

**ABSTRACT:** This review summarizes current information regarding the changes in structure or function that occur in skeletal muscle secondary to spasticity. Most published studies have reported an increase in fiber size variability in spastic muscle. There is no general agreement regarding any shift in fiber type distribution secondary to spasticity. Mechanical studies in whole limbs as well as in isolated single cells support the notion of an intrinsic change in the passive mechanical properties of muscle after spasticity in addition to the more widely reported neural changes that occur. Evidence is presented for changes within both the muscle cell and extracellular matrix that contribute to the overall changes in the tissue. Taken together, the literature supports the notion that, although spasticity is multifactorial and neural in origin, significant structural alterations in muscle also occur. An understanding of the specific changes that occur in the muscle and extracellular matrix may facilitate the development of new conservative or surgical therapies for this problem.

*Muscle Nerve* 29: 615–627, 2004

## STRUCTURAL AND FUNCTIONAL CHANGES IN SPASTIC SKELETAL MUSCLE

RICHARD L. LIEBER, PhD,<sup>1</sup> SUZANNE STEINMAN, MD,<sup>1</sup> ILONA A. BARASH, BS,<sup>1</sup> and HANK CHAMBERS, MD<sup>2</sup>

<sup>1</sup> Departments of Orthopaedic Surgery and Bioengineering, Biomedical Sciences Graduate Group, University of California and Veterans Administration Medical Centers, 3350 La Jolla Village Drive, San Diego, California, 92161, USA

<sup>2</sup> Department of Orthopaedics, Children's Hospital and Health Center, San Diego, California, USA

Accepted 16 February 2004

**S**keletal muscle spasticity is a condition that occurs secondary to upper motor neuron lesions and can result in serious complications for affected individuals. It is known that the function of individuals with spastic muscles is severely compromised due to decreased range of motion, decreased voluntary strength, and increased joint stiffness, but the basic mechanisms underlying the functional deficits that occur after the development of spasticity are not clearly understood. Although the etiology of spasticity is central, most antispasticity therapy is directed toward the peripheral nerves and muscles. As a result, therapeutic interventions involving stretching, casting, splinting, neurectomy, intrathecal baclofen pump placement, botulinum toxin injection, and electri-

cal stimulation of the muscles are only marginally effective.<sup>8,11,62,69,90</sup> It is estimated that the complications of muscle spasticity cost millions of dollars annually in the United States and, as such, represent a significant medical challenge with dramatic economic impact.

Considerable scientific and medical literature exists regarding the etiology and treatment of spasticity. Most research on skeletal muscle spasticity has focused on the nervous system. This is certainly reasonable because the primary lesion leading to spasticity is located in the central nervous system. Thus, many studies measure skeletal muscle electromyographic (EMG) activity, lesion size and shape, and patient gait characteristics. In addition, there is wide discussion of the various surgical procedures used to correct spastic deformities.<sup>44</sup> Far less attention has been directed toward understanding the structural and functional changes in skeletal muscle that occur secondary to spasticity. With a few notable exceptions,<sup>13,14</sup> the properties of skeletal muscle have largely been ignored. Yet, with recent technical advances, it is now possible to characterize many properties of skeletal muscle from patients with spasticity, and it is becoming increasingly clear that there are dramatic changes within skeletal muscle as well as in the nervous system.

**Abbreviations:** CC, contractile component of skeletal muscle; cDNA, complementary DNA; EDL, extensor digitorum longus muscle; EMG, electromyogram; FCU, flexor carpi ulnaris; NADH, nicotinic acid dehydrogenase enzyme; PEC, parallel elastic component of skeletal muscle; SEC, series elastic component of skeletal muscle; TA, tibialis anterior muscle

**Key words:** biomechanics; extracellular matrix; neural control; sarcomere; stiffness; upper motor neuron lesion

**Correspondence to:** R. L. Lieber; e-mail: rlieber@ucsd.edu

© 2004 Wiley Periodicals, Inc. This article is a US Government work and, as such, is in the public domain in the United States of America.  
Published online 14 April 2004 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.20059

Muscle and neural changes are usually related,<sup>79</sup> but recent data have revealed certain changes in muscle that are not easily explained by classic interpretations of the effects of neural changes alone.<sup>23,57,86</sup> It is therefore important to improve our understanding of the structural and functional changes that occur in spastic muscle. Understanding muscle plasticity will thereby improve, because the spasticity “model” appears to be unlike any other plasticity model previously studied. In addition, increased understanding will lend insight into the nature of spasticity itself, as muscles respond in stereotypical fashion to altered neural and mechanical input. Finally, because most of the interventions related to spasticity revolve around treatment of the musculoskeletal system, improved understanding of muscle–tendon unit properties may lead to the development of more rational interventions to treat these patients.

The purpose of this review is to provide a focused presentation of the structural and mechanical changes that occur within skeletal muscles secondary to spasticity. This literature is limited and, among these studies, complete agreement is lacking; this emphasizes the need for a constructive review of the topic.

To create this review, the PubMed database was searched using the following key words: spasticity, skeletal muscle, muscle changes, muscle transformation, fiber type, cerebral palsy, and upper motor neuron lesion. This search yielded over 200 references. These reports were reviewed to yield a total of 24 studies dealing directly with skeletal muscle changes. Using these studies and their associated references, we ultimately found 32 relevant references dealing with structural or functional changes in skeletal muscle secondary to spasticity, which are reviewed here. Of these studies, 17 were mechanical in nature,<sup>1,6,15,21,23,32,57,65,72,73,87,85,88,94,95,102,106</sup> 14 reported muscle properties obtained from biopsies,<sup>3,10,13,17,18,19,36,52,57,60,71,72,83,88</sup> 12 reported joint kinematics or joint kinetics,<sup>1,6,15,21,32,61,72,73,87,85,88,91</sup> 15 reported other aspects of muscle morphology distinct from fiber type or size distributions,<sup>3,13,17,18,19,23,36,52,57,60,71,72,83,84,88</sup> and 9 utilized EMG in their studies,<sup>1,12,13,14,15,19,61,91,95</sup>; several studies were multidisciplinary in nature. The majority of the studies reported findings from patients with static perinatal encephalopathy (cerebral palsy,  $n = 18$ ), but patients suffering from multiple sclerosis ( $n = 3$ ), stroke ( $n = 11$ ), spinal cord injury/upper motor neuron lesions ( $n = 7$ ), and Parkinson’s disease ( $n = 2$ ) were also studied.

## SKELETAL MUSCLE PLASTICITY

It is reasonable to study skeletal muscle properties even though the primary lesion is neural, because muscles respond in a fairly stereotypical manner to the amount and type of activity imposed upon them. For example, the classic studies of the 1960s and 1970s revealed that chronic electrical stimulation of skeletal muscle can progressively transform skeletal muscle cells into a slower phenotype.<sup>20,68,80,81</sup> Although there are subtle differences among muscles in terms of the nature, extent, and time-course of the transformation, the results are surprisingly consistent. There is general agreement that chronic electrical stimulation produces increased capillary density, increased percentage of type I muscle fibers, decreased fiber size (if the stimulation duration is long enough), increased endurance, and decreased strength. This serves as a template that describes the changes that occur in skeletal muscle with increased use. Voluntary exercise, especially when performed for long durations, results in many of the same muscle changes.<sup>82</sup> Spasticity is often thought to result in changes typically seen in increased use models, as is discussed in what follows.

The opposite model, chronic decreased use of skeletal muscle, which can be studied using models of simulated weightlessness,<sup>74</sup> tenotomy,<sup>7</sup> immobilization in a shortened position,<sup>4,59</sup> or spinal cord isolation,<sup>75,76</sup> causes muscle fibers to decrease their size and transform in the direction of the faster phenotype. One of the most extreme examples of such a transformation was reported in a rat spinal cord injury model in which the rats lived about half their lifespan with upper motor neuron lesions and, as a result, converted almost all of their muscle fibers to the fastest phenotype, even in the very slow soleus muscle.<sup>53,55</sup> Similar results have been reported for humans after traumatic spinal cord injury<sup>29</sup> and in other animals as well.<sup>37,38,75,77</sup> It is clear that an analysis of skeletal muscle fiber type distribution can be a useful indicator of the amount and type of activity that a muscle has received over an extended period of time. In addition to fiber type distribution, fiber size provides insights into the extent of fiber use. Increased use of skeletal muscle at high loads produces muscle fiber hypertrophy, whereas decreased use leads to muscle cellular atrophy. Both responses appear to be load-dependent. Thus, fiber size is typically interpreted as an indirect indicator of the amount of force imposed upon a muscle.

