Scientific Advances in the Understanding of Contracture Pathogenesis in Brachial Plexus Birth Injury

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Abstract: Contractures caused by brachial plexus birth injury (BPBI) can limit function and quality of life in affected children and frequently lead to skeletal dysplasia and dislocations. Existing orthopaedic treatments for these contractures do not restore normal function as they fail to address the previously unknown contracture pathophysiology. Recent research in BPBI has shifted the paradigm of contracture pathogenesis from a problem of muscle strength to a problem of muscle length, and from a mechanical to biological realm. Additionally, we have learned mechanisms governing longitudinal muscle growth and how they are perturbed by neonatal denervation. A recent discovery has proven the concept that muscle contractures following BPBI can be pharmacologically prevented by targeting the underlying biological perturbations in neonatally denervated muscles. Although much work must be done before such a pharmacologic strategy can be translated to children, these discoveries hold promise for a new era in the pediatric orthopaedic care of BPBI in which contractures are medically prevented rather than surgically treated.

Key Concepts:
- Contractures in BPBI result from impaired longitudinal growth of neonatally denervated muscle, not muscle strength imbalance.
- Neonatal longitudinal muscle growth does not require activity of muscle stem cells (satellite cells).
- Neonatal denervation impairs longitudinal muscle growth through increased protein degradation.
- Contractures in a mouse model of BPBI can be prevented pharmacologically by inhibiting protein degradation.

Introduction

Muscle contractures are a prominent and disabling feature of many neuromuscular disorders in childhood, including brachial plexus birth injury (BPBI) and cerebral palsy (CP). These contractures can dramatically reduce joint range of motion and limit the functional use of limbs for ambulating, reaching, and other activities of daily living. Furthermore, the muscle contractures alter the physical forces on the developing skeleton, leading to progressive dysplasia and possible subluxation of joints. These contractures are a primary driver of the need for rehabilitative and surgical therapies, assistive devices, and accommodations for daily functioning. However, no existing treatment strategies alter the actual contracture pathology, and instead can worsen function.
by further weakening already abnormal muscles.\textsuperscript{8-11} As a result, the contractures and their secondary skeletal consequences remain unchecked, leading to pain, loss of physical function, and heavy reliance on costly healthcare and supportive services. It is therefore imperative that we gain a better understanding of contracture pathogenesis so we can develop and test novel contracture prevention and treatment strategies.

Over the past decade, a substantial research effort has helped to elucidate the pathogenesis of contractures following BPBI. This collective work has recently led to the proof of concept discovery that contractures can be corrected medically rather than surgically by targeting an underlying biological perturbation in affected muscles. In this review, we will summarize the work from our laboratory and clinical research, along with scientific and clinical work from others. This research, primarily using a mouse model of BPBI, sheds light on the regulation of postnatal muscle growth, the causative mechanisms of contracture formation, and the neurologic circuitry required for postnatal muscle development.

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\caption{Mouse model of NBPI-induced contractures. Intraoperative view of brachial plexus before (A) and after (B) section of the C5 and C6 nerve roots. (C) Immediate postoperative examination demonstrating absent elbow flexion. Four weeks after NBPI, (D) elbow flexion contracture on the operated side (schematic of measurement technique shown), compared to full passive elbow extension on the control side (E) Complete relief of contracture on operated side after excision of the elbow flexor muscles, leaving the joint capsule intact (F, G) Shoulder external rotation contracture on the operated versus control side 4 weeks after NBPI (H, I). **p<0.01, ***p<0.001 (Adapted with permission from Nikolaou et al. 2011)}
\end{figure}
This work also highlights a similar common muscle contracture phenotype between BPBI and CP, suggesting that discoveries in BPBI can potentially be applied to both upper and lower motor neuron disorders in childhood. This opportunity is of particular importance because animal models of contractures in CP have never been successfully created.\textsuperscript{12-14} Furthermore, as many other childhood neuromuscular disorders are plagued by muscle contractures, including congenital myopathies, muscular dystrophies, and spinal muscular atrophy,\textsuperscript{15-17} these findings have possible ramifications across a wide array of disorders.

