

Introduction

- Brachial plexus birth injury (BPBI) is the most common nerve injury in children¹
- 30-40% of injured children experience lifelong arm impairment with limited range of motion and joint dislocation^{2,3}
- BPBI leads to gross changes in muscle^{2,4} and bone⁵ development during rapid postnatal growth
- Muscular changes including contracture² and atrophy⁴ occur with BPBI, but the causes of these changes at the cellular level are not well understood
- We hypothesize that underlying muscle composition changes, including increased fibrosis, contribute to limited arm functionality in patients with BPBI



http://www.childrenshospital.org

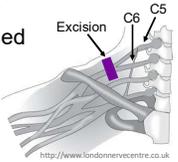
Methods: Study Design

Age

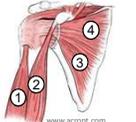
Day 0 Sprague-Dawley rats
 • n = 6 (5 male, 1 female)



Day 5 Postganglionic neurectomy
 • Nerves C5 and C6 excised
 Unaffected-left
 Affected-right



Week 8 Sacrifice
 • Muscles of interest:
 1) Biceps long head
 2) Biceps short head
 3) Lower subscapularis
 4) Upper subscapularis



http://www.criver.com
http://www.londonmevcentre.co.uk
www.acropst.com

Methods: Histology

- Muscles were snap frozen and stored at -80°C
- Samples were cryosectioned to create three frozen sections (10 μm thickness) for each muscle
- Sections were stained with Masson's trichrome and imaged with an EVOS XL light microscope at 20X magnification

Methods: Image Processing

Imaged sections were analyzed using a custom protocol in ImageJ software

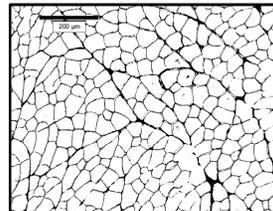
Collagen Detection

- Excessive collagen, stained blue by Masson's trichrome, indicates fibrosis
- Color thresholding used to measure percent of image occupied by collagen

Original Image
blue = collagen



New Image
black = collagen

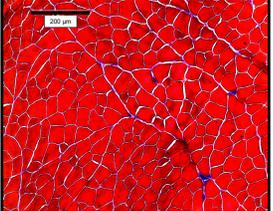


Color threshold

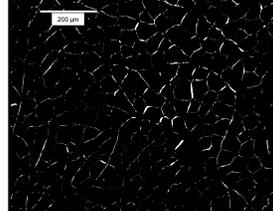
Muscle Tissue Detection

- Healthy muscle fibers are stained red by Masson's trichrome
- Saturation thresholding used to measure percent of image occupied by tissue

Original Image
red = tissue



New Image
black = tissue



Saturation threshold

Calculations and Statistics

- The ratio of collagen to total tissue was calculated using the percentages of collagen and tissue in the image
- Paired t-tests were used to compare collagen content in the affected and unaffected shoulders for each muscle ($\alpha = 0.05$)

Results: Statistical Analysis

Fibrosis was significantly increased in the affected biceps long, biceps short, and lower subscapularis muscles

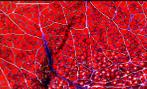
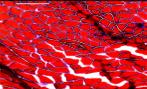
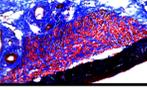
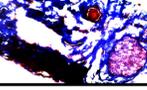
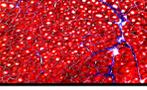
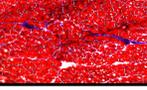
Paired T-tests

Muscle	Unaffected (%)	Affected (%)	p-value
Biceps Long Head	~15	~53.6	$p < 0.0001$
Biceps Short Head	~18	~38.5	$p = 0.0012$
Lower Subscapularis	~18	~22.71	$p = 0.022$
Upper Subscapularis	~18	~20.323	$p = 0.90$

Discussion

- Neurectomy affected fibrosis in innervated muscles to different extents:
 - Biceps muscles demonstrated the greatest increase in collagen, suggesting development of fibrosis
 - Subscapularis muscles demonstrated moderate or no increase in collagen
- Our results confirm previous findings³ suggesting postganglionic neurectomy increases fibrosis in the developing shoulder
- Understanding the impact of neurectomy on underlying muscle changes is the first step toward understanding the development and progression of limited arm function in patients with BPBI
- Future work will compare collagen composition with preganglionic and postganglionic neurectomy
- Additional metrics, muscle fiber size and geometry, will be analyzed and correlated with muscle and bone changes

Results: Representative Images

Muscle	Biceps Long Head	Biceps Short Head	Lower Subscapularis	Upper Subscapularis
Unaffected				
Affected				

References

- Mehlman CT. Neonatal Brachial Plexus Palsy, 589.
- Pöyhliä TH. *Pediatr Radiol* 35, 402, 2005.
- Pondaag W. *Dev Med Child Neurol* 46, 138, 2004.
- Hogendoorn S. *J Bone Joint Surg Am* 92, 935, 2010.
- Pearl ML. *J Bone Joint Surg Am* 80, 659, 1998.
- Nikolaou S. *J Hand Surg Am* 40, 2007, 2015.
- Wagner, EB. *J Appl Physiol* 109, 350, 2010.

Acknowledgments

Funding was provided by NIH R21 HD088893 and the North Carolina State University Office of Undergraduate Research.