JPn Neuroscience
The Journal of Physiology

TOPICAL REVIEW

New perspectives on the development of muscle contractures following central motor lesions

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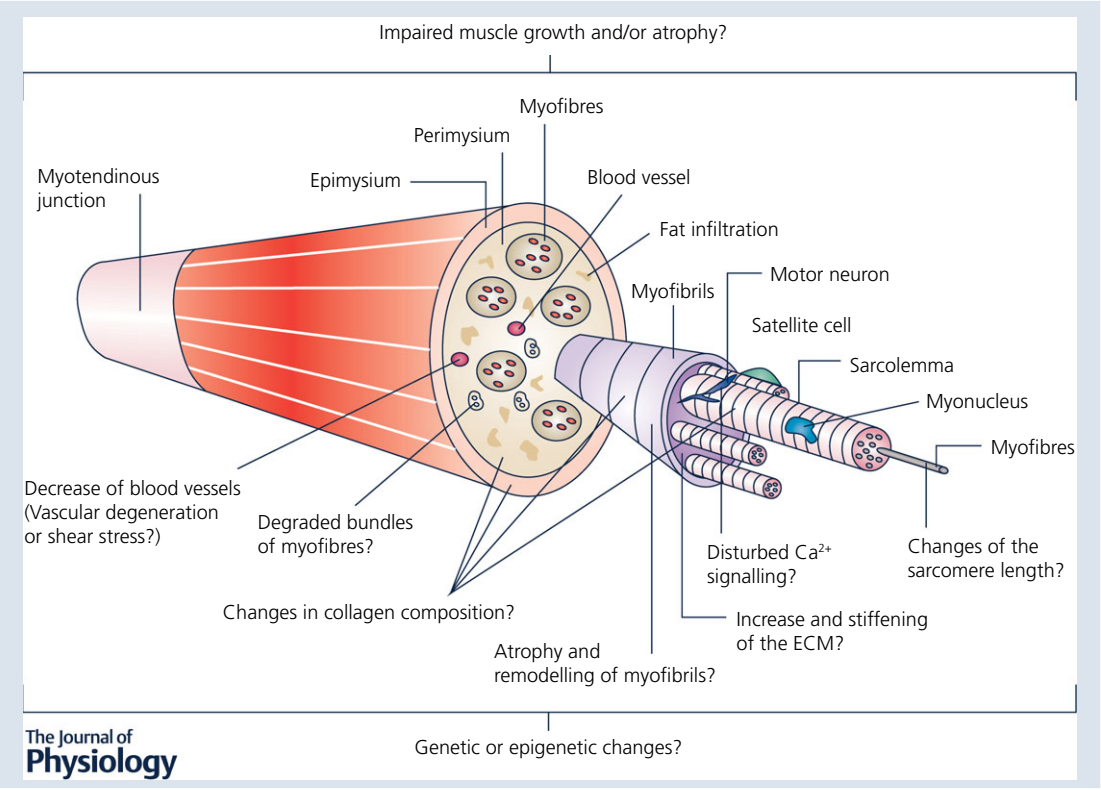

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DOI: 10.1113/JP272767

Abstract Muscle contractures are common in patients with central motor lesions, but the mechanisms responsible for the development of contractures are still unclear. Increased or decreased neural activation, protracted placement of a joint with the muscle in a short position and muscle atrophy have been suggested to be involved, but none of these mechanisms are sufficient to explain the development of muscle contractures alone. Here we propose that changes in tissue homeostasis in the neuromuscular–tendon–connective tissue complex is at the heart of the development of contractures, and that an integrated physiological understanding of the interaction between neural, mechanical and metabolic factors, as well as genetic and epigenetic factors, is necessary in order to unravel the mechanisms that result in muscle contractures. We hope thereby to contribute to a reconsideration of how and why muscle contractures develop in a way which will open a window towards new insight in this area in the future.

(Received 9 May 2016; accepted after revision 18 October 2016; first published online 24 October 2016)
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Abstract figure legend This figure summarizes the current unresolved questions regarding the development of muscle contractures. Central motor lesions cause an adaptation in tissue homeostasis in the neuromuscular–tendon–connective tissue complex. This new state of homeostasis affects several mechanisms in the muscle including growth, atrophy, genetics, epigenetics, chemotransduction, mechanotransduction and vascularization. It is this complex adaptation that we need to understand in order to prevent and treat muscle contractures.

Abbreviations COL, collagen; CP, cerebral palsy; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; IGF-1, insulin-like growth factor 1; miRNA, microRNA; mTOR, rapamycin; p70S6K, ribosomal protein S6 kinase β-1; TRP, transient receptor potential channel.

Introduction

Muscle contractures are defined as unique muscle changes which increase the passive stiffness of the muscle and limit the mobility of the joints without any active force production of the muscles (Smith *et al.* 2011). Muscle contractures are a common complication to lesions of the central motor pathways such as cerebral palsy (CP), stroke and spinal cord injury. The prevalence after stroke has been reported to be 60% of all patients (Sackley *et al.* 2008), 36% in CP patients with upper limb involvement (Makki *et al.* 2014), and 48% following spinal cord injury (Diong *et al.* 2012). Contractures cause the joints of these patients to gradually become fixated in awkward positions, which obstruct normal physical activity. The exact pathology underlying contractures is not clarified and it is still debated whether the increased stiffness involves elastic elements within the muscle fibres, in the extracellular matrix or both. It is also not clarified to what extent ultrastructural changes in the muscle fibres, such as changes in the number and length of sarcomeres, contribute to the alterations in gross muscle anatomy and joint position. This lack of clarity is probably explained by shortcomings of current techniques for measuring tissue stiffness and muscle fibre lengths *in vivo* (Smith *et al.* 2011; Mathewson *et al.* 2014).

It is generally assumed that the development of muscle contractures is related to an abnormally high muscle activity due to spasticity (Gracies, 2005). However, anti-spastic medication has no effect on contractures in many patients with lesions of the central motor pathways (Tedroff *et al.* 2011). Additionally, contractures appear to develop in both stroke and CP without prior evidence of spasticity and, although selective dorsal rhizotomy effectively reduces spasticity, it does not prevent the development of muscle contractures (Tedroff *et al.* 2011). These findings indicate that spasticity cannot be the deciding cause for the development of muscle contractures.

In contrast, both human and animal studies have shown that immobilization, especially in a shortened position, can cause muscle contractures (Trudel *et al.* 1999). A study by Trudel *et al.* showed that hind limb immobilization for various periods ranging from 3 to 32 weeks caused the range of motion (ROM) to decrease simultaneously with an increase in muscle stiffness (Trudel *et al.* 1999). If immobilization alone can lead to development of muscle contractures, then all spinal cord injury patients should in principle develop muscle contractures in all their paralysed muscles. However, this is not the case. Spinal cord injury patients do sometimes develop contractures (Nas *et al.* 2015), but usually in one joint only, depending on the location of their injury.

We consequently have to realize that none of the existing theories adequately explain the development of muscle contractures in all patients and under all circumstances.

This raises two possibilities: Contractures may be more heterogeneous than we have so far realized and may therefore be caused by different mechanisms in different patients and under different circumstances. Alternatively, or complimentarily, contractures may be caused by an interaction between many different factors where no single factor determines their development. Here we will propose that contractures should be seen as an adaptation in tissue homeostasis in the neuromuscular–tendon–connective tissue complex induced by the lesion of the central motor pathways. In the complex network of interaction between different tissues (neural, muscle, connective tissue etc.), different stimuli (neural activity, mechanical factors, growth factors, nutrients, metabolic substances) and different regulatory factors (genetic, epigenetic, metabolic signalling factors), changes in one factor (neural activity) quickly result in adaptive changes in the rest of the network, leading to a new set value of homeostasis (Fig. 1). It is this complex interaction we need to understand in order to unravel the mechanisms that result in contractures in the individual patients and eventually find ways of preventing and treating contractures. In the following we will discuss the mechanisms that have been proposed to be involved in the development of muscle contractures with a focus on the interaction and signalling between different tissues

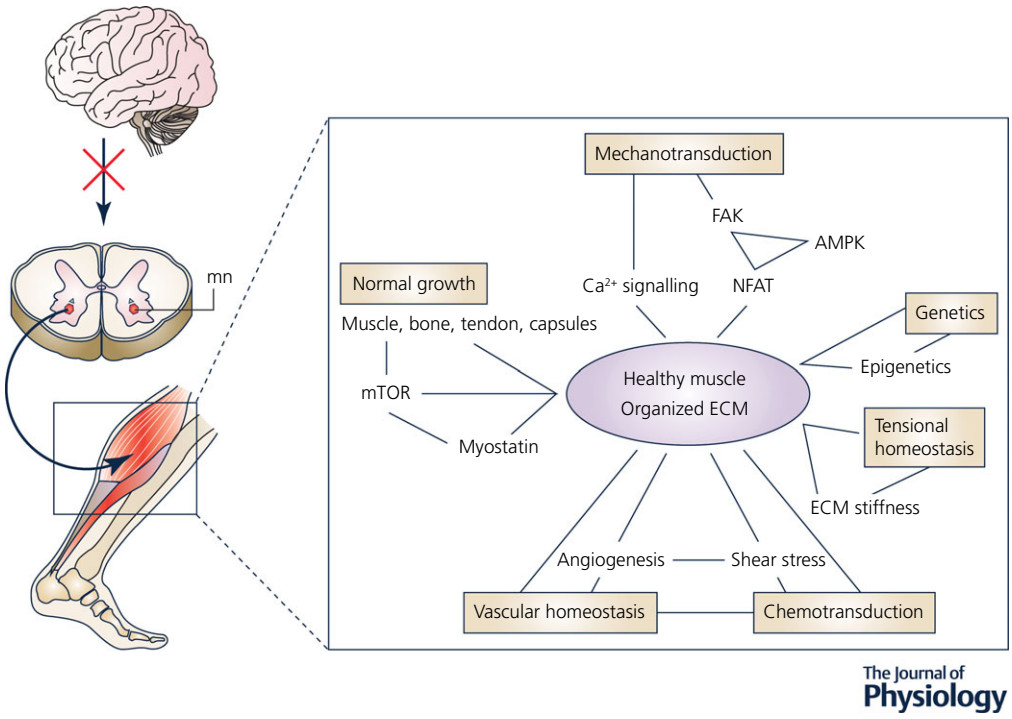

Figure 1. Tissue homeostasis in the neuromuscular–tendon–connective tissue complex
A simplified overview of the different factors involved in the short- and long-term regulation of the triceps surae muscle, tendon and connective tissue. When we use the muscles, neural activity not only signals contraction of the muscle, but also initiates a cascade of signalling factors, which determines the growth of the muscle fibres, the phenotype of the muscle fibres and the properties of the connective tissue, as well as increasing the capillary network in the muscle. As a result of the mechanical stimuli during muscle activity, for instance from the impact of heel strike when we walk, a myriad of different cells are activated, including mechanosensitive fibroblasts, which results in connective and muscle tissue changes. Concomitantly, vascular and metabolic factors are activated in order to meet the energy and metabolic needs of the muscle and connective tissue, when activated. Finally, muscle, tendon and connective tissue elicit signals to the nervous system regarding the present state of the tissue, which is incorporated into the immediate and future neural signalling. The different tissues are thus part of an integrated network that regulates the environment of the cells and maintains a number of key factors (the contractile properties of the muscle, the stiffness of the tissue) within relatively strict limits. Additionally, genetic and epigenetic factors affect the composition of the tissues, thus influencing the capability of the tissue to react and adapt to changes in the tissue homeostasis. This homeostasis is what we observe as a normal and healthy tissue. Alteration in one of the factors involved in the network will inevitably result in adaptive changes throughout the network, in order to maintain homeostasis as far as possible. However, if the alteration is too big the network cannot maintain normal homeostasis and will settle on a new (unhealthy, pathological) set value of homeostasis. This is the state where pathological contractures have developed. In this review we will discuss the possible key contributors to the development of contractures in this homeostatic network. AMPK, 5-AMP-activated protein kinase; FAK, focal adhesion kinase; mn, motor neuron; NFAT, nuclear factor of activated T-cells.

and compartments in the nerve–muscle–tendon–bone complex. It should be noted that we will not address limitations in joint movements caused by alterations of joint flexibility (joint contractures), although similar mechanisms may be involved.

Neural activation: hyper- or hypo-activity?

When contractures develop in neurological disorders, altered neural drive to the muscles is a logical suspect as one of the initial steps in the development of contractures and spasticity is also generally assumed in the clinic to be the main cause of contractures (Hagglund & Wagner, 2011). However, there is little solid scientific evidence to support this idea. One main reason for this is that there is considerable confusion regarding the term spasticity in the literature (Malhotra *et al.* 2009). Spasticity is most commonly defined as a velocity dependent increase of muscle resistance to passive stretch, which implies that hyperactive stretch reflexes have a central pathophysiological role (Malhotra *et al.* 2009). However, a much broader definition, which emphasizes the presence of involuntary muscle activity and which may better reflect the clinical understanding of spasticity, has also been introduced (Malhotra *et al.* 2009). Whereas hyperactive stretch reflexes are unlikely by themselves to result in contractures, the same cannot be said of continuous involuntary muscle activity, which may cause the muscle and joint to be fixed in an undesirable position. This so-called spastic dystonia, which is often seen following stroke and here assumed to interfere with movement ability and lead to abnormal postures, appears to be caused by central mechanisms and is thus unrelated to increased reflex excitability, i.e. spasticity in the original definition (Sheean & McGuire, 2009). Without a clear distinction between these two definitions of spasticity, it is difficult to determine the significance of studies on the pathophysiological role of spasticity for development of contractures. One example is the finding that dorsal rhizotomy has been shown not to have any effect on the development of contractures in children with CP (Tedroff *et al.* 2011). Rhizotomy will certainly reduce sensory input and diminish stretch reflexes, but would have little effect on spastic dystonia if present.

Recently it has been suggested that reduced rather than increased neural drive is more likely to be the key pathophysiological mechanism in the development of contractures (Gough & Shortland, 2012). Reduced neural activation of the muscle may also in its own right cause changes in connective tissue and other elastic structures in the muscle and tendon through its effect on gene expression of the muscle fibres (Smith *et al.* 2012). Although contractures do develop in relation to both denervation (Nikolaou *et al.* 2015) and immobilization (Lake *et al.* 2016), it should be emphasized that there is still no conclusive evidence of the role of atrophy or reduced neural activity in the development of contractures. This may be mainly due to the problems in disentangling the role of neural activity from the role of secondary changes in muscle loading, muscle structure and muscle signalling. Since techniques which allow dissociation between these interacting components are now available and have been validated, there is a great need to apply these to quantify the effect of each component in the contracture process (Bar-On *et al.* 2015). One way of obtaining some information about the role of neural hypo-activity *versus* hyper-activity would be to study a possible relation between contractures and the previous history of muscle activity. To our knowledge no systematic study has so far been performed to document such a relationship in either neurological patients or in animal models. Inactivity models (such as nerve section, limb suspension and Botox injection) are certainly associated with development of contractures (Nikolaou *et al.* 2015; Pingel *et al.* 2016), but it is unclear whether this is due to reduced neural activation, the changes in mechanical loading or the altered joint positions, which are unavoidable consequences of the inactivity.