**Contractures in BPBI: An Unsolved Clinical Problem**

Brachial plexus birth injury (BPBI), occurring in 1.5 per 1000 live births, is the most common nerve injury in childhood.\textsuperscript{18} In the 20-30\% of children without complete neurological recovery,\textsuperscript{19} secondary contractures occur, most notably shoulder internal rotation contractures and elbow flexion contractures.\textsuperscript{6} Historically, the etiology of these contractures has been thought to primarily involve mechanical processes. A commonly cited mechanism for the development of the shoulder internal rotation contracture is muscle imbalance between functioning internal rotators and paralyzed external rotators, leading to static internal rotation joint posturing and ultimate joint contracture.\textsuperscript{6,20} This theory is supported by a magnetic resonance imaging (MRI) study in BPBI patients demonstrating a correlation between the degree of shoulder joint contracture and the ratio of cross sectional area between internal rotator and external rotator muscles.\textsuperscript{21}

In contrast, other MRI studies have demonstrated the degree of contracture to correlate only with atrophy of the subscapularis, an internal rotator.\textsuperscript{22,23} Moreover, muscle imbalance cannot explain the elbow flexion contracture that develops in the setting of elbow flexor paralysis.\textsuperscript{24-26} In fact, the elbow flexion contracture has

**Figure 2. Impaired longitudinal muscle growth after neonatal denervation.** Sarcomeres visualized at 40X oil DIC from (A) control and (B) denervated elbow flexor muscles (brachialis) fixed at symmetric joint angles 4 weeks after NBPI. (C) Significant sarcomere elongation is seen in denervated muscles, suggesting fewer than normal sarcomeres in series, or functionally shortened muscle. When correcting for sarcomere length, longitudinal growth of the denervated biceps and brachialis is impaired by NBPI (D, E). *p<0.05 (Published with permission from CCHMC Orthopedic Surgery)
been shown to correlate on MRI only with atrophy of the brachialis.\textsuperscript{27}

With this uncertainty regarding contracture etiology, it is not surprising that consensus has not been reached on treatment for the different types of upper extremity contractures. Treatment for the shoulder internal rotation contracture following BPBI has varied over the years,\textsuperscript{28-31} with numerous reported surgical techniques variably highlighting the role of the subscapularis,\textsuperscript{8, 10, 32} the pectoralis major,\textsuperscript{33} or the joint capsule.\textsuperscript{34-36} Contemporary techniques to alleviate shoulder contractures most commonly include open or arthroscopic sectioning or lengthening of the subscapularis and/or pectoralis major with or without release of the anterior glenohumeral joint capsule, and with or without concomitant transfer of the latissimus dorsi and/or teres major muscles to the posterior rotator cuff to augment external rotation function.\textsuperscript{8, 32, 35, 37, 38}

Recommendations for treatment of the elbow flexion contracture also vary widely, with proponents of serial casting,\textsuperscript{39, 40} surgical release,\textsuperscript{41, 42} external fixator distraction,\textsuperscript{43} flexor-to-extensor muscle transfers,\textsuperscript{44} and humeral osteotomy.\textsuperscript{45} Regardless of the technique chosen for either the shoulder or elbow contracture, no surgical or nonsurgical treatment has been shown to reliably restore normal range of motion.

Complications can also arise from attempts to treat the contractures in BPBI. For instance, a common and disabling complication of surgical treatment of the internal rotation contracture is loss of active internal rotation function.\textsuperscript{8, 10, 32, 35} Such a loss of internal rotation function has been reported in 42-100\% of cases treated with a variety of subscapularis releases or lengthenings, performed either arthroscopically or open, and with or without concomitant tendon transfers to restore active external rotation function.\textsuperscript{8, 10, 32, 35}

