The Effect of Muscle Unloading on Tendon Structure and Function During Postnatal Development

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SIGNIFICANCE: Neonatal brachial plexus palsy, a condition affecting 1 out of 1000 births, often results in shoulder muscle paralysis leading to permanent shoulder deformities (1). This study aims to investigate the role of muscle forces in postnatal shoulder development in order to enhance our understanding of this condition.

INTRODUCTION: Muscle forces in conjunction with biochemical cues are critical to guiding the development of orthopaedic tissues. Paralysis of the rotator cuff muscles in the murine shoulder at birth results in a phenotype that mimics neonatal brachial plexus palsy (2). The supraspinatus muscle, tendon, and its bony insertion (the “enthesis”) do not develop normally (3). The tendon and its enthesis in particular are severely compromised, putting the rotator cuff at risk for rupture. In this study, we examined the structural and biomechanical consequences of chemically induced muscle unloading on the supraspinatus tendon during postnatal development.

METHODS: Animal Model: 52 CD1 mice were obtained from Charles River Labs. This study was approved by the Division of Comparative Medicine at Washington University. 32 mice received botulinum toxin A (Botox) injections in the left shoulder and saline injections in the right shoulder (1,2). The remaining mice served as normal age-matched controls. Animals were sacrificed 28 and 56 days after birth. Paralysis was maintained until sacrifice with multiple botulinum toxin injections. Collagen Orientation: Supraspinatus tendon-humeral head specimens were fixed, dehydrated, embedded in paraffin, and sectioned at 5 μm. Sections were stained in 0.1% Picrosirius Red solution, destained in 1% acetic acid, counterstained with hematoxylin, and differentiated in 1% HCl in 70% ethanol. Collagen fiber angle distributions were measured near the tendon-bone insertion as described previously (N=4 per timepoint) and angular deviation was calculated (4). Biomechanical Testing: Tendon cross-sectional area (CSA) was determined using a combination of micro-computed tomography measurements and optical methods. The distal humerus was embedded in epoxy and a U-shaped piece of wire was used to prevent growth plate fracture. After removing the muscle, the tendon was glued between two pieces of paper and secured in a tension clamp. Specimens were then tested in a 37 °C saline bath (N=10 per timepoint). Each tendon was subjected to a tare load of 0.05N followed by 5 cycles of force-control preconditioning using a triangle waveform. The tendon was then stretched to 3% strain followed by 5 minutes of relaxation. After returning the tendon to a slack position and allowing 5 additional minutes of recovery, the specimen was pulled in uniaxial tension at a strain rate of 0.5%/sec until failure. Strain was calculated by optically tracking a pattern of tissue stain applied to the surface of the tendon (Figure 1). All data analysis was performed using custom Matlab software.

RESULTS: Angular deviation was significantly higher in the Botox group compared to the Saline group at all timepoints (Fig 1). All data analysis was performed using custom Matlab software.

DISCUSSION: Mechanical cues are necessary for the postnatal development of a mechanically robust tendon. Muscle unloading severely compromised the functional development of the tendons and their bony insertions in the current study.

- Unloaded tendons were weaker and less stiff with no change in tendon size.
- Decreased collagen fiber alignment likely contributes to reduced strength and stiffness in unloaded tendons.
- There were some differences between saline and normal control shoulders. This may be a result of reduced overall activity in the botulinum toxin injected animals compared to normal animals.

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REFERENCES: