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The Sex-Dependent Role of Myostatin Signaling in Contractures Following Neonatal Brachial Plexus Injury

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Abstract:

Purpose: Neonatal brachial plexus injury (NBPI) causes disabling and incurable muscle contractures arising from impaired longitudinal growth of denervated muscles. This growth impairment is caused by dysregulation of muscle proteostasis characterized by increased proteasome-mediated protein degradation. Myostatin (MSTN), a secreted growth differentiation factor and prominent muscle-specific negative regulator of proteostasis, drives muscle protein degradation. MSTN signaling is thus a possible pathway through which

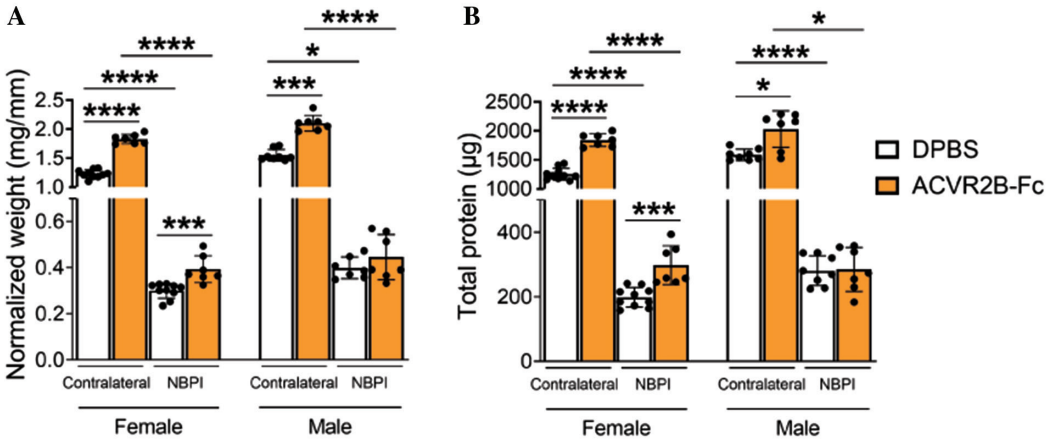

Figure 1. *ACVR2B-Fc increased (**A**) muscle weight and (**B**) protein content of normally innervated (contralateral) biceps in both female and male mice, and enhanced muscle size and protein content of denervated (NBPI) biceps only in female mice. All data are presented as mean ± s.d. *P<0.05, ***P<0.001, ****P<0.0001.*

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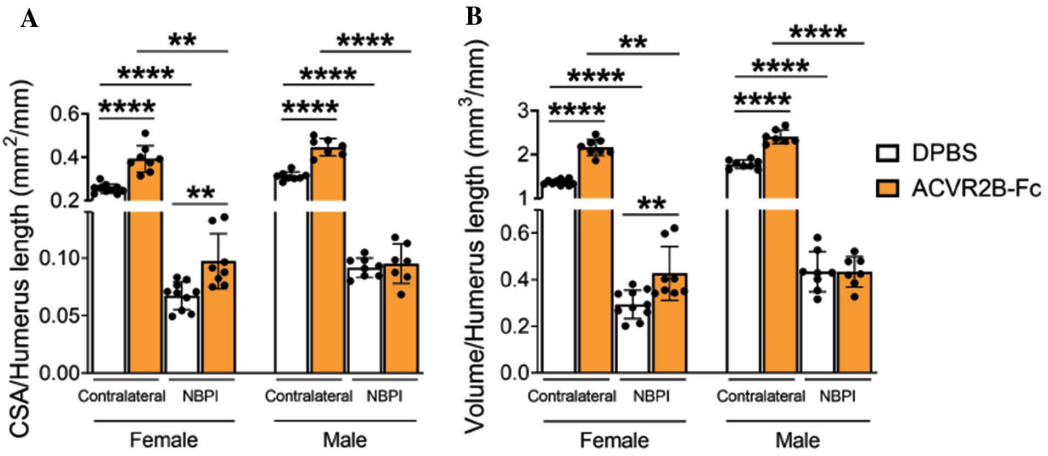

Figure 2. *ACVR2B-Fc increased the* ***(A)*** *cross-sectional area (CSA) and* ***(B)*** *volume of normally innervated (contralateral) brachialis in both female and male mice, and enhanced muscle growth of denervated (NBPI) brachialis only in female mice. CSA and volume are normalized to humerus length to control for effects of overall growth. All data are presented as mean ± s.d. **P<0.01, ****P<0.0001.*

