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Morphological properties of skeletal muscle were compared between wrist flexors and extensors within the same children (n=8, six females, two males; age range 4 to 9y, median age 7y) with wrist muscle imbalance secondary to spastic cerebral palsy (CP). Five patients had hemiplegic CP, two diplegic CP, and one patient had tetraplegic CP. Muscle biopsies were taken during either tendon transfer or tendon lengthening procedures. Analyses included distribution of muscle fibre types, fibre sizes, and expression of developmental myosins. Extensor fibre area was significantly greater than flexor fibre area for type 2A fibres and type 2B fibres but not for type 1 fibres. Coefficient of variation (CV) of fibre size for all three fibre types was greater for flexors compared with extensors. The greatest CV was observed for the type 2A fibres in flexors (38.5 [3.8%]). A wide variation was observed for expression of developmental myosin with the magnitude of the expression being greater, but not statistically significant, in flexors compared with extensors (5.4/mm² vs 0.53/mm²). These data demonstrate that significant secondary myopathy of wrist flexor muscles results from CP.

The orthopaedic management of cerebral palsy (CP) represents a challenging clinical problem (Sutherland et al. 1975, Green et al. 1983, Smyth and Peacock 2000). Musculoskeletal sequelae of CP include gait abnormalities, joint deformities, and joint contractures. Difficulty in treating these problems is partly due to the lack of information available about the tissue changes that occur secondary to CP. Whereas the changes that occur in ligaments, joint capsules, and tendons after contracture are well documented (Akeson et al. 1974, 1977, 1987), there is far less information about muscular changes. In some reports it is claimed that muscle tissue is normal and the contracture is simply secondary to abnormal neural activity (Katz and Rymer 1989). This is a reasonable suggestion in light of the observation that the joint is fixed or restricted in the direction of the strongest muscle. For example, digital flexors, wrist flexors, and ankle plantarflexors may simply overpower their antagonists owing to their larger physiological cross-sectional areas (Wickiewicz et al. 1983; Friederich and Brand 1990; Lieber et al. 1990, 1992).

In other reports, muscle abnormalities, such as fibre type predominance, fibre size variation, and connective tissue proliferation, suggest there may be significant secondary effects of CP on muscle tissue itself (Castle et al. 1979, Rose et al. 1994, Ito et al. 1996). Should specific muscular changes occur secondary to CP, this would provide insights into the disease process. This is because skeletal muscle fibres adapt to the amount and type of neural activity that they receive. Muscles that experience increased use (i.e. chronic electrical stimulation, exercise, etc.) demonstrate a fibre type transformation in the fast-to-slow direction. Conversely, muscles that experience decreased use (i.e. tenotomy, immobilization, space flight, spinal cord injury, etc.) demonstrate a fibre type transformation in the slow-to-fast direction. Thus, changes in fibre type distribution can be used to provide insights into a muscle’s activity history.

If it is true that all muscles are affected equally by CP, clinical management of contractures need not vary as a function of anatomical location. However, it is impossible to know whether CP affects all muscles equally because the three previous studies of muscle fibres type in CP (Castle et al. 1979, Rose et al. 1994, Ito et al. 1996) sampled across numerous different muscles in different individuals, across a wide range of ages, and examined only 200 to 300 fibres per biopsy. Further, controls were lacking in two studies (Castle et al. 1979, Ito et al. 1996) and were used from historical pathological specimens from different muscles in the other (Rose et al. 1994). It is important to make a direct comparison between muscle groups within the same individuals. If different muscle groups demonstrate varying degrees of muscle abnormalities, it can be concluded that muscle groups can respond differentially to the lesion in the central nervous system (CNS). Our null hypothesis was, therefore, that there would be no difference between wrist flexors and extensors in fibre size, fibre size variability, and fibre type distribution. The purpose of this study was to compare skeletal muscle morphological properties between wrist flexors and extensors within the same individuals. We posed the hypothesis that, within the same individual, the morphology of wrist flexor muscles demonstrates greater pathology compared with wrist extensor muscles.
Method

Patients

Children enrolled in this study (n=8, six females, two males; age range 4 to 9y, median age 7y) were scheduled to have tendon transfer or tendon lengthening procedures secondary to CP. Five children had hemiplegic CP, two had diplegic CP, and one had tetraplegic CP (Table I). Surgery was performed at Umeå University Hospital, Uppsala University Hospital, or Karolinska University Hospital, in Sweden. Parents provided informed consent before surgery and children provided their assent when possible. Ethical committees of Umeå University, Uppsala University, the University of California, and the Karolinska Institute approved the study.

