No change in calf muscle passive stiffness after botulinum toxin injection in children with cerebral palsy

ADEL A A ALHUSAINI[1] | JACK CROSBIE[1] | ROBERTA B SHEPHERD[1] | CATHERINE M DEAN[1] | ADAM SCHEINBERG[2]

1 Clinical Rehabilitation Sciences Research Group, Faculty of Health Sciences, The University of Sydney, Lidcombe, NSW, Australia. **2** Department of Rehabilitation, The Children's Hospital at Westmead, NSW, Australia.

Correspondence to Dr Jack Crosbie at Faculty of Health Sciences, The University of Sydney, PO Box 170, Lidcombe, NSW 1825, Australia. E-mail: jack.crosbie@sydney.edu.au

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AIM Stiffness and shortening of the calf muscle due to neural or mechanical factors can profoundly affect motor function. The aim of this study was to investigate non-neurally mediated calf-muscle tightness in children with cerebral palsy (CP) before and after botulinum toxin type A (BoNT-A) injection.

METHOD Sixteen children with spastic CP (seven females, nine males; eight at Gross Motor Function Classification System level I, eight at level II; age range 4–10y) and calf muscle spasticity were tested before and during the pharmaceutically active phase after injection of BoNT-A. Measures of passive muscle compliance and viscoelastic responses, hysteresis, and the gradient of the torque–angle curve were computed and compared before and after injection.

RESULTS Although there was a slight, but significant increase in ankle range of motion after BoNT-A injection and a small, significant decrease in the torque required to achieve plantigrade and 5° of dorsiflexion, no significant difference in myotendinous stiffness or hysteresis were detected after BoNT-A injection.

INTERPRETATION Despite any effect on neurally mediated responses, the compliance of the calf muscle was not changed and the muscle continued to offer significant resistance to passive motion of the ankle. These findings suggest that additional treatment approaches are required to supplement the effects of BoNT-A injections when managing children with calf muscle spasticity.

Cerebral palsy (CP) is a developmental disorder of movement and posture which limits activity and is often accompanied by disturbances of sensation, cognition, communication, perception, and/or behaviour.[1] Spastic CP, either of hemiplegic or diplegic presentation, is commonly associated with hyperreflexia, and the predominant motor impairments of weakness and disordered motor control result from defects in corticospinal (pyramidal) pathways in the developing brain. Also notable are adaptive changes in muscles and other soft tissues that occur over time and are associated with movement limitations.

Calf muscle stiffness, of either neural or mechanical origin, affects motor characteristics in children with CP. Premature and excessive activation of calf muscles, with consequent ankle plantarflexion, has been reported during the stance and terminal swing phases of walking.[2] However, such gait disturbances are not based solely on neural factors. Increased tension of the triceps surae during stance, without associated increase in electromyographic (EMG) activity, has been reported.[3] Reduced dorsiflexion during swing phase has been observed in individuals with spasticity and rigidity, resulting not from abnormal plantarflexor stretch reflex activity nor from recruitment failure of the tibialis anterior muscle, but rather from an increase in passive resistance in the plantarflexors.[4] Increased passive muscle torque can also decrease forward rotation of the tibia over the foot during gait[5] and may change the amount of elastic energy stored in and returned from the soft tissues, potentially impairing gait efficiency.[6]

Injections of botulinum toxin type A (BoNT-A) are often a first-line treatment for focal spasticity involving overactive muscle groups;[7] plantarflexor muscles are the most commonly targeted in children with CP.[8] Few trials have found any significant improvement in the activity and participation domains, as indicated by the International Classification of Functioning, Disability and Health, after BoNT-A injections.[9] However, there is no doubt about the well-documented ability of BoNT-A to reduce spasticity.[10] Legerlotz et al.[11] have also reported histological changes in muscle after BoNT-A injection that may produce changes in the viscoelastic properties, altering the compliance and extensibility of the target muscle. Nevertheless, no investigation has specifically considered the potential for BoNT-A to influence passive muscle characteristics. Tedroff et al.[10] have reported that although BoNT-A can be effective in reducing spasticity over a long period, it does not appear to prevent the development of contractures in

spastic muscles, indicating that contracture development is not solely attributable to increased spasticity.