Taken together, muscle fiber type distribution and muscle fiber size distribution provide insight into the overall type and amount of use imposed

upon a muscle. Therefore, these parameters are often studied in spastic muscle in an attempt to determine its use pattern. However, in spite of the ease of measuring these parameters, they are not very specific and probably provide only a general indicator of muscle use. Excellent reviews of skeletal muscle plasticity and monographs on the subject are widely available in the literature.<sup>66,67,79,82</sup>

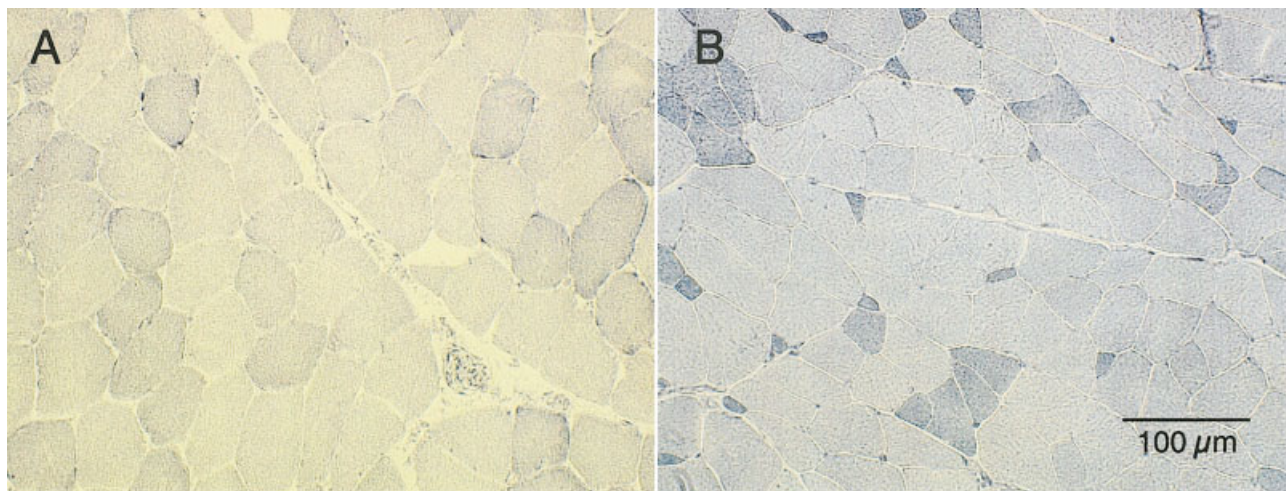
### MUSCLE FIBER TYPE AND FIBER SIZE CHANGES WITH SPASTICITY

Because measurement of muscle fiber size and type is commonly used in the diagnosis of neuromuscular disease<sup>9,16</sup> and also because such studies are relatively easy to perform, it is not surprising that biopsy studies are the most prevalent type of study performed on spastic muscle. Despite their apparent ease, there are methodological concerns that make these studies difficult to interpret definitively. Specific issues that must be addressed in any biopsy study are the fraction of the muscle actually being sampled, the gradient of fiber type and fiber size distribution across and along the muscle, the variability in fiber type and fiber size between muscles, whether different muscles or muscle groups are being used to compare normal subjects and diseased patients, and the variability in clinical presentation of patients.

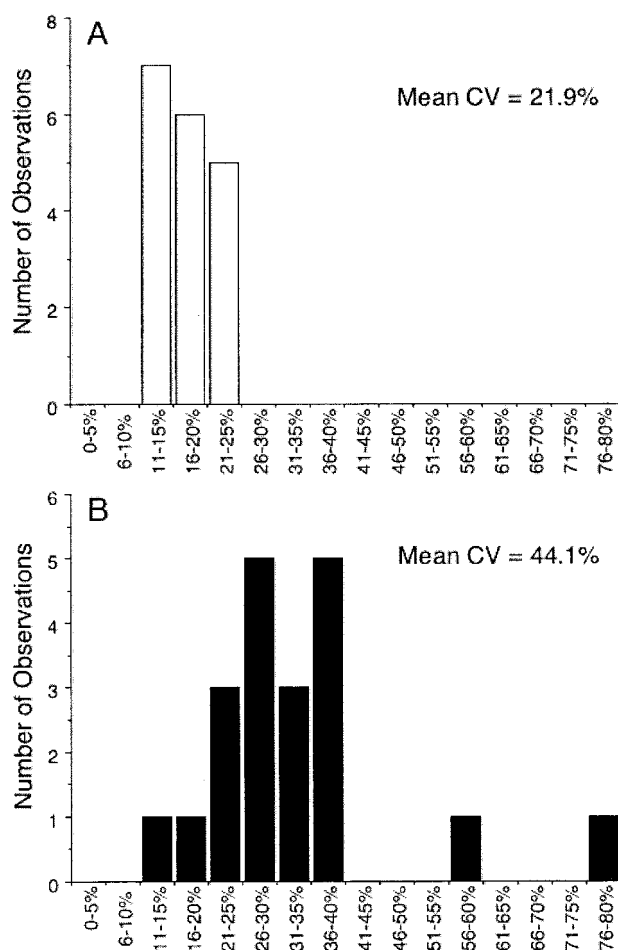
Most of these issues were not addressed by any of the studies reviewed. For example, it was extremely rare to find a study in which the same muscle was measured in populations of individuals with and without spasticity. In almost all of the studies reviewed, due

to practical and ethical considerations, data were reported from different muscles for the two experimental groups, and individuals with and without spasticity were almost always of different age—this was especially true in studies of spasticity secondary to static perinatal encephalopathy (cerebral palsy), where it is almost impossible to obtain normal tissue from children. In at least one study, the investigators endeavored to age-match the groups by using historical pathological specimens from children (which turned out to be normal) but these biopsies were from different muscles as compared with the spastic patients.<sup>72</sup> These factors obviously confound the study of muscle spasticity and make it difficult to generalize results across either age groups or diagnoses.

In spite of such limitations, one common finding in biopsy studies is that fiber size variability is increased in muscles from patients with spasticity. When sectioned, normal skeletal muscle biopsies have tightly packed fibers that form polygons juxtaposed to one another (Fig. 1A). However, most published micrographs of muscle from spastic patients showed abnormalities such as increased fiber size variability, increased numbers of “rounded” fibers, “moth-eaten” fibers, and in some cases increased extracellular space (Fig. 1B).<sup>3,10,13,36,71,72</sup> When fiber size is measured and expressed as coefficient of variation, the value is always higher for spastic muscle samples compared with normal samples (Fig. 2). Fiber size variability is characteristic of numerous neuromuscular disorders as well as occurring in spasticity, so such findings are not very specific.<sup>16</sup> The mechanistic basis for this response is not known. It



**FIGURE 1.** Muscle fiber morphology is abnormal in spastic muscle. Light micrograph of a muscle obtained from a 19-year-old hemiplegic boy. (A) Nonspastic extensor carpi radialis brevis muscle. Histochemical stain of the NADH oxidative enzyme that labels fiber type I and type IIA dark, and type IIB lighter. (B) Spastic flexor carpi ulnaris muscle. Note the spastic muscle demonstrates greater fiber size variability. (Micrographs courtesy of Dr. Eva Pontén, Karolinska Institute, Stockholm, Sweden.)



**FIGURE 2.** Muscle fiber size is more variable in spastic muscle. Histogram of fiber area coefficient of variation in **(A)**, normal muscles from patients ranging from 4 to 13 years of age, and **(B)** spastic muscles from patients ranging from 5 to 14 years of age. Data pooled for type 1 and type 2 fiber coefficients of variation in Table 2 of reference 72.

should be noted that muscle fiber tapering occurs near the end of some fibers and may result in fiber size variation, but this is not considered a pathological state.<sup>64,97,98</sup>

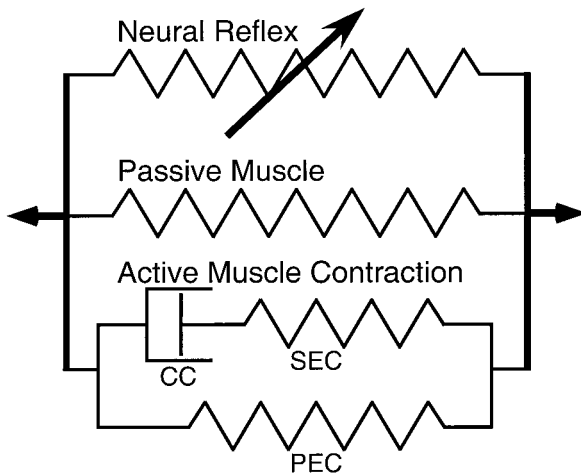
It would be convenient if fiber type distribution changes were consistent among studies, but this is not the case. Because most investigators were predisposed to the idea that spastic muscles were chronically active electrically, it may have been tempting to interpret an increase in type I fiber percentage from spastic patients as evidence of muscle fiber type transformation.<sup>32</sup> Unfortunately, whereas a few studies have indeed reported an increased percentage of type I fibers,<sup>13,36,60</sup> others reported an increased percentage of type II fibers<sup>88</sup> or no change in fiber type distribution.<sup>3,10,71,72</sup> There is thus no agreement that spasticity represents either an increased- or de-

creased-use model, perhaps in part due to the sampling problems inherent to the biopsy procedure itself.<sup>2,16</sup> For example, when fiber type percentages were measured from vastus lateralis biopsies and compared with the fiber type distributions within the entire human vastus lateralis,<sup>47,48</sup> such values were notoriously inaccurate. However, considering the literature as a whole, the results probably indicate that spastic muscle is not simply subjected to chronic increased or decreased activation.

#### BIOMECHANICAL STUDIES OF SPASTIC LIMBS

Most clinicians agree that individuals with spastic muscles present clinically with increased joint stiffness. Numerous attempts have been made to characterize this stiffness objectively, which ultimately led to the classic definition of spasticity by Lance<sup>46</sup> as “a velocity-dependent resistance to stretch.” This definition has enjoyed wide acceptance and, in many cases, is clearly evident in spastic patients. It acknowledges the increase in joint stiffness and further refines this idea by identifying a dynamic component to it. This definition is also consistent with the idea that spasticity is due to an increase in gain of the stretch reflex,<sup>46</sup> which would cause the velocity-dependence. This is the rationale for the therapeutic method of surgical deafferentation that is performed at several centers.<sup>44,99</sup> However, numerous mechanisms could provide an explanation for an “increased resistance to stretch” and do not necessarily involve the nervous system. Although evidence exists for alterations in reflex gain or motor neuron firing threshold,<sup>70,96</sup> the precise description of the increased resistance to stretch and the underlying basis for it are neither routinely studied nor universally agreed upon.

Several laboratories have developed devices to quantify limb stiffness.<sup>40,61,86</sup> In many cases, stiffness is explained in terms of underlying neural and mechanical properties. The basic approach of this type of experiment is to measure dynamic limb stiffness and decompose it into its constituent parts. Resistance to stretch is primarily due to one of three factors: passive muscle stiffness; neurally mediated reflex stiffness; and active muscle stiffness (Fig. 3). The value of the decomposition approach is that the mechanical changes may provide insights into the underlying neuromuscular changes. Increased passive muscle stiffness would presumably be due to fibrosis within the muscle tissue or even a change in the cellular properties of muscle. Increased reflex gain may reflect a change in descending influences on the monosynaptic reflex between muscle spindle



**FIGURE 3.** Graphic representation of the sources that can contribute to stiffness when a muscle is mechanically perturbed. These three major sources include neural reflex (depicted as a variable gain resistor), passive muscle properties (depicted as a resistor), and active muscle properties (depicted as a three-element Hill model<sup>31</sup> with parallel elastic component [PEC], series elastic component [SEC], and contractile component [CC]). In practice, when joint stiffness is measured, some or all of these sources may be contributing to the measured value.

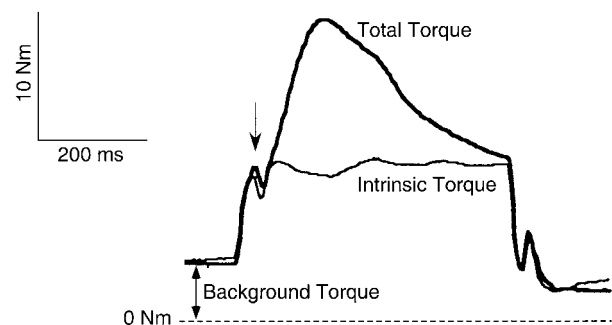
afferents and alpha motor neurons or on polysynaptic stretch reflex pathways. Increased active muscle stiffness may be due to an increase in the number of cross-bridges attached during contraction or to an increase in stiffness per cross-bridge, both of which have been documented<sup>22,39</sup> and have increased our understanding of classic muscle mechanics.<sup>33–35</sup>

Experimentally, total joint stiffness is measured and the fraction of measured stiffness that can be explained by passive muscle, neural reflex, and active muscle contraction is quantified. This is done by placing the patient in a customized instrumented dynamometer in which joint moment is accurately measured during joint angular perturbation. The muscle is activated to a preset level and then, during the perturbation, muscle electromyographic (EMG) activity and limb dynamics are measured. Analysis of these data involves the use of either time- or frequency-domain methods.<sup>61,86</sup> An example of data from this type of experiment is shown in Figure 4 in which a subject voluntarily activates the muscles to generate a plantarflexion moment of  $\sim 5$  Nm (“background torque” in Fig. 4). The ankle is then rapidly dorsiflexed, resulting in abrupt torque increase. The initial portion of this increased torque results from the nonreflex components of the system—passive and active muscle properties as well as the inertial properties of the device. However, after  $\sim 200$  ms, when the EMG indicates significant reflex activity is

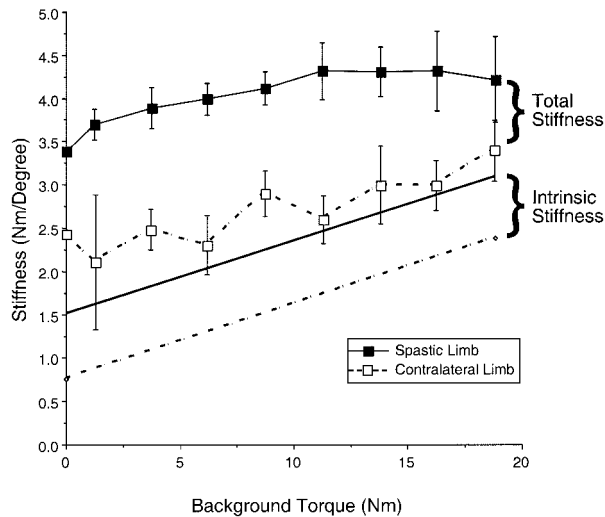
present, total torque increases further, which reflects the intrinsic muscle plus the reflex-mediated stiffness. Finally, in a separate experiment, the same patient receives electrical stimulation of the tibial nerve to cause the plantarflexors to again generate  $\sim 5$  Nm of torque. The ankle is again dorsiflexed and torque measured. This provides an estimate of the nonreflex stiffness increment or intrinsic muscle stiffness. Subtraction of the two curves provides estimates of both the reflex and nonreflex components of stiffness.

Using this methodology, Sinkjaer and Magnusen showed that the total stiffness measured in the spastic leg of hemiparetic patients was greater than the total stiffness measured in contralateral “control” legs (Fig. 5).<sup>85</sup> This was not extremely surprising. What was surprising was that the intrinsic stiffness component of the spastic leg was much higher than that of the contralateral leg, suggesting a difference in the passive mechanical properties of the muscles of the spastic limb compared with the normal limb. The increased passive mechanical stiffness accounted for almost all of the increase in joint stiffness measured. Reflex stiffness did not differ significantly between spastic and contralateral limbs.

A slightly more comprehensive approach uses the same basic method, but generalizes it by implementing a parallel cascade system to decompose stiffness into components that include both static and dynamic factors.<sup>43,61</sup> With this technique, multiple perturbations are imposed at multiple frequen-



**FIGURE 4.** Raw mechanical torque curve illustrating the method for determining stiffness in human subjects during mechanical perturbation of the ankle. Plantarflexors are voluntarily activated to a preset level, in this case about 5 Nm (“background torque”), and then the ankle is rapidly dorsiflexed (down arrow). This results in the “total torque,” which is due to intrinsic muscle properties and reflex properties (thick line). In a separate experiment, using electrical stimulation of the tibial nerve, the rapid dorsiflexion is repeated, which results in the total intrinsic torque (thin line). Stiffness is calculated from these data in terms of torque/angle in units of Nm/degree (c.f. Fig. 5). Zero torque level (0 Nm) shown by dashed line. Calibration bars shown to left of figure. Figure modified from reference 85.