Muscle biopsy

During the surgical procedure, open biopsies (15 mm long and 3 to 8 mm in diameter) were obtained from wrist flexors and extensors. Specimens were mounted in an embedding medium (Tissue Tek, Miles Laboratories, Naperville, Illinois, USA), snap frozen in isopropylene chilled with liquid nitrogen, and stored at −80°C until analyzed. Transverse serial cryosections of thickness 8 to 10 µm were sectioned and stained for myofibrillar ATPase with preincubation at pH 4.3, 4.6, and 10.4 to distinguish between fibre types according to the nomenclature by Brooke and Kaiser (1970). Owing to the relatively small portion of type 2AB and 2C fibres, data for these subtypes are not reported.

Sections were labelled with primary monoclonal antibodies against slow, fast, and developmental myosin heavy chain (MyHC) proteins. Slow myosin was identified using the monoclonal antibody (mAb) A4:840. The N2.261 mAb has a strong affinity for the fast 2A MyHC, less affinity for the slow MyHC, and no affinity for the fast 2X MyHC. The F1.652 mAb identifies the embryonic MyHC, and the MyHCn mAb labels fetal MyHC. ‘Developmental myosin’ was defined as myosin expressed by fibres that were labelled with embryonic or fetal MyHC.

Table I: Characteristics of patients and muscles studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Hand funct.</th>
<th>Muscle studied</th>
<th>Nr of fibres studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>7</td>
<td>Hemiplegic CP</td>
<td>4</td>
<td>FCU, ECU</td>
<td>810</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>6</td>
<td>Hemiplegic CP</td>
<td>4</td>
<td>FCU, ECU</td>
<td>1765</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>9</td>
<td>Tetraplegic CP</td>
<td>0</td>
<td>FCU, ECRB</td>
<td>1019</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>5</td>
<td>Diplegic CP</td>
<td>2</td>
<td>FCU</td>
<td>340</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>5</td>
<td>Hemiplegic CP</td>
<td>5</td>
<td>FCU, ECRBL</td>
<td>1328</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>7</td>
<td>Diplegic CP</td>
<td>2</td>
<td>ECU</td>
<td>269</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>8</td>
<td>Hemiplegic CP</td>
<td>4</td>
<td>FCU, ECRB</td>
<td>1679</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>7</td>
<td>Hemiplegic CP</td>
<td>4</td>
<td>FCU, ECRB</td>
<td>615</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>6.8 (1.4)</td>
<td></td>
<td></td>
<td></td>
<td>978 (573)</td>
</tr>
</tbody>
</table>

FCU, flexor carpi ulnaris; ECU, extensor carpi ulnaris; ECRB, extensor carpi radialis brevis; ECR, extensor carpi radialis longus. Hand funct., hand function (according to House et al. 1981).

Figure 1: Light micrographs from a spastic paired human wrist (a) extensor (extensor carpi radialis brevis) and (b) flexor (flexor carpi ulnaris) muscles from a 9-year-old female with tetraplegic CP. N2.261 monoclonal antibody labels type 2A fibres darkly (long arrows), type 1 fibres lightly (short arrows), and no labelling of type 2B fibres. Both micrographs are shown at same magnification. Note decreased fibre size, increased fibre size variability, and higher proportion of type 2B fibres in flexors compared with extensors, and decreased fibre size in tetraplegic sample.
fetal myosin antibodies or both.

Monoclonal antibodies were used to localize the extracellular matrix laminin chains α2, α5, and β2 to enable automated definition of fibre outlines as previously detailed (Mishra et al. 1995). All antigens were visualized using the peroxidase-antiperoxidase (PAP) technique (Pearse 1961). Morphometric analysis was performed using serial sections stained for myofibrillar ATPase, mAb N2.261, mAb against slow myosin, and the α5 chain of laminin. Four randomly selected areas of the specimens were photographed with a light microscope (Zeiss Axioiophor, Munich, Germany) interfaced with computerized image analysis (IBAS, Kontron Electronic GMBH, Eching, Germany). For the specimens reported in this study, an average of 1151 (333) fibres were analyzed from each biopsy for fibre type, fibre area, fibre percentage, and calculations of coefficient of variation (CV).

### Table II: Characteristics of spastic muscle

<table>
<thead>
<tr>
<th>Parameter of interest</th>
<th>Flexor muscles</th>
<th>Extensor muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 CV</td>
<td>36.1 (9.9)</td>
<td>32.3 (7.6)</td>
</tr>
<tr>
<td>Type 2A CV</td>
<td>39.5 (9.4)</td>
<td>30.7 (7.2)</td>
</tr>
<tr>
<td>Type 2B CV</td>
<td>37.4 (11.9)</td>
<td>29.2 (8.2)</td>
</tr>
<tr>
<td>Type 1 (%)</td>
<td>36.3 (15.4)</td>
<td>40.9 (11.2)</td>
</tr>
<tr>
<td>Type 2A (%)</td>
<td>37.1 (13.7)</td>
<td>48.6 (11.1)</td>
</tr>
<tr>
<td>Type 2B (%)</td>
<td>19.7 (18.6)</td>
<td>7.1 (11.9)</td>
</tr>
<tr>
<td>Developmental myosin (mm²)</td>
<td>5.46 (8.5)</td>
<td>0.53 (0.47)</td>
</tr>
</tbody>
</table>

Data are mean (SD). *Significant difference between flexors and extensors (p<0.05). CV coefficient of variation.

