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High Resolution Muscle Measurements Provide Insights into Equinus Contractures in Patients with Cerebral Palsy

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Abstract

Muscle contractures that occur after upper motor neuron lesion are often surgically released or lengthened. However, surgical manipulation of muscle length changes a muscle's sarcomere length (L_s), which can affect force production. To predict effects of surgery, both macro- (fascicle length (L_f)) and micro- (L_s) level structural measurements are needed. Therefore, the purpose of this study was to quantify both L_s and L_f in patients with cerebral palsy (CP) as well as typically developing (TD) children. Soleus ultrasound images were obtained from children with CP and TD children. L_f was determined and, with the joint in the same position, CP biopsies were obtained, formalin fixed and L_s measured by laser diffraction. Since soleus L_s values were not measurable in TD children, TD L_s values were obtained using three independent methods. While average L_f did not differ between groups (CP=3.6±1.2 cm, TD=3.5±0.9 cm; $p>0.6$), L_s was dramatically longer in children with CP (4.07±0.45 μ m vs. TD=2.17±0.24 μ m; $p<0.0001$). While L_f values were similar between children with CP and TD children, this was due to highly stretched sarcomeres within the soleus muscle. Surgical manipulation of muscle-tendon unit length will thus alter muscle sarcomere length and change force generating capacity of the muscle.

Keywords

cerebral palsy; skeletal muscle; sarcomere; fascicle length; ultrasound

Introduction

Surgical manipulation of muscle length is common in orthopedic surgery procedures, including tendon lengthening, total hip arthroplasty, and tendon transfer. Muscle length changes caused by surgery have functional consequences, since muscles are very length

sensitive. The muscle length-tension curve, or “Blix” curve[1], defines the fraction of maximum muscle force produced as a function of length. Specifically, the sarcomere length-tension curve defines active muscle force as a function of *sarcomere* length (L_s) (Fig. 1). Sarcomeres are microscopic length-sensitive force generators in muscle whose properties are well known[2]. While direct measurement of muscle length intraoperatively and indirect calculation of fascicle length (L_f) using ultrasound are fairly easy, neither of these methods provides insights into a muscle’s L_s . Knowledge of L_s requires use of specialized instrumentation[3].

Because of the length-sensitivity of sarcomeres, muscles will either become stronger or weaker after surgery, depending on how their length is changed relative to their pre-surgical position on the length-tension curve. Specifically, for a muscle at a constant level of neural drive, if L_s is longer than optimal, increasing its length during surgery will *decrease* force production. Conversely, if L_s is shorter than optimal, increasing its length will *increase* force production. For this reason, intraoperative surgical decisions can make a muscle either weaker or stronger depending on starting conditions. Not only does L_s impact muscle function, but the number of sarcomeres in series also has an effect. If two muscles differ only in serial sarcomere number, the muscle with more sarcomeres in series will have greater excursion and will be in a different position on the length-tension curve at a given joint angle compared to the other muscle[4]. Thus, for a surgeon to make a knowledgeable decision about muscle function due to surgery both L_s and serial sarcomere number must be known. While some investigators discuss L_f as if it were a surrogate for L_s [5], it is not. Long fascicles may be associated with short sarcomeres and *vice versa*, leading to different numbers of sarcomeres in series and different excursion and force production characteristics. Therefore, unambiguous evaluation of a muscle’s condition requires simultaneous evaluation of both its L_f and L_s . Since sarcomeric force producing proteins actin and myosin dictate the dimensions of the length tension curve[6], defining the length of these protein filaments is also important to define functional capacity.

One intraoperative situation in which a complete understanding of a muscle’s condition may be especially useful is when performing surgery on patients with contractures due to cerebral palsy (CP). Muscle lengthenings are common in CP, as in surgical correction of equinus contracture[7; 8], and, while this surgery is believed to improve patient function, there is evidence that overcorrection may be a complication[9]. While it is generally believed that fascicles in patients with CP are shorter compared to typically developing (TD) children, the literature is ambiguous[10]. We suggest, based on the discussion above, that this is because L_f data have been interpreted in the absence of any L_s data. Therefore, the purpose of this study was to measure both L_f and L_s in patients with equinus contractures secondary to CP to more appropriately compare fiber length between children with and without CP.

Methods

Study Participants

All participants were recruited from the pediatric hospital’s orthopedic department. The Institutional Review Board approved this study, and all patients and guardians provided informed consent or assent. Patients with CP were undergoing tendon lengthening for ankle

equinus contracture ($n=20$, 7 female, age= 12.1 ± 5.3 years, mean $\pm$ SD) while TD patients were attending a clinic for injuries unrelated to the imaged leg ($n=21$, 11 female, age= 12.4 ± 3.4 years).

Ultrasound Measurements of Fascicle Length

Ultrasound images of the medial aspect of the soleus were obtained with a t3200 Terason portable ultrasound system (Teratech Corporation, Burlington, MA, USA) in B-mode using a 15L4 Linear Array probe (variable frequency 4-15MHz) with constant settings for all patients. Participants sat in one chair and placed their foot on another (Fig. 2A). Tibial length was measured on the medial aspect of the leg between the medial malleolus and tibial plateau. For TD patients, ultrasound images were taken in two ankle orientations: 0° and 30° of plantarflexion. Ultrasound images of patients with CP were obtained in maximum passive dorsiflexion, ranging from 0° to 45° of plantarflexion ($32.8^\circ\pm7.9^\circ$, $n=20$), depending on contracture severity. Images were converted to TIFF format in OsiriX software[11] and quantified using ImageJ[12].

Soleus superficial and deep fascial planes were identified and the distance between them (d) measured perpendicular to the aponeuroses (Fig. 2B). Fascicle angle (θ) corresponding to each d was also measured using ImageJ (Fig. 2C). Five to ten points along the soleus with clearly identifiable fascicles were measured for each patient. L_f was calculated as the hypotenuse of the triangle corresponding to angle (θ) and line between fascial planes (d) as previously described by others[13]. Measurements were reproducible, with intraclass correlation coefficient 0.94 ($n=4$ CP, $n=4$ TD) for measurements made on separate days, similar to those reported elsewhere[14].

Muscle Sarcomere Length Measurements

In children with CP, intraoperative soleus biopsies from the region previously imaged were obtained with custom-made biopsy clamps[15]. Ankles were set, with a goniometer, to the same angle as during ultrasound measurement (maximum dorsiflexion). A small section of tissue was isolated by blunt dissection and clamped with care taken not to disturb its *in vivo* position. Biopsies were fixed for three days in 10% buffered formalin, and stored in fresh phosphate buffered saline until L_s determination. Thin tissue bundles were dissected at 10-15 sites and mounted on a glass slide using Permount mounting media (Fisher Scientific, Waltham, MA, USA) and L_s was measured by laser diffraction as described previously[3] (Fig. 3A). At least 10 measurements were obtained per biopsy and average L_s was calculated for each patient. Because the soleus is almost never exposed during surgery in young TD patients, reported TD L_s are from a previous study[16] ($n=19$) in older adults (see below for comparable values used).

Plantarflexion in the previous study was slightly greater (TD= $49.0^\circ\pm13.8^\circ$, CP= $32.8^\circ\pm7.9^\circ$), therefore, the L_s value from TD patients was mathematically adjusted (from $2.12\text{ }\mu\text{m}$ at 49.0° to $2.17\text{ }\mu\text{m}$ at 32.8°) using parameters from the previous work[16].

We acknowledge that use of elderly cadaveric data for comparison to children is not optimal, but in two years of recruitment we obtained no sarcomere lengths from TD

children's soleus muscle. Thus, we used two other independent approaches to estimate TD L_s values:

1. To establish "normal" human L_s values, we performed a complete summary of all human muscle sarcomere lengths in the literature to calculate the confidence interval for human sarcomere length with the joint in a neutral position. Forty-four L_s values were obtained from six papers published between 1988 and 2011[16-21]. Values were screened for normality and mean and 99.99% confidence interval were calculated.
2. To determine the effect of age on L_s , we compared previously measured sarcomere lengths from younger and older subjects for 5 matched younger (50 ± 6 years, $n=14$ for deltoid, $n=20$ for others) and elderly (89 ± 12 years, $n=12$) rotator cuff muscles[22-24], younger (39.8 ± 11.73 years, $n=5$) and elderly (85.2 ± 17.42 years, $n=10$) pelvic floor muscles ([25] and personal communication), and 28 younger (49.7 ± 5.7 , $n=13$) and elderly (82.5 ± 9.4 , $n=19-20$) lower limb muscles ([16] and personal communication).

For our study, serial sarcomere number (i.e., number of sarcomeres in series within a fascicle) was calculated by combining ultrasound and L_s data as previously described[26]

using the following equation: $N_s = \frac{L_f}{L_s}$ where N_s is serial sarcomere number, L_f is fascicle length, and L_s is sarcomere length. Both theoretical and experimental data demonstrate that serial sarcomere number is the best predictor muscle excursion capability[27; 28].

Data Analysis

Based on experimentally measured L_f variability (σ) of 1.22 cm for CP and 0.90 cm for TD patients, this study was powered at 80% to detect a 0.96 cm difference (~25%) in L_f , L_s , and serial sarcomere number. Comparisons between groups were made using Student's t-test. Significance level (α) was 0.05. Data are represented as mean $\pm$ standard deviation.

Results

Fascicle Length Comparison

Average soleus L_f was similar between CP (3.6 ± 1.2 cm) and TD individuals (3.5 ± 0.9 cm) (Fig. 2D, $p=0.64$). It has been stated that L_f must be normalized to bone length to permit comparison between groups[29], but normalization of soleus L_f to tibial length changed neither significance ($p=0.24$) nor power calculation.

Sarcomere Length Comparison

In contrast to similar L_f observed between groups, L_s for CP was 4.07 ± 0.45 μm , which was dramatically longer ($p < 0.0001$) compared to TD, 2.17 ± 0.24 μm (Fig. 3B). Based on length-tension properties of human sarcomeres, sarcomeres at 4.07 μm would produce much less force than sarcomeres at 2.17 μm , (Fig 1). At a L_s of 4.07 μm , decreasing L_s by tendon lengthening will allow muscle to theoretically generate greater force while, at a L_s of 2.17 μm , decreasing L_s by tendon lengthening will result in decreased force. This logic suggests that tendon lengthening could make TD sarcomeres weaker but sarcomeres from CP

stronger, which may represent a fundamental difference between groups. To ensure that sarcomere length force production predictions were not affected by altered filament length, muscle actin was measured from patients with CP. There was no difference between CP and TD muscle (Supplemental Material).

Independent sarcomere length comparisons supported the use of elderly TD L_s discussed above. Analysis of 44 previously measured sarcomere lengths had mean=2.67 μm and 99.99% CI=2.43-2.91 (Fig. 4). Based on these measurements, CP sarcomere length was highly significantly different from previously measured sarcomere lengths ($p<0.0001$). None of the 36 comparisons between younger and elderly sarcomere lengths in rotator cuff, pelvic floor, and lower limb were significantly different between age groups, with a mean difference between young and old of 0.036 μm . Based on this average young-old difference, the difference between young CP and elderly TD samples, 1.9 μm , is more than 14 standard deviations from the mean and extremely unlikely to be due to age. Thus, using every comparative method that we can envision, children with CP have dramatically longer sarcomeres compared to our estimates of normal soleus L_s or normal L_s in other muscles (Fig. 4).

Serial Sarcomere Number

Combining L_f and L_s data yielded a serial sarcomere number that was 40% lower ($p<0.0001$) in CP ($9,190\pm3,810$) than (using previous L_s data) in TD individuals ($16,040\pm4,160$; Fig. 5). Since serial sarcomere number is an indicator of a muscle's excursion ability, and patients in both groups have similar bony dimensions, patients with CP have fascicles with a greatly decreased capacity for joint rotation. This may represent a fundamental change that limits range of motion in contractures.

Discussion

The purpose of this study was to integrate macroscopic muscle properties (fascicle length) with microscopic properties (L_s) to understand structural changes in muscle contractures that can provide insights into surgical decision-making. Considering only L_f , which is the norm in the literature, muscle function would be predicted to be similar between patients with CP and TD since this value was similar between groups (Fig. 2C). However, sarcomeres are much longer in CP (Fig. 3B), which suggests these muscles would actually have a significant force production deficit (Fig. 1). Using both L_f and L_s values, we calculated that there are fewer serial sarcomeres in patients with CP, which would lead to a significant deficit in muscle excursion. This study clearly demonstrates that, without considering L_s , imaging modalities measuring only macroscopic muscle features may provide incomplete or even misleading information.

Muscle weakness is a well-known feature of CP[30-33], but previous studies have not been able to fully explain weakness based on muscle size or volume measurements [30; 34]. L_f alone in patients with CP also offers no explanation for muscle weakness[34] and while arguments have been made for reduced fiber area leading to aponeurosis contracture and subsequent muscle dysfunction in CP [13], fiber area is only modestly decreased secondary to CP [35; 36]. For these reasons, weakness is often primarily attributed to neuromuscular

control, which is clearly altered in patients with CP[37]. Although we agree that neuromuscular control is impaired in this population, this study demonstrates for the first time a clear candidate for muscle weakness—reduced serial sarcomere number and increased L_s . While L_f is similar between these groups, it is only because sarcomeres in patients with CP are highly stretched.

Previous studies of serial sarcomere number development in mice[38; 39], rats[40], rabbits[41; 42], cats[43], and goats[44] demonstrated that when a muscle is stretched, as during bone growth, serial sarcomeres are added to maintain original L_s . Sarcomere number adaptation has even been shown in a human muscle case of adolescent distraction osteogenesis[26]. As such, the idea that sarcomere number adapts to surgical muscle length change has permeated surgical literature. Data collected in this study, however, suggest that L_s regulation in CP may differ from TD. Strain from bone growth, which would normally cause an increase in serial sarcomere number, does not appear to cause the appropriate sarcomere number increase in CP, leading to a muscle with normal L_f but highly stretched sarcomeres. These findings have profound clinical implications for patients with CP. If surgeons assume that bone growth is accompanied by corresponding L_f changes (due to addition of serial sarcomeres), this will be incorrect. Previous models of force production after surgical intervention, even when taking into account reduced serial sarcomere number, have assumed TD L_s in patients with CP[45]. This assumption leads to inaccurate estimates of force production changes with surgery.

