ABSTRACT: Children with cerebral palsy often have spasticity of both the extensors and flexors, but how and why a flexion contracture of the wrist will develop during growth is not thoroughly understood. In order to understand the muscle adaptations that occur during contracture formation, the relationship between intraoperative sarcomere length and the extent of contracture was measured in 23 children (average age, 14.3 ± 2.9 years) undergoing tendon transfers involving the flexor carpi ulnaris (FCU) or extensor carpi radialis brevis (ECRB) muscles. For both ECRB and FCU, sarcomere lengths measured intraoperatively were longer compared to sarcomere lengths predicted from a regression relationship obtained from "control" patients with radial nerve injury (P < 0.001). The most interesting aspect of the long FCU sarcomere lengths measured was that there was a highly significant correlation between the degree of contracture formation and intraoperative sarcomere length (r² = 0.5, P < 0.005). These data clearly show that greater contracture severity is associated with longer intraoperative FCU sarcomere length. No such correlation was observed for the ECRB. The data suggest that the clinical presentation of the contracture is related to degree of FCU, but not ECRB adaptation, to the central nervous system lesion.


INTRAOPERATIVE MUSCLE MEASUREMENTS REVEAL A RELATIONSHIP BETWEEN CONTRACTURE FORMATION AND MUSCLE REMODELING

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Joint contractures are a complication that often follows upper motor neuron (UMN) lesions. Contractures limit range of motion and patient function and can often be painful. Surgical treatment of contractures often follows conservative management such as splinting, serial casting, baclofen pump, and neurotoxin injection, all of which are used to delay surgery. Surgical correction of flexion contractures involves release or lengthening of the affected muscle–tendon unit or tendon transfers that restore the balance across the joint.28,31 These treatments are relatively effective. However, the ability to treat contractures with more sophisticated conservative or surgical interventions is limited in part by a lack of understanding of the secondary changes that occur in muscle after UMN lesions.

A number of such changes have been documented.16 The most widely reported alteration is a shift in muscle fiber type from the slow to fast phenotype24,25 and an increase in fiber size variability throughout the tissue.21,22 Less widely appreciated are the mechanical changes that occur in affected muscles, especially if spasticity is present. For example, Sinkjaer et al. measured plantarflexion moment during forced ankle dorsiflexion and decomposed the mechanical response into intrinsic, passive, and reflex components. They concluded that the passive stiffness of muscle was altered secondary to UMN lesion.24–26 These data demonstrate that mechanical and functional alterations can arise from the muscle tissue itself even though the nervous system is the site of the pri-
mary lesion. Using a more sophisticated signal processing technique, Mirbargheri et al. demonstrated that passive stiffness properties of plantarflexors from patients with spinal cord injury (SCI) increased as well. These studies provide sufficient evidence to conclude that gross mechanical changes occur in skeletal muscle secondary to spasticity and during development of contractures.

It is difficult to define the structural basis for many of the mechanical changes observed in skeletal muscle after UMN lesion. In a micromechanical study of single cells obtained primarily from spastic flexor carpi ulnaris (FCU) muscles, Friden and Lieber demonstrated that “spastic” cells were intrinsically stiffer and had a shorter resting sarcomere length than normal cells, providing direct evidence for cellular-level changes secondary to spasticity and contracture formation. Alterations in extracellular matrix (ECM) content and quality were also observed in these muscles. The ECM was hypertrophied in terms of area fraction of muscle but its mechanical properties were inferior compared to normal ECM. Intraoperative sarcomere length measurements from these patients revealed another major alteration in wrist flexors, namely the counterintuitive finding that the in vivo FCU sarcomere length was extremely long even though the muscles themselves were shortened. This implies a major remodeling of muscle during contracture development because, under a wide variety of conditions and across age groups, the FCU sarcomere length–joint angle relationship is usually highly conserved.

In an effort to understand the FCU remodeling process that results in contracture formation, we have again measured sarcomere length in patients with cerebral palsy (CP), but this time in patients with a wide range of contracture severities instead of the fixed flexion contractures studied previously. Patients with fixed flexion contractures had no active range of motion with their wrists passively fixed at about 90° of flexion. In this study, we report on patients with a range of contracture severity and provide evidence that the degree of contracture formation is related to the FCU sarcomere length observed in the wrist muscle at the time of surgery.