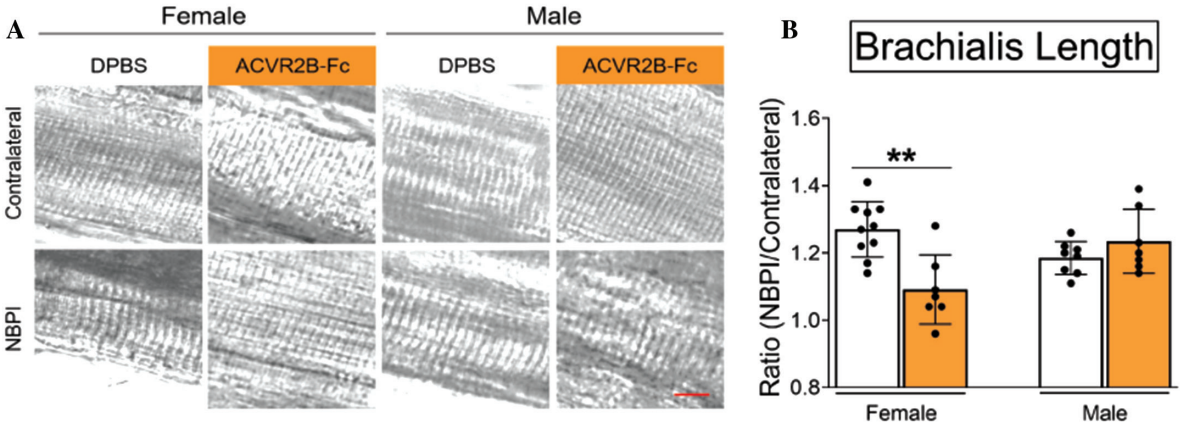

Figure 3. (A) *Representative DIC images of sarcomeres in brachialis muscles and* ***(B)*** *quantitation of sarcomere length showed a reduction in sarcomere overstretch in denervated (NBPI) muscles of female mice with ACVR2B-Fc treatment, indicating an improvement in functional muscle length. In* ***(B)****, sarcomere length on the NBPI side is normalized to the contralateral side, with any value over 1.0 indicating sarcomere overstretch on the NBPI side, and thus, functional muscle shortening. All data are presented as mean ± s.d. **P<0.01. Scale bar: 10 μm.*

denervation impairs muscle growth and a potential muscle-specific molecular target for preventing contractures. This study uses pharmacologic inhibition of MSTN signaling in a mouse model of NBPI to test the hypothesis that NBPI induces contractures through MSTN-dependent impairment of longitudinal muscle growth.

Methods: Unilateral NBPI was generated in postnatal day (P)5 mice. Mice were treated weekly with Dulbecco's phosphate-buffered saline (DPBS) or ACVR2B-Fc, a soluble inhibitory decoy receptor for MSTN, starting at P4. Mice were sacrificed at P33, whereupon elbow and shoulder range of motion were measured to assess contractures. Brachialis

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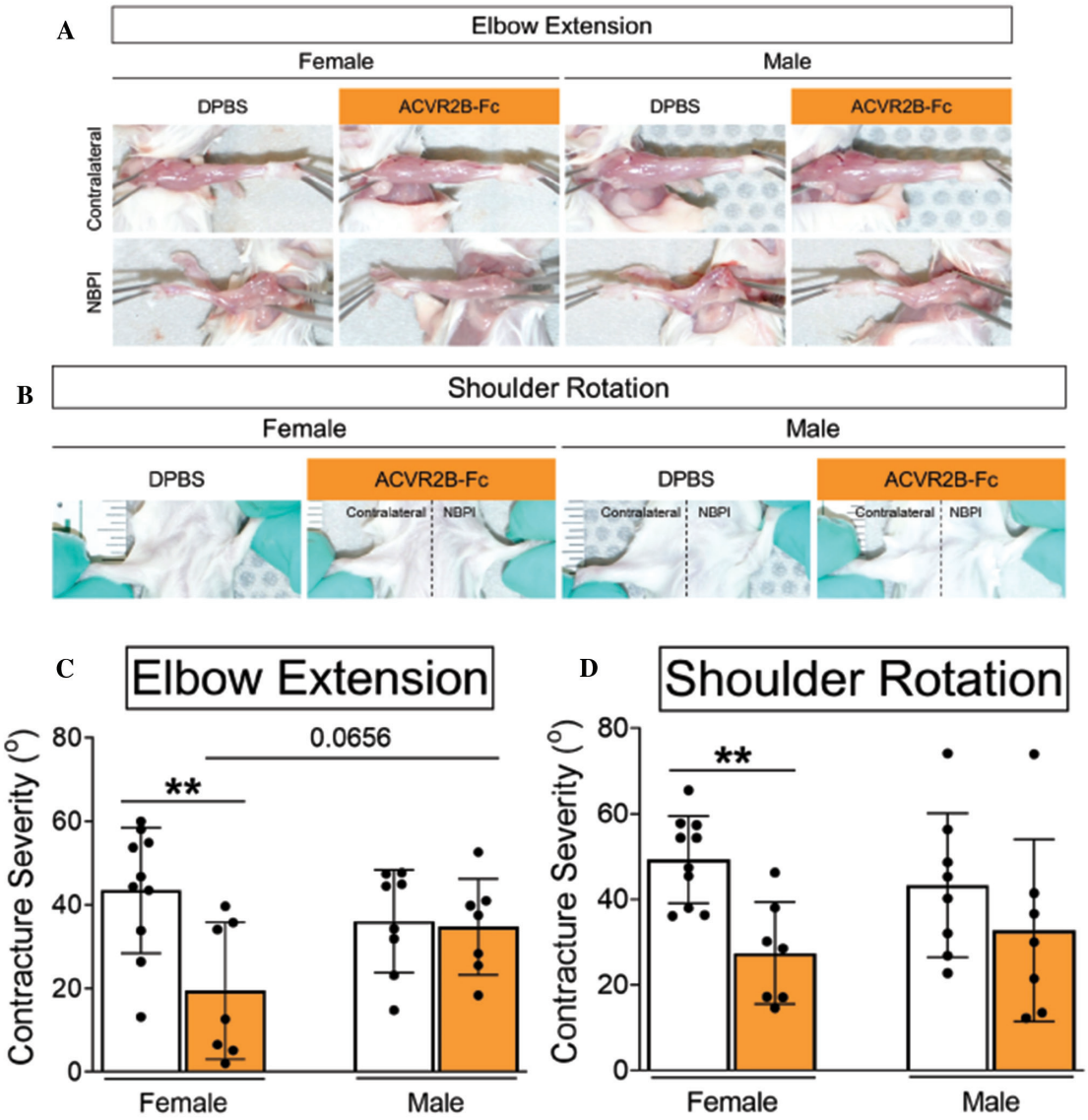