In testing spasticity, clinicians and researchers frequently group the passive mechanical properties together with the neurally mediated phenomena, referring to increased resistance to passive movement as spasticity. However, it is important to distinguish between reflex and non-reflex-mediated stiffness to understand and evaluate reliably the mechanisms of spasticity because this information may have consequences for treatment.[12] It is also important to distinguish between muscle stiffness and joint flexibility, which are different but frequently confused.[13] It is common to infer muscle length and extensibility from joint range of motion, regardless of the amount of force required to cause a change in length. Passive muscle stiffness, however, reflects the resistance of the myotendinous unit to a force that attempts to change its length. The greater the stiffness, the more force required. Stiffness is quantified mechanically by measuring the rate of change of the joint angle with respect to change in applied torque.[14] Therefore, joint flexibility is a component of, but is not the same as, stiffness. In a recent study, we found that the plantarflexor muscles of children with CP were three times stiffer and intrinsically more resistant to stretch than in typically developing children, and that this increased stiffness was non-neurally mediated.[15] Further, these children also exhibited greater stretch-shortening energy loss within the muscle, as demonstrated through substantially greater hysteresis compared with typically developing children. No investigations have explored the effect of intramuscular BoNT-A injections on these characteristics of the muscle, yet it is not certain that any changes to joint flexibility are matched by changes to intrinsic stiffness or hysteresis.

The purpose of the present study was to test the hypothesis that the passive mechanical properties of the calf muscle after BoNT-A injection would exhibit no significant reduction compared with their pre-injection status.

What this paper adds

- Passive intrinsic muscle stiffness and hysteresis were not decreased after injection with BoNT-A.
- Increased range of motion may not indicate reduction in passive stiffness.
- Future interventions may need to focus more on the passive mechanical properties of muscle and not just motoneuron excitability.

METHOD

Participants

We recruited participants through the Child Assessment Centre of The Children's Hospital at Westmead, New South Wales, Australia (Table I). Informed consent was obtained from each child's parent or guardian. The Human Ethics Committees of both the hospital and the University of Sydney approved the study. Over a 2-year period, 16 children with spastic CP (seven females, nine males) were recruited for BoNT-A treatment. All participants were aged between 4 and 10 years and able to walk unaided. Participants were consecutively recruited based on inclusion and exclusion criteria and their willingness to participate. Inclusion criteria for children with CP were the following: diagnosed with spastic CP, affecting one or both lower limbs; able to walk independently on level ground (Gross Motor Function Classification System level I or II); undergoing treatment for gait impairment using BoNT-A injections into the triceps surae. Exclusion criteria were as follows: cognitive problems that could hinder communication or cooperation; unrelated musculoskeletal problems, such as recent acute injury, or congenital structural deformity (e.g. talipes equinovarus) interfering with ankle movement; using systemic anti-spasticity medications or had received phenol injections to lower limbs; previous orthopaedic procedures to lower limbs (soft tissue or bony surgery) or other management of spasticity (dorsal rhizotomy or intrathecal baclofen).

Design

The study was a single-group, prospective, open-label trial with repeated measures. The clinical effect of BoNT-A is generally seen at less than 1 week, works optimally at 1 month, and will go on to produce a clinical effect up to 4 to 6 months.[7] Therefore, we arranged that baseline measurements were taken 1 hour to 1 week before BoNT-A injections; participants were retested 6 weeks after injection, within the 'chemically active' period, to ensure that the muscle was most compliant.

BoNT-A (Botox; Allergan Inc., Irvine, CA, USA) was prepared using the standard procedure and injected at multiple sites in the triceps surae muscle following standard guidelines.[16] The mean dose of BoNT-A was 4.3 units/kg body mass (SD 1.1). The BoNT-A was diluted to a concentration of 50 U/mL saline. The number of sites chosen for injection depended upon the size of muscle mass, but was mainly applied to two: the medial head of the gastrocnemius and the soleus muscle, in each affected leg. The procedure was supported by inhaled nitrous oxide for sedation and topical prilocaine (EMLA; AstraZeneca, London, UK) cream applied at the site of injection 30 to 60 minutes before the procedure.

