



Passive muscle stiffness may be influenced by active contractility of intramuscular connective tissue

Robert Schleip *, Ian L. Naylor, Daniel Ursu, Werner Melzer, Adjo Zorn, Hans-Joachim Wilke, Frank Lehmann-Horn, Werner Klingler

Department of Applied Physiology, Ulm University, Albert-Einstein-Allee 11, 89069 Ulm, Germany

Received 28 July 2005; accepted 15 August 2005

Summary The article introduces the hypothesis that intramuscular connective tissue, in particular the fascial layer known as the perimysium, may be capable of active contraction and consequently influence passive muscle stiffness, especially in tonic muscles. Passive muscle stiffness is also referred to as passive elasticity, passive muscular compliance, passive extensibility, resting tension, or passive muscle tone. Evidence for the hypothesis is based on five indications: (1) tonic muscles contain more perimysium and are therefore stiffer than phasic muscles; (2) the specific collagen arrangement of the perimysium is designed to fit a load-bearing function; (3) morphological considerations as well as histological observations in our laboratory suggest that the perimysium is characterized by a high density of myofibroblasts, a class of fibroblasts with smooth muscle-like contractile kinetics; (4) in vitro contraction tests with fascia have demonstrated that fascia, due to the presence of myofibroblasts, is able to actively contract, and that the resulting contraction forces may be strong enough to influence musculoskeletal dynamics; (5) the pronounced increase of the perimysium in muscle immobilization and in the surgical treatment of distraction osteogenesis indicates that perimysial stiffness adapts to mechanical stimulation and hence influences passive muscle stiffness. In conclusion, the perimysium seems capable of response to mechanostimulation with a myofibroblast facilitated active tissue contraction, thereby adapting passive muscle stiffness to increased tensional demands, especially in tonic musculature. If verified, this new concept may lead to novel pharmaceutical or mechanical approaches to complement existing treatments of pathologies which are accompanied by an increase or decrease of passive muscle stiffness (e.g., muscle fibroses such as torticollis, peri-partum pelvic pain due to pelvic instability, and many others). Methods for testing this new concept are suggested, including histological examinations and specific in vitro contraction tests.

© 2005 Elsevier Ltd. All rights reserved.

Introduction

When skeletal muscles are passively stretched they exhibit measurable resistance even when their motor neurons are quiescent and their

* Tel.: +49 89 346016; fax: +49 89 337927.
E-mail address: info@fasciaresearch.com (R. Schleip).

myofibers are not actively contracting. This behaviour is called passive muscle stiffness and is also referred to as passive elasticity, passive muscular compliance, passive extensibility, resting tension, or passive muscle tone.

Since the discovery of the titin filaments and their highly elastic properties, it has been commonly assumed that passive muscle stiffness depends primarily on these tertiary intramuscular filaments [1]. Yet recent research suggests that passive muscle stiffness is also influenced by muscular connective tissues [2]. This new perspective is supported by conclusions drawn from work involving tenotomy, fasciotomy and aponeurotomy [3] as well as studies on the passive extensibility of skeletal muscle [4].

Based on this previous research, the authors propose the hypothesis that intramuscular connective tissues, and in particular the fascial layer called the perimysium, may be able to actively contract and thereby adapt myofascial tissue stiffness to increased tensional demands, especially in tonic muscles.

Evidence for the hypothesis will be given from five considerations:

1. Tonic muscles contain more perimysium than phasic muscles.
2. The perimysium is designed to serve a load bearing function.
3. The perimysium may be characterized by a high density of myofibroblasts.
4. Myofibroblasts enable fascia to actively contract.
5. The perimysium exhibits a high stiffness adaptability response to mechanical stimulation.

Implications for pathologies with increased or decreased passive muscle stiffness will be elucidated, and concrete steps towards testing the hypothesis will be suggested.

Evidence

Tonic muscles contain more perimysium than phasic muscles

Intramuscular connective tissue is generally divided into three hierarchical and interconnected layers: the endomysium, surrounding individual myofibers; the perimysium, separating and enveloping myofiber bundles; the epimysium, the fascial wrapping for the whole muscle. It is of interest that tonic muscles like the soleus, which are more in-

involved in posture, tend to contain significantly more perimysium than phasic muscles [5,6]. This may be related to the increased oxygen demand and vascularization of tonic muscles, since the perimysium in muscular fasciae contains the highest proliferation of blood vessels and the increased thickness of the perimysium may provide a useful cushioning effect during muscular contraction. On the other hand, the increase in perimysial thickness has also been shown to correlate with a higher passive stiffness [7]. In food science perimysial thickness correlates with raw meat toughness [8,9].