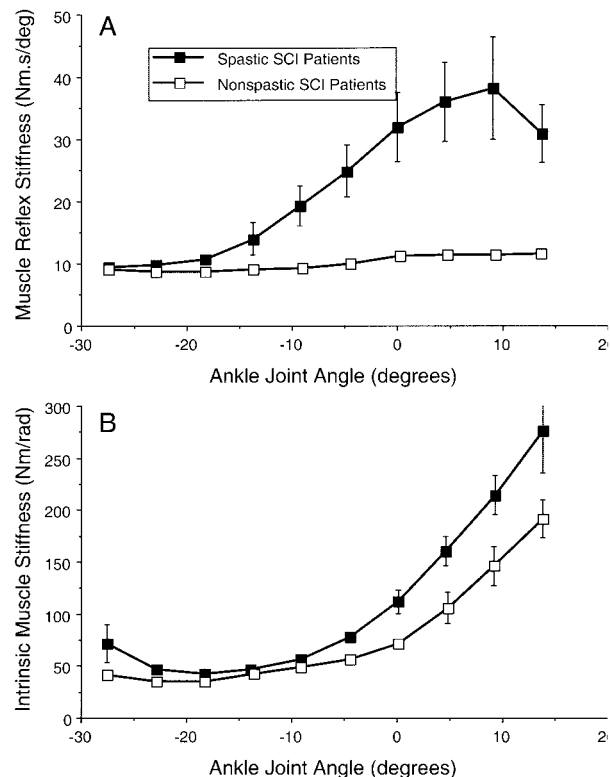
**FIGURE 5.** Increased passive joint stiffness measured in spastic hemiparetic patients. Total measured stiffness is shown for the spastic limb (filled squares) and contralateral nonspastic (open squares) limb. Also shown is the regression line for nonreflex stiffness in the spastic (solid line) and contralateral nonspastic (dashed line) limb. Data were obtained as described in the text. The intercepts of the intrinsic stiffness regression lines indicate passive muscle stiffness, whereas the slopes of the lines represent active muscle stiffness. Passive stiffness was significantly different between groups. This implies structural differences between spastic and contralateral muscles. Data replotted from reference 85.

cies for multiple perturbation sizes, and sophisticated signal processing techniques are used to decompose stiffness into its components. These experiments are performed across a range of joint angles. Mirbagheri et al.<sup>61</sup> used this approach and found that, in spastic spinal cord-injured patients, reflex gain was dramatically increased compared with nonspastic spinal cord-injured patients (Fig. 6A). They also showed that the gain change was a function of joint angle, being more pronounced at dorsiflexed angles—that is, long plantarflexor muscle lengths (Fig. 6A). Intrinsic muscle stiffness, both elastic and viscous, was also increased in spastic subjects, with the largest effect seen at dorsiflexed angles (Fig. 6B). It was very impressive that these investigators also controlled for the inertial properties of the limbs and of the device, revealing no significant inertial effects of either. These two studies provide some of the strongest direct evidence that the intrinsic mechanical properties of skeletal muscles from spastic patients are altered secondary to spasticity. The structural basis of this difference is unknown. The effects reflect only the stiffness changes across a small joint angle, and the relationship to stiffness during large joint displacements is not clear. The

thorough analysis by Mirbagheri et al. highlights the point that it is patently oversimplified to assume that joint stiffness is due to either muscle or nervous system changes, but not both. Certainly both systems are involved, probably to differing extents, depending on the particular disease state, patient age, and time since injury. There can be no denying that, even though the primary lesion in these patients resides within the central nervous system, the peripheral muscular system has adapted in such a way as to increase passive mechanical stiffness.

#### MUSCLE FIBER LENGTH IN SPASTIC MUSCLE

It is probably not an overstatement to assert that skeletal muscle fiber length is the single most functionally important property of a skeletal muscle. Muscle fiber length is the primary determinant of muscle excursion and fiber-length to muscle-length ratio is a strong indicator of the design of a skeletal muscle either for producing high force or high excursion.<sup>27,51,78</sup> Analysis and interpretation of muscle



**FIGURE 6.** Increased intrinsic muscle stiffness and reflex gain demonstrated in spastic limbs. Joint dynamics measured in spastic spinal cord-injured patients (solid squares) compared with nonspastic spinal cord-injured patients (open squares) using frequency-domain analysis. Data were obtained as described in the text. (A) Reflex stiffness. (B) Intrinsic stiffness. Data replotted from reference 61.

fiber length is part of the general field of skeletal muscle architecture.

Unfortunately, the only method to determine muscle fiber length definitively in mammalian muscle is to obtain whole, fixed muscles and to dissect individual fibers or fiber bundles from these fixed muscles. In fact, most studies actually measure the length of a small bundle of fibers, and thus, strictly speaking, most of these lengths are actually fascicle lengths. Numerous studies of this sort have revealed enormous architectural variations among muscles in the upper<sup>5,49,54</sup> and lower<sup>24,101</sup> human limbs. Such invasive studies are not possible in human subjects and it has thus been difficult to obtain reliable information on muscle architectural properties in different human musculoskeletal diseases. However, using ultrasound studies of human muscle,<sup>25,26,42</sup> it is now possible to measure human muscle fascicle length directly both at rest and during exercise. This field is in its infancy but promises to produce much valuable data.

There is a widely held belief among clinicians that the muscle contractures occurring secondary to spasticity are due to a reduction in muscle fiber length and, thus, a decrease in the number of serial sarcomeres within muscle fibers. Much of the basis for this belief is found in the studies from the 1970s by Williams and Goldspink<sup>103,104</sup> and Tabary et al.,<sup>92,93</sup> who observed that rodent soleus muscles add or subtract serial sarcomeres to optimize muscle fiber length to a particular immobilization angle. They observed that immobilization of the soleus in a shortened position led to decreased serial sarcomere number, whereas soleus immobilization in a lengthened position led to an increased serial sarcomere number. In other words, serial sarcomere number always adjusted as needed. This clearly occurs in rodent soleus muscles, but it is inappropriate to extrapolate these results to all muscles in all species under all conditions. This was clearly demonstrated by Edgerton and colleagues, who showed that immobilized rat muscles adapt differentially, depending on the particular muscle immobilized, and the particular length at which they are immobilized.<sup>89</sup> They confirmed the fact that soleus muscles respond readily to the length at which they were immobilized, but their antagonistic muscles (i.e., extensor digitorum longus [EDL] or tibialis anterior [TA]) did not. Both the EDL and TA muscles were much less adaptive to chronic length changes. Unfortunately, these results have been overlooked by many investigators and therefore the widely held belief has persisted that spastic muscle has a decreased serial sarcomere number, secondary to the contracted anatomical po-

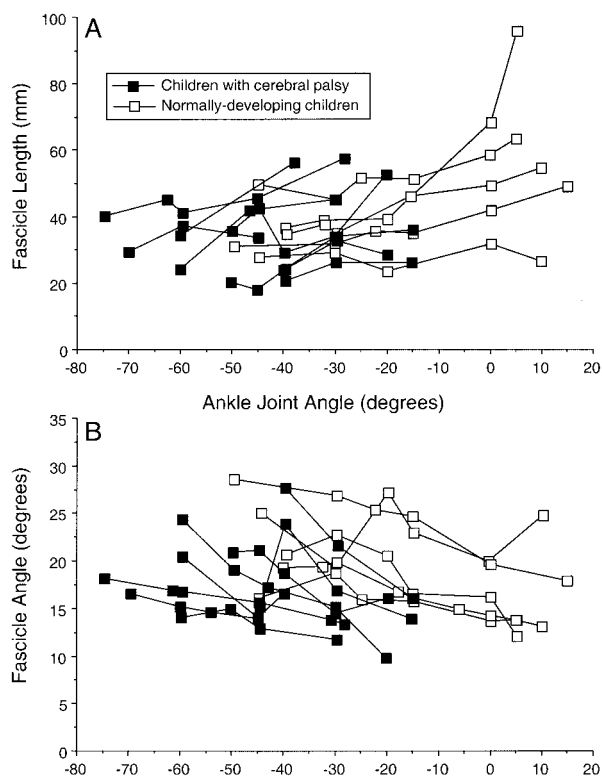
sition of the muscle and therefore shortened *in vivo* length.