Data collection

Computation of range of motion, muscle stiffness, and hysteresis followed a previously described protocol.[15,17] Briefly, this involved using a specially constructed, adjustable ankle measurement device. The device consisted of a footplate hinged to

Table I: Characteristics of participants

	Height, cm Mean (SD)	Body mass, kg Mean (SD)	Age at initial testing (range)	Side tested
GMFCS level I				
Male (*n*=4)	129 (9)	27 (8)	7y 10mo (6y 3mo–10y 1mo)	3 R; 1 L
Female (*n*=4)	119 (12)	26(5)	6y 2mo (4y 10mo–8y)	1 R; 3 L
GMFCS level II				
Male (*n*=5)	124 (16)	24 (7)	7y (4y 4mo–10y 2mo)	1 R; 4 L
Female (*n*=3)	118 (15)	28 (10)	5y 7mo (4y 5mo–7y 8mo)	1 R; 2 L

GMFCS, Gross Motor Function Classification System; R, right; L, left.

a support bracket for the lower leg (Fig. 1), with a standard load cell (X-Tran, 450N; Applied Measurement Australia Ltd, Sydney, NSW, Australia) mounted perpendicular to the footplate, which measured the force applied manually to the footplate during rhythmic passive rotation of the ankle between fully plantarflexed and fully dorsiflexed positions. A handle was attached to allow manual oscillation of the footplate. The load transducer measured uniaxial loading (only one component of the force), so errors were possible owing to the application of 'off-axial' forces; however, these would have been small and we were vigilant in maintaining the angle of force application. Oscillation was conducted at a target speed of 60° per second (to avoid reflex contraction). Passive ankle torque was then computed from the product of applied force and the perpendicular distance from the fixed point of application of the force to the axis of rotation of the footplate. Electrical activity in the ankle dorsiflexor and plantarflexor muscles was monitored at a frequency of 3000 Hz using EMG (Telemyo 2400R G2 system; Noraxon, AZ, Scottsdale, Arizona 85254 USA) to ensure there was no increase in active or reflexive muscle recruitment from the baseline value. We used disposable, self-adhesive silver/silver chloride bipolar surface electrodes (Kendall Medi-Trace Mini 130 Foam ECG Electrodes; Neurotronics, Randwick, NSW, Australia), placing pairs of electrodes parallel to the muscle fibre direction with minimal interelectrode distance. Before electrode placement, the skin was cleaned with isopropyl alcohol. The location of electrodes was based on contemporary recommendations.[18] EMG acquisition enabled monitoring of muscle activity during the test.

Range of motion was obtained by measuring the dorsiflexion and plantarflexion angles using a rotary potentiometer aligned with the axis of the talocrural joint. Sampling of torque and angle was performed at 125 Hz. Participants lay supine with their foot secured in the footplate with Velcro straps (Velcro Australia Pty Ltd, Hallam, Vic., Australia), the knee in a position of maximum available extension and light pressure applied by the researcher's (AA) hand on the thigh above the knee joint to ensure that knee position was maintained. The calf was free of contact and clear of all surfaces and structures.

Force, angle, and EMG data were collected simultaneously using a 16-bit analogue-to-digital converter (DAQCard-6036E; National Instruments, Austin, TX, USA). The application software (PhysioDAQXS version 3.0; The University of Sydney, Australia) consisted of a graphical user interface designed using Borland C++ builder. Access to the data was gained by using National Instruments call-back functions to retrieve data collected by the data acquisition card. The graphical user interface supported the collection, display, and storage of data in real time. The repeatability and linearity of the force and angle signals were confirmed before data collection.

High interrater and intrarater reliability has previously been demonstrated for the measurement of passive torque and ankle displacement using a similar technique (intraclass correlation >0.91).[19] The procedure has been shown to be highly responsive to change in characteristics of soft-tissue stiffness.[20] All children were instructed to keep their legs relaxed and to avoid assisting or resisting the motion during the sinusoidal rotation. During data analysis only those cycles with minimal EMG

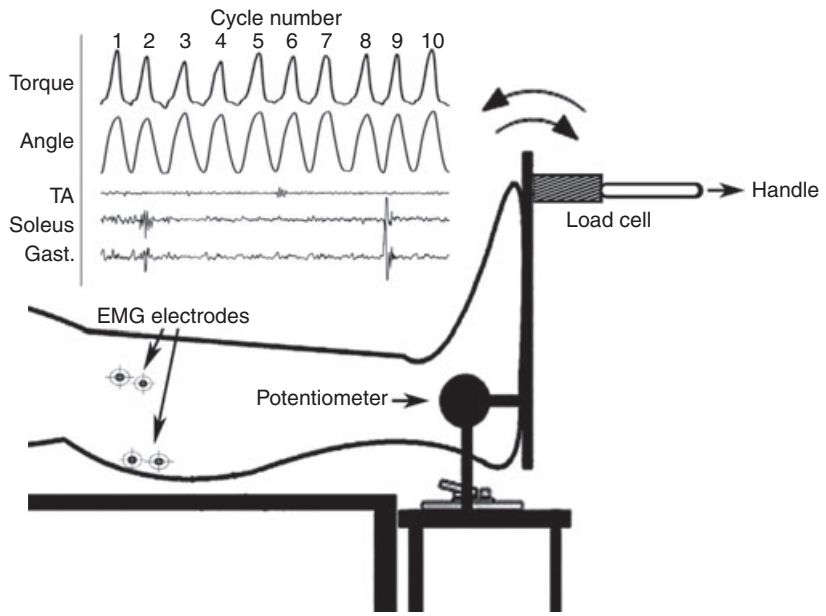

Figure 1: Ankle measurement device and experimental procedure. Electromyography (EMG) electrodes detect muscle activity in medial gastrocnemius, soleus, and tibialis anterior (TA). Applied torque is calculated from the product of the force measured through the load cell and the perpendicular distance to the axis of rotation (potentiometer). The lower leg is clear of the support surface. A typical trace is shown, illustrating 10 cycles of manual oscillation of the ankle. Applied torque is the upper trace and the resultant angular displacement the lower. EMG traces for TA, soleus, and gastrocnemius (Gast.) are shown. In this example, cycles 1, 2, and 9 were rejected because of calf muscle activity, and cycle 6 was rejected because of TA activity. The other cycles were suitable for analysis and the analyzed cycle was randomly selected.

activity (Spike2 software, version 2.09; Cambridge Electronic Design, Cambridge, UK), defined by visual inspection of the signal (Fig. 1), were chosen to ensure that there was no reflex contribution to total stiffness.

Data analysis

We collected force and angle data simultaneously as described previously. We calculated the torque due to the weight of the footplate as a function of the angle, and computed the net torque from the difference between applied torque and footplate effect.

The following variables were computed and tested before and after intervention. (1) Range of motion from maximum dorsiflexion and plantarflexion recorded during passive oscillation of the ankle (total passive range and maximum passive dorsiflexion angle). (2) Net torque required to move the ankle to predetermined dorsiflexion angles (0°, 5°, and 10°), scaled by dividing by body weight and leg length. (3) Passive stiffness of the calf muscles, determined through the gradient of the loading curve (between 0° and 5° dorsiflexion, and between 50% and 75% of the torque required to achieve full available dorsiflexion). (4) Hysteresis, expressed as the area enclosed between the loading and unloading curves (in absolute terms and scaled against the total range of motion for each child).

Stiffness values represent a measure of the intrinsic resistance to stretch of the muscle and its associated soft tissues. Hysteresis is a measure of the mechanical energy absorbed by the muscle, and, therefore, lost to elastic recoil, during the cycle.

Statistical analysis

A paired *t*-test was used to compare means on the same participant over time for the above variables. The alpha level for statistical significance was set at 0.05. Data were examined using Kolmogorov–Smirnov test to ensure normal distribution.

RESULTS

Table II shows the primary outcome variables and the effect of BoNT-A. The sinusoidal movement speed was 59° per second (SD 14) and 58° per second (SD 16) for pre- and post-BoNT-A testing, respectively, with no significant difference between the two. The sinusoidal nature of the motion resulted in a maximum angular velocity during the pre-BoNT-A test of 115° per second (SD 18) and 123° per second (SD 16) during the post-BoNT-A test; these differences were not statistically significant. All variables tested for differences between pre- and post-BoNT-A were found to be normally distributed, as were the difference values themselves.