The perimysium is designed to serve a load-bearing function

The morphology and arrangement of collagen fibers in the perimysium is different from the epi- or endomysium in several ways. It consists mainly of collagen fibers with a fairly large diameter [6]. In the perimysium these fibers exhibit a pronounced crimp formation, and they are arranged in a lattice-like criss-cross orientation with parallel fibers going in two different directions. Muscle contraction or stretch changes the angles of this lattice with respect to the muscle fiber direction, ranging from 80° at short sarcomere length to approximately 20° at high extension. Interestingly, at high extension the collagen crimp formation also straightens out, and it increases again when the muscle tissue is relaxed [10,9].

As outlined in detail by Rowe [9] and Purslow [10], the particular combination of these features supports the assumption, that the perimysium is designed to increase passive muscle stiffness and to serve a load-bearing function by preventing overstretching of the muscle fiber bundles.

The perimysium may be characterized by a high density of myofibroblasts

The most common type of connective tissue cells are fibroblasts. Fibroblasts as well as having other functions are responsible for the production of collagen fibers and other elements of the extracellular matrix. Several phenotypes of fibroblasts exist. Among these, the myofibroblast group is of special interest in tissue contraction. These cells can be identified by their α -smooth muscle actin stress fibers. Their smooth muscle-like contractile kinetics make myofibroblasts well suited for long lasting isometric contractions, and their contraction plays a major role in wound healing as well as in pathological fascial contractures

such as Dupuytren disease, plantar fibromatosis, or frozen shoulder [11].

The presence of myofibroblasts in normal (non-pathological) fascia has already been demonstrated [12–14]. Unfortunately, no quantitative histological examination has yet been published examining possible differences in myofibroblast density between the epi-, peri- and endomysial fascial layers. Nevertheless the following observations can be made.

The density of myofibroblasts in connective tissues has been reported to be greater in both the more crimped areas as well as in the more vascularized regions [15,16]. The correlation to vascularization may be related to a stimulatory effect of the mast cell product heparin on myofibroblast development [17,18]. It is of interest that the perimysium is not only the intramuscular layer which exhibits the most crimp formation, but it also contains the most blood vessels [19,20]. It can therefore be postulated, that the density of myofibroblasts is probably higher in the perimysium than in either the endo- or epimysial fascial layers.

Preliminary results from histological examinations of rat soleus muscle in our laboratory support this assumption. They show a striking increase in the density of myofibroblasts in the perimysium. No other fascial layer that we have observed to this date (in non-pathological conditions) seems to have such a high density of contractile cells [21].

Myofibroblasts enable fascia to actively contract

As has originally been suggested in this journal [22], several lines of reasoning and experimental findings support the notion, that fascia is able to actively contract and consequently influence musculoskeletal dynamics. While this new concept was originally only offered as a justified hypothesis, it has now been repeatedly confirmed by several laboratories. Masood and Naylor [18,23,24] reported that superficial and deep lumbar fascia from rats as well as from guinea pigs contracted in response to in vitro application of the myofibroblast stimulant mepyramine as well as to the smooth muscle agonists adenosine and angiotensin II. Contractions started within several minutes and were in a dose dependent, reproducible and reversible manner. The smooth muscle relaxing substances nifedipine and EDTA as well as the microtubule disrupting substance cytochalasin-D exhibited a relaxing effect. A relaxing response

in porcine lumbar fascia to the substance glyceryl-trinitrate (a NO donor and smooth muscle relaxant) has been reported by Schleip et al. [12]. Malata found that mepyramine-induced contractions in rat subcutaneous fascia were enhanced by previous incubation with heparin [25]. Using an immunohistochemical analysis of 39 tissue samples from the thoracolumbar fascia of 11 human donors (ages 19–76 years), Schleip et al. [12] demonstrated the widespread presence of myofibroblasts in all samples, with an average density of 79 cells/mm² in his longitudinal sections.

Taken together, these findings confirm that fascial tissues can actively contract, and that their contractility appears to be driven by myofibroblasts. The question as to whether or not these active fascial contractions could be strong enough to exert any significant impact on musculoskeletal dynamics has previously been addressed in this journal [22] the following way: taking the greatest measured force of in vitro fascial contractions and extrapolating that to an average size of the superficial layer of the thoracolumbar fascia in humans, the resulting contraction force can amount to 38 N, which may be a force strong enough to influence biomechanical behaviour, such as in a contribution to paraspinal compartment syndrome or in the prevention of spinal segmental instability [26].