METHODS

Children were recruited for this study (average age, 14.3 ± 2.9 years) because they were receiving tendon transfers (n = 23) involving the FCU muscle. Tendon transfers were into the extensor carpi radialis brevis (ECRB) or the extensor carpi radialis longus (ECRL) muscle. All patients had CP and had been treated conservatively using a variety of treatments such as splinting, occupational therapy, botulinum toxin injection, and serial casting. Parental consent and patient assent was obtained in accordance with our institutional review boards.

The severity of contracture formation was estimated for each child based on the extent to which the wrist could be passively extended by the therapist. Passive wrist extension was measured by the therapist to the nearest 5° with a goniometer that estimated the angle between the radius and third metacarpal, fingers flexed. By convention, negative angles signify wrist flexion whereas positive angles signify wrist extension.

Intraoperative Measurements. During the surgical procedure, sarcomere length was measured by laser diffraction. The device was calibrated using diffraction gratings of 2.50 μm and 3.35 μm placed at the location of the muscle fiber bundle. To measure sarcomere length intraoperatively, the FCU sheath was incised in the distal third of the muscle (i.e., just distal to the region that was denoted “P3” in a previous publication) and the small fiber bundle was isolated along its natural fascicular plane by atraumatic blunt dissection. Based on the dimensions of the laser beam (1/e² = 0.7 mm) and fiber bundle, we estimate that the diffraction pattern represents the spatial average of several million sarcomeres. Previous studies have provided evidence that this single sarcomere length measurement technique is indeed representative of the entire muscle. The laser device was inserted beneath the fiber bundle and approximated into the normal plane of the muscle, taking care not to artificially stretch the muscle. Sarcomere length was calculated by measuring the distance between the ±first-order diffraction line using the grating equation, nλ = dsinθ, where θ is diffraction angle, λ is the laser wave length (0.632 μm), and n is the diffraction order number. As the ±second-order diffraction line was consistently more difficult to obtain, it was not used for sarcomere length determination. The geometrical configuration of the device within the muscle has been previously defined (c.f. Figure 1 in Lieber and Friden) and, in practice, yields a sarcomere length resolution of ~0.2 μm.

Sarcomere length was measured at a single location for each subject using a metal triangle-shaped template to position the wrist in one of three defined positions: (1) 30° extension was one endpoint, as this is a “normal” wrist-carrying angle during grip, with the consequence that patients with the most extreme flexion contractures were excluded; (2) 30°
flexion was the other endpoint, as this is typically the shortest muscle length at which sarcomere length can reliably be obtained without the muscle going slack; and (3) sarcomere length was measured with the wrist in neutral to estimate the anatomical sarcomere length operating range of the FCU muscles using a reasonable amount of intraoperative time.

After FCU sarcomere length measurement, the ECRB was exposed via a volar–radial incision at the level of the pronator teres muscle insertion on the radius. The overlying fascia was removed, exposing ECRB muscle fibers and enabling sarcomere length measurement in the same three positions, as previously described. It was not always possible to obtain clear diffractions for all muscles in all wrist positions due to surgical time limitation, intramuscular connective tissue, or inability to isolate atraumatically a muscle fiber bundle.

**Data Analysis.** There are no sarcomere length data in the literature from truly normal patients to which the data obtained for either the FCU or the ECRB could be compared. For comparison, “control” FCU sarcomere lengths were used from previously published data in patients with radial nerve injuries where denervated wrist and digital extensor function was being restored by a “control” FCU. After combining those two data sets (n = 9 and 17 data points, respectively) the “control” FCU sarcomere length–joint angle relationship yielded was:

$$Y (\mu m) = 0.0013 \cdot \theta + 3.45$$ (1)

where $Y$ is the intraoperative sarcomere length and $\theta$ is the wrist-joint angle. These combined data are plotted as filled circles in Figure 1A. Similarly, for comparison, “control” ECRB sarcomere lengths were used from previously published data obtained from patients with “tennis elbow,” who were undergoing surgical lengthening of the distal muscle–tendon junction. After combination of the two data sets (n = 14 and 21 data points, respectively) the “control” ECRB relationship yielded was:

$$Y (\mu m) = -0.0075 \cdot \theta + 3.01$$ (2)

where $Y$ is the intraoperative sarcomere length and $\theta$ is the wrist-joint angle. These combined data are plotted as filled circles in Figure 1B.

