MECHANICAL PROPERTIES OF SPASTIC MUSCLE CELLS AND THEIR CORRELATION WITH CLINICAL SEVERITY IN CHILDREN WITH CEREBRAL PALSY
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INTRODUCTION:
Cerebral palsy (CP) is a non-progressive neuromuscular disorder caused by an upper motor neuron lesion. It results from pre-, peri- or post-natal insult, most commonly associated with prematurity. CP affects 2 in 1000 live births annually (5) and is thus one of the most common chronic disabling conditions of childhood. While most studies focus on the neurologic basis of spasticity secondary to CP, there is strong evidence that the passive mechanical properties of skeletal muscle are also altered (1-4). However, it is not clear whether these mechanical changes are due to changes in the muscle cell, extracellular matrix (ECM) or a combination of the two. Thus, the purpose of this study was to compare the mechanical properties of single skeletal muscle fibers from normal children and those with CP.

METHODS:
This prospective study obtained biopsies from children with cerebral palsy (n=10; age=11.0±3.1) or healthy children (n=10; age=13.8±2.3) undergoing surgery. Informed consent was obtained from the parents of all patients. Procedures were performed under the auspices of the Human Research Protection Program at the University of California, San Diego and Children’s Hospital, San Diego. For all children with CP, the Gross Motor Function Classification Score (GMFCS) was determined (ranging from 1-5 with 1 representing full ambulatory ability) (6).

After biopsies were obtained, they were immediately pinned to cork board and placed in a relaxing solution for 60 minutes containing (mM): imidazole 59.4, KCH$_2$(mM): imidazole 59.4, KCH$_2$O$_4$ (86.0), CatCK/CH$_2$O$_4$ (0.13), Mg$_2$(KCH$_2$O$_4$)$_2$ (10.8), KEGTA (5.5), KH$_2$PO$_4$ (1.0), NaN$_2$ATP (5.1), and 50 µM leupeptin to prevent hyper-contraction and proteolytic degradation. Biopsies were stored at -20 °C in storage solution containing KPr, EGTA, MgCl$_2$, imidazole, NaN$_2$ATP, NaN$_2$, glutathione, glycerol and leupeptin until analyzed (no more than 3 weeks). Single fibers were dissected from the biopsy and transferred to an experimental chamber for mechanical testing. One end of the fiber was tied to a micromanipulator and the other end to a force transducer using 9-0 suture. Resting fiber length and diameter were measured and the fiber was transilluminated by a He-Ne laser to measure sarcomere length. A clean diffraction pattern confirmed fiber orientation. Each fiber was elongated in 250 µm increments after which stress-relaxation was allowed for 2 minutes and sarcomere length and tension were recorded. Fibers were elongated to mechanical failure. Ultimate sarcomere length, ultimate stress and strain, and tangent modulus were calculated from the slope of the stress-strain relationship from single muscle cells. Spastic fibers were significantly stiffer than normal fibers (p<0.0005; Fig 1). Collagenase treatment, which selectively digests extracellular collagen, significantly decreased the tangent modulus in both spastic and normal fibers, yet spastic fibers still had a significantly higher tangent modulus compared to normal fibers (p<0.01; Fig 2). These mechanical differences observed were not explained based on either differences in myosin heavy chain composition or the child’s age.

Importantly, there was a significant difference in tangent modulus between children in the three different GMFCS categories obtained (p<0.05), and further, there was a significant positive correlation between GMFCS score and modulus (p<0.05; Fig. 3).

DISCUSSION:
This study has demonstrated that some of the increased stiffness observed clinically in children with CP can actually be attributed to the muscle fibers themselves. Spastic fibers were stiffer compared to normal (Fig. 1) and this difference could not be explained by differences in extracellular collagen since differences persisted, even after digestion with Type IV collagenase (Fig. 2). The difference was not explained by shorter sarcomere length in the spastic fibers, different fiber types or age. These data thus suggest a significant remodeling of the muscle cell’s cytoskeletal network, a change that may implicate intermediate filaments, microtubules, or even the giant intramuscular protein, titin, in the etiology of muscle spasticity.

One might suggest that such an increased stiffness in spastic fibers was an unimportant microcellular phenomenon. However, we have shown here for the first time, a correlation between the microcellular mechanics and clinical severity in CP as indicated by the GMFCS score. Not only was there a difference in stiffness between GMFCS scores but there was an increasing stiffness with increasing clinical severity (Fig. 3). It is thus possible that children with CP may actually be working “against” their own muscles during movement. This may partially explain the increased energetic cost of ambulation observed in CP (7). The cause of this increased stiffness in the spastic fibers remains to be determined. Future studies are required in order to determine the structural basis for the altered mechanical properties in spastic muscle cells.

REFERENCES

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