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, but the structural elements responsible for increased stiffness are 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 have confounded 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 semitendinosus (ST).

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 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 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. Fibers stress vs. sarcomere length curves were linearly fit. Fiber bundles were measured in the same way as single fibers but with a quadratic fit (Fig. 1).

Single fibers were homogenized in sodium dodecyl sulfate-vertical agarose gel electrophoresis (SDS-VAGE) sample buffer used in gel electrophoresis [4] to determine titin size. Titin mass was calculated by regression based on three standard lanes.
Sarcomere lengths were determined in vivo using specialized biopsy clamps. After the muscle was exposed the leg was placed in 90° of hip and knee flexion and the biopsy clamp was engaged. The clamped biopsy was then freed and placed in a fixing solution for 3 days. Sarcomere length was determined by laser diffraction after fixation.

RESULTS

To compare stiffness across samples and muscles, tangent stiffness at a sarcomere length of 4.0 µm was calculated (Fig. 2A, 2B). A 3-way ANOVA was run with specimen scale, muscle, and condition with significant main effects (p < 0.05). There was also a significant condition by scale interaction, suggesting a CP-dependent difference in bundle properties. Post Hoc tests demonstrated that, in contrast to previous studies in the upper extremity [2], stress was equal between control and contractured fibers at all sarcomere lengths for both gracilis and semitendinosus muscles (Fig. 2A). However, when fiber bundles were considered, contractured muscle was stiffer than control muscle (p<0.05). Sarcomere strain experienced by the muscle in vivo was also investigated. At a position of 90° of hip and knee flexion CP muscles had significantly longer sarcomere lengths than predicted by model data for controls (Fig. 3). This means that bundles in vivo at the same joint configuration would produce much larger stiffness for CP (Fig. 2C). Titin molecular weight has been linked to passive stiffness of skeletal muscle fibers [5]. However, there was no 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. This is compounded by the fact that CP muscles have longer sarcomere lengths in vivo. These results implicate a major role of the ECM in the increased passive stiffness in joint contracture, rather than titin mechanics.

REFERENCES