Biomechanical Basis of Shoulder Osseous Deformity and Contracture in a Rat Model of Brachial Plexus Birth Palsy

Dustin L. Crouch, PhD, Ian D. Hutchinson, MD, Johannes F. Plate, MD, PhD, Jennifer Antoniono, Hao Gong, MS, Guohua Cao, PhD, Zhongyu Li, MD, PhD, and Katherine R. Saul, PhD

Investigation performed at the Department of Orthopaedic Surgery and Rehabilitation, Wake Forest School of Medicine, Winston-Salem, North Carolina

Background: The purpose of this study was to investigate the relative contributions of two proposed mechanisms, strength imbalance and impaired longitudinal muscle growth, to osseous and postural deformity in a rat model of brachial plexus birth palsy (BPBP).

Methods: Thirty-two Sprague-Dawley rat pups were divided into four groups on the basis of surgical interventions to induce a strength imbalance, impaired growth, both a strength imbalance and impaired growth (a combined mechanism), and a sham condition in the left forelimb. Maximum passive external shoulder rotation angle (ER\text{max}) was measured bilaterally at four and eight weeks postoperatively. After the rats were killed at eight weeks, the glenohumeral geometry (on microcomputed tomography) and shoulder muscle architecture properties were measured bilaterally.

Results: Bilateral muscle mass and optimal length differences were greatest in the impaired growth and combined mechanism groups, which also exhibited >15° lower ER\text{max} (p < 0.05; four weeks postoperatively), 14° to 18° more glenoid declination (p < 0.10), and 0.76 to 0.94 mm more inferior humeral head translation (p < 0.10) on the affected side. Across all four groups, optimal muscle length was significantly correlated with at least one osseous deformity measure for six of fourteen muscle compartments crossing the shoulder on the affected side (p < 0.05). In the strength imbalance group, the glenoid was 5° more inclined and the humeral head was translated 7.5% more posteriorly on the affected side (p < 0.05).

Conclusions: Impaired longitudinal muscle growth and shoulder deformity were most pronounced in the impaired growth and combined mechanism groups, which underwent neurectomy. Strength imbalance was associated with osseous deformity to a lesser extent.

Clinical Relevance: Treatments to alleviate shoulder deformity should address mechanical effects of both strength imbalance and impaired longitudinal muscle growth, with an emphasis on developing new treatments to promote growth in muscles affected by BPBP.

Disclosure: One or more of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of an aspect of this work. None of the authors, or their institution(s), have had any financial relationship, in the thirty-six months prior to submission of this work, with any entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. In addition, no author has had any other relationships, or has engaged in any other activities, that could be perceived to influence or have the potential to influence what is written in this work. The complete Disclosures of Potential Conflicts of Interest submitted by authors are always provided with the online version of the article.
Deformity is characterized by contracture with limited passive range of motion of shoulder abduction and external rotation. Osseous deformity is strongly correlated with internal rotation contracture, so it is likely that postural and osseous deformities share a common mechanical basis.

The prevailing hypothesized mechanism of shoulder deformity is strength imbalance between muscles that internally and externally rotate the shoulder. The infraspinatus, supraspinatus, and teres minor muscles, which augment shoulder external rotation on the basis of their moment arms, are paralyzed following a C5–C6 injury. Consequently, if unimpaired, the subscapularis and pectoralis major muscles would act unopposed, presumably leading to the development of an internal rotation contracture. A second, recently proposed mechanism suggests that impaired longitudinal growth of paralyzed muscles increases the passive force acting on the shoulder. Fibers from the subscapularis muscle, which primarily contributes to internal rotation of the shoulder, were found to be mechanically stiffer and functionally shorter in infants with BPBP. Additionally, in a mouse model of BPBP, the subscapularis muscle in the affected forelimbs was significantly shorter, smaller, and more fibrotic than the muscle in the unimpaired forelimbs.

Evidence supporting the role of either deformity mechanism in the development of shoulder deformity is not consistent across studies. For instance, higher ratios of pectoralis major to external rotator muscle cross-sectional area, indicative of strength imbalance, were associated with increased severity of the glenohumeral deformity. However, excision of the infraspinatus and teres minor in a mouse model of BPBP did not produce internal rotation contractures. Despite evidence of impaired subscapularis and biceps growth and their association with contracture, the prevalence of external rotation contracture is relatively low, even though external rotator muscles (i.e., the supraspinatus and infraspinatus) are frequently involved. Establishing a clearer causal relationship between the two mechanisms and shoulder deformity may guide clinical treatment decisions for BPBP.

We conducted a prospective cohort study in a rat model of BPBP to determine the extent to which two deformity mechanisms—strength imbalance and impaired longitudinal muscle growth—contribute to shoulder deformity following BPBP. We allowed both deformity mechanisms to contribute to shoulder deformity independently and in concert. The study hypothesis was that muscle changes associated with strength imbalance would be more strongly related to shoulder deformity than those associated with impaired longitudinal muscle growth.

Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee. Thirty-two five-day-old Sprague-Dawley rat pups (Harlan Laboratories, Indianapolis, Indiana) were divided into four groups on the basis of surgical interventions designed to permit strength imbalance or impaired longitudinal muscle growth, or both mechanisms, to contribute to shoulder deformity. The number of rats per group was chosen to detect a difference of 4° of glenoid version between groups (within-group standard deviation = 2.1°) on the basis of previously obtained computed tomography (CT) measurements, with a power of 0.80 using analysis of variance (ANOVA). All surgeries were performed in the left forelimb while the animals were anesthetized with inhaled isoflurane. The animals were closely monitored and given butorphanol tartrate to relieve pain when necessary. In the strength imbalance group, the posterior aspect of the shoulder was injected with botulinum neurotoxin A (BOTOX; Allergan, Irvine, California) to inactivate muscles while leaving their nerves intact, on the basis of a previous BPBP model generated using botulinum toxin. Our strength imbalance model differs from a previous one in which external rotator muscles were excised. In the impaired growth group, rats underwent a neurectomy, used previously to induce BPBP, of the brachial plexus upper trunk by transverse infraclavicular incision through the pectoralis major muscle. To reduce imbalance between internal and external rotator muscles, botulinum neurotoxin A was injected in the anterior aspect of the shoulder. Botulinum toxin injections were administered when the rats were five days old (1 U/kg dose in a 0.1-mL volume) and at four weeks postoperatively (6 U/kg dose in a 0.1-mL volume) to maintain chemodenervation in the target muscles. Both mechanisms were permitted to contribute to deformity in the combined group in which rats underwent an upper trunk neurectomy only, which is most analogous to the clinical condition. The sham group received infraclavicular incisions and saline solution injections to the anterior and posterior aspects of the shoulder.

Range of Motion

At four and eight weeks postoperatively, we measured the maximum passive external shoulder rotation angle (ERmax) bilaterally. Time points correspond to approximately 3.1 and 5.8 years of postnatal human musculoskeletal development, by which time shoulder deformity is well established clinically. The ERmax was measured using a custom test fixture while the animals were under anesthesia with inhaled isoflurane (Fig. 1). The forelimb was placed in 90° of shoulder abduction and 90° of elbow flexion, with the limb oriented ventrally at a neutral shoulder rotation posture. During the examination, electromyographic (EMG) signals were recorded from the pectoralis major muscle using stainless-steel fine wire electrodes inserted percutaneously into the muscle belly. The individual who performed the measurements was not blinded to treatment group.

![Custom test fixture used to measure the maximum passive external shoulder rotation angle (ERmax). The forearm was fixed against a rotating arm (A) that was connected by an axle to a 1-in (2.54-cm) circular disc (B). A 10-g weight hung from the disc rim (C) applied a constant external rotational force at the shoulder. The ERmax was measured using a protractor (not shown).](image)
The individual performing the measurements was blinded to treatment group but not to side (right or left).