**Statistical Analysis.** All assumptions of regression analysis (normal distribution of independent variable and residuals) were explicitly tested prior to parametric analysis. Significance level ($\alpha$) was set to 0.05 and statistical power ($1-\beta$) exceeded 80% for all nonsignificant results. To determine analytically whether measured FCU or ECRB sarcomere lengths were significantly different from the “control” data ensembles, several approaches were taken. First, the measured sarcomere length at each of the three intraoperative joint angles ($-30$, $0$, and $+30$) was subtracted from that predicted by the regression relationships at the corresponding joint angle and then a one-sample $t$-test determined whether this difference was significantly different from zero. In other words, for each muscle, three one-sample $t$-test comparisons were made. This was the most “powerful” method for determining whether measured intraoperative sarcomere lengths were signifi-
Table 1. Flexor carpi ulnaris intraoperative sarcomere length–passive wrist extension relationships

<table>
<thead>
<tr>
<th>Intraoperative joint angle (°)</th>
<th>Regression equation</th>
<th>Coefficient of determination ($r^2$)</th>
<th>Significance level ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−30</td>
<td>$Y = -0.021 \cdot \theta + 5.57$</td>
<td>0.28</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>0</td>
<td>$Y = -0.032 \cdot \theta + 6.46$</td>
<td>0.50</td>
<td>$P &lt; 0.005$</td>
</tr>
<tr>
<td>30</td>
<td>$Y = -0.025 \cdot \theta + 6.44$</td>
<td>0.57</td>
<td>$P &lt; 0.001$</td>
</tr>
</tbody>
</table>

*Equations represent the relationship between the extent of passive wrist extension ($Y$, in degrees) and FCU intraoperative sarcomere length measured at the selected joint angle ($\theta$, in μm).

Interestingly different from theoretical predictions. Second, regression analysis was performed on the sarcomere length–joint angle relationship calculated individually for each subject, and average slope was compared to the slope of the “control” data shown as regression lines in Figure 1. This was the best method to compare the slope of the “control” data to the average slope of the experimental data set. Third, the regression slopes between spastic and “control” data were compared statistically using analysis of covariance (ANCOVA), which compared average sarcomere length using joint angle as the covariate. ANCOVA is another method for comparing slopes of the two data sets that includes the variability of the “control” data, unlike the regression method presented earlier, which reduces the “control” data to a single equation. In practice, both methods yielded identical results for slopes comparison but are presented for completeness. In what follows, data are presented as mean ± standard error, except where noted.

RESULTS

All patients included in the study were operated upon due to a decreased ability to extend the wrist voluntarily, impairing grip. Normal active wrist extension maximum was 90° of extension in these children, whereas in our experimental cohort the active wrist extension maximum was $-30 ± 12°$ of flexion. Several patients were excluded as their wrist flexion contractures were so severe that the wrist could not be extended to 30°. In 3 cases, patients were operated upon but, for unknown reasons, it was not possible to obtain diffraction patterns from their muscles. For FCU measurements, it was not possible to obtain clear diffraction patterns at 0° in 3 patients (yielding a sample size of 17 for FCU), and for ECRB measurements it was not possible to obtain clear diffraction patterns at 0° in 7 patients (yielding a sample size of 13 for ECRB muscles).

For FCU muscles, sarcomere lengths measured intraoperatively were extremely long at all three joint angles (4.2 ± 0.3 μm, 4.6 ± 0.3 μm, and 5.1 ± 0.2 μm; Fig. 1A) compared to those predicted by the regression relationship at the corresponding joint angles (3.1 μm, 3.5 μm, and 3.8 μm, respectively). The difference between sarcomere length measured in the neutral position and that predicted by the regression equation was 1.1 ± 0.3 μm, which was significantly different from zero (Fig. 1A, $P < 0.001$). Similar differences were observed for both the flexed and extended angles (1.1 ± 0.5 μm and 1.3 ± 0.2, respectively; $P < 0.001$) and there was no significant difference among angles for the difference relative to the regression prediction.

The most interesting aspect of the long FCU sarcomere lengths measured was that there was a highly significant correlation between the degree of contracture formation (as indicated by the extent of passive wrist extension) and intraoperative sarcomere length measured with the wrist in either flexion, neutral, or extension (Table 1 and Fig. 2A; $r^2 = 0.50, P < 0.005$). These data show that greater contracture severity is associated with longer intraoperative sarcomere length. For the wrist in the neutral position, this relationship is well described by the equation:

$$Y (\mu m) = -0.032 \cdot \theta + 6.46$$

where $Y$ is intraoperative sarcomere length in neutral (in μm) and $\theta$ is the extent of passive wrist extension (in degrees), with analogous statistically significant regression relationships obtained for other wrist-joint angles (Table 1). The level of significance decreased and coefficient of determination increased with increasing wrist extension (Table 1).

