

# Structural and mechanical alterations in spastic skeletal muscle

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Spasticity, a neurological problem secondary to an upper motor neuron lesion, has a significant effect on skeletal muscle. The upper motor neuron lesions may be secondary to a cerebral vascular accident, head injury, spinal cord injury, or degenerative diseases such as multiple sclerosis, or perinatal brain injuries such as cerebral palsy. Functional ability in these patients can be severely compromised but the basic mechanisms underlying these deficits are not clearly understood. In this review we evaluate the current evidence in the literature that suggests that skeletal muscle tissue itself is altered in spastic conditions. Experimental studies were evaluated that included a variety of methods encompassing joint mechanics, tissue mechanics, and muscle morphology. Taken together, the literature strongly supports the assertion that 'spastic muscles' are altered in a way that is unique among muscle plasticity models and inconsistent with simple transformation due to chronic stimulation or disuse. Further studies are required to detail the intra- and extracellular modifications of skeletal muscle that occur secondary to spasticity so that novel therapeutic treatments can be developed for this impairment.

Spasticity can be a disabling and often painful condition that occurs secondary to upper motor neuron lesions such as stroke, head or spinal cord injury, multiple sclerosis, or cerebral palsy (CP). Clinically, spasticity is associated with increased muscle tone, stiffness, exaggerated reflexes, and eventual joint contractures. These, combined with decreased voluntary motor strength, balance deficits, and impaired motor control, lead to considerable functional limitations. The pathophysiology underlying alterations in skeletal muscle (if any) that contribute to these functional deficits is not clearly understood.

Because of its central etiology, most research on spasticity has focused on the nervous system. Some investigators have proposed that alterations in the nervous system might have secondary effects on skeletal muscles.<sup>1,2</sup> However, far less attention has been directed toward characterizing the structural and functional changes in skeletal muscle that occur secondary to spasticity. Although muscular and neural changes are usually related,<sup>3</sup> recent data have demonstrated that muscular changes in spasticity cannot be explained by classic interpretations of the effects of neural changes alone. Initial discussions on the changes in skeletal muscle secondary to spasticity were presented in the context of the chronic electrical stimulation model, but this has turned out to be inaccurate.<sup>4</sup> In addition, there is still no animal model that accurately mimics the changes observed in human muscle that has been subjected to chronic spasticity. It is important, therefore, to improve our understanding of the alterations that occur in human muscle. For this review, skeletal muscle that has been subjected to spasticity will be referred to as 'spastic muscle'.

The purpose of this review is to provide a focused presentation of the structural and mechanical changes that occur in skeletal muscles secondary to spasticity. A more

comprehensive version of this review has previously been published<sup>4</sup> and was presented as the Mac Keith lecture at the American Academy of Cerebral Palsy and Developmental Medicine (September 2004).

The lack of agreement in the admittedly limited literature emphasizes the need for a review on this topic which could lead to the development of rational therapeutic approaches for patients with spasticity. To create this review, 33 relevant references dealing with structural and/or functional changes in skeletal muscle secondary to spasticity were examined. Of these studies, some of which were multidisciplinary in nature, 18 reported mechanical data, 14 reported muscle properties obtained from biopsies, 13 reported joint kinematics and/or joint kinetics, and 16 reported other aspects of muscle morphology, distinct from fiber type or size distributions. Most of the studies reported findings from patients with CP ( $n=19$ ), but others included patients diagnosed with multiple sclerosis ( $n=3$ ), stroke ( $n=11$ ), spinal cord injury/upper motor neuron lesions ( $n=7$ ), and Parkinson's disease ( $n=2$ ).

### Skeletal muscle plasticity

It seems reasonable to study skeletal muscle properties in disorders of neural origin because muscles respond in a fairly stereotypical manner to the amount and type of activity imposed on them. Chronic electrical stimulation can progressively transform skeletal muscle cells into a slower phenotype<sup>5-8</sup> with all accompanying phenotypic changes, including increased capillary density, increased percentage of type I (so-called 'slow-twitch') muscle fibers, decreased fiber size (if the stimulation duration is long enough), increased endurance, and decreased strength. This model has served as a useful template to describe the changes that occur in skeletal muscle upon increased use.