## Muscle Architecture

Ten muscles crossing the glenohumeral joint were dissected bilaterally from the fixed specimens: deltoid, pectoralis major (sternoclavicular head), supraspinatus, infraspinatus, spinodeltoid (not found in humans), subscapularis, teres major, teres minor, biceps brachii, and triceps (long head). Compartments of the biceps brachii (long and short heads), deltoid (anterior and posterior), subscapularis (upper and lower), and pectoralis major (sternal and clavicular heads) were sectioned along connective tissue that delineated the compartment boundaries. Mass, muscle-tendon length, muscle length, and sarcomere length were measured directly for each muscle. Mass was measured on a digital scale with a resolution of 0.1 µg. Before muscle mass was measured, extramuscular tendon and connective tissue were removed and muscles were blotted dry. Muscle-tendon length, muscle length, and sarcomere length were measured using digital calipers with a resolution of 0.01 mm. Sarcomere length was measured using a 5.0-mW HeNe laser with a wavelength of 633 nm (Thorlabs, Newton, New Jersey). The laser diffraction projected light bands, and the spacing between the bands was measured using calipers. Optimal muscle length ($L_{m}^{0}$) was computed by normalizing muscle length by sarcomere length$^{25}$:

$$L_{m}^{0} = L_{m} \left( \frac{2.4 \mu m}{L_{s}} \right)$$

In the equation, $L_{m}$ and $L_{s}$ are muscle length and sarcomere length, respectively. Optimal sarcomere length was assumed to be 2.4 µm for rat skeletal muscle$^{25}$. The individual performing the measurements was blinded to treatment group but not to side (right or left).

## Data Analysis

Paired Student t tests were performed to compare $ER_{max}$, muscle properties, and bone geometry between the affected (left) and unaffected (right) forelimbs for each group. As an estimate of internal-external rotation strength imbalance, we calculated the ratio between internal rotator (pectoralis major and subscapularis) and external rotator (supraspinatus, infraspinatus, and spinodeltoid) muscle mass. The strength ratio was compared among groups using a one-way ANOVA with a post hoc Tukey test to identify between-group differences. Finally, data were combined across groups to determine, using the Pearson correlation coefficient (r), whether optimal muscle length correlated with $ER_{max}$ and anatomical bone geometry measurements from the affected sides. Comparisons were significant at p < 0.05.
Source of Funding
This work was supported by a grant from the Pediatric Orthopaedic Society of North America and the Orthopaedic Research and Education Foundation. The funding sources did not play a role in this investigation.

Results
Muscle Mass
Nine of ten dissected muscles were significantly atrophic on the affected side in the impaired growth and combined groups, which received an upper trunk neurectomy (Fig. 2). In the impaired growth group, biceps and triceps muscle mass was a mean of 55% (p < 0.001) and 48% (p = 0.006) lower, respectively, on the affected side. Likewise, in the combined group, biceps and triceps muscle mass was a mean of 77% (p < 0.001) and 54% (p = 0.002) lower, respectively, on the affected side. In the strength imbalance group, the mass of the supraspinatus, infraspinatus, and spinodeltoid muscles, which externally rotate the shoulder, was 19% (p = 0.008), 8% (p < 0.001), and 50% (p < 0.001) lower, respectively, on the affected side. In the sham group, the pectoralis major had significantly lower muscle mass on the affected side, presumably because of splitting the pectoralis major as part of the neurectomy procedure. The ratio of internal rotator to external rotator muscle mass was significantly higher in the strength imbalance group than in the other three groups (p < 0.001) (Fig. 3).

Optimal Muscle Length
Bilateral optimal muscle length differences, an indicator of impaired longitudinal muscle growth, were largest in the impaired growth and combined groups, which underwent neurectomy (Fig. 4). In the impaired growth group, the mean optimal muscle length was significantly shorter (p < 0.05) on the affected side for two internal rotator muscles (pectoralis major [14%], subscapularis [13%], and teres major [11%]) and one external rotator muscle (supraspinatus [4%]). In the strength imbalance group, spinodeltoid and teres major optimal muscle lengths were significantly shorter on the affected side.