Similar to the FCU results, ECRB sarcomere lengths measured intraoperatively were extremely long at all three joint angles (4.6 ± 0.5 μm, 3.9 ± 0.2 μm, and 3.6 ± 0.2 μm; Fig. 1B) compared to those predicted by the regression relationship at the corresponding joint angles (3.2 μm, 3.1 μm, and 2.8 μm, respectively). The difference between sarcomere length measured in the neutral position and
that predicted by the regression equation was 0.94 ± 0.21 μm, which was significantly different from zero (Fig. 1B; \( P < 0.001 \)). Similar differences were observed for both the flexed and extended angles (1.4 ± 0.3 μm and 0.8 ± 0.2, respectively; \( P < 0.005 \)) and there was no significant difference among angles for the difference relative to the regression prediction (Fig. 1B). However, in contrast to the FCU results, there was no correlation between contracture severity (as indicated by the clinical presentation of limit of passive wrist extension) and sarcomere length measured intraoperatively for any of the wrist positions investigated (Table 2). Thus, whereas both FCU and ECRB showed excessively long sarcomere lengths, the clinical presentation of the patient appeared to be dominated by the condition of the FCU.

**DISCUSSION**

The main result of this study is that both FCU and ECRB sarcomere lengths measured intraoperatively are significantly longer compared to “control” values (Fig. 1). Second, it was demonstrated that, for FCU (Fig. 2A), but not ECRB (Fig. 2B), intraoperative sarcomere length was highly correlated with the degree of contracture. Because these data were obtained across a range of contracture severities, we suggest that they provide insight into the progression of contracture as they change from mild to severe. As the more severe contractures are the ones with the greatest limitation to passive extension and as contractures progress from less to more severe, by analyzing a cross-section of contractures of various severities we are, in a sense, observing the “process” of contracture formation. Contracture “progression” is the context in which these data are interpreted and is consistent with our clinical experience, because all of these contractures progressed from mild to severe.

**No Evidence That Contracture Formation Is Due to Muscle Fiber Shortening.** The data suggest that, as contracture severity increases, FCU sarcomere length increases. This appears to be counterintuitive because it is obvious from intraoperative measurement or gross clinical inspection that, as contracture se-
verity increases, FCU muscle–tendon unit length actually decreases. The most intuitive explanation for the progressive FCU sarcomere length increase is that contracture progression involves a progressive decrease in serial sarcomere number in FCU fibers. This loss in serial sarcomere number would then “pull” the wrist into flexion. The ultimate effect would be an increase in FCU sarcomere length (as seen), whereas other structures (e.g., joint capsule, antagonistic muscles) would oppose wrist flexion. In order for sarcomere length to increase by 1.2 μm (as shown in Fig. 1) due to a decrease in sarcomere number, sarcomere number would have to change markedly. The normal value for serial sarcomere number in the human FCU is obtained by dividing FCU nominal fiber length (47.5 mm) by the sarcomere length used for normalization of these data (2.5 μm), yielding a sarcomere number of ~19,000. For fibers to maintain their length but sarcomere length to increase to ~3.7 μm, sarcomere number would have to decrease to a new value of ~13,000—a 30% decrease (47.5 mm/3.7 μm). This decrease in sarcomere number would be easily observed as a 30% increase in the slope of the sarcomere length–joint angle relationship, which, as described in what follows, was not observed.

