Passive Mechanical Properties of Muscle in Hamstring Contractures of Children with Spastic Cerebral Palsy

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INTRODUCTION:
Children with spastic cerebral palsy (CP) often develop hamstring contractures, however, the mechanism of contracture formation is not known. Contractures represent a resistance of muscle to increased length. The structural elements responsible for this increased stiffness are also not known [1]. Previous work showed that single fibers from “contractured” muscle tissue has increased passive stiffness that could lead to the overall increased stiffness of the muscle [2]. Interestingly, the opposite result was observed when scaled to bundles of muscle fibers, as bundles from typically developing (TD) children were stiffer than contractured bundles [3]. Unfortunately, these samples were compared across a variety of muscles which may confound the results. To avoid potential complications associated with comparing among different muscles, the purpose of the current study was to investigate passive mechanical properties of two specific hamstring muscles involved in gait—gracilis (GR) and semitendinous (ST).

Hamstring contractures limit knee extension and are suggested as a contributing factor of crouch gait in patients with CP [4]. Despite new therapies, current best practices are unable to prevent contractures. Further understanding of the mechanism of contracture and the elements responsible for them could lead to new therapies to prevent contracture development and improve muscle function.

METHODS:
Biopsies were obtained during hamstring lengthening surgery for patients with CP and from the hamstring autograft used in ACL reconstruction surgery for TD patients. All procedures were performed with full IRB approval from UCSD. Biopsies were removed and placed directly into a glycerol relaxing solution. Single fibers were dissected in chilled relaxing solution and transferred to a loading chamber at room temperature. The fiber was attached via suture to a force transducer on one end and a motor arm on the other end. Muscle fiber sarcomere length was measured by laser diffraction and monitored using a photodiode. Fiber length was set to the minimum length that produced a measurable force. The motor stretched the fiber in approximately 0.25 µm sarcomere length increments. Force was continuously measured over 2 minute time interval while the fiber underwent stress-relaxation. Stretches were repeated ~10 times or over approximately 2.5 µm sarcomere length range. Data were fit to a viscoelastic model containing a spring in parallel with a series spring and dashpot. Fiber bundles were measured in the same way as single fibers but with a strain dependent parallel spring which required a quadratic stress-strain fit (Fig. 1).

Single fibers were homogenized in sodium dodecyl sulfate-vertical agarose gel electrophoresis (SDS-VAGE) sample buffer used in gel electrophoresis [5]. The gel regression relationship was calculated based on the three standard lanes containing human soleus titin and rat cardiac titin.

RESULTS:
To compare stiffness across samples and muscles, tangent stiffness at a sarcomere length of 4.0 µm was calculated (Fig. 2). A 3-way ANOVA was run with scale, muscle, and condition with significant effect of p < 0.05. Each main effect was significant indicating differences between fibers and bundles, gracilis and semitendinosus, and CP compared to TD. There was also a significant interaction for condition/scale, indicating that CP is differentially effects bundles and muscle/scale indicating that ECM is different between gracilis and semitendinosus. Post Hoc tests demonstrated that in contrast to previous studies in the upper extremity [2], our data revealed that stress was equal between control and contractured fibers at all sarcomere lengths. This was true for both gracilis and semitendinosus muscles (Fig. 2). However, when the fiber bundles were measured, contractured muscle was stiffer than control muscle (p<0.05), with a greater difference in gracilis (Fig. 1). These differences in stiffness increased at longer sarcomere lengths.

Figure 2: Tangent modulus of CP (filled bars) and control muscle (open bars) from gracilis and semitendinosus muscles. Tangent modulus is defined at a sarcomere length of 4.0 µm. Results show that CP fibers are similar to control, but CP bundles are significantly stiffer than controls (p < 0.05 for GR and ST bundles)

Titin molecular weight has been linked to passive stiffness of skeletal muscle fibers [6]. However, there was no statistically significant difference in titin molecular weight between CP and control fibers in either muscle tested (CP: 3784 +/- 27 kDa; TD: 3747 +/- 21 kDa).

DISCUSSION:
These results demonstrate that the passive mechanics of gracilis and semitendinosus muscle cells themselves are not altered in contracture, but that the ECM connecting fibers together is altered and becomes stiffer in CP muscle. Because fiber bundles have a non-linear stress strain relationship, this difference becomes more pronounced at greater sarcomere lengths. These results implicate a major role of the ECM in the increased passive stiffness in joint contracture, rather than titin. These data may provide a target for therapeutic intervention in the treatment of muscle contractures in CP and other upper motor neuron lesions.

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REFERENCES: