DECREASED LOADING DURING POST-NATAL DEVELOPMENT IMPAIRS MINERALIZATION AT THE SUPRASPINATUS TENDON ENTHESION

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INTRODUCTION: A number of studies have shown that the stress environment influences the development and maintenance of musculoskeletal tissues. For example, bone responds to increased load by producing matrix and to decreased load by resorbing matrix [e.g., 1]. Tendon responds to compressive loads by producing proteoglycan [e.g., 2]. The current study uses an animal model to explore the role of the stress environment on the post-natal formation of the supraspinatus tendon enthesis. We hypothesized that mechanical loading is necessary for mineralization to occur at the tendon enthesis.

METHODS: Animal model: 40 CD-1 neonatal mice were used in this study. Supraspinatus intramuscular injections of botulinum toxin A (BOTOX, Allergan Inc) were used to paralyze the left shoulders of 20 animals within 24 hours of birth. The supraspinatus muscles of right shoulders were injected with an equal volume of saline to serve as contralateral controls. A botulinum dose of 0.05U per gram body weight was used, delivered intramuscularly in a 10μl volume. Effective dosage and volume was determined in pilot studies. Injections were repeated every 3 days to maintain local paralysis in the left shoulder. Mice were sacrificed 14, 21, 28, and 56 days after birth. Mice were graded for paralysis by two observers once a day until sacrifice. Grading was based on degree of observed paralysis of the experimental forelimb compared to the contralateral forelimb on a scale of 0-3 (0=full abduction, 1=partial abduction, 2=no abduction, 3=full limb paralysis). Analysis of bone and muscle: Humeral head and supraspinatus muscle were evaluated at each timepoint using micro computed tomography (N=5 per group per timepoint). To evaluate mineralization and bone architecture, bone volume, trabecular thickness, and trabecular spacing were measured. To evaluate the muscle, supraspinatus volume and cross-sectional area were measured. A repeated measures ANOVA followed by a Least Squares Differences test was used to compare groups. After scanning, specimens were fixed, decalcified, paraffin embedded, and sectioned at 5μm. Slides were processed for tartrate resistant acid phosphatase (TRAP) immunohistochemistry to identify osteoclasts [3].

RESULTS: Gross observations: 90% of botulinum injected shoulders were paralyzed within 24 hours after birth. Paralysis was maintained until sacrifice. The average grade for paralyzed shoulders was 1.64 ± 0.19 (average ± standard deviation). Muscle changes: The volume and maximum cross-sectional area of the supraspinatus muscles that were injected with saline were significantly larger than those of the contralateral botulinum injected muscles at all timepoints except 14 days (Fig. 1). Bone changes: Bone changes were most dramatic at the insertion of the supraspinatus tendon (Fig. 2). Bone volume and trabecular thickness in the humeral head was higher in the saline side compared to the botulinum side (Fig. 3–4). Trabecular spacing was lower in the saline side compared to the botulinum side (Fig. 5). Bone volume and trabecular thickness were significantly lower in the saline group compared to the normal group at 28 and 56 days. There were no differences between the saline and normal groups for trabecular spacing. TRAP positive staining was high in both groups through the early timepoints. Staining intensity at the insertion and in the humeral head decreased dramatically over time in the saline group. Staining intensity remained high in the botulinum group through 56 days (Fig. 6).

DISCUSSION: During normal insertion site development, chondrocytes proliferate, become hypertrophic, and finally mineralize to form a transition between tendon and bone. Consistent with normal development, saline injected shoulders had mature insertion sites by 21 days with a fully mineralized enthesis. In comparison, the insertion of the botulinum injected shoulders showed a marked delay in the mineralization process. There were no differences in mineralization between groups 14 days post-natally, indicating that genetic factors may dominate joint development at early stages. However, mineralization was clearly impaired at later time points due to the decreased loading, indicating that mechanical factors may dominate joint development at later stages.