Indirect inference of muscle fiber length in spastic patients was provided by Tardieu and colleagues,<sup>94,95</sup> who reported a steeper passive torque-angle relationship and change in the range of active torque generation in children with cerebral palsy. They interpreted these data as indicating decreased muscle fiber length. Although their results are consistent with the idea of decreased muscle fiber length, they did not directly demonstrate any change in fiber length. As described in what follows, muscle stiffness can change for a variety of structural reasons, only one of which is altered fiber length. Apparently, this limitation is not widely appreciated.

Recently, Shortland et al. directly measured the architectural properties of the medial gastrocnemius of children with static perinatal encephalopathy using ultrasound.<sup>84</sup> Fascicle length and fascicle angle were measured in these children and in normally developing children. As expected, fascicle length increased with dorsiflexion (Fig. 7A). The ankle angles over which children with cerebral palsy were measured was biased toward plantarflexion due to equinus contracture, but the lengths of the fascicles themselves were not significantly different between the two groups. They found no evidence of fascicle length change in the children with encephalopathy (cerebral palsy), contrary to their expectation. Because this is the only such study in the literature, it is premature to conclude that this result is generally applicable to all spastic muscles. However, it must be emphatically stated that the only direct measurement of fiber length that has ever been performed in children with spastic diplegia has shown fiber length to be normal.

The change in fascicle angle with ankle angle was similar for both groups, but absolute fascicle angles were greater in nonspastic limbs compared with spastic limbs (Fig. 7B). The investigators posited that differences in absolute fascicle angles between groups were probably secondary effects due to the atrophy of the spastic muscles. Atrophy and fascicle angle had been shown previously to be highly correlated in animal models of immobilization-induced atrophy,<sup>30</sup> and hypertrophy and fascicle angle were highly correlated during power training in humans.<sup>41</sup>

A second form of evidence for normal fiber length in spastic muscle was reported for children with chronic wrist flexion contractures.<sup>52</sup> In that study, Lieber and Fridén used intraoperative laser diffraction to measure flexor carpi ulnaris (FCU) sarcomere length. Sarcomere length is an excellent



**FIGURE 7.** No change in muscle fascicle length measured in children's medial gastrocnemius muscles. **(A)** Muscle fascicle length. **(B)** Muscle fascicle angle. Filled symbols represent measurements on spastic limbs of children with cerebral palsy, whereas open symbols represent measurements on limbs of normally developing children. Abscissa represents tibiotarsal angle. Data replotted from reference 84.

predictor of skeletal muscle relative force and can thus be used to predict muscle function.<sup>28</sup> Sarcomere length was measured in these children with their wrists flexed and was compared to patients with radial nerve injuries who were undergoing FCU tendon transfer procedures.<sup>50,56</sup> The latter group of patients (average age 32–55 years) had a normally innervated FCU and were thus thought to provide “normal” control values. Sarcomere length measured with the wrist flexed was dramatically longer in children with wrist flexion contractures ( $3.48 \pm 0.44 \mu\text{m}$ ) compared to the patients with radial nerve injury, with the wrist in the same position ( $2.41 \pm 0.31 \mu\text{m}$ ). These data demonstrate that, whereas the spastic wrist flexion contractures resulted in a shortened muscle–tendon unit, the sarcomeres within the fibers were actually lengthened. Furthermore, the investigators rotated the wrist joints of the children over the permitted range and found that the slope of the FCU sarcomere length–joint angle relationship was the same compared to the patients with radial nerve injury. Because the slope of the sarcomere

length–joint angle relationship reflects fiber length within the muscle, these data indicate that fiber length in the two groups was the same. This result seems a bit counterintuitive in that muscles that were shortened had fibers within them that were lengthened. Thus, children with spastic wrist flexion contractures had muscles with normal fiber length, albeit the sarcomeres within the fibers were highly stretched for an unknown reason. This high stretch may have been due to lack of growth of the muscle with bone growth, or some other as yet unidentified mechanism. The conclusion from both the intraoperative sarcomere length study and the ultrasound study is that there is no evidence for fiber length change due to spasticity. These two studies represent the only direct measurements of fiber length in human spastic muscle.

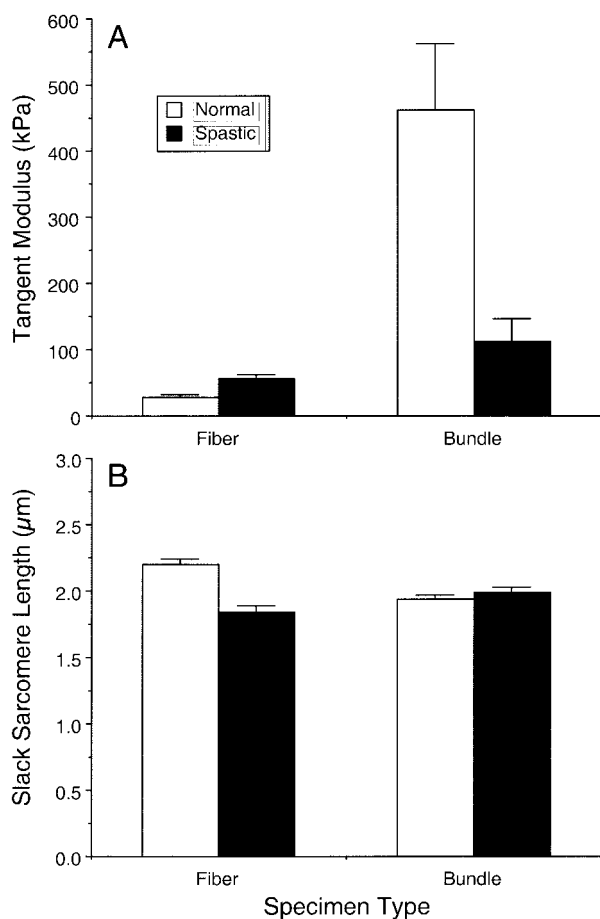
#### MECHANICAL CHANGES IN SPASTIC MUSCLE TISSUE

The indirect biomechanical studies just described demonstrate that spastic muscles have altered intrinsic mechanical stiffness. What structures might be responsible for this altered stiffness? Comprehensive answers cannot be provided at this time. However, two recent studies indicated that the passive mechanical properties of isolated muscle cells and small muscle fiber bundles were altered secondary to spasticity. These two studies provide a view of the complex interactions between muscle cells and the extracellular matrix that may result from spasticity.

To compare directly the intrinsic passive mechanical properties of normal and spastic muscle cells (i.e., muscle cells obtained from biopsies of patients with spastic limbs), a novel method was developed in which muscle biopsies were removed from patients at the time of reconstructive surgery (secondary to cerebral palsy for the spastic muscle) or during surgical repair (radial nerve injury or trauma for the normal muscle) and were placed in a relaxing solution designed to prevent hypercontraction of the muscle fibers.<sup>105</sup> Then, using high-powered microscopic illumination, segments of individual cells were dissected free of the surrounding connective tissue and placed into a micromechanical testing apparatus that enabled passive elongation of the muscle cell with simultaneous measurement of intracellular sarcomere length.<sup>23</sup> Muscle cells were elongated in  $250\text{-}\mu\text{m}$  increments and the relationship between muscle passive stress and sarcomere length was quantified. The slope of the single cell stress–strain curve was then used to calculate fiber elastic modulus as previously described in detail.<sup>23</sup>

Interestingly, the tensile modulus of muscle cells from spastic patients was over twice that of neuro-muscularly intact patients (left panel of Fig. 8A), demonstrating that the intrinsic passive stiffness of individual spastic muscle cells was increased. In addition, the resting sarcomere length of spastic cells (i.e., the length of the sarcomeres when the muscle cell was completely unloaded), was significantly shorter in spastic muscle cells compared with normal cells (left panel of Fig. 8B). Internal cytoskeletal structures set resting sarcomere length in muscle cells.<sup>100</sup>

These two findings demonstrate that the structures within the muscle cell responsible for setting resting sarcomere length and determining cellular elastic modulus are altered in spastic muscle. The



**FIGURE 8.** Increased stiffness of single cells and fiber bundles in muscles from children with cerebral palsy. Biomechanical properties measured from single cells (left panels) and muscle fiber bundles (right panels) of normal (open bars) and spastic (filled bars) subjects. **(A)** Tangent modulus calculated from the linear portion of the sarcomere length–stress relationship. **(B)** Slack sarcomere length measured prior to mechanical testing. Data replotted from references 23 and 57.

