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Excitation-contraction coupling and mechano-sensitivity in denervated skeletal muscles

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Abstract

The Skeletal muscle atrophy can be defined as a wasting or decrease in muscle mass and muscle force generation owing lack of use, ageing, injury or disease. Thus, the etiology of atrophy can be different. Atrophy in denervated muscle is a consequence of two factors: 1) the complete lack of motoneuron activity inducing the deficiency of neurotransmitter release and 2) the muscles disuse. The balance of the muscular functions depends on extra- and intra-muscular signals. In the balance are involved the excitation-contraction coupling (ECC), local growth factors, Ca^{2+}-dependent and independent intracellular signals, mechano-sensitivity and mechano-transduction that activate Ca^{2+}-dependent signaling proteins and cytoskeleton-nucleus pathways to the nucleus, that regulate the gene expression. Moreover, retrograde signal from intracellular compartments and cytoskeleton to the sarcolemma are additional factors that regulate the muscle function. Proteolytic systems that operate in atrophic muscles progressively reduce the muscle protein content and so the sarcolemma, ECC and the generation of the force generation. In this review we will focus on the more relevant changes of the sarcolemma, excitation-contraction coupling, ECC and mechano-transduction evaluated by electrophysiological methods and observed from early- to long-term denervated skeletal muscles. This review put in particular evidence that long-term denervated muscle maintain a sub-population of fibers with ECC and contractile machinery able to be activated, albeit in lesser amounts, by electrical and mechanical stimulation. Accordingly, this provides a potential molecular explanation of the muscle recovery that occurs in response to rehabilitation strategy as transcutaneous electrical stimulation and passive stretching of denervated muscles which are developed as a result of empirical clinical observations.
Key Words: Excitation-contraction coupling; L-type Ca^{2+} current; Mechano-transduction; Skeletal muscle; Long-term denervation

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Skeletal muscle atrophy can be defined as a wasting or decrease in muscle mass and muscle force generation owing lack of use, ageing, injury, or disease. Thus, the etiology of atrophy can be different. Atrophy resulting from disuse (muscle unloading, immobilization, bed rest, and spaceflight) and described as acute atrophy, is readily reversible by exercise and is the consequence of the lack of muscle activity that reduced the gene activation and protein synthesis. The age-related loss of muscle mass and strength, sarcopenia, may be considered to be chronic. Muscle atrophy resulting from chronic disease rather than disuse is described as cachexia and generally arises either from damage of motoneuron or from muscle disease. Both causes can be the result of either genetic abnormality of nerves or muscles, or systemic disease pathology. In motoneuron pathology muscle

atrophy is the consequences of affected signal from nerves (action potential rate, neurotransmitter release). Muscle pathology involves genetic abnormality with lack or affected myofibrillar contractile or cytoskeleton proteins. Atrophy in denervated muscle is a consequence of two factors: 1) the complete lack of motoneuron activity inducing the deficiency of neurotransmitter release and 2) the muscles disuse. In accord, a severe atrophy and degeneration of leg muscles tissue does not occur in patients with upper motor neuron lesions even 20 years after thoracic-level of spinal cord injury, SCI [38]. In such SCI the lower motoneuron (LMNs) activity is reduced but it is still present, in a certain degree. In contrast, atrophy of leg muscles is particularly severe when the injury destroys the LMNs and, therefore, the contacts between neurons and muscle. During the first days after SCI, human muscles become less excitable by transcutaneous electrical stimulation (functional electrical stimulation, FES) because the nerve degenerates and so FES can not act by activating the motoneurons. Instead, FES can only operate by direct activation of the less excitable muscle fibres so that, after nerve injury the chronaxie becomes longer, being that of the denervated skeletal muscle [7,28]. The progress of muscle atrophy in denervated muscle is time dependent. In mammals changes of the passive electrical properties of the sarcolemma [15], in transverse tubules organization [68], sarcolemmal Ca^{2+} current, and the ability to sustain tension during prolonged contractions [19] were just observed in the first weeks after muscle denervation. Muscle atrophy advances faster in the early weeks after denervation but then progresses slower and it may reach a steady-state that is maintained for long time [2,62]. Within months after complete injury of the conus medullaris and cauda equina, the muscles are no longer excitable by commercially available electrical stimulators. This is because they have undergone severe disorganization of the sarcolemmal proteins, excitation-contraction coupling (ECC) apparatus, cytoskeleton, metabolic activity and contractile elements (i.e. myofibrils). Finally, after several years of LMN denervation, muscle fibers are almost completely replaced by adipose and fibrous tissues [37,52], therefore muscle degeneration superimposes to muscle atrophy. We have to take into account that many aspects of the early responses to denervation of limb skeletal muscles are similar in all mammalian species investigated in detail. If, however, later stages of denervation are examined there are indications (although no hard evidence) of differences between muscles and species particularly in the time course of the atrophy (i.e. alteration after a few months in rat can be observed up to one year in humans) [2,6,52]. Skeletal muscle atrophy involves many protein systems; in this review we will focus on the more relevant changes of the excitation-contraction coupling, ECC, and mechano-transduction observed in early- and long-term denervated skeletal muscles.