The perimysium exhibits a high stiffness adaptability response to mechanical stimulation

Several examples demonstrate that the perimysium adapts more readily to changes in mechanical tension than other intramuscular connective tissues. Skeletal deformities are sometimes treated by a surgical reconstruction process known as distraction osteogenesis. Unfortunately, the treatment is frequently accompanied by increased muscle stiffness. It has been shown that this effect correlates with a significant increase in perimysial thickness happening in response to the increased tissue stretch. A faster lengthening rate (1.4 mm/day in the tibialis anterior of skeletally immature rabbits) leads to more severe tissue stiffness than a slower rate (0.7 mm/day); even though no major cellular pathologic abnormalities were detected [27].

Another example is the effect of immobilization. When a muscle is immobilized in a shortened position it becomes stiffer. This correlates with an increase in intramuscular fascia, which can already be documented within two days of immobilization.

While the endomysium remains unaffected during this time, a significant thickening of the perimysium can be seen in the first few days; and it is this change in perimysial thickness (and therefore also in absolute perimysial stiffness) which is considered to be responsible for the rapid muscular stiffening during the first week [28]. It is of interest that the process of myofibroblast-induced stiffening of fascia seems to have a U-shaped responsiveness to mechanostimulation, since both an absence, as well as an unusually high amount of mechanical tension can lead to increased tissue stiffening. The existence of similar windows of responsiveness to mechanostimulation is nevertheless not unusual among connective tissue cells [29,30].

It can be assumed that these perimysial adaptations involve the functioning of fibroblasts, since these cells regulate both collagen density and matrix constitution, as well as having the capacity to contract. Among the different types of fibroblasts, it is most likely the myofibroblast group which is responsible for the rapid increase in tissue thickness (within two days in the last example above). Although regular turn-over times for collagen by normal fibroblasts would take much longer; myofibroblast-facilitated tissue contractions such as in wound healing or pathological scarring often demonstrate similar rapid changes in tissue density as have been observed here. Due to their complexity and relatively recent discovery, many aspects of myofibroblast are still not yet understood. Similar to the group of smooth muscle cells, it seems that many different phenotypes of myofibroblasts exist, with significant differences in both receptor expression and contractile behaviour [17]. In spite of this, it seems clear that in vivo myofibroblast-facilitated tissue contractions, sometimes also called 'contractures' (to indicate a more long lasting tissue change), usually involve aspects of smooth muscle-like cellular contraction as well as an increased and altered connective tissue remodeling [11].

Conclusions

Tonic muscles contain more perimysium than phasic muscles. Their increased passive muscle stiffness makes them nutritionally the tougher meat. The specific collagen arrangement of the perimysium supports a load bearing function, i.e., an increase in perimysial stiffness is expected to increase muscular stiffness. It can be assumed that the perimysium is characterized by a high density of myofibroblasts, a group of fibroblasts responsi-

ble for rapid tissue contractions. Perimysial fibroblasts respond readily to changes in mechanical stimulation and their response leads to significant changes in passive muscle stiffness. It is suggested that active contractility of perimysial myofibroblasts – similar to the tissue contractions in wound healing and pathological fascial contractures – enables the perimysium to respond to significant changes in mechanical stimulation with an increase in its stiffness. It is our hypothesis, that intramuscular connective tissues, particularly the perimysium and especially in tonic muscles, may be able to actively contract and thereby adapt muscle stiffness to altered tensional demands. As a secondary and a slower reacting adaptation system, this may assist the primary and more rapid adjustments of the neuromuscular system.

Implications

A multitude of pathologies are accompanied and complicated by increased passive muscle stiffness. Examples range from torticollis and other muscle fibroses, to Parkinson's rigor, ankylosing spondylitis, neck or back pain associated with chronic muscular tightness, to muscle shortness in rehabilitation. While the primary cause of the pathology is clearly outside the field of this paper, daily motor performance is frequently impeded by secondary changes in passive muscle stiffness, particularly in tonic muscles. Our hypothesis suggests, that – aside from myogenic changes – an increase in the perimysial stiffness may be a result of myofibroblast-facilitated contraction of this fascial layer. If verified, this could open new avenues for novel mechanical or pharmaceutical approaches that would complement existing treatments. In spastic muscular dystrophy, for example, the soleus is often chronically shortened, which makes walking difficult. This is usually treated either surgically or with various mechanical stretching approaches. We suggest that treatment with super-slow manual deep tissue techniques which are geared towards sensing and influencing cellular contractility, may be helpful in this and similar conditions. Such techniques are commonly practiced by osteopaths and by practitioners of the Rolfing method of deep tissue manipulation [31]. Treatment could be further assisted nutritionally with substances like L-arginine that tend to increase the presence of nitrous oxide [32] and, therefore, may increase myofibroblast relaxation. Pharmaceutical treatment with relaxin could be explored for its related antifibrotic effect [33].

