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STRETCHED SARCOMERES CONTRIBUTE TO EQUINUS CONTRACTURE IN PATIENTS WITH CEREBRAL PALSY

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INTRODUCTION

It is often stated that muscle fascicles in children with cerebral palsy (CP) are shortened compared to those of typically developing (TD) children, but ultrasound data provide conflicting results [1]. We believe that this conflict occurs because a crucial missing piece of information, muscle sarcomere length, cannot be measured by ultrasound, which thus cannot capture the underlying adaptation in CP. These adaptations in architectural properties, specifically the *in vivo* muscle sarcomere length, can have a significant impact on the passive mechanical environment of muscle. Two muscles that appear identical by ultrasound may have very different mechanical characteristics and sarcomere length distributions. Therefore, the purpose of this study was to quantify fascicle length, sarcomere length, and serial sarcomere number in TD children and those with CP, as well as to characterize the passive mechanical environment of these muscles. Based on previous work by others, we hypothesized that serial sarcomere number would be smaller in children with CP than in TD children, and passive mechanical tangent stiffness would be higher in patients with CP.

METHODS

Subjects included children with CP undergoing tendon lengthening (n=20) aged 12.1±5.3 years and TD children visiting the clinic for non calf sports-related injuries (n=21) aged 12.4±3.4 years. All procedures were approved by the local institutional review board and parental consent and child's assent was obtained prior to enrollment.

Multiple ultrasound images were taken of the soleus muscle, and the tibial length of each participant was measured to account for differences in size. Average muscle fascicle length was calculated as described previously [2] using the distance between fascial planes and fascicle angle. Multiple measurements were made and the average was used for each child. In children with CP, intraoperative soleus biopsies were then obtained with clamps placed over a dissected section of muscle in the same region as had been measured by ultrasound. Samples were divided and one section was placed in glycerinated muscle relaxing solution while the other was fixed in formalin. Sarcomere lengths of fixed muscle were measured by laser diffraction [3]. TD sarcomere lengths (n=19) were obtained from a previous study from our lab [4] and adjusted for ankle angle. Ankle angle was recorded for both ultrasound images and biopsies, and TD ultrasound measurements were made at the average ankle angle value of patients with CP.

Passive mechanical testing was carried out on samples preserved in relaxing solution. Muscle fibers or bundles of fibers including surrounding connective tissue were dissected from the biopsy and placed in a chamber filled with cold relaxing solution. They were then tied to a motor arm on one end and to a force transducer on the other. Using a custom made LabView program, samples were stretched in increments of 0.25 μm/sarcomere over a sarcomere length range of ~2-4.5 μm. A three-minute relaxation period was provided after each stretch. Force and sarcomere length were measured for each stretch, and fiber or bundle stress and tangent stiffness with respect to sarcomere length were calculated at each sarcomere length.

RESULTS AND DISCUSSION

All data are presented as mean±SD. No significant difference ($p>0.6$) was found between the average soleus fascicle length of patients with CP (3.6±1.2 cm) and TD individuals (3.5±0.9 cm) (Fig. 1). This result was not affected by normalization for tibial length, so results are shown without normalization. Sarcomere length, however, was significantly longer ($p<0.0001$) in patients with CP (4.07±0.45 μm) compared to TD patients (2.17±0.24 μm; [2]) (Fig. 2).

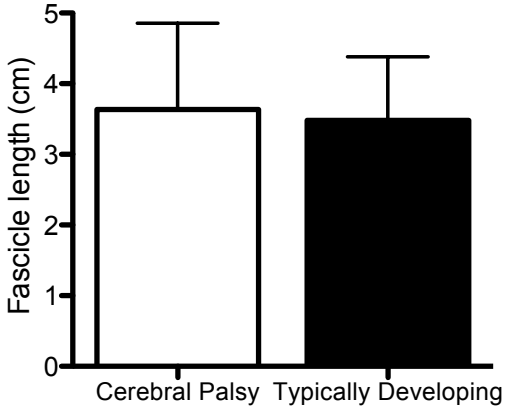

Figure 1: Fascicle lengths were not significantly different between patients with and without CP ($p>0.6$).