1. Skeletal muscle fiber type plasticity

Skeletal muscles functions are plastic, that is depending on their activity they can reversibly change from normal to hypertrophic and atrophic state. The maintenance of skeletal muscle depends upon the imbalance between these two states. Atrophic conditions share the commonality of an imbalance resulting in reduced protein synthesis and increased protein breakdown/proteolysis, which in turn results in reduced muscle mass and muscle fiber size. The imbalance involves the gene activation and, as a consequence, the whole synthesis of proteins of sarcolemma, sarcoplasmic reticulum, extra- and myofibrillar-cytoskeleton, sarcomeric and of the mitochondria.

Mammalian skeletal muscles express particular isoforms of myosin heavy chain (MyHC) and are classified as slow (type I) and fast (type II) fibers; the latter can be further subdivided into three subsets: type IIA, IIX and IIB. These fibers also differ in oxidative enzyme and mitochondrial content, which is higher in type I and IIA (fast oxidative) fibers than in type IIX (fast intermediate) and IIB (fast glycolytic) fibers. Velocity of contraction is associated with the type of MyHC isoforms, whereas resistance to fatigue is related to oxidative enzyme expressed. In accord, type I fibers show low velocity of shortening and high resistance to fatigue, and type IIB fibers show high velocity of shortening and low resistance to fatigue. Fiber type composition can be modulated by nerve activity. For example, muscle inactivity tends to shift MyHC expression and fiber type properties, following the compulsory pathway I→IIA→IIX →IIB, whereas increased activity and functional overload promote a change in the opposite direction: IIB→IIX→IIA→I [12,57]. So, atrophy of denervated muscles can be reduced by increasing their activity as by passive movements or FES. However, the above shift of MyHC expression may be different in slow respect to fast muscle and the adaptive changes subsequent to unloading may be more dependent on the muscle function (involving mainly postural muscles) than on the fiber type [12,14,49].

2. Proteolytic systems in atrophic muscles

The skeletal muscle protein loss in atrophic muscle involves three major proteolytic systems [34]: one calcium dependent (the cytosolic calcium-dependent calpain system) [33,53] and two calcium independent (the lysosomal proteases, i.e., cathepsins, and the ATP-dependent ubiquitin-proteasome system). [42].

Factors such as TNF-α, IL-1 and IL 6, glucocorticoids, myostatin, and reactive oxygen species can induce muscle protein loss under specified conditions. The different etiology of muscle atrophy is put in evidence

by the diverse involvement of these factors in the genesis of atrophy. Unloading-induced atrophy does not require myostatin. Thus, whereas myostatin may contribute to atrophy in cachexia it is not required for disuse atrophy. Treatment of unloaded rats with an inhibitor of glucocorticoids, RU-38486, also did not inhibit disuse atrophy. Thus glucocorticoids do not appear to be required for disuse atrophy whereas in the case of cachexia, glucocorticoids seem to be a contributing factor to muscle wasting. There is no evidence that TNF-α or other cytokines are involved in disuse atrophy no difference was found in TNF-α protein levels in unloaded muscle. However, in cachexia TNF-α and other cytokines such as IL-1 and IL-6 are increased and administration of TNF-α can induce cachexia [43]. Thus, with cachexia, but not with disuse, cytokines are key triggers of muscle wasting. Finally, the NF-α transcription factor complex has been implicated in muscle atrophy attributable to both disuse and cachexia, but the specific family members involved in the two types of atrophy are distinct [34,39]. These are important findings that show differences in the molecular signaling for different types of muscle and, therefore, that there may be more specific molecules to target in the development of therapies related to denervated muscle wasting.