Although serial sarcomere number loss is an intuitively appealing explanation, we have no evidence that it is the cause of contracture. If the most severe contractures are those with the smallest serial sarcomere number, this would be manifest as a higher slope in the sarcomere length–joint angle relationship for the most severe contractures. For the entire data set, a correlation between the slope of the sarcomere length–joint angle relationship and contracture severity would be observed. In addition, a difference in the sarcomere length–joint angle slope for these muscles would be measured relative to “control” FCU muscles. To test these predictions, we analyzed the sarcomere length–joint angle relationships of all subjects and found no relationship between the slope of the sarcomere length–joint angle relationship and the severity of contracture. The average slope of this relationship for all experimental subjects was 0.017 ± 0.003 μm/° which is very close to the theoretical value of 0.015 μm/° predicted for the normal FCU18 or the regression slope of 0.013 μm/° measured for the muscles in Figure 1A. Also, it is virtually identical to the slope of the sarcomere length–joint angle relationship reported previously by Lieber and Fridén in FCU muscles of patients with severe wrist flexion contractures (0.017 ± 0.005 μm/°).14 Furthermore, ANCOVA demonstrated no significant difference between the slopes of the sarcomere length–joint angle relationship measured for experimental subjects compared to the “controls” plotted in Figure 1. Finally, if sarcomere number were to decrease to the extent required for the measured sarcomere length increase, the slope of the sarcomere length–joint angle relationship would have to exceed 0.025 μm/°, which is beyond the 95% confidence interval of the slopes measured for the experimental subjects. Taken together using a variety of analytical methods, we could not provide evidence for altered fiber length in these subjects. The statistical power of this conclusion ranges from 68% (for the ANCOVA analysis) to 83% (for the linear regression analysis).

No Other Evidence of Fiber Length Decrease in Cerebral Palsy. Apart from sarcomere length data analysis, our experimental results are consistent with the findings of Smeulders et al., who, based on intraoperative measurement of FCU muscle length–tension relationships, concluded that there was no fiber length change in muscles of children with CP.27 They reported that the width of the active length–tension curve (an indirect measurement of fiber length) was similar to that expected for the observed wrist range of motion measured. Our data are also consistent with ultrasound measurement of fascicle length in gastrocnemius muscles of CP patients who were compared to normally developing children.25 Thus, in contrast to previous assertions,30,32 there is currently no evidence in the literature that muscle fiber length is shortened in contracture or in spastic skeletal muscle.

Possible Explanations for Sarcomere Length Change with Contracture. What mechanism would cause a sarcomere length increase in these muscles if fiber length is apparently not changing? The only way for the slope of the sarcomere length–joint angle relationship to remain constant with increasing sarcomere length is if the FCU fiber length:moment arm ratio remains constant. As previously outlined, there are only three gross structural explanations for an increased sarcomere length with an identical slope of the fiber length:moment arm ratio (see Fig. 3 in Lieber and Fridén14). First, tendon or muscle length could decrease, resulting in long resting sarcomere lengths with maintenance of the sarcomere length–joint angle slope. Second, sarcomere number could increase in proportion to an increase in average moment arm. Finally, sarcomere number could decrease in proportion to a decrease in average moment arm. Of these possibilities, we view muscle shortening as the most probable because no dra-
matic changes in the absolute magnitude of the moment arm were seen clinically. At this time, based on the resolution of tools available, the relatively imprecise measurement of joint angle obtained in this study, and the lack of continuous sarcomere length data, it is not possible to distinguish among these possibilities. These three possibilities presume that the fundamental subcellular sarcomere dimensions of filament length, Z-disk structure, and cytoskeletal structure remain constant. The extent to which this is true remains to be determined.

Clinical Presentation Dominated by Muscle Physiological Cross-Sectional Area. A secondary result of this study is that, apparently, the FCU muscle changes determined the clinical presentation of contracture severity even though the ECRB appeared to also be adapting in terms of sarcomere length. This is not surprising in light of the fact that the FCU in particular and the wrist flexors in general have a very high physiological cross-sectional area (PCSA) compared to the ECRB in particular and the wrist extensors in general. Specifically, the FCU has a PCSA (3.4 cm²) that is about 25% greater than that of ECRB (2.7 cm²) and the combined wrist and digital flexors have a PCSA (19.2 cm²) that is almost twice that of the combined wrist and digital extensors (10.8 cm²). Thus, if flexors and extensors were similarly affected, flexors would clearly dominate the clinical presentation. This might explain the clinical presentation of most joints of the upper extremity where spasticity results in a posture that is dominated by the functional group with the highest PCSA, namely wrist flexors, elbow flexors, and internal rotators.

Although there are almost no comparative data available within subjects, between muscle groups, with regard to sarcomere length changes, FCU appears to be more severely affected than ECRB. There is the intriguing report in the literature that wrist flexors are more severely affected by CP compared to wrist extensors, which is consistent with this finding. The underlying basis for this observation is not known but we suggest that contracture formation results from the inappropriate architectural adaptation of upper extremity muscles in response to UMN lesion. The mechanistic explanation for this alteration in sarcomere length remains to be determined.

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