A mismatch between muscle and bone length?

Muscle growth and atrophy. Impaired muscle growth and muscle atrophy have been suggested in a number of studies in later years to be a key to the development of muscle contractures, at least in children with CP, but possibly also in adults with stroke and spinal cord injury (Gough & Shortland, 2012; Aze *et al.* 2016). Individuals with CP have smaller muscles than healthy individuals, and both the muscle belly length and the cross sectional area are reduced (Gough & Shortland, 2012). This appears to be related to reduced growth of the muscle, possibly caused by reduced neural activation and disuse, since reduced muscle volume is not observed until children are older than 15 months (Herskind *et al.* 2016). Muscle atrophy and associated structural muscle changes also develop quickly in adults following stroke (Aze *et al.* 2016) and spinal cord injury (Qin *et al.* 2010). Some studies have suggested that the shortening of the muscle belly observed in individuals with CP is the result of shorter fascicle length in the muscle (Matthiasdottir *et al.* 2014), but other studies have failed to confirm this (Mathewson *et al.* 2015). Muscle growth and atrophy mainly relate to the diameter of the muscle fibres (number of sarcomeres in parallel), but because of the pennate structure of most muscles, the diameter of the fibres contributes significantly to the total muscle length – in the case of the soleus muscle, increase of fibre diameter contributes up to 80% of the growth in muscle length (Gough & Shortland, 2012). Muscle atrophy may therefore cause muscles to be too short in relation to the bone length, and the consequent stretching and stress on

the sarcomeres may trigger the development of contractures (Gough & Shortland, 2012).

Increases of muscle mass (hypertrophy) and decreases in muscle mass (atrophy) are controlled by anabolic and catabolic responses, respectively. One key regulator of muscle mass and metabolism that stimulates protein synthesis is rapamycin (mTOR). mTOR modulates protein synthesis in muscle through two distinct pathways, the PHAS-1 pathway and the ribosomal protein S6 kinase β-1 (p70S6K) pathway (Glass, 2005). Knockout models for p70S6K have shown that a lack of this gene results in a significantly smaller cross sectional area of muscle cells (Ohanna *et al.* 2005). Furthermore, insulin like growth factor 1 (IGF-1) is of crucial importance in muscle growth. IGF-1 induces an increase of muscle mass by stimulating the phosphatidylinositol-3 kinase (PI3K)/AKT (protein kinase B) pathway. This activation results in downstream activation of targets required for protein synthesis (Glass, 2005). The downstream signalling of IGF-I through the AKT pathway is antagonized by myostatin. A blockage of myostatin results in a tremendous increase of muscle mass (13–30%) (Whittemore *et al.* 2003). Interestingly, children with CP have shown an upregulation of both IGF-1 and myostatin, indicating an increased turnover in muscles from children with CP compared to muscle tissue from two normally developing children (Smith *et al.* 2009). Increased muscle turnover has been observed in several conditions presenting muscle atrophy. Thus, the increased turnover in CP muscles observed by Smith *et al.* is consistent with muscle atrophy in children with CP. However, atrophy alone is not likely to explain the development of the passive stiffness observed in muscle contractures, since other conditions causing muscle atrophy such as sarcopenia do not develop increased muscle stiffness despite of tremendous loss of muscle mass.

Bone growth. Although reduced muscle growth and muscle atrophy may lead to a mismatch between muscle length and bone length in children with cerebral palsy this does not necessarily indicate that bone growth is not also affected. The bone density is strongly dependent on weight bearing, and individuals with cerebral palsy show significant abnormalities in bone growth, including delayed maturation, diminished linear growth and low bone density (Henderson *et al.* 2005*b*). Similar changes occur in stroke patients and in subjects with spinal cord injury (Sato *et al.* 2004; Liu *et al.* 2008). In children with spinal cord injury, regional osteopenia was furthermore associated with loss of muscle mass (Liu *et al.* 2008). Children with severe CP develop clinically significant osteopenia throughout their lives, and it has been shown that the reduction of bone minerals is related to decreased growth rate of the bone, rather than to genuine gross losses of bone minerals, as seen with ageing (Henderson *et al.* 2005*b*). Furthermore, the delayed skeletal maturation in CP was found to be correlated with a diminished linear growth (height) and decreased bone density (Henderson *et al.* 2005*a*). Another interesting observation by Stevenson *et al.* was a diminished linear bone growth, decreased bone density, and delayed skeletal maturation in the affected leg of hemiplegic CP persons when compared to the unaffected leg (Stevenson *et al.* 1995). This indicates that the impaired bone growth is a local response.

Role of muscle micro-architecture. Evidence from animal experiments has suggested that the number of sarcomeres in a muscle is regulated according to the joint position (Tamai *et al.* 1989). Immobilization in elongated position thus increases the number of sarcomeres in series (Goldspink *et al.* 1974), whereas a decrease of the number of sarcomeres is seen with immobilization in shortened position (Williams & Goldspink, 1984). The addition/decrease of sarcomeres appears to be independent of neural activation (Goldspink *et al.* 1974). This adaptation of the sarcomeres may take place in order to maintain the most optimal muscle function in a given position (Tamai *et al.* 1989). There is, furthermore, accumulating evidence that suggests that sarcomeres appear to be stretched and therefore longer in CP muscles compared to typically developed muscles (Smith *et al.* 2011; Mathewson *et al.* 2015), although there is not general agreement about this (Smeulders *et al.* 2004). An unresolved, but important, question is whether other circumstances in the muscle are responsible for an inadequate adaptation of sarcomere length in muscle contractures.