Postural Deformity
The mean ERmax was 26° (p = 0.03) and 23° (p = 0.004) lower in the affected limbs in the impaired growth group at four and eight weeks, respectively, compared with the unaffected side (Fig. 5). Likewise, the mean range of motion was 17° (p = 0.006) and 11°...


(p = 0.10) lower in the affected limbs in the combined group at four and eight weeks, respectively. The strength imbalance groups had significantly higher ER\text{max} at four weeks, possibly because of increased passive external rotation forces with impaired growth of the spinodeltoid. No muscle activity was observed in the EMG recordings during the passive external shoulder rotation trials.

**Osseous Deformity**

Bilateral glenohumeral geometry differences were most pronounced in the impaired growth and combined groups. The glenoid was more declined in the impaired growth and combined groups on the affected side (mean inclination, \(-52.3^\circ\) [p = 0.07] and \(-56.1^\circ\) [p = 0.02], respectively) than on the unaffected side (mean inclination, \(-37.4^\circ\) and \(-38.4^\circ\), respectively) (Fig. 6). Conversely, in the strength imbalance group, the affected glenoid was less declined than the unaffected glenoid (mean inclination, \(-33.1^\circ\) and \(-38.4^\circ\), respectively; p = 0.01). The affected glenoid was 20.7° more anteverted in the impaired growth group (p = 0.11), but 7.7° more retroverted in the combined group (p = 0.10), compared with the unaffected glenoid. In the impaired growth and combined groups, the humeral head was translated more inferiorly on the affected side (mean, 0.86 and 1.03 mm inferior translation, respectively) than on the unaffected side (mean, 0.10 [p = 0.07] and 0.09 mm [p = 0.09] superior translation, respectively) (Fig. 7). In the strength imbalance group, the humeral head was translated more posteriorly on the affected side (mean, 61.5%) than on the unaffected side (mean, 69%; p = 0.04).

**Correlation Between Optimal Muscle Length and Shoulder Deformity**

Across all groups, optimal muscle length was significantly correlated with at least one shoulder osseous deformity measure on the affected side for the posterior deltoid, spinodeltoid, subscapularis, teres major, and long head of the biceps muscles.
and combined groups, the humeral head was translated more inferiorly on the affected side than on the unaffected side (p = 0.07) and combined (p = 0.02) groups, compared with the unaffected side. Glenoid version and inclination were similar between the sham group and the unaffected sides.

Discussion

Both impaired longitudinal muscle growth and strength imbalance mechanisms are capable of producing shoulder deformity following BPBP, but strength imbalance has long been considered the predominant deformity mechanism. In our study, the strength imbalance group exhibited the highest ratio of internal-external rotator muscle mass across all groups. In three-day-old mice, delayed addition of serial sarcomeres (impaired longitudinal growth) in denervated soleus muscle was attributed to reduced muscle excursion as a consequence of denervation. It is also possible that certain conditions, including strength imbalance and severe weakness, may effectively immobilize muscles in a lengthened or shortened position, potentially altering optimal muscle lengths and contracture. Our findings corroborate those of a previous muscle excision-induced strength imbalance mouse model. Altered cross-sectional area or antagonist muscle mass ratios only indicate an imbalance of force-generating capacity, and may not appropriately reflect the actual mechanical loading of the glenohumeral joint. Increased muscle activity of dually innervated unimpaired muscles could alter glenohumeral mechanical loading, but whether such loads are sufficient to cause deformity remains unknown.