Figure 4. *(**A, B**) Representative images of forelimbs, and (**C, D**) quantitative analysis of passive elbow extension and shoulder rotation, respectively, revealed that ACVR2B-Fc treatment reduces the formation of elbow and shoulder contractures in female mice after denervation (NBPI). In (**C, D**), contracture severity is calculated as the difference in passive elbow extension or shoulder rotation between the NBPI side and the contralateral side. All data are presented as mean ± s.d. **P<0.01.*

muscles were prepared for microCT assessment of cross-sectional area (CSA) and volume, then processed for sarcomere length measurement as a readout of longitudinal muscle growth. Total protein content in biceps was assessed via Bradford assay. Protein degradation in triceps was quantified via 20S proteasome β1 subunit activity assay.

Results: In female mice, myostatin inhibition with ACVR2B-Fc enhanced muscle weight and total protein content of denervated biceps (Figure 1), increased cross-sectional and volumetric growth of denervated brachialis (Figure 2), preserved longitudinal growth in denervated brachialis (Figure 3), and reduced elbow and shoulder contracture severity in the denervated limbs

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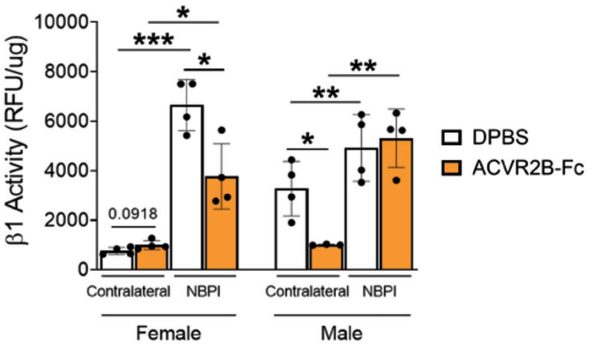

Figure 5. *Sex-specific improvements with ACVR2B-Fc were associated with reduced β1 subunit activity of the 20S proteasome in denervated (NBPI) muscles of female mice. All data are presented as mean ± s.d. *P<0.05, **P<0.01, ***P<0.001.*

(Figure 4). Analysis of protein dynamics demonstrates the improvements in female denervated muscles were associated with a reduction in proteasome β1 subunit activity (Figure 5). However, no such improvements in denervated muscles occurred in male mice with ACVR2B-Fc. Conversely, myostatin inhibition increased muscle weight, CSA, volume, and total protein content of the normally innervated, contralateral control muscles in both male and female mice (Figures 1 and 2).

Conclusion: Our findings reveal MSTN as a driver of impaired muscle growth and contracture formation in female but not male mice. This sex dimorphism lies in the pathophysiology of denervation-induced contractures rather than drug pharmacodynamics, given the sex-independent effects of MSTN inhibition on normally innervated muscle. Future studies must rigorously interrogate the contributions of sex in contracture pathophysiology.

Significance: Our discovery identifies MSTN as a signaling target for modulating contractures in females and highlights the overlooked role of sex in the pathophysiology of acquired neuromuscular disorders.

Level of Evidence: Not Applicable

FDA: The FDA has not cleared the following pharmaceuticals and/or medical devices for the use described in this presentation.

Disclaimer: The authors have no conflicts of interest to disclose.

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