# Silicon Nitride Cantilevers for Muscle Myofibril Force Measurements

**CNF Project Number: 1255-04**

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*Primary CNF Tools Used: GCA 5X stepper, Photolith spinners, Oxford 81 ion etcher*

**Abstract:**

**Spastic cerebral palsy (CP) is associated with increased passive muscle stress/stiffness in muscles, fascicles and fibres. However, crucial information on passive stress/stiffness on the sarcomere/myofibril level is missing. Previous research has shown that the sarcomere, the basic contractile unit of skeletal muscle, is overstretched in spastic muscle tissue compared to normal, and operates at long sarcomere lengths. At these increased lengths, the overstretched sarcomeres would have low active force-generating capacity and high passive forces, which agrees with the clinical situation whereby muscles are not only tight but also weak. Adductor longus muscle biopsies from children with CP and from typically developing children were analyzed for their *in vivo* sarcomere lengths, passive stress/stiffness, titin isoforms, and titin abundance and we found *in vivo* sarcomere lengths were increased and passive stress/stiffness and titin abundance were reduced, in CP muscle compared to controls. We conclude CP myofibrils are more compliant than control myofibrils, contrary to reports at higher structural levels. This increased compliance is caused by a reduction in the abundance of titin in CP sarcomeres. Because of the increased *in vivo* sarcomere length in CP, passive forces at functional muscle/sarcomere lengths are greater in children with CP compared to controls. Titin loss appears to be an adaptive response reducing high passive forces in CP muscles, but is insufficient to bring passive stresses to control levels, *in vivo*.**

**To measure muscle forces in the nano-Newton range, silicon nitride cantilever pairs were manufactured using the GCA 5x-stepper photolithography system and the Oxford 81 ion etching system at the CNF, and then used in our lab in Canada.**

## Summary of Research:

The aim of this research was to investigate passive properties of single CP myofibrils to see if an increase in passive stress/stiffness mirrors what has been previously reported in CP muscles, fascicles and fibres. The isolated myofibril is devoid of passive structural elements outside of the sarcomere, such as the extracellular matrix (including collagen), and so this preparation provides crucial insight into the mechanics of sarcomeres and titin in CP. The molecular spring titin is an important structural element within the sarcomere, tethering the thick filament (myosin) to the Z-discs. This protein centers the thick filament within the sarcomere and is thought to account for the majority of passive force generated when sarcomeres are stretched [1].

## Methods:

Biopsies of operated adductor longus muscle were obtained and either held at the *in vivo* length for later *in vivo* sarcomere length determination, stored in a special rigor solution for generation of myofibrils, or frozen for titin analysis.

To obtain myofibrils, samples were homogenized and placed in the testing chamber [2]. The testing protocol was a ramp-hold-return design and every myofibril was lengthened (0.1 μm/sarcomere/second) from slack length (< 2.0 μm) to a sarcomere length (SL) of 2.4, 2.8, 3.2, 3.6 and 4.0 μm, sequentially. Steady-state stress and SL were measured at the end of a 1-minute hold. The cantilevers used were manufactured at the CNF and had a stiffness of 75 nN/μm.

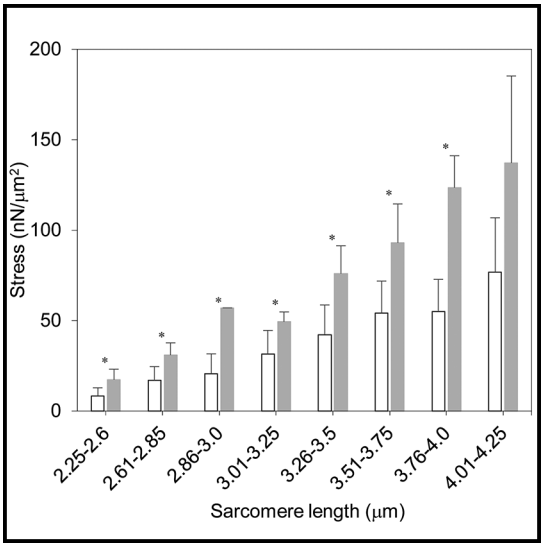


*Figure 1: Passive stress generation versus sarcomere length for CP and control adductor longus. Mean stress ± SD for CP (white) and control (grey) are significantly different at all sarcomere lengths tested, except for the longest range (> 4 μm). (*p < 0.05). The non-significant result at the longest sarcomere lengths is explained by the reduced number of observations.*

Titin isoforms were determined using 2% agarose-strengthened polyacrylamide electrophoresis gels. To assess the total titin content in the tissues, a ratio of titin to nebulin was calculated. Nebulin is a large protein found in association with the thin filament; a single nebulin molecule spanning each thin filament, thus the titin/nebulin ratio provides a measure of the abundance of titin relative to the contractile thick and thin filaments in a sarcomere.

## Results:

For the CP participants, 46 adductor longus myofibrils were isolated and analyzed. For the controls, 8 myofibrils were isolated and analyzed. At all matched sarcomere length ranges (< 4 μm) passive steady-state stress was significantly lower in CP compared to control myofibrils (Figure 1). The elastic modulus for CP was 98 ± 45kPa, significantly lower than in controls (166 ± 22kPa, p = 0.0005).

Titin molecular weights for CP and control tissues were 3611 ± 41kDa and 3615 ± 8kDa, respectively (no difference: p = 0.76). The ratio of titin-nebulin content for CP was 1.47 ± 0.37 and for control was 3.26 ± 0.16 (p = 0.004). This difference is indicative of a reduced titin content relative to an unchanged nebulin content within the sarcomeres of the CP muscle. *In vivo* sarcomere lengths were much greater in CP than in typically developing children (3.6 μm versus 2.7 μm).

## Discussion and Conclusions:

Passive stresses are much lower in CP myofibrils compared to typically developing control myofibrils. This finding is in contrast to results found on the single fibre, fascicle and muscle level, and as such, appears to be an adaptation to reduce an already excessive passive force in spastic muscles. Despite the much lower stresses in CP compared to control myofibrils at matched sarcomere lengths, passive stresses at *in vivo* sarcomere lengths are much greater in CP than in typically developing children because the CP sarcomeres are over-stretched (3.6 μm versus 2.7 μm). A loss in titin content in CP muscle was found to be associated with decreases in the passive peak stress and elastic modulus of CP sarcomeres, which may be an adaptive response resulting in a more compliant myofibril, to partially offset the high passive stresses experienced at long *in vivo* sarcomere lengths of CP patients [3].

## References:

[1] Bartoo, M. L., Linke, W. A., and Pollack, G. H. Basis of passive tension and stiffness in isolated rabbit myofibrils. Am. J. Physiol. 273, C266-276 (1997).

[2] Joumaa, V., Rassier, D.E., Leonard, T.R., Herzog, W. The origin of passive force enhancement in skeletal muscle. Am. J. Physiol. Cell Physiol. 294, C74-78. https://doi.org/10.1152/ajpcell.00218.2007 (2008).

[3] Smith, L. R., Lee, K. S., Ward, S. R., Chambers, H. G., and Lieber, R. L. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased *in vivo* sarcomere length. J. Physiol. 589, 2625-2639 (2011).