New approaches may also be possible for pathologies with a decreased myofascial stiffness, such as peri-partum pelvic pain due to pelvic instability, fibromyalgia, or back pain due to spinal segmental instability.

It is therefore suggested, that this hypothesis be tested via histological examination and via in vitro contraction tests. For the histological examination, we recommend taking muscular tissue sections which include the endo-, peri- and epimysium, treating these sections with immunohistochemistry for α -smooth muscle actin, and then performing a quantitative analysis. Our prediction is that a much higher quantity of contractile cells will be found in the perimysium than in the epi- or endomysium, especially in the tonic muscles.

For the in vitro contraction tests the isometric superfusion bath protocol of Pipelzadeh and Naylor [14] is suggested, as this method has the advantage of continually washing the tissue and seems more sensitive for measuring slow tissue responses than the classical organ bath method [34]. Using mepyr-amine or other myofibroblast stimulants, perimysial tissues from tonic and phasic muscles could be tested for a contractile response to these agents. It would also be of interest to apply this protocol to strips of tonic muscle tissue which include myofibers as well as the connected endo-, peri- and epimysium. Given a contractile response, further discriminative tests could then be performed, for example the application of nifedipine or other smooth muscle relaxants, or treating enzymatically skinned myocells with the same myofibroblast contraction stimulants for comparison. If it could then be shown, that tonic muscles can be stimulated by myofibroblast agents to contract and relax in a reproducible and reversible manner without active changes in their myofibers, our hypothesis should prove to be a fertile ground for both new therapeutic directions and further research.

