

INFERIOR MECHANICAL PROPERTIES OF SPASTIC MUSCLE DUE TO HYPERTROPHIC BUT COMPROMISED EXTRACELLULAR MATRIX MATERIAL

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INTRODUCTION:

Cerebral palsy (CP) is a neuromuscular disorder, caused by a brain lesion in the pyramidal and extra pyramidal systems and is one of the most common chronic disabling conditions of childhood. Physical signs include loss of balance, decreased proprioception, dysfunctional motor control and joint contracture secondary to muscle spasticity (1). While numerous clinical studies of spastic muscle have been reported, only one directly measured cellular (2) but none measured tissue properties. The present study was specifically designed to compare the properties of cellular and extracellular matrix components of spastic muscle tissue.

METHODS:

All patients included in this study or their parents provided informed consent for the muscle biopsies that were obtained secondary to planned surgical procedures. Procedures were performed with the full approval of the Human Ethical Committee at Göteborg University as well as the Committee on the Use of Human Subjects in Research at the University of California, San Diego and V.A. Medical Centers. Patients with spasticity suffered from cerebral palsy, whereas "control" patients were receiving surgery for nonneuromuscular disorders such as fracture repair, joint fusion, or tendon repair.

Biopsies were excised and immediately placed into a muscle-relaxing solution composed of (mM): EGTA (7.5), potassium propionate (170), magnesium acetate (2), imidazole (5), creatine phosphate (10), ATP (4), leupeptin (17 µg/ml), and E64 (4 µg/ml), to prevent protein degradation.

Single muscle cells (n=40 normal, 15 spastic) or small bundles (n=22 normal, 12 spastic) were dissected from biopsies while in chilled relaxing solution under 40X magnification with epiillumination using microsurgical forceps and transferred into an experimental chamber filled with relaxing solution. The specimen was then secured on either side to 125 µm titanium wires using two 9-0 silk suture loops and one wire was secured to a ultrasensitive force transducer while the other was secured to a micromanipulator. Structural integrity of the bundles treated in this way was excellent as evidenced by clear striation patterns and distinct, periodic laser diffraction patterns.

Each specimen was elongated in 250 µm increments after which stress-relaxation was permitted for 2 minutes and sarcomere length, tension, and diameter (to permit stress calculations) were measured. Specimens were elongated until mechanical failure occurred.

At the conclusion of the mechanical test, bundle were pinned at rest length into a small plastic cassette and frozen in liquid nitrogen-cooled isopentane (-159°C) and stored at -80°C until analyzed. Cross-sections (10 µm thick), taken from the mid-portion of the tissue block, were cut on a cryostat at -25°C to quantify muscle and ECM morphology. Muscle fiber size within each bundle was quantified by manually tracing the outline of each cell within the best tissue section.

Data were grouped by specimen type (fiber vs. bundle) and tissue type (normal vs. spastic) and analyzed by two-way analysis of variance (ANOVA). Data were screened for normality and skew to justify the use of parametric statistics. *Post hoc* multiple t-tests were used to make specific comparisons between bundles and cells of a given type and between like specimens of different types.

RESULTS:

For both normal and spastic muscle, tangent modulus was significantly greater in bundles compared to single fibers (p<0.0001); Fig. 1), but the difference was much more dramatic for normal muscle compared to spastic muscle (filled vs. hatched bars in Fig. 1).

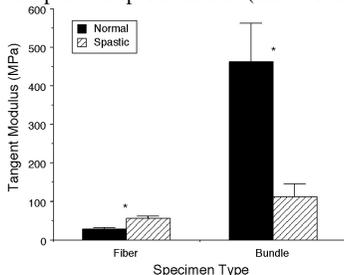


FIGURE 1: Tangent modulus calculated from the linear portion of the sarcomere length-stress relationship from single cells and muscle fiber bundles of normal (filled bars) and spastic (hatched bars) subjects. Asterisks indicate p<0.05 between normal and spastic specimens.

Whereas spastic muscle bundle modulus was only twice the single fiber modulus, normal muscle bundle modulus was over 15 times greater than the modulus of a normal single muscle cell (Fig. 1). Two-way ANOVA of tangent modulus revealed a significant effect of tissue type (p<0.01), a significant effect of specimen type (p<0.0001) and a highly significant interaction term (p<0.005). These data clearly reveal a dramatic difference in the behavior of spastic muscle bundles compared to normal muscle fiber bundles and a more subtle difference between single cells of the two types.

Spastic muscle fibers were only one-third the size of normal fibers (Fig. 2A). However, since the spastic and normal bundles were approximately the same size, we calculated that, only about 40% of the spastic bundle was occupied by muscle fibers whereas 95% of the normal bundle was occupied by muscle fibers (Fig. 2B).

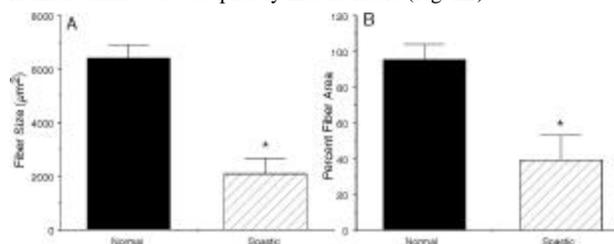


FIGURE 2: Morphological properties measured from muscle bundles of normal (filled bars) and spastic (hatched bars) subjects. (A) Muscle fiber area was digitized from light micrograph cross-sections. (B) The percentage of bundle cross-sectional area occupied by muscle fibers. Note that a much larger proportion of spastic muscle bundles is occupied by extracellular material. * indicates significant difference between normal and spastic muscle types.

DISCUSSION:

The most significant mechanical difference between normal and spastic muscle was the higher tangent modulus of small bundles compared to single cells (Fig. 1). In normal muscle, the addition of the ECM dramatically increased modulus from ~28 kPa to ~470 kPa, a fifteen-fold increase (filled bars, Fig. 1). This is especially impressive in light of the calculation in the current study that the ECM of normal muscle made up only 5% of the specimen cross-section (Fig. 2B). Considering the muscle and ECM to be acting mechanically in parallel, the tangent modulus of the normal muscle's ECM is calculated to be ~8.5 GPa, extremely stiff for mammalian connective tissue which is reported to have a modulus of from 1 to 3 GPa (3,4). Surprisingly, spastic muscle bundles had a modulus that was only twice the fiber modulus (hatched bars, Fig. 1). Apparently, this was due to the relatively small area fraction of stiff muscle cells (Fig. 1) embedded in a large quantity of low-quality ECM (Fig. 2B). Again, considering the muscle and ECM to be acting in parallel, the tangent modulus of the spastic ECM is calculated to be only ~0.20 GPa, 45 times lower than that calculated for normal ECM and much lower than any literature value reported for connective tissue. The structural basis for the inferior mechanical properties of the spastic ECM is not known. Future studies are required to determine whether spastic ECM changes its properties in response to the altered muscle properties or *vice versa*. However, these data clearly demonstrate dramatic alterations of both muscle and ECM properties in response to upper motor neuron lesion.

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