**Figure 3.** Muscle imbalance alone does not contribute to contracture formation. (A) Relationship between passive external rotation and length of the subscapularis 4 weeks following C5-6 excision versus external rotator excision. Denervation by C5-6 excision (open diamonds) causes subscapularis shortening correlating with contracture severity, whereas muscle imbalance by external rotator excision (closed diamonds) leads to normal subscapularis length and no contractures. (B) Isolated neonatal denervation of the elbow flexor muscles by BPI (C5-6 excision) causes elbow flexion contractures, whereas creation of muscle imbalance by excision of the triceps does not. *p<0.05 (Adapted with permission from Nikolaou et al. 2011, Weekley et al. 2012)
A similar complication had been noted following subscapularis release during total shoulder arthroplasty, prompting Cleeman et al.\textsuperscript{46} to develop a technique of subscapularis release in which only the superior tubular tendon (STT) is released. This technique was subsequently adapted for BPBI patients by arthroscopically sectioning only the upper portion of the subscapularis tendon. A retrospective review\textsuperscript{47} of 15 BPBI patients who underwent this release found significant improvements in passive external rotation, global shoulder function on the Mallet scale,\textsuperscript{48} and importantly, preserved or improved active internal rotation in all patients. This finding raised a problem, however. The upper subscapularis receives its innervation from the upper trunk (C5-6) whereas the lower subscapularis receives innervation from the posterior cord (C5-7).\textsuperscript{49} Thus, a typical upper trunk BPBI will disproportionately denervate the upper

\textbf{Figure 4. Comparison of contractures in pre- vs. post-ganglionic NBPI.} Schematic diagram of (A) postganglionic and (B) preganglionic NBPI, with efferent (red), afferent (green) and sympathetic (blue) neurons depicted. Dashed lines indicate Wallerian degeneration of axons (denervation). (C) Preganglionic injury preserves a significantly greater proportion of parvalbumin\textsuperscript{+} (afferent) axons in the musculocutaneous nerve (MCN). (D) preganglionic NBPI does not cause elbow contractures whereas postganglionic NBPI does. PRE: preganglionic, POST: postganglionic. (Adapted with permission from Nikolaou et al. 2015)
subscapularis muscle belly, from which the STT arises. The fact that isolated STT release relieved the contracture following BPBI suggests that a denervated muscle, not a functional muscle, is causing the contracture.

This observation challenged the muscle imbalance theory of contracture pathogenesis and led instead to an alternate hypothesis: that neonatal denervation impairs longitudinal muscle growth during a period of rapid skeletal growth, leading to relative muscle shortening and contracture. This hypothesis, which could explain the paradoxical elbow flexion contracture that follows elbow flexor paralysis, could also explain why contractures in both BPBI and CP progressively worsen with skeletal growth. Indeed, prior work in animal models of lower limb neonatal denervation has demonstrated altered postnatal muscle growth in mass, cross-sectional area, and fiber number. However, the effect of neonatal denervation on longitudinal muscle growth or joint range of motion remains unknown.

Figure 5. Clinical evaluation of abduction contracture and coronal glenohumeral dysplasia. (A) Clinical photograph demonstrating the Putti sign due to glenohumeral abduction contracture requiring scapular tilt to adduct the arm to the thorax. (B) MRI assessment of glenohumeral abduction contracture in the setting of abnormal scapular tilt. (C, D) Three-dimensional MRI scans of unaffected (C) and affected (D) shoulders used to evaluate 3D deformity, revealing coronal plane as well as axial plane deformities caused by NBPI. (Adapted with permission from Eismann et al. 2015, Eismann et al. 2016)
The Role of Impaired Longitudinal Muscle Growth in Contractures

In order to test the hypothesis of impaired longitudinal growth causing contractures, Nikolaou et al. developed an animal model of BPBI.\textsuperscript{56} Other animal models of BPBI had previously been reported,\textsuperscript{57-59} with shoulder and elbow contractures phenotypically similar to those in humans. However, no studies using animal BPBI models had attempted to identify biological mechanisms of contracture formation. In this new model of BPBI,\textsuperscript{56} (Figure 1) unilateral extraforaminal C5 and C6 nerve root excision in 5-day-old mice produced paralysis mimicking the human phenotype of upper trunk BPBI. Four weeks following BPBI, the operated limb demonstrated significant elbow flexion and shoulder internal rotation contractures also mimicking the human phenotype.

With this model, several important questions regarding contracture pathophysiology could be answered. First, it was unknown whether the tissue responsible for the contracture was the muscle or the joint capsule, pathology of which causes other forms of elbow contractures.\textsuperscript{60} Notably, the elbow flexion contractures in the BPBI mouse model could be completely relieved by excision of the denervated elbow flexor muscles, leaving the joint capsule intact.\textsuperscript{56} This finding suggests that BPBI-induced contractures are a problem of tightness of denervated muscle.

