure 2).

Figure 2.

3.2 Muscle stiffness, strain and fascicle strain

There was also a significant time × group interaction effect for muscle stiffness ($F_{1, 11} = 57.73, p < 0.001$). Muscle stiffness decreased from pre- to post-intervention in the experimental group, whilst in the control group, muscle stiffness increased during the intervention period (Figure 3). For muscle strain there was a significant interaction effect between time and group ($F_{1, 11} = 35.54, p < 0.001$). Muscle strain increased from pre- to post-intervention in the experimental group. For fascicle strain there was also a significant interaction effect ($F_{1, 11} = 235.64, p < 0.001$). From pre- to post-intervention, fascicle strain increased in the experimental group and decreased in the control group (Figure 4). There was no change in resting fascicle length from pre- to post-intervention ($F_{1, 11} = 0.66, p = 0.44$).
3.3 Tendon stiffness and strain

No significant changes in tendon stiffness were observed in either group, pre- to post-intervention ($F_{1, 11} = 0.028, p = 0.86, \eta^2 = 0.03$) (Figure 3). In addition, there were no significant changes in tendon strain in either the experimental or control group, pre- to post-intervention ($F_{1, 11} = 2.69, p = 0.13, \eta^2 = 0.20$).

3.4 Findings from follow-up

To assess whether any effects of stretching were maintained after the cessation of the stretching intervention, three participants from the experimental group and three from the control group also took part in one further follow-up session, during which we measured muscle and tendon stiffness. In the subset of experimental participants, muscle stiffness was still reduced four weeks after the intervention compared to baseline; however the values were closer to the baseline values (Figure 5). For tendon stiffness, no considerable differences were observed before and after the intervention (Figure 5). For the control group, there was also no considerable change in muscle and tendon stiffness from pre- to post-intervention, or from post-intervention to the follow-up session (Figure 5).
4. Discussion

The overall purpose of this study was to examine the mechanical adaptations resulting from a six week passive stretching intervention of the triceps surae muscle-tendon unit in children with spastic CP. Our findings demonstrated that six weeks of passive stretching increased maximal passive dorsiflexion angle and also reduced joint stiffness. Additionally, there was a reduction in muscle stiffness, with concurrent increases in muscle and fascicle strain. No changes to the mechanical properties of the tendon and resting length of the fascicles were found following stretching.

The stretching intervention elicited an increase in maximal passive ankle dorsiflexion from 6 deg to 9 deg, accompanied by a 32% decrease in passive joint stiffness. This finding has previously been reported in healthy populations following stretching (Kubo et al., 2002; Nakamura et al., 2012). Nakamura et al. (2012) reported a 13% reduction in passive joint stiffness following four weeks (120 s per day). Similarly, in children with spastic CP, O’Dwyer et al. (1994) showed a reduction in passive joint stiffness after 30 minutes of stretching, three times per week for six weeks, which is consistent with the findings from this study.

The most significant findings in the present study were that changes in joint stiffness were accompanied by a 12% reduction in muscle stiffness, confirming the clinical assumption in this regard. These are the first data to show a clear tissue-dependent response to stretching exercise in this population. Importantly, they indicate that the spastic muscle can respond to long-term stretching. It should be noted that the measures of muscle stiffness made in this study are somewhat conceptual. “Muscle stiffness” as measured here, contains not only stiffness of the triceps surae muscles, but all surrounding tissues, the contribution of which
cannot be known. Despite this limitation, the findings of “muscle stiffness” are in line with the finding of a concurrent reduction in joint stiffness and no alterations in tendon stiffness. A lack of alteration in the tendon’s stiffness suggests that passive stretching does not provide an effective stimulus to alter the mechanical properties of the tendon in children with spastic CP. This is consistent with previous research in healthy populations (Kubo et al., 2002; Mahieu et al., 2007).

The changes in muscle stiffness were accompanied by changes in muscle and fascicle strain. On average, we observed an increase in muscle (23%) and fascicle strain (13%) post-stretch in the experimental group. The present results are of substantial importance, showing that the muscle offers a greater plasticity in response to passive stretching than the tendon. The results from the follow-up session showed a trend towards increased muscle stiffness towards baseline, three weeks after the intervention, although some reduction in stiffness compared to pre-intervention did still exist.

The factors which contribute to muscle and joint stiffness in CP are not fully understood, but given that a decrease in muscle stiffness was observed after six weeks of passive stretching, it is interesting to speculate as to the possible mechanisms underpinning the change. One mechanism is a change in the mechanical properties of the series elastic component. Although no changes were observed in the tendon, it cannot be ruled out that changes in other tissues occurred such as changes in myofilaments or titin (Prado et al., 2005). Changes may also have occurred within the parallel elastic component. The endomysium, perimysium and epimysium are thought to substantially influence passive resistance to stretch (Gajdosik, 2001), although it is not possible to directly measure these in humans. Given that muscle strain showed a greater change post-stretch, than the fascicle, changes are likely to have
occurred in the parallel elastic component and intra-muscular connective tissue, which is shown to contribute to increased passive stiffness in CP (Smith et al., 2011). In addition, fascicle strain also increased suggesting some alterations in intra-fascicular structures (Prado et al., 2005), fibre-based connective tissues (Prado et al., 2005), or the number of serially-arranged sarcomeres within fibres (Tabary et al., 1981). Of these, a change in in-series sarcomere number is often speculated to underpin changes in muscle or fascicle extensibility (Gajdosik, 2001) and, in fact, increases in sarcomere number were observed after long-term, intense muscle strain was imposed by tibial lengthening in humans (Boakes et al., 2007). Nonetheless, the present data are not consistent with this finding because there was no change in absolute resting fascicle length. In the absence of firm evidence in this and other studies, it suggests that this volume of passive stretching was not sufficient to alter sarcomere number in children with spastic CP.

Neural factors could contribute to the reduction in stiffness. For example, a reduction in stretch reflex gain or an increase in the threshold of the stretch reflex could theoretically reduce muscle stiffness, although there has been no conclusive evidence of a change in these parameters in CP following stretching. More frequently, alterations in stretch tolerance has been reported involving a change in the perception of stretch. However, given that our post-measurements were made at least 48 hours after the last stretch session, and the fact that some reductions in stiffness could still be observed three weeks after the intervention, suggests that at least some of the alterations seen here were a result of changes in the mechanical properties of the muscle and fascicles.

Collectively, the results of this study confirm the clinical assumption that passive stretching can reduce joint stiffness for children with CP. Moreover, the present study shows for the
first time that such reductions were associated with changes in the mechanical properties of muscle but not the tendon. This is an important finding, which suggests that application of currently employed long term stretching can potentially affect spastic MTU function by reducing muscle stiffness. Future research should specifically focus on applying these findings in clinically relevant contexts.

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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Figure 1. Maximum dorsiflexion angle in the experimental and the control groups, at pre- and post-intervention.

Figure 2. Change (Δ) in joint stiffness pre- to post-intervention in the experimental and control groups (values are mean (SD), **p < 0.01).

Figure 3. Change (Δ) in muscle and tendon stiffness pre- to post-intervention in the experimental and control groups (values are mean (SD), **p < 0.01).

Figure 4. Changes in muscle strain (left), fascicle strain (right) in the experimental group and control groups, pre- to post-intervention (values are mean (SD), **p < 0.01, *p < 0.05).

Figure 5. Changes in muscle stiffness (left) and tendon stiffness (right) in the experimental group and control group, post-intervention and follow-up (values are mean (SD), *p < 0.05).
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