Another recent work has shown a role for calpains in muscle atrophy resulting from sepsis. Sepsis induced by cecal puncture leads to Z-band disintegration, increased release of myofilaments, and increased mRNA of calpain 1, calpain 2, and calpain 3 in extensor digitorum longus muscle [70]. However, treatments of rats with dantrolene (an inhibitor of qγ, L-CaC and RYR1/CRC and, in turn, of ECC; see below) attenuate these changes by inhibiting the release of calcium from the sarcoplasmic reticulum. The caspases, proteins involved in apoptosis that have specific proteolytic activity, were recently shown to have a role in disease-related muscle atrophy [10,18]. The caspases have been proposed to have a role in the initial step in myofibrillar proteolysis by cleavage of actomyosin. In this way, the caspases may be similar to the calpains in making myofibrillar proteins available for ubiquitination.

Denervation of muscle (often with associated aberrant reinnervation) is regarded as a potent trigger to sarcopenia, both associated with ageing and neuromuscular disorders [59], and it has recently been examined at the molecular level [50]. The use of IGF-I electroporation, recombinant human IGF-I and the peptide representing mechano-growth factor (MGF) have all been considered for ameliorating such neuromuscular conditions, including muscular dystrophy and motor neuron disease [41].

3. The balance of the muscular functions depends on extra-muscular and muscular signals

The balance between hypertrophy and atrophy is regulated by extra-muscular and muscular signals.

The first extra-muscular signal is the motoneurones activity others are hormonal as catecholamines, insulin, thyroid hormones, sex hormones, glucocorticoids. Various growth factors stimulate satellite cell proliferation during muscle hypertrophy and regeneration [22,32].

Skeletal muscle itself is a major contributor to a variety of metabolic conditions since autocrine–paracrine loops involving growth factors released by the muscle fibers themselves are implicated in activity-dependent muscle fiber atrophy-hypertrophy balancing. For example, insulin-like growth factor 1 (IGF-1) splicing variants produced by muscle cells are upregulated during muscle hypertrophy induced by overload, stretch or stretch combined with electrical stimulation. Another autocrine–paracrine growth factor is sphingosine 1-phosphate, S1P [11,16,23,24,70]. Finally, there are locally generated negative regulators of muscle functions as IL-1, IL-6 and TNF-α.

4. Ca^{2+}-dependent and independent muscular intracellular signals

We have to take in consideration the role of calcium handling that is concerned in many Ca^{2+}-dependent processes as troponin C, TnC, involved in the cross-bridge activation and force generation; calmodulin involved in L-CaC inactivation. The intracellular $[Ca^{2+}]$ results from ryanodine receptors (RyRs) opening, Na^{+}/Ca^{2+} excengers and Ca^{2+}-pumps expressed in sarcolemmal and sarcoplasmic reticulum membrane [8,12,13].

Calcium-dependent calpain system involved in Calpain substrates are proteins that are involved in the assembly and scaffolding of myofibrillar proteins such as titin, vinculin, C-protein, nebulin, and others. Finally, the intra-sarcomeric cytoskeleton protein Titin is a Ca^{2+}-dependent protein capable of producing a passive tension during sarcomere contraction [36]. It is, in fact, a large elastic proteins linking the thick filaments to the Z-disc, so that the sliding of myosin filaments induces changes in the elastic domain of the protein.

Other Ca^{2+}-dependent proteins are involved in paths activating the gene expression as the Ca^{2+}-calmodulin dependent Calcineurin [40,56]. Calcium/calmodulin-dependent protein kinase IV (CaMKIV) has been identified as a major regulator of mitochondrial activity [50].