Impaired longitudinal muscle growth, observed clinically, has been associated with contracture in previous animal models of BPBP. Likewise, groups that underwent neurectomy (impaired growth and combined groups) also exhibited the most pronounced impaired longitudinal growth of internal rotator muscles and the most severe internal rotation contractures (limited ER$_{\text{max}}$ on the affected side). Additionally, low optimal muscle length was significantly correlated with osseous shoulder deformity for external rotator (posterior deltoid and spinodeltoid) and internal rotator (teres major and subscapularis) muscles across all groups. In three-day-old mice, delayed addition of serial sarcomeres (impaired longitudinal growth) in denervated soleus muscle was attributed to reduced muscle excursion as a consequence of denervation. It is also possible that certain conditions, including strength imbalance and severe weakness, may effectively immobilize muscles in a lengthened or shortened position, potentially altering optimal muscle lengths. Since excursion is regarded as an important sarcomere-regulating factor, passive stretching by application of a cast or passive manipulation of the upper limb may reduce contracture severity in children with BPBP; however, more research is needed to quantify its efficacy.

The strength imbalance group, in which the spinodeltoid and teres major muscles had both significantly lower mass and optimal length on the affected side, included aspects of a combined mechanism model, albeit with higher internal-external rotator imbalance and fewer muscles with impaired growth than observed in the combined group. Although the strength imbalance mechanism was...
not purely isolated as we intended, our principal finding remains that more extensive impaired longitudinal muscle growth was associated with more severe shoulder deformity, while more pronounced strength imbalance conditions were not. Additionally, muscle changes presumably induced by chemodenervation, along with absent EMG activity during range-of-motion measurements, provide insight into the variable outcomes achieved when botulinum toxin has been used to relieve contractures in children with BPBP.

The combined group exhibited glenoid retroversion similar to clinical cases, but the glenoid was antverted in the impaired growth group. Glenoid anteversion was possibly related to botulinum toxin injections that the group received in the anterior aspect of the shoulder. Additionally, the subscapularis, which applies a posteriorly directed force on the glenoid, was significantly shorter in the combined group but not in the impaired growth group, possibly explaining why glenoid retroversion was observed only in the combined group.

To our knowledge, inferior humeral head translation, observed in the impaired growth and combined groups, has not been previously investigated in clinical cases. Observations of inferior translation corresponded with impaired growth of muscles that depress the humerus on the basis of their lines of action at the glenohumeral joint, including the pectoralis major (impaired growth and combined groups) and subscapularis (combined group only). Similarly, significant posterior humeral head translation in the strength imbalance group was possibly due to impaired growth of the spinodeltoid and the teres major, which apply a posteriorly directed force on the humerus on the basis of their lines of action.

We observed global patterns of muscle atrophy, although we expect that interventions were more localized. Similarly, children with incomplete BPBP may present with globally impaired growth of the affected upper limb. Weakness or paralysis of muscles directly impaired by the interventions could contribute to limb disuse and global atrophy. It is also possible that injury induced by neurectomy and chemodenervation was more widespread than intended, given the challenges of operating on rat pups.

There were several limitations of our study. Although shoulder neuromusculoskeletal anatomy between rats and humans is similar, glenohumeral mechanical loading differs between the species since rats are lifelong quadrupeds, while infants may crawl only within their first year. In the sham group, muscle changes were induced by splitting the pectoralis major, but these changes did not appear to contribute substantially to shoulder deformity in the sham group, while deformity was more pronounced in the groups that underwent neurectomy. It was difficult to clearly define the musculotendinous junctions of some muscles, notably the biceps, which may have contributed to measurement error of in situ muscle lengths used to compute optimal muscle lengths. High within-group variability in the study outcomes may be due to variable spontaneous neuromuscular recovery or surgery effects. Since the investigators were not blinded to treatment group when measuring ERmax and bone geometry, these values may have been influenced by detection bias.

In summary, impaired longitudinal muscle growth was more strongly associated with severe shoulder deformity than were strength imbalance conditions in rats that underwent neurectomy. Clinical management of BPBP must address the clear and substantial role that impaired longitudinal muscle growth appears to play in the development of shoulder deformity.

References