most obvious candidate for this structure is the giant intracellular cytoskeletal protein, titin.<sup>45</sup> Titin has been demonstrated in frog skeletal muscles to bear almost the entire elastic load during passive elongation and is a significant component that bears the passive load in human muscle.<sup>58</sup> Can the titin protein be altered secondary to spasticity? There are no definitive data, but there is circumstantial evidence to suggest that it is possible. For example, it has been demonstrated, based on the differences in cDNA sequences, that titin can exist in multiple isoforms between heart and skeletal muscle and even among various skeletal muscles.<sup>45</sup> It is known that the titin isoform in heart muscle is much stiffer and much shorter than the titin isoforms in most skeletal muscles. Furthermore, it has also been demonstrated that the titin isoform can change within heart muscle under pathological conditions, specifically, ischemia-induced cardiomyopathy. This condition is usually accompanied by elevated left ventricular end-diastolic pressure, which follows from increased myocardial stiffness resulting from upregulated collagen expression. However, in addition to collagen proliferation, a “switch” from a stiff to a more compliant isoform of titin was documented.<sup>63</sup> It is therefore reasonable to speculate that titin isoform may be altered in skeletal muscles of patients with spastic limbs, although definitive evidence of this change has not been reported.

Because surgical reconstructive procedures are often performed on spastic muscles to restore or augment function, it is of interest to know whether the increased resistance to stretch that is “felt” by surgeons in the operating room may be explained by differences in the elastic properties of muscle cells within spastic muscle. To investigate this point, small bundles of muscle cells were subjected to the same procedure as described earlier for single cells and their elastic moduli were measured.<sup>57</sup> These small bundles of cells (5–50 fibers) contained the same types of muscle cells that were tested previously, but they were also ensheathed by the connective tissue matrix of the muscle tissue. Several interesting findings emerged. First, the tangent modulus measured in bundles was significantly greater than the same modulus measured in single cells (Fig. 8A). However, the difference was much more pronounced for normal muscle bundles than spastic muscle bundles. Whereas spastic muscle bundle modulus was increased by only about twofold over the single fiber modulus, in normal muscle the modulus was increased over 16 times compared with the modulus of the normal isolated muscle cell. These data demonstrate a clear difference in the mechanical properties

of spastic muscle tissue bundles compared with normal muscle fiber bundles. The differences were even more impressive when the structural differences between the two bundle types are considered—only 40% of the spastic muscle bundle cross-sectional area was occupied by muscle fibers, whereas 95% of normal muscle bundle was occupied by muscle fibers. Morphologically, there was a large amount of poorly organized extracellular material in spastic bundles compared with normal bundles. One can calculate the mechanical properties of the extracellular matrix material in the bundles by subtracting the single cell modulus from whole bundle modulus. When this is done, it is seen that the extracellular matrix of the spastic muscle has a modulus of  $\sim 0.2$  GPa, whereas normal muscle has a modulus of  $\sim 8$  GPa—about 40 times greater. These data demonstrate that, although spastic muscle contains a larger amount of extracellular matrix material, the quality of that material is much lower compared with normal muscles. It is of interest that the spastic muscle model with high fiber stiffness in a compliant extracellular matrix shows the opposite adaptation as the ischemic heart muscle, which possesses a very stiff extracellular matrix and compliant fibers. The only other explicit description of extracellular matrix changes in spastic muscle was based on biochemical measurement of collagen concentration, which increased dramatically in spastic muscle.<sup>3</sup>

#### **FUTURE DIRECTIONS**

We have presented the evidence from the literature that muscle from patients who develop spasticity is dramatically altered. Although the primary lesion leading to spasticity is within the central nervous system, there is no doubt that the peripheral musculature has become abnormal. This conclusion is based on results using a variety of experimental methods in a variety of diseases across a wide range of patient ages, so the general conclusion cannot be dismissed. The following alterations occur in spastic muscle: (1) altered muscle fiber size and fiber type distribution; (2) proliferation of extracellular matrix material, measured both morphologically and biochemically; (3) increased stiffness of spastic muscle cells and, to a lesser extent, spastic muscle tissue; and (4) inferior mechanical properties of extracellular material in spastic compared to normal muscle. Although some specific muscular changes have been reported, sophisticated biomechanical experiments have demonstrated that the changes that occur secondary to spasticity are complex, involv-

ing various components of the neural and muscular systems. These effects are joint angle- and, likely, movement-dependent.

Improvement in the quality of life of patients with spasticity will depend on a new understanding of both the changes that occur secondary to spasticity and the development of rational interventions to either prevent these changes or reverse them. The importance of this problem to society was recently highlighted by the National Institutes of Health, which held a summit on the topic and issued a Request for Applications that would address this problem.

Details of the structural changes that occur in spastic muscle, as well as mechanistic explanations for how these changes occur, are lacking. Basic questions that must be addressed in this field follow logically from the material presented herein. What are the proteins that are altered within spastic muscle cells and the extracellular matrix of spastic muscle tissue? Specifically, are contractile proteins altered in a different way compared with cytoskeletal proteins? Are the focal adhesion molecules that integrate a muscle cell with the extracellular matrix altered secondary to spasticity? Do spastic muscles develop a compliant extracellular matrix material and then compensate by causing muscle fibers to become stiffer, or do spastic muscle fibers stiffen secondary to spasticity and then the extracellular matrix material becomes more compliant in response to fiber changes? Is the signaling between the extracellular matrix and skeletal muscle cells altered secondary to spasticity? Do spastic muscle cells retain their ability to adapt using mechanisms observed in normal muscle such as sarcomere number alteration, stress-induced hypertrophy, and regeneration via satellite cell proliferation?

In addition, there are important clinically relevant questions that remain largely unexplored. Is there a difference in the muscle response to different causes of spasticity? Is there an effect of the age at which the spasticity is acquired on muscle properties?

These are the types of questions that must ultimately be answered to develop rational surgical and rehabilitation strategies to treat patients who suffer from this devastating malady.

This work was supported by NIH grants AR40539 and HD044822, the United Cerebral Palsy Foundation, Allergan, Inc., and the Department of Veterans Affairs. We thank Gauri Kelekar for her skillful assistance in digitizing the data for the figures.