Is it all a question about the right tension in the cell?

Cells exist in a three-dimensional environment in which they are continuously exposed to mechanical and physical stimuli. Tensional homeostasis is a pervasive concept in mechano-biology and it has received compelling attention as a way of defining the interplay between the external and internal mechanical state of cells (Banes *et al.* 1995). Tensional homeostasis describes the state in which cells maintain defined levels of tension within their surroundings despite mechanical perturbations that could change tension (Banes *et al.* 1995).

Lamin A and myosin II have previously been described as prime candidates for biological tension sensors (Dingal & Discher, 2014). These coiled-coil proteins assemble into structural networks and transduce mechanical signals between the extracellular matrix (ECM) and the nucleus (Dingal & Discher, 2014). Lamin A is an intermediate filament protein that is found in various cell nuclei. It contributes to nuclear stiffness and nuclear stability (Dingal & Discher, 2014). When culturing cells in soft

to stiff environments the expression of lamin A increases tremendously (30-fold) in stiff environments, while lamin B is only affected to a minor degree (Broers *et al.* 1997). Myosin II is associated with the extracellular matrix stiffness. High matrix stiffness increases the tension (stress) in the cell, and myosin II responds to the matrix stiffness by increasing in amount and assembling into stress fibres (Rehfeldt *et al.* 2012). When disrupting the tensional homeostasis by inhibiting myosin, the cells lose the ability to connect with and sense focal adhesions. Elevated tension within the focal adhesions have shown to increase integrin clustering and focal adhesion kinase (FAK) phosphorylation, and these molecular changes induce subsequently a cascade of signalling activation of the Rho pathway (Fig. 1) (Vogel & Sheetz, 2009). Several pathologies, including arteriosclerosis, muscular dystrophies and potential central nervous system disorders, can either be a result of a disruption of the tensional homeostasis, caused by altered forces at the cell or tissue level, a perturbed response to the mechanical stimuli, or altered material properties of the ECM (Jaalouk & Lammerding, 2009). Under static conditions, the gene expression response varies dramatically depending on shear stress and the viscosity of the extracellular matrix in osteoblast cultures (Sikavitsas *et al.* 2003). Furthermore, ECM-generated mechanical tension can trigger the synthesis of ECM proteins in fibroblasts (Kessler *et al.* 2001). On the other hand, reduced mechanical tension in the ECM decreases the expression of ECM building proteins in aged skin (Rittie & Fisher, 2015). It seems that tensional homeostasis is a very sensitive system, and that small alterations of the tension homeostasis can lead to severe consequences both at cell and tissue level. It has previously been proposed that muscle contractures are a result of increased stiffness of the extracellular matrix (Smith *et al.* 2011). A disruption of tension homeostasis due to progressive extracellular matrix stiffening in the muscle cells could be involved in the development of muscle contractures. However, no research has yet been carried out to elaborate whether the tensional homeostasis is disturbed in muscle cells of patient groups that are predisposed for developing muscle contractures. Future studies will hopefully clarify whether tensional homeostasis due to either alterations of the forces within the cell, a perturbed response to the mechanical stimuli, or stiffening of the extracellular matrix is involved in the development of muscle contractures.

Is the calcium signalling of the muscle cell impaired in muscle contractures?

Intracellular calcium concentration regulates signalling mechanisms, which control various biological processes crucial in the development and regeneration of the muscle (Fig. 1). The calcium signal is unique in a spatiotemporal pattern, and the responses to the calcium signal can either cause short-term effects such as gene transcription, signal transduction, contraction (Fig. 2) and secretion, or long term regulation of fertilization, proliferation, migration, differentiation, apoptosis and necrosis (Benavides Damm & Egli, 2014). A continuous increase of the calcium concentration can lead to cellular damage and an activation of proteases (Batchelor & Winder, 2006). Consequently, it is necessary that the calcium homeostasis is maintained to ensure a proper function of the cell needed in the development, repair and regeneration of healthy muscle tissue (Benavides Damm & Egli, 2014). The calcium homeostasis is distorted in both CP (Smith *et al.* 2009, 2012) and muscular dystrophy (Vallejo-Illarramendi *et al.* 2014). Smith *et al.* observed an upregulation of parvalbumin, a calcium binding protein and the voltage-dependent L-type calcium channel subunit β-1 (CACNB1) in wrist muscles of individuals with CP, indicating pathological activation of the ryanodine receptors and increased calcium levels in CP (Smith *et al.* 2009). Furthermore, a transcriptional analysis of hamstring muscles of individuals with CP showed that several targets involved in calcium handling were significantly changed in CP, with the exception of parvalbumin, which was not significantly altered (Smith *et al.* 2012). In muscular dystrophy, changes in calcium signalling have been reviewed thoroughly (Vallejo-Illarramendi *et al.* 2014). In brief, the lack of the structural protein dystrophin causes membrane fragility of the sarcolemma and increases basal intracellular Ca^{2+} levels. These increased calcium levels induce activation of the protease calpain, protein degradation, mitochondrial permeability transition pore opening, and subsequently fibre death and necrosis. A blockage of transient receptor potential channels (TRP) reduces the extracellular Ca^{2+} influx (Formigli *et al.* 2009). Whether subjects with CP suffering from muscle contractures would benefit from therapeutic approaches towards decreasing intracellular calcium levels has to our knowledge never been investigated.

