Effect of Postganglionic and Preganglionic Brachial Plexus Birth Injury on Muscle Fibrosis

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INTRODUCTION: Brachial plexus birth injury (BPBI) is the most common nerve injury in children [1], resulting in lifelong arm impairment in up to 30% of affected children [2]. Injury occurs during difficult childbirth when excessive stretching of the neck and shoulder damages the brachial plexus nerve bundle, leading to altered musculoskeletal development and ultimately gross morphological changes at the glenohumeral joint [3]. Clinically, nerve damage results from either nerve rupture or avulsion [4]. Nerve rupture results in weakness, disuse, and shoulder contractures [5], while nerve avulsion results in weakness and disuse without shoulder contracture [6]. While injury morbidities are well studied, the underlying mechanisms behind them are not. Muscle composition may play a role in loss of function at a macrostructural level due to muscle stiffening from fibrosis. We hypothesized that the biceps brachii and subscapularis muscles, known contributors to elbow and shoulder contracture [6,7], have increased collagen content following both nerve rupture and avulsion injuries.

METHODS: All animal work was performed under an approved IACUC protocol. Sprague Dawley rat pups were separated into three groups. The sham neurectomy group (n=8) underwent sham surgery without nerve injury. The postganglionic neurectomy group (n=11) underwent C5 and C6 nerve root excision distal to the dorsal root ganglion via incision of the pectoralis major [5], mimicking nerve rupture. The preganglionic neurectomy group (n=12) underwent C5 and C6 nerve root excision proximal to the dorsal root ganglion via a supraclavicular incision [6], mimicking nerve avulsion. Neurectomies were performed at postnatal day 3-5 on one forelimb (affected) with the contralateral forelimb (unaffected) serving as a control. After sacrifice at week 8, affected and unaffected biceps brachii and subscapularis muscles were harvested and dissected into biceps short and long head and upper and lower subscapularis. These specimens were snap-frozen and cut transversely (10 µm thick). Sections were stained with a Masson's trichrome kit, imaged at 20X, and analyzed for percentage of collagen using ImageJ (National Institutes of Health). The % collagen raw values were compared between affected and unaffected muscles separately for each group using paired t-tests and affected/unaffected ratios of % collagen content were compared between groups using one-way ANOVA with Tukey's posthoc tests (alpha=0.05).

RESULTS: Analyses are ongoing, and a subset of data are presented here (n=6-7/group). Within the postganglionic group, some muscles in the affected forelimb tended to have greater amounts of collagen than those in the unaffected forelimb, specifically the biceps short head (+59.5%, p=0.067) and lower subscapularis (+30.5%, p=0.11) (Fig. 1a). Within the preganglionic group, the biceps long head tended to have greater collagen content on the affected side than on the unaffected side (+60.4%, p=0.058) (Fig. 1b). Within the sham group, collagen content was not significantly different between the affected and unaffected side for any of the muscle specimens. Affected/unaffected ratios of collagen content for postganglionic and preganglionic were not significantly different from that of sham (Fig. 2). However, the mean values of the ratios were consistently higher in the postganglionic and preganglionic group than in sham.

DISCUSSION: This work shows a trend for some muscles to develop fibrosis after both postganglionic and preganglionic injuries. Similar amounts of increased collagen content were previously reported in the affected biceps brachii following a postganglionic injury in mice [8]. Our study is the first to examine collagen content in the subscapularis of the affected forelimb following BPBI, a muscle that has been implicated as a major contributor to the shoulder contracture observed clinically [4]. Some muscles show a trend towards more amounts of collagen being deposited in the postganglionic and preganglionic groups than in the sham and may be found significant when the entire dataset is completed. The collagen content data for the postganglionic and preganglionic groups follow a similar trend, in contrast to our recent findings in the macrostructural changes seen at the joint, which showed group differences such as relatively less glenoid deformity (i.e., declination) and greater range of motion for preganglionic than postganglionic in these same rats [9]. Together, these findings suggest that gross deformities in the glenohumeral joint are not primarily driven by the development of muscle fibrosis, at least in the biceps brachii or subscapularis muscles.

SIGNIFICANCE: Advancing our understanding about changes in the underlying muscle and bone tissue surrounding the glenohumeral joint following BPBI will lend insight into the mechanisms by which shoulder and postural deformities occur and is a critical first step for developing better, more effective treatments.

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ACKNOWLEDGMENTS: Support was provided by NIH R21HD088893.

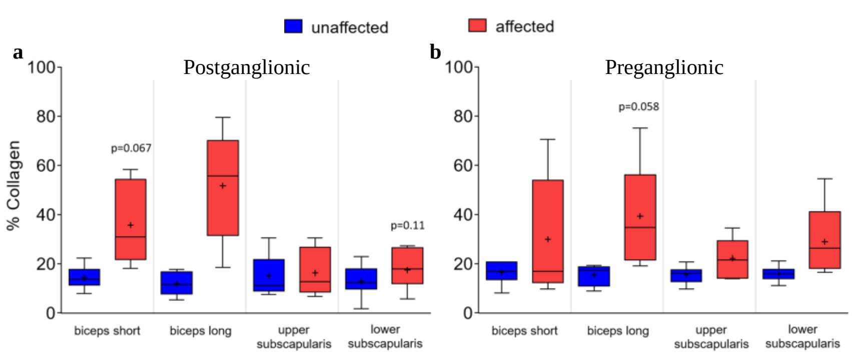

Figure 1. a) Postganglionic injury: Affected biceps short head and lower subscapularis tended to have a greater amount of collagen than unaffected. **b)** Preganglionic injury: Affected biceps long head tended to have a greater amount of collagen than unaffected. + mean value.

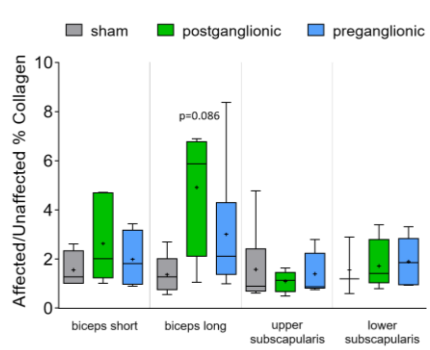

Figure 2. The biceps long head tended to have a greater increase in collagen in the postganglionic group compared to sham. + mean value.