## REFERENCES

- Berger W, Horstmann G, Dietz V. Tension development and muscle activation in the leg during gait in spastic hemiparesis: independence of muscle hypertonia and exaggerated stretch reflexes. *J Neurol Neurosurg Psychiatry* 1984;47:1029–1033.
- Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* 1975;35:609–616.
- Booth CM, Cortina-Borja MJ, Theologis TN. Collagen accumulation in muscles of children with cerebral palsy and correlation with severity of spasticity. *Dev Med Child Neurol* 2001;43:314–320.
- Booth FW, Kelso JR. Effect of hind-limb immobilization on contractile and histochemical properties of skeletal muscle. *Pflugers Arch* 1973;342:123–238.
- Brand PW, Beach RB, Thompson DE. Relative tension and potential excursion of muscles in the forearm and hand. *J Hand Surg (Am)* 1981;3A:209–219.
- Broberg C, Grimby G. Measurement of torque during passive and active ankle movements in patients with muscle hypertonia. A methodological study. *Scand J Rehabil Med* 1983;9:108–117.
- Buller AJ, Lewis DM. Some observations on the effects of tenotomy in the rabbit. *J Physiol (Lond)* 1965;178:326–342.
- Cadenhead SL, McEwen IR, Thompson DM. Effect of passive range of motion exercises on low goniometric measurements of adults with cerebral palsy. *Phys Ther* 2002;82:658–669.
- Carpenter S, Karpati G. Pathology of skeletal muscle. New York: Churchill Livingstone; 1984.
- Castle ME, Reyman TA, Schneider M. Pathology of spastic muscle in cerebral palsy. *Clin Orthop Rel Res* 1979;142:223–233.
- DeDeyne PG. Application of passive stretch and its implications for muscle fibers. *Phys Ther* 2001;81:819–827.
- Dietz V, Berger W. Interlimb coordination of posture in patients with spastic paresis. Impaired function of spinal reflexes. *Brain* 1984;107:965–978.
- Dietz V, Ketelsen UP, Berger W, Quintern J. Motor unit involvement in spastic paresis. Relationship between leg muscle activation and histochemistry. *J Neurol Sci* 1986;75:89–103.
- Dietz V, Quintern J, Berger W. Electrophysiological studies of gait in spasticity and rigidity. *Brain* 1981;104:431–449.
- Dietz V, Trippel M, Berger W. Reflex activity and muscle tone during elbow movements in patients with spastic paresis. *Ann Neurol* 1991;30:767–779.
- Dubowitz V, Brooke MH. Muscle biopsy: a modern approach. Philadelphia: W. B. Saunders; 1973.
- Edstrom L. Histochemical changes in upper motor lesions, parkinsonism and disuse. Differential effect on white and red muscle fibres. *Experientia* 1968;24:916–917.
- Edstrom L. Selective changes in the size of red and white muscle fibers in upper motor neuron lesion and Parkinsonism. *J Neurol Sci* 1970;11:523–550.
- Edstrom L. Relation between spasticity and muscle atrophy pattern in upper motor neurone lesions. *Scand J Rehabil Med* 1973;5:170–171.
- Eisenberg BR, Salmons S. The reorganization of subcellular structure in muscle undergoing fast-to-slow type transformation. A stereological study. *Cell Tissue Res* 1981;220:449–471.
- Engsberg JR, Ross SA, Olree KS, Park TS. Ankle spasticity and strength in children with spastic diplegic cerebral palsy. *Dev Med Child Neurol* 2000;42:42–47.
- Ford LE, Huxley AF, Simmons RM. The relation between stiffness and filament overlap in stimulated frog muscle fibres. *J Physiol (Lond)* 1981;311:219–249.
- Fridén J, Lieber RL. Spastic muscle cells are shorter and stiffer than normal cells. *Muscle Nerve* 2003;27:157–164.
- Friederich JA, Brand RA. Muscle fiber architecture in the human lower limb. *J Biomech* 1990;23:91–95.
- Fukunaga T, Ichinose Y, Ito M, Kawakami Y, Fukashiro S. Determination of fascicle length and pennation in a contracting human muscle in vivo. *J Appl Physiol* 1997;82:354–358.
- Fukunaga T, Ito M, Ichinose Y, Kuno S, Kawakami Y, Fukashiro S. Tendinous movement of a human muscle during voluntary contractions determined by real-time ultrasonography. *J Appl Physiol* 1996;81:1430–1433.
- Gans C. Fiber architecture and muscle function. In: Holloszy J, editor. Exercise and sport science reviews. Vol. 10. Lexington, MA: Franklin University Press; 1982. p 160–207.
- Gordon AM, Huxley AF, Julian FJ. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J Physiol (Lond)* 1966;184:170–192.
- Grimby G, Broberg C, Krotkiewska I, Krotkiewski M. Muscle fiber composition in patients with traumatic cord lesion. *Scand J Rehabil Med* 1976;8:37–42.
- Heslinga JW, Huijting PA. Effects of growth on architecture and functional characteristics of adult rat gastrocnemius muscle. *J Morphol* 1990;206:119–132.
- Hill AV. The mechanics of active muscle. *Proc R Soc Lond B* 1953;141:104–117.
- Hufschmidt A, Mauritz KH. Chronic transformation of muscle in spasticity: a peripheral contribution to increased tone. *J Neurol Neurosurg Psychiatry* 1985;48:676–685.
- Huxley AF. Muscular contraction. *J Physiol (Lond)* 1974;243:1–43.
- Huxley AF, Simmons RM. Proposed mechanism of force generation in vertebrate striated muscle. *Nature* 1971;233:533–538.
- Huxley HE, Simmons RM, Faruqi AR, Kress M, Koch M. Millisecond time-resolved changes in x-ray reflections from contracting muscle during rapid mechanical transients, recorded using synchrotron radiation. *Proc Natl Acad Sci USA* 1981;78:2297–2301.
- Ito M, Araki A, Tanaka H, Tasaki T, Cho K, Yamazaki R. Muscle histopathology in spastic cerebral palsy. *Brain Devel* 1996;18:299–303.
- Jiang B, Roy R, Edgerton R. Expression of a fast fiber enzyme profile in the cat soleus after spinalization. *Muscle Nerve* 1990;13:1037–1049.
- Jiang B, Roy RR, Edgerton VR. Enzymatic plasticity of medial gastrocnemius fibers in the adult chronic spinal cat. *Am J Physiol* 1990;259:C507–C514.
- Julian FJ, Morgan DL. Tension, stiffness, unloaded shortening speed and potentiation of frog muscle fibres at sarcomere lengths below optimum. *J Physiol (Lond)* 1981;319:205–217.
- Katz RT, Rymer WZ. Spastic hypertonia: mechanisms and measurement. *Arch Phys Med Rehabil* 1989;70:144–151.
- Kawakami Y, Abe T, Fukunaga T. Muscle-fiber pennation angles are greater in hypertrophied than in normal muscles. *J Appl Physiol* 1993;74:2740–2744.
- Kawakami Y, Ichinose Y, Fukunaga T. Architectural and functional features of human triceps surae muscles during contraction. *J Appl Physiol* 1998;85:398–404.
- Kearney RE, Stein RB, Parameswaran L. Identification of intrinsic and reflex contributions to human ankle stiffness dynamics. *IEEE Trans Biomed Eng* 1997;44:493–504.
- Keenan ME. The orthopaedic management of spasticity. *J Head Trauma Rehabil* 1987;12:62–71.
- Labeit S, Kolmerer B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* 1995;270:293–296.
- Lance JW. Symposium Synopsis. In: Feldman RG, Young RR, Koella WP editors. Spasticity: disorder of motor control. Chicago: Year Book Medical; 1980. p 485–494.