The opposite model, chronic decreased use of skeletal muscle, which can be studied using models of simulated weightlessness,<sup>9</sup> tenotomy,<sup>10</sup> immobilization in a shortened position,<sup>11,12</sup> or spinal cord isolation,<sup>13,14</sup> causes muscle fibers to decrease their size and transform in the direction of the faster phenotype (i.e. type II or 'fast-twitch' fibers).<sup>15-17</sup> Thus, an analysis of the distribution of skeletal muscle fiber type may be a useful indicator of the amount and type of activity that a muscle has received over an extended period of time.

Muscle fiber size provides an insight into the extent of fiber use. Increased use of skeletal muscle at high loads produces muscle fiber hypertrophy, whereas decreased use yields muscle cellular atrophy. Both responses appear to be load dependent. Thus, fiber size is typically interpreted as an indirect indicator of the chronic force level imposed upon a muscle. Therefore, fiber type and size are often measured in spastic muscle in an attempt to determine its use pattern. Although these parameters are easily quantified, they are relatively non-specific and probably only provide a general indicator of muscle use. Excellent reviews of skeletal muscle plasticity and monographs on the subject are available in the literature.<sup>1,3,18-20</sup>

### Muscle fiber type and fiber size changes with spasticity

The most prevalent type of analysis of spastic muscle tissue involves muscle biopsies, although recurrent methodological concerns<sup>4</sup> make interpretation of these studies difficult. When sectioned, normal skeletal muscle biopsies have tightly packed fibers that form polygons juxtaposed to one another,

whereas muscle from patients with spasticity tends to show abnormalities such as increased variability in fiber size, increased numbers of 'rounded' fibers, 'moth-eaten' fibers, and in some cases, increased extracellular space.<sup>21-26</sup> Variability in fiber size (i.e. large and small fibers within the same muscle) is characteristic of numerous neuromuscular disorders and not necessarily specific to spasticity.<sup>27</sup>

Some biopsy studies report an increased percentage of type I fiber type<sup>21,24,28</sup> in muscle from patients with spasticity. Fewer report an increased percentage of type II fiber,<sup>29</sup> and others have found no change in the distribution of fiber types.<sup>22,23,25,26</sup> Thus, there is no general agreement that spasticity represents either an increased or decreased use model. That there is no general agreement on this issue must be due, in part, to the sampling problems that are inherent in the biopsy procedure itself.<sup>27,30</sup> However, when taking the literature as a whole, the results appear to indicate that spastic muscle is not simply subjected to chronic increased or decreased electrical activity.

### Biomechanical studies of spastic limbs

Clinically, patients with spastic muscles present with increased joint stiffness. Numerous attempts to characterize this stiffness objectively ultimately led to the classic definition of spasticity presented by Lance in which he stated that spasticity is 'a velocity-dependent resistance to stretch'.<sup>31</sup> This definition has enjoyed wide acceptance. It acknowledges the increase in joint stiffness and further refines this idea by identifying a dynamic component to the 'resistance to stretch' (i.e. stiffness). This definition is also consistent with the idea that spasticity is due to an increase in the gain of the stretch reflex,<sup>31</sup> which would cause the velocity dependence.

However, numerous mechanisms could explain the 'increased resistance to stretch' which do not involve the nervous system and, in many cases, stiffness is explained in terms of underlying neural *and* mechanical properties. Several laboratories have developed devices that use a combination of dynamometry, electromyography (EMG), and electrical nerve stimulation to study limb stiffness.<sup>32-34</sup> The basic approach of this type of experiment is to measure dynamic limb stiffness and break it down into its constituent parts to quantify the relative contributions of the three factors thought to be responsible for resistance to stretch. These are: (1) passive muscle stiffness (presumably due to fibrosis or muscle fiber properties); (2) neurally mediated reflex stiffness (presumably due to descending influences on the monosynaptic reflex between the muscle spindle afferents and the alpha-motor neurons); and (3) active muscle stiffness (presumably due to the number of cross-bridges attached during contraction or an increase in stiffness per cross-bridge, both of which have been previously documented<sup>35,36</sup>).