5. Mechano-sensitivity, mechano-transduction and cytoskeleton-nucleus pathways

Many evidences suggest a significant role of physical forces as passive stretching or active contraction in exercise, in the development and maintenance of

skeletal muscle mass and in the onset and maintenance of several muscle disorders [1,16,54]. About the mechanisms by which mechanical stress modulates muscle physiology and pathophysiology there is a growing list of signaling pathways that are activated in response to mechanical stimulation in skeletal muscle cells. These include the Ca^{2+}-independent and Ca^{2+}-dependent signaling molecules [24,30] and the cytoskeleton [35]. Sarcomeres are connected to the sarcolemma by extrasarcomeric cytoskeletal proteins forming a morphological and functional unit. Among the extrasarcomeric proteins, ~~is~~ desmin [47] ~~that~~ links the sarcolemma to sarcomeres at Z-disk and the costameric proteins such as vinculin, spectrin, talin, distrophin [20,21,46], which, being localized in rib-like bands beneath the sarcolemma, connect the cytoskeleton to the cell surface. Interestingly, Desmin also links to the nuclear envelop, so it is a path that connect the sarcolemma to nucleus and is involved in mechano-trasduction regulating the gene activation [65,67]. This mechanical path parallels other signals (described above) acting directly on the nucleus and involving Ca^{2+}-activated molecules, such as the Ca^{2+}calmodulin-dependent calcineurin [9,40,56].

Mechanical signaling path to the nucleus involves the integrity of the sarcoglican-anchoring protein-actin cytoskeleton [29,30,35]. The mechanical tension of actin filaments is regulated by a Ca^{2+}-independent Rho path and by its contraction via the Ca^{2+}-dependent activation of the non sarcomeric myosin II [23,24]. In myoblasts and myotubes mostly Ca^{2+} entry in the sarcoplasma depends on the activity of stretch-activated-Ca^{2+} channels (SACs) of the sarcolemmal membrane [23]. These are cationic channels whose activation determines an influx of Na^+ and Ca^{2+} ions that, in turn, causes intracellular Ca^{2+} increase and the fiber depolarization. In adult, differentiated skeletal muscle fibers the most important Ca^{2+} signal for sarcomeric contraction came from ECC. The intracellular [Ca^{2+}] increase is important as a signal to the nucleus about the entity and type of muscle contraction.

Disturbed intracellular Ca^{2+} signaling is involved in muscle atrophy. The altered Ca^{2+} homeostasis is related to a leaky plasma-membrane and loss of its mechanical properties causing fragility and less stiffness, depending on the anomalous cytoskeleton organization [2,8,13]. This in turn affects not only the properties of plasma-membrane ionic channels but also the gene expression. The result is an increase of the SAC and a decrease of the L-CaC expression. Moreover, L-CaC become mechano-sensitive being more activated by stretch. In other words denervated fibers regress and express SACs and L-CaC as in myoblasts/myotubes.

6. Excitation-contraction coupling in normal skeletal muscles

In skeletal muscle fibres the generation of the action potential in the sarcolemma causes the excitation-contraction coupling (ECC) by triggering a series of events that ends with the contraction of the fibre [51,58]. Action potential propagates into the fiber via the T tubular system, TT, where the dihydropyridine receptors (DHPR) lie arranged in tetrads. Each unit of the tetrad is also a voltage operated L-type Ca^{2+} channel (L-CaC), consisting of five subunits: α1, α2, β, γ and δ. The pore-forming unit α1 has an amino acidic sequence organized in four repeated domains (I, II, III, IV), each containing six transmembrane segments (S1-S6). The S4 segments contain positively charged amino acid residues. When the TT is depolarized by the action potential the charged S4-segment senses the new potential and moves. This movement, recorded by electrophysiological techniques, is called intramembrane charge movement (ICM). ICM determines the movement of the loop between the II and III domain. Consequently, the coupled ryanodine receptor type 1/Ca^{2+}-release channel (RyR1/CRC) of the sarcoplasmic reticulum (SR) opens and the luminal Ca^{2+}, stored in the SR, can flow into the myoplasm. The Ca^{2+} released by RyR1/CRC opens the uncoupled Ca^{2+}-dependent RyRs, type 2 and 3/CRCs via Ca^{2+}-induced Ca^{2+} release mechanism determining a further Ca^{2+} release. The total Ca^{2+} released reached the [Ca^{2+}]i able to activate the acto-myosin coupling and so the cross-bridge contraction.