The next question was whether the muscle tightness resulted from fibrosis (muscle too stiff) or impaired longitudinal growth (muscle too short).\textsuperscript{61} Fibrosis, or increased extracellular collagen accumulation, has been postulated to contribute to contractures in both BPBI and CP due to findings on muscle biopsies after contractures have formed.\textsuperscript{22, 62} However, when the mouse model was used to investigate fibrosis during contracture development, it was found that fibrosis occurs after the onset of contractures, collagen content does not correlate with contracture severity, and pharmacologic reduction of fibrosis after denervation does not prevent contractures.\textsuperscript{61} Therefore, fibrosis is not a causative mechanism.

\textbf{Figure 6. Muscle length assessment in human subjects with BPBI.} The Zebrascope microendoscope uses laser illumination to visualize muscle fibers between two 20-gauge needles (A) The needles are attached to a handheld microscope and inserted percutaneously into muscle in vivo (B) to visualize sarcomeres in real time. Sarcomere images from needle microendoscopy of unaffected (C) and affected (D) biceps muscles at symmetric joint positions. (E) Quantification of sarcomere lengths among five subjects with BPBI. Note the elongation of sarcomeres on the affected side, consistent with fewer sarcomeres in series, and consistent with animal model data. \textit{*p<0.05 (Published with permission from CCHMC Orthopedic Surgery)}
These findings warranted further attention to the effect of neonatal denervation on postnatal longitudinal muscle growth.\textsuperscript{56} Further work in the mouse model found that 4 weeks after BPBI, denervated muscles exhibited elongated sarcomeres compared to contralateral control muscles when controlling for joint position, indicating fewer sarcomeres in series\textsuperscript{63} (Figure 2). This degree of sarcomere overstretch indicates an approximately 30\% deficiency in functional muscle length and is consistent with sarcomere elongation seen in muscles responsible for contractures in humans with cerebral palsy,\textsuperscript{62, 64} suggesting a potential final common pathway of muscle contractures in BPBI and CP.

The next question to address was whether the impaired longitudinal growth of the denervated muscle is a direct effect of denervation or a result of the muscle’s altered mechanical environment. Neonatal mice were therefore used to test the isolated effect of muscle strength imbalance at the shoulder and elbow by surgically excising the external rotators and triceps, respectively, finding that muscle imbalance alone was insufficient to cause shoulder internal rotation or elbow flexion contractures or impair longitudinal growth of the subscapularis or elbow flexor muscles\textsuperscript{65} (Figure 3). Conversely, neonatal denervation of the elbow flexors (preserving triceps innervation) was sufficient to cause elbow flexion contractures.\textsuperscript{65} In fact, a direct correlation was found between denervation and contracture severity. When the model was adapted to modulate the degree of denervation severity by varying the type of nerve injury (C5-6 neurotomy and repair, C5-6 neurotomy, C5-6 excision and C5-T1 excision), it was found that the degree of biceps and brachialis shortening and elbow flexion contracture correlated with the degree of axon loss in the denervated musculocutaneous nerve.\textsuperscript{65} These findings support a direct effect of denervation on longitudinal muscle growth and rule out an effect of muscle imbalance alone.

Figure 7. Muscle stem cells do not contribute to longitudinal muscle growth or contractures. (A, B) Pax7 staining reveals an abundance of satellite cells in denervated biceps muscles 3 weeks following postganglionic NBPI. The incorporation of 5'-bromo-2'-deoxyuridine (BrdU) into the nuclei of Pax7+ cells further indicate an enhanced capacity for proliferation post-NBPI. (C, D) Similarly, detection of BrdU+ nuclei within dystrophin-stained fibers demonstrates that NBPI increases the addition of stem cell nuclei to the growing myofiber. (E, F) Targeted deletion of Myomaker in satellite cells at P0 successfully inhibits myonuclear accretion in both normal and denervated muscles at P33. (G, H) Despite the loss of muscle stem cell nuclei in these mice, both sarcomere length in brachialis muscles and elbow range of motion were not altered after 4 weeks of NBPI. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bar: 100 µm. (Adapted with permission from Nikolaou et al. 2019)
However, it remained unknown what type of innervation is required for longitudinal muscle growth. Muscle is innervated by three neuron types: efferent (motor), afferent (sensory), and sympathetic (autonomic). One would be tempted to assume that efferent neurons are the drivers of muscle growth and development, as motor paralysis is the most conspicuous phenotype of muscle denervation. However, another clue from the clinic challenges this assumption.

The vast majority of C5-6 injuries in children are post-ganglionic nerve root ruptures, but pre-ganglionic C5-6 avulsion injuries can occur with breech deliveries. Al-Qattan found a “notable absence” of contractures in patients with pre-ganglionic C5-6 avulsions, consistent with our clinical experience as well. Furthermore, clinical research has shown that functional recovery of muscle reinnervated by nerve transfer surgery following nerve root avulsion is superior to that following the same surgery in the setting of nerve root rupture.68 It is possible, therefore, that preganglionic injury is protective against contractures and other secondary muscular effects of neonatal paralysis. While root avulsions and ruptures both cause complete motor denervation, pre-ganglionic avulsion injuries preserve the muscles’ afferent and sympathetic innervation, as the dorsal root ganglia and sympathetic ganglia remain connected to the muscle. Therefore, the contracture phenotype may be modulated by afferent and/or sympathetic innervation rather than efferent innervation or muscle activity.

In neonatal mice, when pre- and post-ganglionic C5-6 BPBI were performed (Figure 4), both injuries caused similar total denervation, but the pre-ganglionic injury preserved significantly more muscle afferent axons in the musculocutaneous nerve. Consistent with clinical observations, elbow flexion contractures occurred only following post-ganglionic injury, and less severe contractures occurred at the shoulder following pre-ganglionic than post-ganglionic injury. Since efferent denervation and muscle paralysis are present in both

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**Figure 8. Muscle protein degradation elevated after neonatal denervation.** (A, B) Western blot and assessment of signal intensity reveal elevated levels of K48-polyubiquitinated proteins in denervated muscle at multiple weeks after postganglionic NBPI. (C) Muscle ring-finger protein-1 (MuRF1) transcript levels, and (D) catalytic activity of the 20S proteasome are increased at 2 weeks post-NBPI. *P < 0.05, ***P < 0.001, ****P < 0.0001. (Adapted with permission from Nikolaou et al. 2019)
groups but contractures occur in only one group, these data rule out a role for efferent innervation or muscle activity in longitudinal muscle growth or contractures.

**Validating the Impaired Longitudinal Muscle Growth Theory in Humans**

At this point in the scientific investigations, animal model data supported denervation-dependent muscle growth impairment rather than muscle activity imbalance as the cause of contractures. But do these findings hold up to clinical scrutiny in patients with B PBI?

Based on the impaired muscle growth theory, any denervated muscle could cause a contracture, including the glenohumeral abductors, which are innervated by C5 and thus are denervated in BPBI. In a clinical study, magnetic resonance imaging (MRI) was used to quantify glenohumeral abduction contractures in children with BPBI and investigate their relationship with muscle atrophy. Twenty-five of 28 patients with residual shoulder weakness were found to have glenohumeral abduction contractures (10-65°) that were associated with greater abductor atrophy than adductor atrophy (Figure 5A, B). Not surprisingly, a separate 3-dimensional MRI study found glenohumeral dysplasia following BPBI to occur in the coronal plane as well as the axial plane (Figure 5C, D). Furthermore, the glenohumeral abduction contracture produces abnormal scapular tilt that has subsequently been found to confound existing axial MRI measurements of glenohumeral dysplasia. Additionally, others have highlighted the external rotation contracture that exists in BPBI even without prior internal rotation contracture release, again consistent with denervated muscles (C5-innervated external rotators) causing contractures. These findings are therefore consistent with the theory that denervated muscles are responsible for contractures and deepen our previously simplistic view of shoulder sequelae following BPBI.

Another strategy to test the relevance of animal model data to the human condition involved using a validated pharmacologic approach.