The main limitation of this study is the lack of age-matched soleus L_s data from TD children. (IRB approval was obtained but no possible patients presented over a 2-year period.) The primary comparative data for TD L_s were obtained from elderly cadaveric studies[16]. While using age-matched control soleus data would be ideal, the difference in L_s between groups is so great that we believe the overall effect is not in question, only the magnitude. However, acknowledging this limitation, we implemented two independent methods to determine the degree to which soleus L_s values measured were abnormal. First, we compiled 44 human sarcomere lengths from 6 previously published reports (see Methods; Fig. 4). Our average L_s value of 4.07 μm exceeds the 99.99% confidence interval by 1.16 μm . In fact, given the normal distribution of sarcomere lengths generated by this data set, the probability that a 4.07 μm L_s would be considered normal is only 0.016%. Second, we have performed studies of sarcomere length in cadaveric tissues in older and younger subjects for 5 rotator cuff muscles[22-24], 3 pelvic floor muscles[25], and 28 lower limb muscles[16]. For all 36 muscles studied, sarcomere lengths were relatively short [mean = 2.74 ± 0.33 μm ; $n=72$ groups (1 younger and 1 elderly per muscle)] and were never significantly different in paired muscles as a function of age. These data agree with previous studies, in which increasing L_f during development occurred mostly by sarcomere number increase[38; 39]. Therefore, we do not expect a dramatic L_s difference between children and adults, but instead L_f differences. Given the exceedingly low probability that a sarcomere length of 4.07 μm could be obtained from normal human muscle and the lack of an age effect, we are confident our conclusion that soleus L_s in CP is very long is justified.

This is not the first study to report long sarcomeres in patients with CP, which was demonstrated in forearm[17; 46] and hamstring[35]. It is, however, the first study to

measure both L_s and L_f in the same muscle of the same patient. Without knowing both values, it is impossible to determine how many sarcomeres the muscle has in series, and this serial sarcomere number is what actually determines excursion capability of a muscle. Our results clearly demonstrate the importance of knowing both values for proper surgical decision-making. Patients with CP have highly stretched fascicles that appear normal by ultrasound but have highly stretched sarcomeres at the microscopic level. Because L_s regulation appears impaired in these patients, therapies designed under the assumption of normal sarcomere number addition may not have the expected effect. Stretching, for example, might increase muscle weakness if it makes already-stretched sarcomeres even longer. Taken together, these data highlight the importance of a comprehensive understanding of a muscle's macroscopic and microscopic properties for appropriate surgical decision-making. Future studies must be performed to determine the biological basis for these muscular abnormalities with the hope that novel therapies could be developed to resolve contractures using state-of-the-art biological approaches.

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Figure 1.

Muscle length-tension curve derived for human muscle. This curve was created using filament length data from a previous report[18] based on the sliding filament theory elucidated by Gordon et al.[2] This graph compares theoretical force production for a muscle from a patient with CP (open circle) vs. TD (X), which shows that muscles from patients with CP compared to TD patients are located on very different positions of the sarcomere length-tension curve.

Figure 2.

Method for fascicle length measurement in human subjects. A) Ultrasound leg positioning. B) Distance measurement from superior to inferior soleus fascial planes on an ultrasound image. Red line on the image indicates the location of a measurement of d . C) Fascicle angle measurement with respect to the upper fascia plane. D) Ultrasound soleus fascicle length measurements of patients with CP and patients with typical development demonstrate that fascicle lengths were not significantly different between the two groups ($p=0.64$).

Figure 3.

A) Photograph of the laser diffraction method for measuring muscle sarcomere length. Zeroth, first, and second order diffraction lines are highlighted in the image. B) Soleus sarcomere length from patients with CP and elderly TD patients were significantly different ($p < 0.0001$). Soleus sarcomere length for patients with CP were measured in the group of patients whose fascicle lengths were measured by ultrasound, while TD values were taken from a previous report from our laboratory which used cadaveric specimens[16].

Figure 4.

Comparison of forty-four previously published sarcomere length values from across the body[16-21] (black circles) to measured CP soleus sarcomere length (red square). Black bars represent the mean $\pm$ 99.99% confidence interval.

Figure 5.

Calculated serial sarcomere number in the soleus of CP patients ($p < 0.0001$). Patients with CP had nearly half the number of serial sarcomeres as patients with TD, which predicts highly reduced excursion.