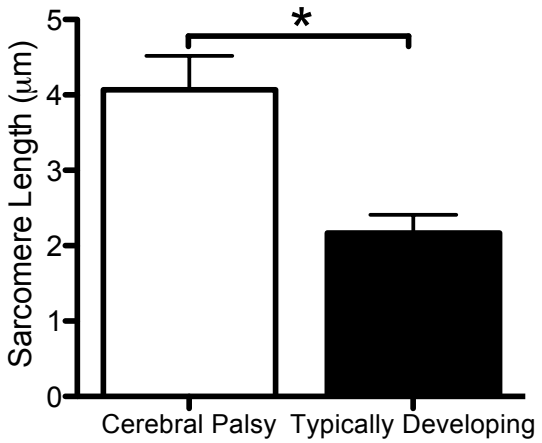

Figure 2: Sarcomere length was significantly longer in patients with CP ($p<0.0001$) [4].

Because of the long sarcomere length in patients with CP, calculated average serial sarcomere number was significantly lower ($p<0.0001$) in these patients (9,190±3,810) compared to TD (16,040±4,160) individuals (Fig. 3). Thus, muscle fibers in patients with CP have the same fascicle length as those of TD participants because their sarcomeres are highly stretched.

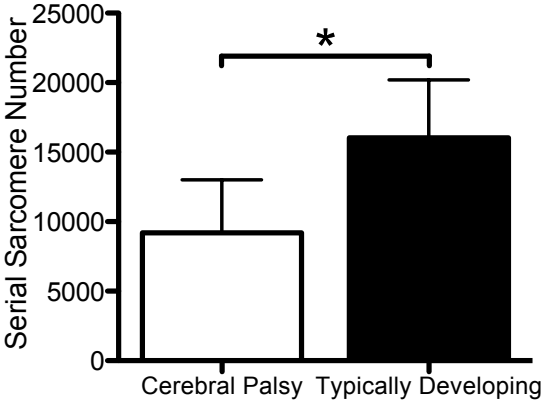

Figure 3: Serial sarcomere number is nearly doubled in TD participants as compared to those with CP ($p<0.0001$).

Passive mechanical testing of muscle fibers and bundles showed that both bundles and fibers are significantly stiffer at the in vivo sarcomere length of patients with CP (4.07 μm) than of TD individuals (2.17 μm). CP fibers have a tangent modulus of 21.04±15.1 kPa/μm compared to the TD value of 2.51±4.6 kPa/μm, and CP bundles have a modulus of 24.92±16.5 kPa/μm compared to 0.34±7.3 kPa/μm for TD bundles ($p<0.001$).

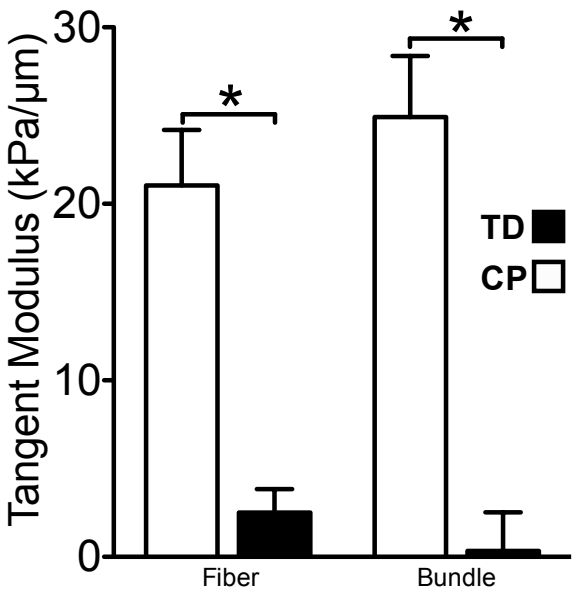

Figure 4: At *in vivo* sarcomere lengths, the passive stiffness of CP fibers is significantly higher than that of TD fibers, and CP bundle stiffness is significantly higher than that of TD bundles ($p<0.001$).

CONCLUSIONS

Our data demonstrate for the first time that, while fascicle length is similar between TD participants and those with CP, sarcomere number is significantly decreased in CP. At the long sarcomere length measured in patients with CP, the passive force produced by muscle is much higher. These data together suggest that the contractures observed in CP may be caused by highly stretched sarcomeres that create very high joint deforming forces.

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