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**Figure 9. Pharmacologic prevention of contractures in BPBI using the proteasome inhibitor, bortezomib.** (A) Forelimb images showing the lack of contractures after NBPI in mice treated with 0.3 mg/kg bortezomib beginning at P8. (B, C) Contracture severity in the elbow and shoulder from the mice shown in (A). The dotted line represents the severity in saline controls. (D) Sarcomere length in the brachialis shows that 0.3 mg/kg bortezomib preserves muscle length. ***p<0.001, ****p<0.0001 (Adapted with permission from Nikolaou et al. 2019)
computational model of the human shoulder to model various forms of BPBI, altering muscle strength and/or muscle length.\textsuperscript{73} This investigation found that the 30\% reduction in longitudinal muscle growth discovered in the mouse model is sufficient to explain the loss of human shoulder passive range of motion as well as altered glenohumeral pressures and glenohumeral dysplasia, with a greater effect than altered muscle strength alone.

Finally, a novel technique of needle microendoscopy\textsuperscript{74} has been used to visualize sarcomeres in affected and contralateral unaffected muscles in human subjects with BPBI-induced elbow flexion contractures\textsuperscript{75} (Figure 6). In these subjects, isokinetic torque was also measured to assess functional strength and length of bilateral elbow flexor muscles. Denervated muscles were found to have sarcomere overstretch identical to that seen in the mouse model, as well as reduced isokinetic torque over an even more reduced arc of motion, demonstrating that contractures in humans are indeed associated with short, weak muscles.

It must be noted that, despite the animal and clinical data suggesting that the contractures are not caused by muscle activity imbalance, some authors have advocated botulinum toxin injection for treating shoulder contractures in BPBI, extrapolating from its use in reducing muscle tightness affected by cerebral palsy and motivated by the assumption that internal rotation contractures in BPBI are caused by overly strong internal rotator muscles.\textsuperscript{76} These authors have demonstrated success in reducing posteriorly dislocated shoulders in BPBI using botulinum toxin injection in internal rotator muscles combined with shoulder immobilization in external rotation. However, the ability to reduce and immobilize a shoulder in external rotation immediately following botulinum toxin injection implies that such shoulders lack a true contracture, since botulinum toxin does not exert immediate effects. Importantly, recent work has shown failure of this botulinum toxin strategy to be directly proportional to the pre-intervention internal rotation contracture,\textsuperscript{77} suggesting that botulinum toxin is itself ineffective against established contractures. This finding is again consistent with contractures resulting from muscles with length deficits rather than strength imbalance.

Importantly, the findings of impaired longitudinal muscle growth causing contractures in the original mouse model have also been directly replicated in multiple laboratories using similar rodent models.\textsuperscript{78, 79} Furthermore, subsequent clinical research by other authors\textsuperscript{80, 81} has supported the contribution of impaired longitudinal muscle growth to contracture formation. Most recently, a scoping review\textsuperscript{82} directly weighing the scientific and clinical evidence regarding the relative contributions of muscle imbalance versus impaired longitudinal muscle growth in contracture pathogenesis concluded that impaired growth is the predominant cause of contractures.

How is Longitudinal Muscle Growth Impaired?

Given this preponderance of evidence that contractures result from impaired longitudinal muscle growth, we must understand the causative biological mechanisms in order to potentially correct them pharmacologically. However, we must first understand the mechanisms governing normal longitudinal muscle growth, which are surprisingly not well elucidated. In general, muscle grows by two basic processes: (1) fusion of muscle stem cells (satellite cells) to growing multinucleated myofibers (myonuclear accretion), and (2) protein synthesis within the myofibers. However, the contributions of these mechanisms to longitudinal muscle growth have never been experimentally dissected. Myonuclear accretion is a logical candidate, since it is unique to neonatal muscle growth,\textsuperscript{83} and since satellite cell depletion has been found following long-term denervation\textsuperscript{84} or in longstanding contractures from cerebral palsy.\textsuperscript{85-88} These latter findings, obtained from specimens after contractures have formed, have sparked enthusiasm for stem cell therapies to correct contractures.
In contrast, a mouse model of BPBI was used to investigate and manipulate satellite cell function during the neonatal time period of contracture development in order to ascertain causation rather than correlation.