47. Lexell J, Downham D, Sjøstrom M. Distribution of different fiber types in human skeletal muscles. A statistical and computational study of the fiber type arrangement in m. vastus lateralis of young, healthy males. *J Neurol Sci* 1984;65:353–365.
48. Lexell J, Downham D, Sjøstrom M. Distribution of different fibre types in human skeletal muscles. Fibre type arrangement in m. vastus lateralis from three groups of healthy men between 15 and 83 years. *J Neurol Sci* 1986;72:211–222.
49. Lieber RL, Fazeli BM, Botte MJ. Architecture of selected wrist flexor and extensor muscles. *J Hand Surg [Am]* 1990;15A:244–250.
50. Lieber RL, Fridén J. Intraoperative measurement and biomechanical modeling of the flexor carpi ulnaris-to-extensor carpi radialis longus tendon transfer. *J Biomech Eng* 1997;119:386–391.
51. Lieber RL, Fridén J. Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve* 2000;23:1647–1666.
52. Lieber RL, Fridén J. Spasticity causes a fundamental rearrangement of muscle–joint interaction. *Muscle Nerve* 2002;25:265–270.
53. Lieber RL, Fridén JO, Hargens AR, Feringa ER. Long-term effects of spinal cord transection of fast and slow rat skeletal muscle. II. Morphometric properties. *Exp Neurol* 1986;91:435–448.
54. Lieber RL, Jacobson MD, Fazeli BM, Abrams RA, Botte MJ. Architecture of selected muscles of the arm and forearm: anatomy and implications for tendon transfer. *J Hand Surg (Am)* 1992;17A:787–798.
55. Lieber RL, Johansson CB, Vahlsing HL, Hargens AR, Feringa ER. Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. I. Contractile properties. *Exp Neurol* 1986;91:423–434.
56. Lieber RL, Pontén E, Fridén J. Sarcomere length changes after flexor carpi ulnaris-to-extensor digitorum communis tendon transfer. *J Hand Surg (Am)* 1996;21A:612–618.
57. Lieber RL, Runesson E, Einarsson F, Fridén J. Inferior mechanical properties of spastic muscle bundles due to hypertrophic but compromised extracellular matrix material. *Muscle Nerve* 2003;28:464–471.
58. Magid A, Law DJ. Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* 1985;230:1280–1282.
59. Maier A, Cockett JL, Simpson DR, Saubert CI, Edgerton VR. Properties of immobilized guinea pig hindlimb muscles. *Am J Physiol* 1976;231:1520–1526.
60. Marbini A, Ferrari A, Cioni G, Bellanova MF, Fusco C, Gemignani F. Immunohistochemical study of muscle biopsy in children with cerebral palsy. *Brain Dev* 2002;24:63–66.
61. Mirbagheri MM, Barbeau H, Ladouceur M, Kearney RE. Intrinsic and reflex stiffness in normal and spastic, spinal cord injured subjects. *Exp Brain Res* 2001;141:446–459.
62. Moseley AM. The effect of casting combined with stretching on passive ankle dorsiflexion in adults with traumatic injuries. *Phys Ther* 1997;77:240–247.
63. Neagoe C, Kulke M, del Monte F, Gwathmey JK, de Tombe PP, Hajjar RJ, Linke WA. Titin isoform switch in ischemic human heart disease. *Circulation* 2002;106:1333–1341.
64. Ounjian M, Roy RR, Eldred E, Garfinkel A, Payne JR, Armstrong A, Toga AW, Edgerton VR. Physiological and developmental implications of motor unit anatomy. *J Neurobiol* 1991;22:547–559.
65. Parker DF, Carriere L, Hebestreit H, Bar-Or O. Anaerobic endurance and peak muscle power in children with spastic cerebral palsy. *Am J Dis Child* 1992;146:1069–1073.
66. Pette D. *Plasticity of muscle*. New York: Walter de Gruyter; 1980.
67. Pette D. *The dynamic state of muscle fibers*. Berlin: Walter de Gruyter; 1990. 729 p.
68. Pette D, Smith M, Staudt H, Vrbova G. Effects of long-term electrical stimulation on some contractile and metabolic characteristics of fast rabbit muscles. *Pflügers Arch* 1973;338:257–272.
69. Pohl M, Ruckriem S, Mehrholz J, Ritschel C, Strik H, Pause MR. Effectiveness of serial casting in patients with severe cerebral spasticity: a comparison study. *Arch Phys Med Rehabil* 2002;83:784–790.
70. Powers RK, Marder-Meyer J, Rymer WZ. Quantitative relations between hypertonia and stretch reflex threshold in spastic hemiparesis. *Ann Neurol* 1988;23:115–124.
71. Romanini L, Villani C, Meloni C, Calvisi V. Histological and morphological aspects of muscle in infantile cerebral palsy. *Ital J Orthop Traumatol* 1989;15:87–93.
72. Rose J, Haskell WL, Gamble JG, Hamilton RL, Brown DA, Rinsky L. Muscle pathology and clinical measures of disability in children with cerebral palsy. *J Orthop Res* 1994;12:758–768.
73. Ross SA, Engsborg JR. Relation between spasticity and strength in individuals with spastic diplegic cerebral palsy. *Dev Med Child Neurol* 2002;44:148–157.
74. Roy R, Bello M, Bouissou P, Edgerton R. Size and metabolic properties of fibers in rat fast-twitch muscles after hindlimb suspension. *J Appl Physiol* 1987;62:2348–2357.
75. Roy RR, Pierotti DJ, Flores V, Rudolph W, Edgerton VR. Fibre size and type adaptations to spinal isolation and cyclical passive stretch in cat hindlimb. *J Anat* 1992;180:491–499.
76. Roy RR, Sacks RD, Baldwin KM, Short M, Edgerton VR. Interrelationships of contraction time,  $V_{max}$ , and myosin ATPase after spinal transection. *J Appl Physiol* 1984;56:1594–1601.
77. Roy RR, Talmadge RJ, Hodgson JA, Oishi Y, Baldwin KM, Edgerton VR. Differential response of fast hindlimb extensor and flexor muscles to exercise in adult spinalized cats. *Muscle Nerve* 1999;22:230–241.
78. Sacks RD, Roy RR. Architecture of the hindlimb muscles of cats: functional significance. *J Morphol* 1982;173:185–195.
79. Salmons S, Henriksson J. The adaptive response of skeletal muscle to increased use. *Muscle Nerve* 1981;4:94–105.
80. Salmons S, Streter FA. Significance of impulse activity in the transformation of skeletal muscle type. *Nature* 1976;263:30–34.
81. Salmons S, Vrbova G. The influence of activity on some contractile characteristics of mammalian fast and slow muscles. *J Physiol (Lond)* 1969;201:535–549.
82. Saltin B, Gollnick PD. Skeletal muscle adaptability: significance for metabolism and performance. In: *Handbook of physiology*. Baltimore: American Physiological Society; 1983. p 539–554.
83. Scelsi R, Lotta S, Lommi G, Poggi P, Marchetti C. Hemiplegic atrophy. Morphological findings in the anterior tibial muscle of patients with cerebral vascular accidents. *Acta Neuropathol* 1984;62:324–331.
84. Shortland AP, Harris CA, Gough M, Robinson RO. Architecture of the medial gastrocnemius in children with spastic diplegia. *Dev Med Child Neurol* 2002;44:158–163.
85. Sinkjaer T, Magnussen I. Passive, intrinsic and reflex-mediated stiffness in the ankle extensors of hemiparetic patients. *Brain* 1994;117:355–363.
86. Sinkjaer T, Toft E, Andresassen S, Hornemann BC. Muscle stiffness in human ankle dorsiflexors: intrinsic and reflex components. *J Neurophysiol* 1988;60:1110–1121.
87. Sinkjaer T, Toft E, Larsen K, Andresassen S, Hansen HJ. Non-reflex and reflex mediated ankle joint stiffness in multiple sclerosis patients with spasticity. *Muscle Nerve* 1993;16:69–76.
88. Sjöström M, Fugl-Meyer AR, Nordin G, Wahlby L. Post-stroke hemiplegia; crural muscle strength and structure. *Scand J Rheumatol* 1980;7:53–67.
89. Spector SA, Simard CP, Fournier M, Sternlicht E, Edgerton VR. Architectural alterations of rat hindlimbs skeletal muscles immobilized at different lengths. *Exp Neurol* 1982;76:94–110.

90. Steffen TM, Mollinger LA. Low-load, prolonged stretch in the treatment of knee flexion contractures in nursing home residents. *Phys Ther* 1995;10:886–896.
91. Svantesson U, Takahashi H, Carlsson U, Danielsson A, Sunnerhagen KS. Muscle and tendon stiffness in patients with upper motor neuron lesion following a stroke. *Eur J Appl Physiol* 2000;82:275–279.
92. Tabary JC, Tabary C, Tardieu C, Tardieu G, Goldspink G. Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J Physiol (Lond)* 1972;224:231–244.
93. Tabary JC, Tardieu C, Tardieu G, Tabary C, Gagnard L. Functional adaptation of sarcomere number of normal cat muscle. *J Physiol (Paris)* 1976;72:277–291.
94. Tardieu C, de la Tour HE, Bret MD, Tardieu G. Muscle hypoe extensibility in children with cerebral palsy: I. Clinical and experimental observations. *Arch Phys Med Rehabil* 1982;63:97–102.
95. Tardieu G, Tardieu C, Colbeau-Justin P, Lespargot A. Muscle hypoe extensibility in children with cerebral palsy: II. Therapeutic implications. *Arch Phys Med Rehabil* 1982;63:103–107.
96. Thilmann AF, Fellows SJ, Garms E. The mechanism of spastic muscle hypertonus. Variation in reflex gain over the time course of spasticity. *Brain* 1991;114:233–244.
97. Trotter JA. Dynamic shape of tapered skeletal muscle fibers. *J Morphol* 1991;207:211–223.
98. Trotter JA, Purslow PP. Functional morphology of the endomysium in series fibered muscles. *J Morphol* 1992;212:109–122.
99. Vaughan CL, Berman B, Peacock WJ. Cerebral palsy and rhizotomy. A 3-year follow-up evaluation with gait analysis. *J Neurosurg* 1991;74:178–184.
100. Wang K, McCarter R, Wright J, Beverly J, Ramirez-Mitchell R. Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin–myosin composite filament is a dual-stage molecular spring. *Biophys J* 1993;64:1161–1177.
101. Wickiewicz TL, Roy RR, Powell PL, Edgerton VR. Muscle architecture of the human lower limb. *Clin Orthop Rel Res* 1983;179:275–283.
102. Wiley ME, Damiano DL. Lower-extremity strength profiles in spastic cerebral palsy. *Dev Med Child Neurol* 1998;40:100–107.
103. Williams P, Goldspink G. The effect of immobilization on the longitudinal growth of striated muscle fibers. *J Anat* 1973;116:45–55.
104. Williams P, Goldspink G. Changes in sarcomere length and physiological properties in immobilized muscle. *J Anat* 1978;127:459–468.
105. Wood DJ, Zollman J, Reuban JP, Brandt PW. Human skeletal muscle: properties of the “chemically skinned” fiber. *Science* 1975;187:1075–1076.
106. Young JL, Mayer RF. Physiological alterations of motor units in hemiplegia. *J Neurol Sci* 1982;54:401–412.