Figure 2 shows a schematic overview including several factors affecting calcium homeostasis in the cell. A dysregulation of any of these factors shown in Fig. 2 can lead to disturbances in the muscle remodelling, cell cycle progression, growth, differentiation, proliferation and apoptosis, which can have fatal consequences for the muscle tissue.

Cellular mechano-sensing. The cells sense physical forces through cell–cell interactions and cell substrate interactions and translate the mechanical input into biochemical signals. Myosin and the giant protein titin are believed to be the main players in mechano-sensing through the sarcomere itself (Gautel, 2011), and the kinase

in the M-band is believed to play a role in this (Gautel, 2011). In spastic CP patients, a reduced resting length of the sarcomeres has been observed. This was accompanied by a doubling of the elastic modulus compared to normal muscle cells (Friden & Lieber, 2003). The same observation was made in spastic spinal cord injury patients (Olsson *et al.* 2006). Friden and Lieber suggested that titin isoforms may be critical to the structural changes in the muscles of spastic patients (Friden & Lieber, 2003). Furthermore, titin also binds calcium, and the protein charge on titin is dependent on the calcium concentration, showing a sharp transition at a pCa of 6.8. This indicates that titin could be the component controlling the calcium dependence along the total length of a sarcomere (Coomber *et al.* 2011). One can therefore speculate if titin is involved in the development of contractures.

Whether any of these various processes with calcium regulation and mechano-sensing are impaired or affected in muscle contractures is yet to be discovered. We propose that this particular area should achieve meticulous attention in future research into the origin of muscle contractures. If the calcium signalling either downstream or upstream is involved in the initiation of muscle contractures, or if binding of calcium to titin is impaired, this would open completely new opportunities for the treatment of muscle contractures.

Is impaired micro-vascularization involved in development of contractures?

Blood flow to the skeletal muscle is determined by mechanical, neural and metabolic forces (Uchida *et al.* 2015). The micro-vascularization of skeletal muscles is crucial for tissue maintenance, repair and remodelling (Fig. 1) (Uchida *et al.* 2015). Even a short-term blockade of the blood supply to a skeletal muscle causes significant injuries to the muscle cell. Furthermore, denervated skeletal muscles have been shown to