### Statistics

CV expressed as a percentage, was used as the indicator of fibre area variability for the different fibre types in each specimen based on the equation CV=(s/X)×100%, where X is the sample mean and s is the sample standard deviation (SD). Abnormally high CV represents a pathological but non-specific abnormality in skeletal muscle. Morphological parameters between flexors and extensors were compared by one-way analysis of variance (ANOVA). Fibre size, as a function of age and functional group, was analyzed by linear regression. Significance level was chosen as 0.05. Data are presented in the text as mean (SD) unless otherwise noted.

### Results

Sixteen biopsies were obtained from eight individuals. In two cases the technical quality of fixation was inadequate, resulting in the loss of one flexor sample and one extensor sample. Therefore, the data set consisted of seven flexor samples and seven extensor samples from eight individuals. In only two cases were samples not paired. Even with this lack of pairing, there was no significant difference between groups in terms of age (p>0.4) which covered the narrow range of 5 to 9 years (Table I).

The most obvious qualitative result was an apparent decrease in fibre size and increase in size variability in spastic flexors compared with spastic extensors (Fig. 1). Extensor fibre area was significantly greater than flexor fibre for type 2A fibres (p<0.05) and type 2B fibres (p<0.05) but not for type 1 fibres (p>0.4).

CV of fibre size for the fast fibre types was greater for flexors compared with extensors. The greatest CV was observed for the type 2A fibres in flexors (39.5 [9.4%]), which was significantly greater than the CV for the type 2A fibres in extensors (30.7 [2.7%], p<0.05, Table II) with a similar result observed for type 2B fibres (p<0.05, Table II). For type 1 fibres, the CV in area was greater in flexors compared with extensors but this difference was not significant (p>0.3, Table II).

Surprisingly, developmental myosins were expressed in both the flexors and extensors of these spastic muscles (Table II). These isoforms are normally only expressed in fetal, neonatal, denervated, or regenerating skeletal muscle (Periasamy et al. 1985). Tremendous variation was observed for expression of developmental myosins with the magnitude of the expression of being much greater in flexors compared with extensors (5.4/mm² compared with 0.55/mm², Table II). The variability was such that this difference did not achieve statistical significance (p>0.2).

There was a significantly greater percentage of type 2B fibres in flexors compared with extensors (p<0.05, Table II) but no significant difference in fibre type percentages for the type 1 and type 2A fibre types (Table II).

In an effort to suggest potential causative parameters for the surprising observation of developmental myosin expression, multiple linear regression was performed using fibre area, fibre number, fibre percentage, and fibre CV for all three fibre types, as well as patient age, in their ability to predict the percentage of developmental myosins expressed (Draper and Smith 1981).
This analysis highlights unique associations between variables and eliminates variables that are highly correlated. The only parameter of the 13 tested that actually entered the regression model was the percentage of type 2B muscle fibres ($p<0.005$, yielding a relatively high $r^2$ value of $0.65$).

Finally, there was a significant correlation between age and fibre area for all fibre types observed (Fig. 3). Although all relations were significant ($p<0.05$) the linearity of the relation was greatest for type 2A fibres ($r^2=0.59$, Fig. 3b) and lowest for type 1 fibres ($r^2=0.24$, Fig. 3a). In addition, no significant difference was observed for the regression relations between flexors and extensors. These data indicate that although significant size differences occurred with flexor fibres being smaller than extensor fibres (Fig. 2), both muscle groups were growing at approximately the same rate.

Discussion
This study compared skeletal muscle morphological properties between wrist flexor and wrist extensor muscles of children with CP. The aim was to investigate potential specific changes in muscle, secondary to spasticity, that may predispose a muscle to flexion contracture. Because wrist flexor muscles are stronger as a group (Lieber et al. 1990), it is possible that hyperactivity in the nervous system simply activates both flexors and extensors and the flexors overpower the extensors, bringing the wrist into flexion. This is potentiated by the fact that the wrist flexor moment arm is greater than the extensor moment arm in wrist flexion (Loren et al. 1996). However, we obtained strong support for the idea that wrist flexors are preferentially affected by CP. Evidence for this is that the flexors muscles were generally composed of fibres that were significantly smaller compared with the extensors, and that the variability of fibre size was greater for flexors compared with extensors. In addition, more developmental myosins were expressed in flexors compared with extensors, although this difference was not statistically significant. Taken together, these observations point to a significant secondary myopathy of wrist flexor muscles that results from CP. This finding is completely consistent with previous reports of significant structural and functional alterations in both the muscle cells and the extracellular matrix measured in tissue from children with CP (see Lieber and Fridén 2002, Fridén and Lieber 2003, Lieber et al. 2003).