References

- [1] Magid A, Law DJ. Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* 1985;230:1280–2.
- [2] Maas H, Baan GC, Huijing PA. Intermuscular interaction via muscular force transmission: effects of tibialis anterior and extensor hallucis length on force transmission from rat extensor digitorum longus muscle. *J Biomech* 2001;34:927–40.
- [3] Huijing PA. Muscle as a collagen fiber reinforced composite: a review of force transmission in muscle and whole limb. *J Biomech* 1999;32:329–45.
- [4] Gajdosik RL. Passive extensibility of skeletal muscle: review of the literature with clinical implications. *Clinic Biomech* 2001;16:87–101.
- [5] Williams PE, Goldspink G. Connective tissue changes in immobilised muscle. *J Anat* 1984;184(2):343–50.
- [6] Borg TK, Caulfield JB. Morphology of connective tissue in skeletal muscle. *Tissue Cell* 1980;12(1):197–207.
- [7] Mutungi G, Ranatunga KW. The viscous, viscoelastic and elastic characteristics of resting fast and slow mammalian (rat) muscle fibres. *J Physiol* 1996;496(3):827–36.
- [8] Bendall JR. The elastin content of various muscles of beef animals. *J Sci Food Agric* 1967;18:553–8.
- [9] Rowe RW. Collagen fibre arrangement in intramuscular connective tissue. Changes associated with muscle shortening and their possible relevance to raw meat toughness measurements. *J Food Technol* 1974;9:501–8.
- [10] Purslow PP. Strain-induced reorientation of an intramuscular connective tissue network: implications for passive muscle elasticity. *J Biomech* 1989;22(1):21–31.
- [11] Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol* 2003;200:500–3.
- [12] Schleip R, Klingler W, Lehmann-Horn F. Active contraction of the thoracolumbar fascia – indications of a new factor in low back pain research with implications for manual therapy. In: Vleeming A, Mooney V, Hodges P, editors. *The proceedings of the fifth interdisciplinary world congress on low back and pelvic pain*, Melbourne; 2004. ISBN 90-802551-4-9.
- [13] Masood N. An in vitro pharmacological investigation of the contractility of rat and guinea pig dorsal subcutaneous fascia. PhD dissertation, School of Pharmacy, University of Bradford, UK; 1996.
- [14] Pipelzadeh MH, Naylor IL. The in vitro enhancement of rat myofibroblast contractility by alterations to the pH of the physiological solution. *Eur J Pharmacol* 1998;357:257–9.
- [15] Murray MM, Spector M. Fibroblasts distribution in the anteromedial bundle of the human anterior cruciate ligament: the presence of α -smooth actin-positive cells. *J Orthop Res* 1999;17(1):18–27.
- [16] Beon M, Harley RA, Wessels A, Silver RM, Ludwicka-Bradley A. Myofibroblast induction and microvascular alteration in scleroderma lung fibrosis. *Clin Exp Rheumatol* 2004;22(6):733–42.
- [17] Schmitt-Graff A, Desmouliere A, Gabbiani G. Heterogeneity of myofibroblast phenotypic features: an example of fibroblastic cell plasticity. *Virchows Arch* 1994;425(1):3–24.
- [18] Masood N, Naylor IL. Effect of adenosine on rat superficial and deep fascia and the effect of heparin on the contractile responses. *Br J Pharmacol* 1994;113:112.
- [19] Bogusch G. Development of the vascular supply in rat skeletal muscles. *Acta Anat (Basel)* 1984;120(4):228–33.
- [20] Sakamoto Y. Histological features of endomysium, perimysium and epimysium in rat lateral pterygoid muscle. *J Morphol* 1996;227(1):113–9.
- [21] Schleip R, Klingler W, Zorn A. News from the European fascia research project. www.somatics.de/FasciaResearch/News.html [posted 28.05.05, accessed 02.07.05].
- [22] Schleip R, Klingler W, Lehmann-Horn F. Active fascial contractility: fascia may be able to contract in a smooth muscle-like manner and thereby influence musculoskeletal dynamics. *Med Hypotheses* 2005;65(2):273–7.
- [23] Masood N, Naylor IL. The in vitro reactivity of fascia from the rat and guinea-pig to calcium ions and mepyr-amine. *Br J Pharmacol* 1994;112:416P.
- [24] Masood N, Naylor IL. Effect of N6-cyclopentyladenosine, N6-cyclohexyladenosine, and N6-2-phenylisopropyladenosine on rat superficial and deep fascia. *Br J Pharmacol* 1995;116:269P.
- [25] Malata CM, Masood N, Naylor IL. Role of mast cells in wound contraction. *Br J Surg* 1994;81(5):765.

- [26] Wilke HJ, Wolf S, Claes LE, Arand M, Wiesend A. Stability increase of the lumbar spine with different muscle groups. A biomechanical study. *Spine* 1995;20(2):192–8.
- [27] De Deyne P, Meyer R, Paley R, Herzenberg JE. The adaptation of perimuscular connective tissue during distraction osteogenesis. *Clin Orthop Relat Res* 2000;379: 259–69.
- [28] Williams PE, Goldspink G. Connective tissue changes in immobilised muscle. *J Anat* 1984;138(2):343–50.
- [29] Brand RA, Stanford CM. How connective tissues temporally process mechanical stimuli. *Med Hypotheses* 1994;42(2): 99–104.
- [30] Neidlinger-Wilke C, Wilke HJ, Claes L. Cyclic stretching of human osteoblasts affects proliferation and metabolism: a new experimental method and its application. *J Orthop Res* 1994;12(1):70–8.
- [31] Grodin AJ, Cantu RI. Soft tissue mobilization. In: Basmajian JV, Nyberg R, editors. *Rational manual therapies*. Baltimore: Williams & Wilkins; 1993.
- [32] Moss MB, Brunini TM, Soares De Moura R, et al. Diminished L-arginine bioavailability in hypertension. *Clin Sci (Lond)* 2004;107(4):391–7.
- [33] Heeg MH, Koziolok MJ, Vasko R, et al. The antifibrotic effects of relaxin in human renal fibroblasts are mediated in part by inhibition of the Smad2 pathway. *Kidney Int* 2005;68(1):96–109.
- [34] Naylor IL, Coleman DJ, Coleman RA, Cross SE, Foo ITH. Reactivity of nodular cells in vitro: a guide to the pharmacological treatment of Dupuytren's contracture. In: Berger A, Delbrück A, Brenner P, Hinzmann R, editors. *Dupuytren's disease: pathobiochemistry and clinical management*. Berlin: Springer; 1994. p. 139–50.

Available online at www.sciencedirect.com