Table II: Primary outcome variables and the effect of botulinum toxin type A (BoNT-A)

Variables	Pre BoNT-A Mean (SD)	Post BoNT-A Mean (SD)	*p*
Total range of motion (°)	64 (12.5)	68 (12.7)	0.037[a]
Maximum dorsiflexion angle (°)	10 (12.3)	13 (11.8)	0.142
Torque at 0°	9.9 (2.8)	8.9 (2.2)	0.012[a]
Torque at 5°	11.9 (3.8)	11.1 (3.3)	0.022[a]
Torque at 10°	13.2 (4.4)	12.5 (3.8)	0.19
Stiffness between 0° and 5° (×1000)	2.24 (0.98)	2.29 (0.97)	0.843
Stiffness between 50% and 75% of maximum torque	1.92 (0.75)	1.86 (0.91)	0.726
Hysteresis, Nm/degree	2.14 (1.16)	1.98 (0.71)	0.480
Scaled hysteresis	0.033 (0.017)	0.029 (0.01)	0.262

[a]Statistically significant result ($p<0.05$).

Range of motion

The overall range of motion from full plantarflexion to full dorsiflexion increased significantly after BoNT-A injection, but with an average change of only 4°. Despite this increase in total range of motion, maximum dorsiflexion was not significantly different after BoNT-A injection (Fig. 2). Using a comparable value for applied torque in each pair of data, the amplitude of ankle dorsiflexion was not significantly different.

Torques at predetermined joint angles

There was a significant decrease (about 10–11.8% of initial values) in the torques required to produce both the plantigrade position (0°) and 5° of dorsiflexion. However, there was no significant change when dorsiflexion was increased to 10°.

Passive mechanical stiffness

The intrinsic stiffness of the calf muscle and associated tissues did not change after BoNT-A injection, neither when calculated between 0° and 5° dorsiflexion nor when related to relative proportions of the torque required to produce full displacement (Fig. 2).

Hysteresis

There was no significant difference in either the absolute or the scaled values of hysteresis because of BoNT-A injection.

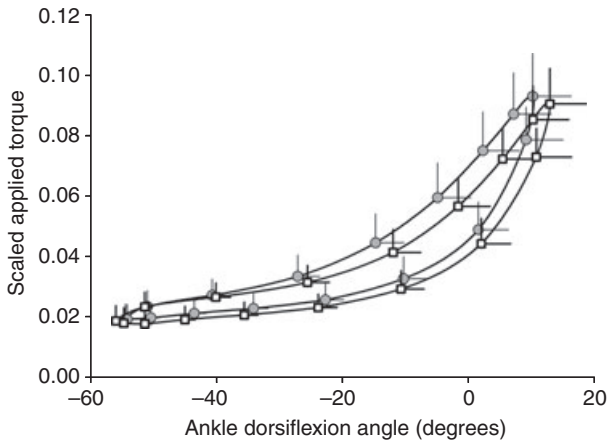

Figure 2: Ensemble-averaged curves for torque–angle relations before (filled circles) and after (open squares) botulinum toxin type A injection, showing one complete load–unload cycle averaged for all participants. Net torque values have been scaled by dividing by the product of the participant's weight and leg length because the children were of different physical size. Error bars represent positive 95% confidence intervals for torque and angle for the entire group (n=16).

DISCUSSION

To our knowledge this study is the first to quantify the effect of BoNT-A on the passive mechanical properties of the ankle plantarflexor muscles in children with CP. Our objective was not to investigate the effect of BoNT-A on spasticity, as this has previously been well documented; rather, it was to determine whether length, compliance, and energy recovery during stretch-shortening of the calf muscles changed after BoNT-A injection. Understanding the adaptive modifications in stiffness and length of the plantarflexor muscles occurring in early development in children with CP may help disclose their role during functional performance. The results of this study showed that although the children with mild CP had gained a slight increase in angular displacement after BoNT-A injection, passive intrinsic muscle stiffness and hysteresis in the plantarflexor muscles were not decreased. Our data before and after BoNT-A injection indicate concordance with an earlier study which indicated that children with CP exhibited around three times as much passive stiffness in the calf muscle than age-matched typically developing children.[15]

