The effects of denervation and reinnervation on functional muscle length and elbow flexion contracture following neonatal brachial plexus injury

INTRODUCTION:
The pathophysiology of secondary joint contractures following neonatal brachial plexus injury (NBPI) is not completely understood, especially that of the paradoxical elbow flexion contracture that occurs following paralysis of the elbow flexors. Our previous work in a mouse model found shortened elbow flexor muscles to be responsible for the elbow flexion contracture following NBPI, although the contractures varied widely among mice, as they do in humans. The current study uses four methods of surgical brachial plexus injury in a neonatal mouse model to modulate denervation and reinnervation potential in order to test the hypothesis that muscle shortening and contractures depend on the degree of denervation and reinnervation of the elbow flexors.

METHODS:
Following Institutional Animal Care and Use Committee approval, 42 CD-1 mice underwent surgical extraruminal brachial plexus injury at 5 days of age. The mice were divided into 4 groups: Excision (C5, C6 nerve root excision), Neurotomy (C5, C6 nerve root neurotomy), Repair (C5, C6 nerve root neurotomy and immediate fibrin glue repair), and Global (C5-T1 nerve root neurotomy). The contralateral limb was used as a control for all mice. Motor function was assessed immediately post-operatively to confirm isolated upper-trunk paralysis in the first three groups and complete paralysis in the Global group. At four weeks post-surgery, motor function was graded with the observer blinded to surgery group, the mice were then sacrificed, and range of motion at the elbow was measured using a validated digital photography technique with the observer blinded to the surgery group.

Functional muscle length of the biceps and brachialis was measured in 22 mice (5-7 mice per group) following sacrifice 4 weeks post-surgery. To control for joint position, both forequarters were removed and fixed in formalin while secured to cork cubes at 0° shoulder abduction and 90° elbow flexion. Following fixation, the biceps and brachialis were removed for muscle belly length measurement and then digested in 15% H2SO4 for dissociation into fiber bundles for sarcomere length measurement under 40x oil DIC microscopy.

Denervation and reinnervation were assessed in the remaining 20 mice (5 mice per group) following sacrifice at four weeks post-surgery. Both forelimbs were removed and fixed in Bouin’s solution and decalcified in 0.5M EDTA prior to embedding in paraffin. The brachium was sectioned transversely for immunohistochemical staining with anti-neurofilament-H to quantify musculocutaneous nerve (MCN) axons and with anti-GAP-43 to detect axonal growth cones indicating reinnervation within the MCN. All measurements were performed by observers blinded to the surgery group.

RESULTS:
Motor recovery of elbow flexion occurred most reliably in the Repair group, and least reliably in the Excision and Global groups (p=0.027, Fisher exact test, Fig 1a). Conversely, elbow flexion contractures were most severe in the Excision and Global groups and least severe in the Repair group (p=0.029, t-test, Fig 1b). Across all groups, mice without recovery of active elbow flexion had significantly more severe elbow flexion contractures than did mice with elbow flexion recovery (p=0.047, t-test). No contractures occurred in mice with elbow flexion recovery, using >10° difference from control to categorically define elbow flexion contracture.

In the 22 mice that underwent functional muscle length measurement, brachialis sarcomeres were significantly longer (indicating functional muscle shortening) on the operated than control side (p=0.001, R2 =0.29, Fig 2a). Mice with functional recovery had normal brachialis sarcomere lengths. Similarly, brachialis sarcomere elongation, relative to the control side, correlated significantly with the degree of elbow flexion contracture (p=0.001, R2 =0.29, Fig 2b). Overall brachialis length, corrected for sarcomere length, did not correlate with elbow flexion contracture, nor did biceps corrected length or sarcomere length.

DISCUSSION:
The current study describes the first mouse model to modulate the severity and recovery potential of neonatal brachial plexus injury, using physiologically and anatomically relevant extraruminal nerve injury and repair methods. In our model, elbow flexion functional recovery, confirmed with immunohistochemical quantification of reinnervation within the musculocutaneous nerve, was improved by brachial plexus repair.

More importantly, however, the current study demonstrates a quantitative link between neonatal denervation, functional muscle shortening, and contractures following NBPI. In the setting of persistent paralysis of the elbow flexors, brachialis functional shortening occurs and correlates with elbow flexion contracture. Similarly, quantitative elbow flexor denervation correlates with elbow flexion contracture, and reinnervation of the elbow flexors prevents elbow flexion contractures. These findings provide not only an opportunity to develop novel preventative and treatment strategies for contractures following NBPI, but also an avenue for further investigation into the molecular mechanisms governing nerve-muscle interaction during postnatal skeletal muscle growth and development.