Detriments in humeral bone microstructure and mineralization following brachial plexus birth injury

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INTRODUCTION: Brachial plexus birth injury (BPBI), the most common nerve injury in children, affects 1-3 in every 1000 births [1]. Sequelae depend on the injury location relative to the dorsal root ganglion. Nerve rupture (postganglionic injury) causes muscle weakness, disuse, and contractures [2], and nerve avulsion (preganglionic injury) leads to muscle weakness and disuse without contracture [3]. Using rat models of these injury locations, we previously showed that postganglionic injury results in scapular deformities and preganglionic injury results in reduced humeral head growth [4]. However, the effect of injury location on humeral microstructure and dynamic bone formation rate is unclear. We hypothesized that both injuries would be detrimental to trabecular microstructure, mineralization, and bone formation rate, with more severe changes in postganglionic than preganglionic BPBI.

METHODS: Under an approved IACUC protocol, Sprague Dawley rat pups were placed in three groups: sham (n=15), postganglionic (n=26), and preganglionic (n=28). At postnatal day 3-5, neurectomies were performed on one side (affected), with the contralateral side (unaffected) serving as a control. C5 and C6 nerve roots were excised either distal (postganglionic) [2] or proximal (preganglionic) [3] to the dorsal root ganglion. At 8 weeks rats were sacrificed, and affected and unaffected scapulae and humeri were harvested, fixed, and stored at 4°C. Bones were scanned using micro-computed tomography (micro-CT, 10-µm voxels). Humeral head regions of interest in the epiphysis and metaphysis were evaluated for standard trabecular bone metrics, including bone volume fraction (BV/TV), bone mineral density (BMD), tissue mineral density (TMD), trabecular number (Tb.N), thickness (Tb.Th), and separation (Tb.Sp), and connectivity density (Conn.D) [5]. After micro-CT, humeri were embedded in PMMA, sectioned transversely at the diaphysis, polished, imaged, and analyzed for standard dynamic histomorphometry metrics, including mineralizing surface per bone surface (MS/BS), mineral apposition rate (MAR), and bone formation rate (BFR/BS) [6]. Affected-to-unaffected ratios of metrics were compared between groups using one-way ANOVA with Tukey's posthoc tests (alpha=0.05, GraphPad).

RESULTS: A subset of the humeri data are presented here for both trabecular microstructure (n=5/group) and dynamic histomorphometry (n=3-4/group). In the humeral metaphysis, trabecular bone microstructure was unaffected by BPBI. In the epiphysis, trabecular bone showed detriments following preganglionic injury relative to sham, including lower BV/TV (-25.3%, p=0.046), BMD (-26.4%, p=0.049), and Tb.N (-21.2%, p=0.016) and greater Tb.Sp (+27.0%, p=0.031) (Fig. 1). Preganglionic also tended to have lower TMD (-4.2%, p=0.067) and Conn.D (-17.2%, p=0.097). Although some trends were observed, trabecular metrics were not significantly different between postganglionic and sham in these preliminary results. On the endosteal surface of the humeral diaphysis, initial results show a trend for reducing mean MS/BS and BFR/BS following post- and preganglionic injury vs. sham (Fig. 2) that need to be confirmed with the full sample size.

DISCUSSION: Trabecular bone in the humeral epiphysis failed to develop a robust microstructure following preganglionic BPBI. While trabecular detriments in the proximal humerus were previously reported following postganglionic injury [7], our study is the first to report trabecular detriments following preganglionic injury, which experiences more stunted humeral growth than postganglionic [4]. Trabecular mineralization, not previously measured, also tended to be lower in preganglionic BPBI. Ongoing analyses will confirm whether trends for decreased bone formation rates following BPBI persist, another metric not previously examined. Our data show that preganglionic injury causes similar or worse mineralization and microstructural detriments in the humerus compared to postganglionic injury, consistent with our macrostructural changes in the humerus in these same animals.

SIGNIFICANCE: This study provides insight into the effect of nerve injury location on underlying trabecular microstructure and mineralization in the humerus, which contribute to the load-bearing function of the shoulder. We present new, previously unknown information about trabecular bone changes with preganglionic injury, and bone formation rates with both injuries, contributing to the understanding of BPBI progression in the humerus. Examining underlying factors driving these differences may inform better treatments to limit the development of deformities following BPBI.

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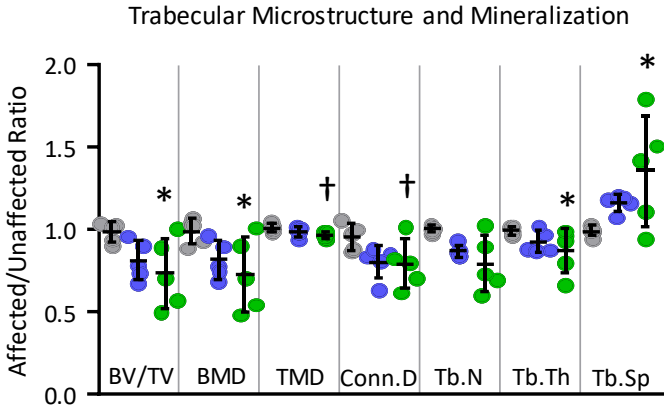

Figure 1. In the humeral epiphysis, detriments in many trabecular metrics were observed with preganglionic injury. *p<0.05 vs. sham, †p<0.1. + mean value.

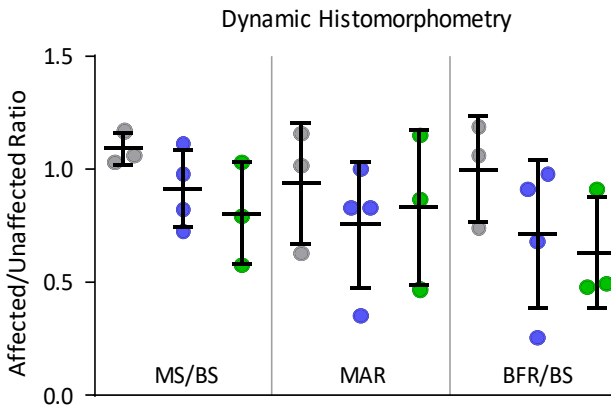

Figure 2. On the endosteal surface, bone formation and mineralization show reducing trends with post- and preganglionic injury vs. sham.