First, investigators asked if in vivo alterations in satellite cell populations were present during contracture development. Two weeks following BPBI, satellite cells were found to comprise a greater than normal proportion of nuclei in the denervated muscles. Similarly, investigators found increased rates of satellite cell proliferation post-BPBI, as well as increased rates of satellite cell fusion with growing myofibers, using DNA labeling techniques and genetically manipulated mice to track satellite cell fate (Figure 7A-D). These results collectively demonstrate that myonuclear accretion is not impaired in denervated muscle.

These surprising findings raised the question of whether myonuclear accretion is even necessary for longitudinal muscle growth. To answer this question, myonuclear accretion was inhibited through neonatal ablation of satellite cell fusion using satellite cell-specific genetic deletion of Myomaker, a protein required for muscle stem cell fusion. Surprisingly, preventing myonuclear accretion did not impair longitudinal muscle growth or...
cause contractures (Figure 7E-H), definitively ruling out a role for myonuclear accretion in longitudinal muscle growth or contractures.

Having ruled out myonuclear accretion as relevant to longitudinal muscle growth and contractures, attention was turned to the other major process of muscle growth: protein synthesis. In denervated muscle post-BPBI, RNA-sequencing and gene ontology analysis revealed upregulation of 336 genes related to muscle development and structure, with no such processes substantially downregulated. Similarly, tracking puromycin incorporation into newly synthesized polypeptides found increased rates of protein translation in denervated muscle during contracture development. These data rule out reduced protein synthesis as a causative mechanism for contractures.

Since the overall accumulation of protein in growing muscle requires an anabolic balance between protein synthesis and protein degradation, a logical next question was whether protein degradation was elevated in neonatally denervated muscle. Initial experiments focused on the ubiquitin-proteasome pathway, which accounts for 90% of the protein breakdown in adult denervation-induced muscle atrophy. Indeed, denervated muscle 1-3 weeks post BPBI demonstrated elevated levels of proteins marked with ubiquitin for degradation, increased expression of muscle-specific drivers of protein degradation, and increased activity of the proteasome’s proteolytic enzymes (Figure 8). These findings implicate elevated protein degradation as a potential mechanism in contracture pathogenesis.

**Proof of Concept: A Cure Is Possible**

Pharmacologic manipulation of protein degradation was then used to definitively test the role of protein degradation in longitudinal muscle growth and contractures. Following BPBI, mice were treated with varied doses of the FDA approved drug, bortezomib, a highly specific proteasome inhibitor, along with appropriate controls. With blinded assessment 4 weeks following BPBI, bortezomib was found to completely prevent contractures (Figure 9).

Bortezomib worked in a dose-dependent manner and also rescued sarcomere length, indicating preservation of longitudinal muscle growth. Although this drug is not FDA-approved for use in muscle disorders, and concerns exist regarding cumulative toxicity, this finding confirms the role of proteasome-mediated protein degradation in longitudinal muscle growth and contracture formation, and provides the proof of concept that a biological therapy can indeed prevent the mechanical contracture phenotype (Figure 10). This discovery therefore represents a major potential advance in the treatment of neuromuscular contractures, as the first ever strategy to prevent contractures by correcting the causative molecular mechanism.

**Summary**

This body of scientific research, prompted by a serendipitous clinical discovery in BPBI, has elucidated a biological mechanism of childhood neuromuscular contractures and identified a novel successful treatment strategy to medically prevent them. Further work must be aimed at identifying relevant muscle-specific regulators of protein degradation to more specifically target this therapy. Elucidating the relative roles of afferent and sympathetic innervation may help to identify potential therapeutic targets in their molecular cross-talk with muscle. Furthermore, important questions must be answered regarding translational parameters, such as treatment timing and its relationship with reinnervation before the stage can be set for clinical trials in humans. Nonetheless, this complementary basic science and clinical work over the past decade has changed the paradigm of neuromuscular contractures from a mechanical to a biological realm, and from a problem of muscle strength to a problem of muscle length. Additionally, these scientific findings have shed light on the biology and neurologic control of postnatal muscle growth, with impact on BPBI and potentially a wide variety of other pediatric neuromuscular disorders, including CP.
References


