# Using Collagenase to Decrease Inherent Muscle Stiffness in Muscle Biopsies from Children with Cerebral Palsy

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## Summary

Cerebral palsy (CP) is associated with significant muscle stiffness, primarily due to increased collagen deposition. Collagen, a tough structural protein, can be cut with collagenase, an enzyme, to reduce stiffness and has safely done so in other collagen-dense conditions such as Dupuytren's and Peyronie's. Our study demonstrates that collagenase significantly decreases stiffness in isolated muscle bundles from children with CP without affecting the structural integrity or mechanical function of their fibers. These findings suggest that collagenase may provide a safe and effective therapeutic strategy for reducing muscle stiffness in CP.

## Introduction

Cerebral palsy (CP) is characterized by increased muscle stiffness, largely resulting from a dense extracellular matrix abundant in collagen [1]. Current clinical interventions, such as surgical release and botulinum toxin injections, offer only temporary relief and may cause adverse side effects. Thus, it is vital to identify a long-term treatment that decreases CP-associated muscle stiffness and maintains muscle contractile properties without introducing harmful side effects.

The dense extracellular matrix in CP is predominantly composed of collagen, which can be degraded by collagenase. This enzyme has been used as a treatment in collagen-dense conditions, such as Dupuytren's and Peyronie's contractures [2]. However, its potential to reduce collagen content, and consequently muscle stiffness, in CP has not been systematically investigated. Therefore, this study aimed to investigate the effect of collagenase on fascicle stiffness and fiber mechanical properties of muscle biopsies isolated from children with CP. We hypothesized that targeted collagenase incubation reduces fascicle stiffness, without altering the mechanical properties of single fibers.

## Methods

*Fascicle stiffness:* Muscle fascicles were isolated from 11 adductor longus biopsies from patients with CP. Each fascicle was mounted between a length controller and a force transducer on a mechanical testing system. Stress-relaxation tests were performed at strains ranging from 1%-7.5% before and after a 45-minute collagenase (350U/mL) incubation at 37°C. Peak and steady-state forces and stiffness were measured and normalized to fascicle cross-sectional area to obtain stresses and Young's modulus (stiffness).
*Fiber mechanical properties*: Skinned single fibers (2–4 per biopsy) were prepared from 9 semitendinosus and 1 semimembranosus biopsies from patients with CP. The fibers were chemically activated to achieve maximal force production (average sarcomere length ~2.4 µm, pCa 4.2) both with and without a 45-minute collagenase incubation at 37°C. Calcium sensitivity was assessed over a range of pCa values from 7.0 to 4.2. Fibers were stepwise stretched from a sarcomere length of 2.4-3.4µm (0.2µm/step). Outcome measures for fibers included active stress, pCa50, Hill coefficient, peak and steady-state passive stress.

## Results and Discussion

After collagenase incubation, Young's modulus decreased in the fascicles significantly ($p < 0.0001$) across all strain values tested, by an average of 51.1±5.7%, (Figure 1A). In contrast, collagenase incubation did not significantly affect active stress $pCa_{50}$, the Hill coefficient, and passive stresses ($p = 0.464$, 0.164,0.910, and 0.0977 to 0.426 across the various SLs, respectively, Figure 1B) in single fibers.

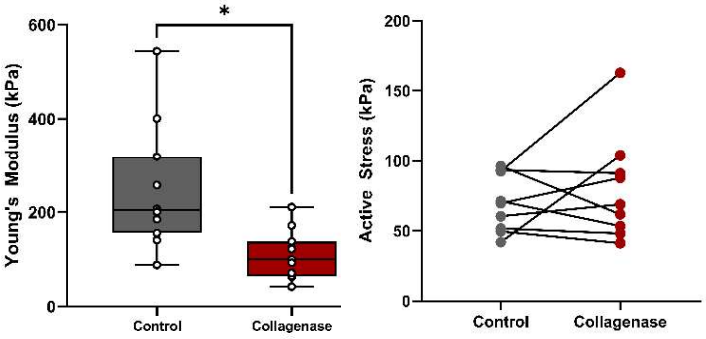


**Figure 1**: Decrease in Young's modulus (stiffness) of muscle fascicles from individuals with cerebral palsy after collagenase incubation (A, $p < 0.0001$), without affecting active force production in single fibres (B).

Collagenase reduced stiffness while preserving active stress, $pCa_{50}$, cooperativity of calcium binding, and titin elasticity. These results suggest that collagenase could be used to decrease the stiffness in CP muscles without compromising contractile ability.

## Conclusions

Current treatments for increased stiffness found in CP muscles can cause transient pain, fibrosis and fat infiltration. Collagenase could mitigate the adverse effects of these treat ments while providing similar relief seen in other collagenase treatments for fibrotic diseases. This could also provide an alternative to the aggressive and stigmatized interventions of surgery or botulinum neurotoxin. If efficacious, this treatment could influence how clinicians approach the treatment of specific CP symptoms and restore mobility in these patients.

## References

**[1]** Howard et al. (2021) Dev Med & Child Neurol. **64**(3): 289-95.
**[2]** Hurst et al., (2009). N Engl J Med, **361**(10), 968–979.