It is tempting to speculate that the decrease in fibre area of the flexors represents disuse of the flexor muscles. At first glance, this does not appear to be consistent with several studies that have reported increased electromyographic activity for spastic muscle. However, chronic overuse of skeletal muscle caused by chronic electrical stimulation does result in a
decreased muscle fibre size (Salmons and Henriksson 1981). Therefore, decreased fibre size could indeed provide support for the idea of spasticity as representing either an increased- or decreased-use model. However, our preliminary conclusion that spasticity represents a decreased-use model is based on the observation of an increased percentage of type 2B fibres, which is the direction of the fibre type shift that occurs in disuse (Lieber 1986a). If spasticity had represented a chronic overuse model, there would have been a shift in fibre type toward type 1 (Lieber 1986b).

It should be noted that there is no general consensus in the literature that spastic muscles increase their proportion of type 2B fibres. For example, Ito et al. (1996) presented data suggesting a dominance of type 1 fibre, as did Rose et al. (1994). However, one of the problems in both of those studies was that paired comparisons within individuals were not made and only 200 to 300 fibres were quantified in each biopsy. In fact, even the ‘control’ and ‘spastic’ muscles were often completely different muscles. In addition, the age range of children from whom the specimens were sampled was relatively broad in both studies (8 to 12y) compared with the current study (5y). Given the large variation among muscles in fibre type distribution and changes that may occur with development, it is not surprising that general trends did not emerge from these earlier studies.

In patients with CP the stretch reflex is more easily elicited in wrist flexors compared with wrist extensors during occupational therapy and personal care, etc. During these intermittent large reflex contractions of the flexors, it is possible that some muscle fibres are maximally stimulated while others are not. These different stimuli may help to explain the increased fibre area variation in flexors compared with the extensors but more studies are required to establish this relation. Rose et al. (1994) showed that energy expenditure during walking was correlated with an increase in fibre size variability (CV), supporting the idea that a severe spastic condition is associated with high CV in children with CP (Rose et al. 1994).

The increased percentage of type 2 fibres, and especially of the type 2B fibre subtype, could be reflected in a change of speed of the wrist muscles. However, movement speed is not only influenced by fibre type. Brain damage, especially of the basal ganglia, may lead to inefficient and slow motor patterns such as co-contraction (Forssberg et al. 1999). Neither is fatigue only determined by muscle fibre type. Clinical ‘fatigue’ could emerge from the CNS, the neural synapses, and/or the muscle cells themselves (Fitts 1994). Nevertheless, the increased occurrence of the easily fatigable type 2B fibres could, at least partly, account for the increased fatigability of the arm in children with CP (Brown et al. 1987).

It has been asserted that spastic muscles fail to grow and that this represents a causal factor that can lead to the ultimate contracture observed (Simon and Ryan 1992). However, our data do not support this assertion. We quantified a significant difference between fibre size and age for all fibre types (Fig. 3) in both spastic flexors and extensors. In fact, the regression relation for flexors indicated a fibre area increase of approximately 175µm² per year (p<0.0001, r²=0.45), whereas the extensors increased at approximately 216µm² per year (p<0.001, r²=0.41).

The preferential involvement of flexors compared with extensors may have surgical implications. First, the idea of using a wrist extensor as a donor muscle in tendon transfer is strengthened because these muscles show less severe involvement and, therefore, would probably perform well after transfer. Second, electrical stimulation, believed to benefit extensors during postoperative rehabilitation, would appear to be reasonable because extensor muscles show only minor abnormalities that would not prevent them from responding to the stimulation. Of course, stimulation treatment would be more effective if the very high passive tension of the flexors were first decreased by flexor tendon lengthening or injection of botulinum toxin. It would be interesting to know if some or all of the myopathic changes observed were reversed by these surgical procedures. The expression of developmental myosins showed considerable variability and, therefore, it would be interesting to determine whether there is a correlation between their occurrence and possible therapeutic treatment. Further studies are required to determine the adaptive properties of flexors and extensors after surgery.

In conclusion, this study has demonstrated a preferential involvement of wrist flexors compared with wrist extensors in spasticity. Because muscle properties reflect the pattern of use imposed upon them, the data are consistent with a decreased level of use in wrist flexors compared with wrist extensors and a myopathic response of the muscle to the chronically changed environment. These data support the concept that, although spasticity is neural in origin, with the primary lesion being in the CNS, significant skeletal muscle myopathic changes occur secondary to this lesion.

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