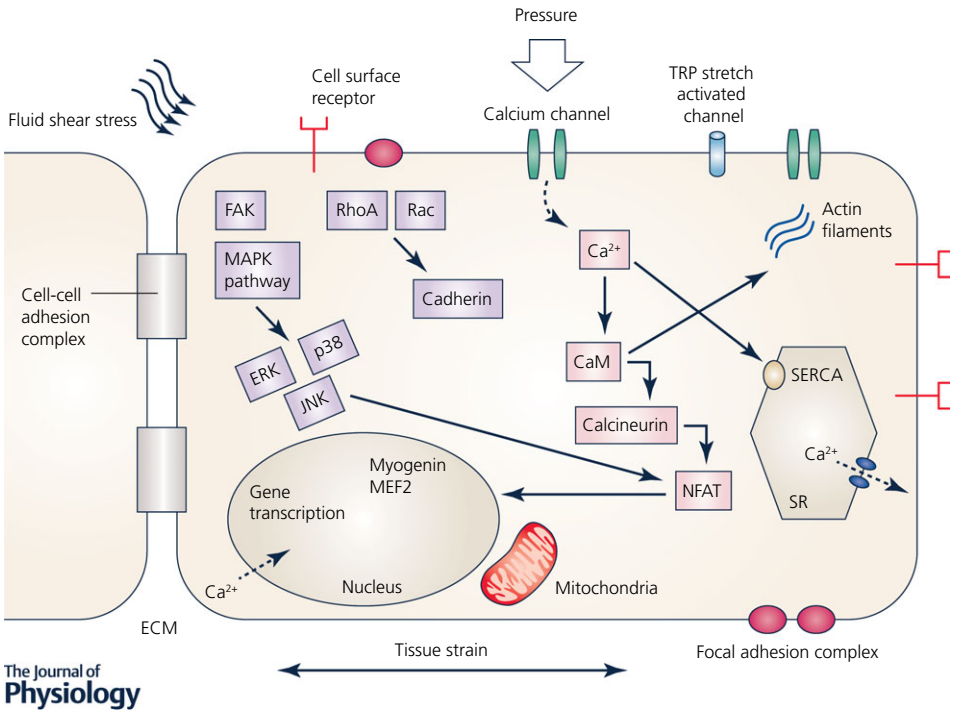

Figure 2. Mechanotransduction in muscle tissue
Mechanical loading is sensed through a diverse group of membrane-anchored mechano-sensors, including stretch-activated ion channels, focal adhesion complexes and cell surface receptors. This mechanical sensation is then converted into biochemical signals by triggering several downstream signalling cascades in the cytoplasm. Elevated tension increases integrin clustering and phosphorylation of the focal adhesion kinase FAK, which triggers the mitogen-activated protein kinase (MAPK) sub-pathways and the Rho pathways: the extracellular signal-regulated kinases (ERK), c-Jun NH-2-terminal protein kinases (JNKs) and p38 MAPKs. Elevated tension within the focal adhesions increases integrin clustering and FAK phosphorylation, and these molecular changes induce subsequently a cascade of signalling activation of the Rho pathway including Rac and cadherin. Calcium binds to camodulin and activates calcineurin which dephosphorylates the nuclear factor of activated T-cells (NFAT). This initiates gene transcription of muscle remodelling transcription factors including myogenin and MEF2. The sarcoplasmic reticulum represents the internal calcium stores. The release of calcium is controlled by inositol-1,4,5-trisphosphate and the reuptake of calcium is controlled by the sarco-endoplasmic reticulum ATPase pumps (SERCA).

express devascularization of the tissue accompanied by degeneration and necrosis of muscle cells (Borisov *et al.* 2000). Why denervation causes capillary necrosis is still unclear. Possible reasons for this development could be either vascular degeneration or increased shear stress.

Vascular degeneration. Patients suffering from amyopathic lateral sclerosis and axonal demyelinative neuropathies suffer from poor vascular perfusion of the muscle tissue caused by necrotic capillaries (Carpenter & Karpati, 1982). An investigation of biceps brachii muscle of seven spastic patients and seven healthy control participants demonstrated the same in muscle contractures. Individuals with CP had 38% lower capillary density than the healthy control subjects (Ponten & Stal, 2007). The authors suggest that the low proportion of oxidative fibres and the low oxidative capacity and low capillary supply could explain that muscles with contractures show increased fatigue (Ponten & Stal, 2007). Stroke patients also show reduced capillarization in the paretic side when compared to the non-paretic side (Prior *et al.* 2009). Reduced capillarization affects various physiological processes, including oxygen uptake and glucose metabolism (Ponten & Stal, 2007; Prior *et al.* 2009). However, the $\dot{V}_{O_2peak}$ does not correlate with reduced capillarization in stroke patients, which indicates that the restricted mobility limits the stroke patients more than the reduced capillarization of their muscles (Prior *et al.* 2009).

Shear stress. The vascular system is continually exposed to stress. The blood flow applies shear stress, blood pressure and muscle contraction apply compression, and finally strains of the surrounding muscles apply tension (Fig. 2) (Egginton, 2009). Intracellular signalling pathways can be activated by any of these haemodynamic forces, and can thereby regulate proliferation, apoptosis, adhesion and matrix degradation (Egginton, 2009). Increased shear stress causes hyperpolarization of the cell membrane, whereas decreased shear stress causes depolarization (Chatterjee & Fisher, 2014). Endothelial cells sense the shear stress associated with blood flow and the resultant mechano-signalling initiates downstream signalling events to regulate the vascular diameter and vessel tone (Chatterjee & Fisher, 2014). In endothelial cells this concept has been described as chemotransduction (Lopez-Barneo *et al.* 1988). A dysregulation of the chemotransduction in endothelial cells can lead to angiogenesis. In other patient groups where the micro-vascularization is poor, such as in intermittent claudication patients, exercise is the most effective therapy, since it increases the oxidative and vascular capacity of the muscles, indicating an improvement of the micro-vascularization with exercise (Hiatt *et al.* 1990). It is crucial for the development and maintenance of the micro-vascularization that the chemotransduction is highly regulated and shut down or upregulated as required.

Whether chemotransduction is impaired or altered in muscle contractures is still unsolved, but it is very likely that the vascularization of the muscle tissue is affected by muscle contractures, since the muscle function and activity level is reduced in the affected muscles. A better understanding of micro-vascularization in connection with this particular patient group, and a possible effect of a reduced blood flow, could possibly lead to several new opportunities of treatments for patients with muscle contractures. It would also help to understand one of the connections to exercise therapy, since exercise is known to cause an increase in vascularization.

The impact of genetics and epigenetics on the development of contractures

Genetics. In regard to the cause of muscle contractures it is the general opinion that muscle contractures are not caused by a gene defect, but that the disease mainly develops depending on the muscle activation and/or the lack thereof. However, some genetic diseases develop muscle and joint contractures, for example Ullrich congenital muscular dystrophy (UD) (Yonekawa & Nishino, 2015). UD was first described in 1930 by Otto Ullrich and is a gene mutation of collagen 6 alpha-1 (*COL6A1*), collagen 6 alpha-2 (*COL6A2*) or collagen 6 alpha-3 (*COL6A3*) (Yonekawa & Nishino, 2015). The disease has clinical–pathological features of a muscle disorder and connective tissue disorganization, and causes progressive joint and muscle contractures, preventing normal physical activity (Yonekawa & Nishino, 2015). Collagen VI is an extracellular protein, forming a distinct microfibrillar network in most interstitial connective tissues, and it has a pivotal role in maintaining skeletal muscle integrity and function (Bonnemann, 2011). Since mutations of collagen type 6 can cause contractures, it is tempting to elaborate whether patients with different contractures might show differences in gene variants of either COL6 or other crucial collagen types, or connective tissue components, responsible for the integrity and functionality of the muscle tissue. Furthermore, will these patients show different epigenetic regulations of these genes (Fig. 1)?