Using this methodology, Sinkjaer and Magnussen<sup>37</sup> showed that the total stiffness measured in the spastic leg of patients with hemiplegia was greater than the total stiffness measured in contralateral 'control' legs. What was surprising was that 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.

Mirbagheri et al.,<sup>33</sup> using a similar, yet more comprehensive approach, found that, in spastic muscle in spinal-cord-injured patients, reflex gain was dramatically increased compared with spinal-cord-injured patients with non-spastic muscles. Intrinsic muscle stiffness, both elastic and viscous, was also increased

in patients with spasticity. These studies provide strong evidence that alterations in both neural input and in the intrinsic mechanical properties of skeletal muscle contribute to spasticity, albeit to differing extents, depending on the particular disease state, patient age, and time since injury.

### **Muscle fiber length in spastic muscle**

Fiber length is probably the single most functionally important property of skeletal muscle. It is the primary determinant of muscle excursion, and the ratio of fiber length to muscle length is a strong indicator of whether a skeletal muscle is designed for producing high force or high excursion.<sup>38–40</sup> There is a widely held belief within the clinical community that muscle contractures that occur 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. Although early studies, such as those performed on the rat soleus muscle by Williams and Goldspink<sup>41,42</sup> and in the cat soleus muscle by Tabary et al.,<sup>43,44</sup> seemed to lend evidence to this theory, later work, such as that by Edgerton and colleagues,<sup>45</sup> showed that not all muscle groups respond to lengthening or shortening by increasing or decreasing serial sarcomere number respectively.

Because of the difficulty in obtaining whole fixed muscles for analysis, direct architectural data on spastic muscle is scarce. Indirect inference of muscle fiber length in patients with spasticity was provided by Tardieu and colleagues,<sup>46,47</sup> who studied passive torque–angle relations in children with CP and had findings consistent with decreased muscle fiber length. In another indirect study, Smeulders et al.<sup>48</sup> examined the force–length relation in the flexor carpi ulnaris (FCU) of children with chronic wrist flexion contracture and provided indirect evidence that overstretching of sarcomeres was not the cause of the contracture in these spastic muscles. It is important to note that these studies did not directly demonstrate any change in fiber length or sarcomere length.

Recently, Shortland et al.<sup>49</sup> directly measured the architectural properties of the medial gastrocnemius of children with CP using ultrasound. Fascicle length and fascicle angle were measured in normally developing children and children with spastic CP. They found no evidence for fascicle length change in children with spasticity, contrary to their expectation. As this is the only such study in the literature, it is premature to conclude that this result is generally applicable to all spastic muscles.

Estimates of fiber length in human spastic muscle were also made by Lieber and Fridén,<sup>50</sup> who used intraoperative laser diffraction to compare FCU sarcomere length in children with chronic wrist flexion contracture with FCU sarcomere length in controls without spasticity. The authors concluded that the children with spastic wrist flexion contractures had muscles with normal fiber length, although the sarcomeres within the fibers were highly stretched.<sup>50</sup> It is important to note that the only direct measurements of fiber length that have ever been performed in children with spastic diplegia have suggested that fiber length is normal.

### **Mechanical changes in spastic muscle tissue**

Two recent studies reported that the intrinsic passive mechanical properties of isolated muscle fibers and small bundles of muscle fibers were altered secondary to spasticity. These studies provide a view of the complex interactions between muscle cells and the extracellular matrix that may result from

spasticity. In the first study, investigators microscopically dissected single muscle fibers from surgical biopsies<sup>51</sup> of spastic and non-spastic muscle. Using a sophisticated micromechanical testing apparatus,<sup>52</sup> the investigators were able to measure intracellular sarcomere lengths and the intrinsic mechanical stiffness of single muscle fibers. Interestingly, the muscle fibers from patients with spasticity were over twice as stiff (based on tangent modulus) as the fibers from the patients without spasticity. Furthermore, the resting sarcomere length (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. These two findings suggest that the structures within the muscle cell responsible for setting resting sarcomere length<sup>53</sup> and determining cellular stiffness are altered in spastic muscle.

The most obvious candidate for this structure is the giant intracellular cytoskeletal protein, titin.<sup>54</sup> In frog skeletal muscles, titin has been demonstrated to bear almost the entire elastic load during passive elongation, and bears significant passive load in human muscle.<sup>55</sup> There are no definitive data demonstrating that titin is actually altered secondary to spasticity but there is circumstantial evidence to suggest that it is possible. It is known, based on the differences in complementary DNA sequences, that titin can exist in multiple isoforms in heart and skeletal muscles.<sup>54</sup> It is also known that the titin isoform in heart muscle is substantially stiffer and shorter than the titin isoforms in most skeletal muscles. Furthermore, it has been demonstrated that the titin isoform can change within heart muscle under pathological conditions.<sup>56</sup> As an example of the ability of the titin isoform to change, ischemia-induced cardiomyopathy increases myocardial stiffness secondary to up-regulated collagen expression. In addition to collagen proliferation, however, investigators discovered a ‘switch’ from a compliant to a stiffer isoform of titin.<sup>56</sup> It is, therefore, reasonable to speculate that titin isoforms may be altered in skeletal muscles of patients with spastic limbs, although definitive demonstration of this change has not been reported. Investigations into the alterations that occur in titin in spastic skeletal muscle certainly seem warranted.

In another study,<sup>57</sup> interesting properties of the extracellular matrix of spastic muscle emerged. The investigators used the micromechanical testing apparatus described above, but instead of testing the stiffness of single fibers, they tested small bundles of muscle (i.e. 5 to 50 fibers embedded in extracellular matrix) from patients with and without spasticity. Understandably, the non-spastic muscle bundles were stiffer than the isolated non-spastic single fibers, and the spastic bundles were stiffer than the isolated spastic single fibers. These results seem logical, given that the bundles contained extracellular matrix which should contribute to bundle stiffness. The surprising result was that even though the spastic single fibers were stiffer than the non-spastic single fibers, the non-spastic bundles were remarkably stiffer than the spastic bundles. This finding is even more impressive given that, on histological cross section, the *non-spastic* bundles appeared to contain substantially less extracellular matrix than the spastic bundles. Morphologically, there was a large amount of poorly organized extracellular material in spastic bundles compared with normal bundles. The authors concluded that, although spastic muscle contains a larger amount of extracellular matrix material within it, the quality of that material is poor compared with that in normal muscles.

The extracellular matrix of skeletal muscle is made up of several different collagen types (the most abundant being types I, III, IV, V, and VI, with at least seven other types identified<sup>58</sup>), as well as various proteoglycans and other glycoproteins. Histopathological studies<sup>22,23,57</sup> have demonstrated a generalized increase in extracellular connective tissue in spastic muscle. However, the specific changes in quantity and quality of collagen types and other connective tissue structures remain largely unexplored. The only explicit description of extracellular matrix changes in spastic muscle was based on biochemical measurement of collagen concentration, which increased dramatically in spastic muscle.<sup>22</sup>

A compelling question based on these mechanical studies is: do spastic muscles develop a compliant extracellular matrix material and then try to compensate by causing muscle fibers to stiffen, or do spastic muscle fibers stiffen secondary to spasticity and then the extracellular matrix material becomes more compliant in response to fiber changes? Clearly, further investigation is warranted.

### Conclusions

Although the primary lesion leading to spasticity lies within the central nervous system, muscle in patients with spasticity is also dramatically altered. This conclusion is based on results from studies using a variety of experimental methods in a number of diseases, across a wide range of patient ages. To summarize, we have made the case for the following alterations in spastic muscle: (1) altered muscle fiber size and fiber type distribution; (2) proliferation of extracellular matrix material, measured morphologically and biochemically; (3) increased spastic muscle cell stiffness and, to a lesser extent, spastic muscle tissue; and (4) inferior mechanical properties of extracellular material in spastic compared with normal muscle.