The total gain in dorsiflexion range was only about 2.5° on average and was not statistically significant. Commonly, the effect of BoNT-A injections on range of motion is reported to be of short duration, with contracture continuing to develop despite any reduction in stretch reflex hyperactivity. For example, Tedroff et al.[10] performed a prospective long-term follow-up study and reported a bilateral increase in dorsiflexion range of motion corresponding to 4 to 7° after the early injections. However, after an initial improvement following the early injections, range of motion decreased as soft tissues reverted to their original status and any additional range was lost. Such a lack of benefit after a good initial treatment response may explain why a substantial proportion of children with CP discontinue BoNT-A injections.[21] The significant 10 to 12% differences that we detected in the present study in initial values for the torque required to produce 0° and 5° displacement may be a direct response to the improved range of motion. BoNT-A injections have been reported to bring about some histological changes within the muscles. Dodd et al.[22] demonstrated a shift in myosin heavy-chain composition from faster to slower isoforms in rat muscle after injection with various doses of BoNT-A injections. Similarly, Legerlotz et al.[11] found that BoNT-A leads to slower myosin heavy-chain isoform composition and reduces titin content by 18% in juvenile rat gastrocnemius muscle. These histological reactions were associated with changes in the passive stiffness and hysteresis of the muscle. Similar changes might be found in children with CP, and might explain some of our findings; however, there appears to be little significant alteration in the stiffness characteristics.

In this study we attempted to mimic some elements of normal gait by using a sinusoidal movement that was similar in angular velocity and range of motion to the stance phase of walking,[23] while not stimulating reflex hyperactivity in the calf muscles. This was designed to partition the contribution of the passive elements but was not meant to reproduce all the characteristics of load-bearing gait. We found that the muscle continued to demonstrate a stiff and short response, even after BoNT-A injection, in keeping with the results of an investigation of ankle kinematics during walking.[24]

A limitation of this study is the small number of participants. However, when we explored the power of the analysis to determine the likelihood of type II error among our non-significant results, we discovered that, to find a statistically significant difference between the pre- and post-BoNT-A results for stiffness of the calf muscles (with α=0.05 and β=0.2), a sample in excess of 2750 would have been required. Similarly, to find a statistically significant difference in hysteresis values, a sample of 475 would have been required. Even in such cases, the 'clinical' effect would be less than 3% reduction in stiffness and 7% in hysteresis. On that basis, we do not feel that the sample size is a major threat to our results.

A further limitation is the difficulty in ensuring that each child received the same stretch velocity, given the fact that there were some differences in available range of motion. The aim of applying stretch at the same frequency, which could be standardized, meant that there could be some variance in the actual velocity of passive movement. However, these velocities were relatively low in all cases; some research has indicated that changes in the passive mechanical properties of the calf muscle would not be expected at angular velocities less than 180° per second, a value that we did not reach in any of our tests.[25] We were satisfied that any differences found in the tissue compliance in each individual child were not attributable to a difference in the rate at which the calf muscle was loaded and unloaded or to variation of the protocol.

The in vivo length of the plantarflexor muscles is difficult to measure; however, indirect measures of angular displacement in response to applied torque can substitute for the force–length variables.[17] Our protocol did not allow differentiation between the mechanical properties of the calf muscle and those of the Achilles' tendon and other connective tissues. Nor could we exclude the contribution of other tissues, such as skin, ligament, joint capsule, and cartilage. However, these last structures generally contribute to stiffness only at the end of the dorsiflexion range.[26] Because we measured the gradient of the loading curve between plantigrade and 5° of dorsiflexion, short of the limit of the participants' dorsiflexion, we believe that our values for stiffness exclude any substantive contribution of these ancillary tissues and that the myotendinous unit is the major impediment to passive ankle joint dorsiflexion.[27] Although the intention in this study was to measure the myotendinous unit stiffness, a recent study by Zhao et al.[28] has shown that the muscle belly, rather than the tendinous elements, offers greater resistance to passive stretching.

Further studies are needed to investigate the postnatal growth and development of calf muscles and the adaptive effects of the movement restriction, which results from motor impairments, on the muscle's passive mechanical properties (principally stiffness and hysteresis). Both of these adaptations would negatively affect the development of functional motor control. A better understanding of the adaptations and changes in the biomechanical properties of the myotendinous unit may help us gain insight into motor impairment in

children with CP, and this may facilitate the development of novel rehabilitation methods.

In conclusion, we have found that children with CP who received BoNT-A injected into the calf muscle displayed a slight increase in ankle range; however, the intrinsic stiffness of the calf muscle did not significantly change. Thus, although BoNT-A clearly reduces stretch hyperactivity, lack of response at the activity and participation levels may be related, among other things, to the unchanged intrinsic stiffness of the muscle. Further studies are required to determine the most functionally effective clinical method of minimizing the development of adaptive increases in intrinsic muscle stiffness. In addition, more studies are needed to measure the effect of BoNT-A on muscle morphology, growth, and development if we wish to optimize function in children with CP.

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