Epigenetics. The most extensively studied mechanisms in epigenetics are DNA methylation, histone acetylation and histone methylation. DNA methylation is the most stable modification of the chromatin structure, providing long-term regulatory directions for transcriptional control and generally instructions for responsiveness (Siegfried *et al.* 1999). DNA methylation occurs at CpG islands (regions of repeated cytosine-guanine pairs) at the 5′

position of the cytosine ring (Siegfried *et al.* 1999). In contrast histone acetylation is a transient and enzymatically controlled process like phosphorylation that generally governs gene activation (Clayton *et al.* 2006). Histone methylation can activate or suppress (silence) transcription in a context dependent manner (Clayton *et al.* 2006). There is increasing appreciation for the contributions of genetic and epigenetic regulations to muscle development, regeneration and function (Baar, 2010). Although a detailed description of these epigenetic regulatory mechanisms is beyond the scope of this review, it is opportune to mention that epigenetics could play a significant role in muscle function, integrity and pathology. Regarding muscle function, several studies have investigated the involvement of epigenetics regarding fibre type differentiation within the muscle (reviewed by Baar, 2010). Furthermore, recent reports highlight the influence of epigenetic mechanisms in several diseases including muscular dystrophies (Colussi *et al.* 2008). The genetic disorder Duchenne muscular dystrophy (DMD), the most severe muscular dystrophy, is caused by mutations of the dystrophin gene. The pathology of the disease is characterized by a rapid progression of muscle degeneration, loss of ambulation and early death (Lopez-Hernandez *et al.* 2015). Previous studies have shown an involvement of epigenetic mechanisms in the development of the disease (Cacchiarelli *et al.* 2011). Studies on monozygotic twins have also shown a different penetrance of facioscapulohumeral muscular dystrophy (FSHD), indicating a strong epigenetic contribution to the pathology of FSHD (Griggs *et al.* 1995). Whether epigenetic mechanisms are involved in muscle contractures is still unknown, but since there is a very high inter-individual variability in the severity of the condition, it is very likely that epigenetic mechanisms are involved in the regulation of the severity of contractures, and maybe even in the initiation of the latter. The discovery of epigenetic regulatory mechanisms in muscle contractures could offer new promising targets of pharmacological treatments for patients with contractures.

MicroRNAs. MicroRNAs (miRNAs) are a group of evolutionary conserved nucleotide non-coding RNAs involved in the regulation of post-transcriptional gene expression (Liu & Bassel-Duby, 2015). miRNAs can silence specific downstream target mRNA through an incorporation of miRNA into target mRNAs with imperfect base pairing causing translational inhibition and RNA degradation (Liu & Bassel-Duby, 2015). This fine-tuning process of RNA expression causes down-regulations of the target mRNAs and protein levels (Liu & Bassel-Duby, 2015). miRNAs influence various biological events, including cell death, differentiation, proliferation and cell growth (Liu & Bassel-Duby, 2015). Recent studies have demonstrated that miRNAs may play critical roles in diseases affecting skeletal muscle, including hereditary spastic paraplegia (Henson *et al.* 2012), muscular dystrophies (Liu & Bassel-Duby, 2015), and amyotrophic lateral sclerosis (ALS) (Campos-Melo *et al.* 2013). Cacchiarelli *et al.* 2010 observed a higher expression of miR-31 in muscle biopsies of DMD patients when compared to healthy muscle. The authors conclude that the higher expression of miR-31 indicates insensitive regeneration and a disability of Duchenne myoblasts to complete differentiation (Cacchiarelli *et al.* 2010). Furthermore, post mortem spinal cord specimens from ALS patients revealed that the expression of several miRNAs was significantly altered (Campos-Melo *et al.* 2013). Dysfunctions of skeletal muscle mitochondria have been suggested to be involved in the progression of severity of ALS. However, no studies have so far investigated whether muscle contractures show alterations of miRNA expression. Since miRNAs are emerging as key players in regulating several muscle diseases, it is of tremendous importance to clarify whether miRNAs are involved in the development of muscle contractures. If alterations of miRNA expression cause skeletal muscle maladaptation during the development of muscle contractures, they could offer potential miRNA-based therapies to benefit skeletal muscle health.

Conclusion

In this review we have summarized several of the factors which are likely to contribute to the development of contractures in patients with central motor lesions, including neural activation, mismatch between bone and muscle growth, mechanotransduction, tensional homeostasis, micro-vascularization, genetics and epigenetics. A framework of factors triggering contracture is appearing, but all the factors and processes involved need to be examined further in relation to the triggering of muscle contractures, and we are convinced that a comprehensive investigation of these topics will open the door to a better understanding of why muscle contractures develop. This may then hopefully pave the way for a more optimal, patient-focused treatment of this heterogeneous patient group.

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Additional information

Competing interests

None declared.

Funding

This project was funded by the Danish Research Council (DFF-1333-00197) and the Elsass Foundation.

Acknowledgements

We thank Dr Adrian Harrison for his help and great scientific discussions about this work.