Improvement of the quality of life of patients with spasticity depends on creating a new understanding of muscular changes that occur secondary to spasticity, and the development of rational interventions to either prevent these changes or reverse them. Innovative research addressing the questions raised in this review will be essential to achieve these goals.

### Future research

The detailed structural changes that occur in spastic muscle as well as mechanistic explanations for how these changes occur are lacking. Therefore, questions that must be addressed in this field, following logically from the material presented in this review, include: (1) What are the proteins that are altered within spastic muscle cells and the extracellular matrix of spastic muscle tissue? 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? (2) Do spastic muscles develop a compliant extracellular matrix material and then try to compensate by causing muscle fibers to stiffen, or do spastic muscle fibers stiffen secondary to spasticity and then the extracellular matrix material becomes more compliant in response to fiber changes? (3) Is the signaling between the extracellular matrix and skeletal muscle cells altered secondary to spasticity? How is this signaling different between heart and skeletal muscle in response to other pathological conditions? (4) Do spastic muscle cells retain their ability to adapt using mechanisms observed in normal muscle, such as alteration to sarcomere number, stress-induced hyper-

trophy, and regeneration through satellite cell proliferation? In addition, important clinically relevant questions that are largely unexplored are: (1) Is there a difference in the muscular response to different spasticity etiologies? Specifically, what is the effect of other movement disorders associated with spasticity (dystonia, athetosis, ataxia, etc.), on muscle properties? (2) Does the age at which spasticity is acquired affect muscle properties?

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### References

- Hufschmidt A, Mauritz KH. (1985) Chronic transformation of muscle in spasticity: a peripheral contribution to increased tone. *J Neurology Neurosurg Psychiatry* **48**: 676–685.
- Dietz V, Berger W. (1995) Cerebral palsy and muscle transformation. *Dev Med Child Neurol* **37**: 180–184.
- Salmons S, Henriksson J. (1981) The adaptive response of skeletal muscle to increased use. *Muscle Nerve* **4**: 94–105.
- Lieber RL, Steinman S, Barash IA, Chambers H. (2004) Structural and functional changes in spastic skeletal muscle. *Muscle Nerve* **29**: 615–627.
- Salmons S, Streeter FA. (1976) Significance of impulse activity in the transformation of skeletal muscle type. *Nature* **263**: 30–34.
- Eisenberg BR, Salmons S. (1981) The reorganization of subcellular structure in muscle undergoing fast-to-slow type transformation. A stereological study. *Cell Tissue Res* **220**: 449–471.
- Pette D, Smith M, Staudte H, Vrbova G. (1973) Effects of long-term electrical stimulation on some contractile and metabolic characteristics of fast rabbit muscles. *Pflugers Arch* **338**: 257–272.
- Salmons S, Vrbova G. (1969) The influence of activity on some contractile characteristics of mammalian fast and slow muscles. *J Physiol (Lond)* **201**: 535–549.
- Roy R, Bello M, Bouissou P, Edgerton R. (1987) Size and metabolic properties of fibers in rat fast-twitch muscles after hindlimb suspension. *J Appl Physiol* **62**: 2348–2357.
- Buller AJ, Lewis DM. (1965) Some observations on the effects of tenotomy in the rabbit. *J Physiol (Lond)* **178**: 326–342.
- Maier A, Cockett JL, Simpson DR, Saubert CI, Edgerton VR. (1976) Properties of immobilized guinea pig hindlimb muscles. *Am J Physiol* **231**: 1520–1526.
- Booth FW, Kelso JR. (1973) Effect of hind-limb immobilization on contractile and histochemical properties of skeletal muscle. *Pflugers Arch* **342**: 123–138.
- Roy RR, Pierotti DJ, Flores V, Rudolph W, Edgerton VR. (1992) Fibre size and type adaptations to spinal isolation and cyclical passive stretch in cat hindlimb. *J Anat* **180**: 491–499.
- Roy RR, Sacks RD, Baldwin KM, Short M, Edgerton VR. (1984) Interrelationships of contraction time, V<sub>max</sub>, and myosin ATPase after spinal transection. *J Appl Physiol* **56**: 1594–1601.
- Grimby G, Broberg C, Krotkiewska I, Krotkiewski M. (1976) Muscle fiber composition in patients with traumatic cord lesion. *Scand J Rehabil Med* **8**: 37–42.
- Lieber RL, Fridén JO, Hargens AR, Feringa ER. (1986) Long-term effects of spinal cord transection of fast and slow rat skeletal muscle. II. Morphometric properties. *Exp Neurol* **91**: 435–448.
- Lieber RL, Johansson CB, Vahlsing HL, Hargens AR, Feringa ER. (1986) Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. I. Contractile properties. *Exp Neurol* **91**: 423–434.
- Pette D. (1990) *The Dynamic State of Muscle Fibers*. Berlin: Walter de Gruyter.
- Pette D. (1980) *Plasticity of Muscle*. New York: Walter de Gruyter.
- Saltin B, Gollnick PD. (1983) Skeletal muscle adaptability: significance for metabolism and performance. In: Peachy LD, editor. *Handbook of Physiology*. Baltimore: American Physiological Society. p 539–554.
- Dietz V, Ketelsen UP, Berger W, Quintern J. (1986) Motor unit involvement in spastic paresis. Relationship between leg muscle activation and histochemistry. *J Neurol Sci* **75**: 89–103.

22. Booth CM, Cortina-Borja MJ, Theologis TN. (2001) Collagen accumulation in muscles of children with cerebral palsy and correlation with severity of spasticity. *Dev Med Child Neurol* **43**: 314–320.
23. Castle ME, Reyman TA, Schneider M. (1979) Pathology of spastic muscle in cerebral palsy. *Clin Orthop* **142**: 223–233.
24. Ito M, Araki A, Tanaka H, Tasaki T, Cho K, Yamazaki R. (1996) Muscle histopathology in spastic cerebral palsy. *Brain Dev* **18**: 299–303.
25. Romanini L, Villani C, Meloni C, Calvisi V. (1989) Histological and morphological aspects of muscle in infantile cerebral palsy. *Ital J Orthop Traumatol* **15**: 87–93.
26. Rose J, Haskell WL, Gamble JG, Hamilton RL, Brown DA, Rinsky L. (1994) Muscle pathology and clinical measures of disability in children with cerebral palsy. *J Orthop Res* **12**: 758–768.
27. Dubowitz V, Brooke MH. (1973) *Muscle Biopsy: A Modern Approach*. Philadelphia: WB Saunders. p 92–108.
28. Marbini A, Ferrari A, Cioni G, Bellanova MF, Fusco C, Gemignani F. (2002) Immunohistochemical study of muscle biopsy in children with cerebral palsy. *Brain Dev* **24**: 63–66.
29. Sjöström M, Fugl-Meyer AR, Nordin G, Wahlby L. (1980) Post-stroke hemiplegia: crural muscle strength and structure. *Scand J Rheumatol* **7**: 53–67.
30. Bergstrom J. (1975) Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* **35**: 609–616.
31. Lance JW. (1980) Symposium synopsis. In: Feldman RG, Young RR, Koella WP, editors. *Spasticity: Disorder of Motor Control*. Chicago: Year Book Medical Publishers. p 485–494.
32. Katz RT, Rymer WZ. (1989) Spastic hypertonia: mechanisms and measurement. *Arch Phys Med Rehabil* **70**: 144–151.
33. Mirbagheri MM, Barbeau H, Ladouceur M, Kearney RE. (2001) Intrinsic and reflex stiffness in normal and spastic, spinal cord injured subjects. *Exp Brain Res* **141**: 446–459.
34. Sinkjaer T, Toft E, Andresassen S, Hornemann BC. (1988) Muscle stiffness in human ankle dorsiflexors: intrinsic and reflex components. *J Neurophysiol* **60**: 1110–1121.
35. Ford LE, Huxley AF, Simmons RM. (1981) The relation between stiffness and filament overlap in stimulated frog muscle fibres. *J Physiol (Lond)* **311**: 219–249.
36. Julian FJ, Morgan DL. (1981) Tension, stiffness, unloaded shortening speed and potentiation of frog muscle fibres at sarcomere lengths below optimum. *J Physiol (Lond)* **319**: 205–217.
37. Sinkjaer T, Magnussen I. (1994) Passive, intrinsic and reflex-mediated stiffness in the ankle extensors of hemiparetic patients. *Brain* **117**: 355–363.
38. Gans C. (1982) Fiber architecture and muscle function. In: Holloszy J, editor. *Exercise and Sport Science Reviews*. Vol. 10. Lexington, MA: Franklin University Press. p 160–207.
39. Sacks RD, Roy RR. (1982) Architecture of the hindlimb muscles of cats: functional significance. *J Morphol* **173**: 185–195.
40. Lieber RL, Fridén J. (2000) Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve* **23**: 1647–1666.
41. Williams P, Goldspink G. (1973) The effect of immobilization on the longitudinal growth of striated muscle fibers. *J Anat* **116**: 45–55.
42. Williams P, Goldspink G. (1978) Changes in sarcomere length and physiological properties in immobilized muscle. *J Anat* **127**: 459–468.
43. Tabary JC, Tabary C, Tardieu C, Tardieu G, Goldspink G. (1972) Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J Physiol (Lond)* **224**: 231–244.
44. Tabary JC, Tardieu C, Tardieu G, Tabary C, Gagnard L. (1976) Functional adaptation of sarcomere number of normal cat muscle. *J Physiol (Paris)* **72**: 277–291.
45. Spector SA, Simard CP, Fournier M, Sternlicht E, Edgerton VR. (1982) Architectural alterations of rat hindlimbs skeletal muscles immobilized at different lengths. *Exp Neurol* **76**: 94–110.
46. Tardieu G, Tardieu C, Colbeau-Justin P, Lespargot A. (1982) Muscle hypoe extensibility in children with cerebral palsy: II. Therapeutic implications. *Arch Phys Med Rehabil* **63**: 103–107.
47. Tardieu C, de la Tour HE, Bret MD, Tardieu G. (1982) Muscle hypoe extensibility in children with cerebral palsy: I. Clinical and experimental observations. *Arch Phys Med Rehabil* **63**: 97–102.
48. Smeulders MJC, Kreulen M, Hage JJ, Peter AH, Van der Horst CMAM. (2004) Overstretching of sarcomeres may not cause cerebral palsy muscle contracture. *J Orthop Res* **22**: 1131–1335.
49. Shortland AP, Harris CA, Gough M, Robinson RO. (2002) Architecture of the medial gastrocnemius in children with spastic diplegia. *Dev Med Child Neurol* **44**: 158–163.
50. Lieber RL, Fridén J. (2002) Spasticity causes a fundamental rearrangement of muscle-joint interaction. *Muscle Nerve* **25**: 265–270.
51. Wood DJ, Zollman J, Reuban JP, Brandt PW. (1975) Human skeletal muscle: properties of the 'chemically skinned' fiber. *Science* **187**: 1075–1076.
52. Fridén J, Lieber RL. (2003) Spastic muscle cells are shorter and stiffer than normal cells. *Muscle Nerve* **27**: 157–164.
53. Wang K, McCarter R, Wright J, Beverly J, Ramirez-Mitchell R. (1993) Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin-myosin composite filament is a dual-stage molecular spring. *Biophys J* **64**: 1161–1177.
54. Labeit S, Kolmerer B. (1995) Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270**: 293–296.
55. Magid A, Law DJ. (1985) Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* **230**: 1280–1282.
56. Neagoe C, Kulke M, del Monte F, Gwathmey JK, de Tombe PP, Hajjar RJ, Linke WA. (2002) Titin isoform switch in ischemic human heart disease. *Circulation* **106**: 1333–1341.
57. Lieber RL, Runesson E, Einarsson F, Fridén J. (2003) Inferior mechanical properties of spastic muscle bundles due to hypertrophic but compromised extracellular matrix material. *Muscle Nerve* **28**: 464–471.
58. Vuokko K. (2002) Intramuscular extracellular matrix: complex environment of muscle cells. *Exerc Sport Sci Rev* **30**: 20–25.