ICM involves three groups of charges with different voltage thresholds, voltage dependence and function. In fact, in normally polarized skeletal muscle fibers, ICM has been resolved into three components, an early qß, evoked by any depolarizing step, and two delayed components, qγ and qh [11,21] evoked only by voltage steps more positive than a certain threshold (about –56 and –38 mV in frog and rat, respectively). The time course of qß shows a monotonic decay and it seems not to be involved in ECC since the total amount of charge moved is not affected by pharmacological interventions directed at L-CaC such as Cd^{2+}, nifedipine or alkanols. The hump–form charges qγ and qh can be resolved into two different ICM components by evaluating the voltage dependence of the amount of charge moved by depolarizing steps above the voltage threshold. In particular, the qγ movement triggers the opening of the coupled RyRs/CRCs, allowing a high Ca^{2+} flux from the SR into the myoplasm and promoting muscle contraction. The qh charge mobilization move slower than qγ and determines the opening of the sarcolemmal L-CaC that allows Ca^{2+} influx (I_{Ca}) from the external medium into the myoplasm. This may further enhance ECC by acting on RyRs/CRCs via Ca^{2+}-induced Ca^{2+} release. So, DHPR/L-CaC has a dual function being a voltage

sensor for RYR1/CRC activation and a functional L-type Ca^{2+} channel.

What is the physiological significance of the skeletal muscle L-type Ca^{2+} current? Single contractions of skeletal muscle fibers do not require Ca^{2+} current through L-CaC because in skeletal muscle-type EC coupling the mechanical interaction between the voltage sensor for EC coupling (i.e., L-CaC) and the RYRs/CRCs determines a high Ca^{2+} release channels from the SR. Thus, skeletal muscle fibers produce normal twitches in the temporary absence of external Ca^{2+} [66]. In contrast, during tetanic contractions, I_{Ca} is involved in maintaining force. In accord, since normal daily activities require sustained activation of skeletal muscles, Ca^{2+} influx through L-CaC should play an important role. Ca^{2+} entry can enhance contraction directly, or entering Ca^{2+} may be sequestered into the SR and thereby increase the amount of Ca^{2+} available for release in response to subsequent action potentials, which would lead to an increase in contractile force.

Distinct kinetic models are required to account for L-CaC channel activation in frog [26] and mammalian muscle [11,45]. However, in all the models the charge particles involved in the opening of L-CaC move coordinately because of the interaction between them. All the models involved various steps, slow and fast, activated at different voltage threshold. The consequence is that a depolarization state of the fibers determines a faster opening of L-CaC.

Finally, also the inactivation of L-CaC is accomplished by various steps and is regulated by at least three mechanisms that concur to reduce the Ca^{2+} entry: 1) Ca^{2+} depletion of the TT system that is regulated by the TT volume; 2) voltage-dependent [27] and 3) Ca^{2+}-dependent inactivation. This latter is modulated by Ca^{2+}-calmodulin [66]. This L-CaC inactivation mechanisms may be important for fine-tuning regulation of intracellular [Ca^{2+}] and consequently of skeletal muscle function.

7. Reciprocal interaction between L-CaC and RYR1/CRC

The ECC is a simple one-way interaction between two proteins that is the voltage-sensing promotes the RyR/CRC activation via mechanical and Ca^{2+}-dependent coupling. The idea to be considered is that manipulations of the RyR should reciprocally influence the behavior of any charge components with which it makes allosteric contact [4] and should spare only charge components that are not supposed to originate in the L-CaC and/or that are scarcely involved in ECC. Indeed, experimental results suggests a more complex functional communication. A reciprocal interaction, or cross-talk, is possible between these two proteins. Thus, in addition to 'receiving' the orthograde ECC signal from the L-CaC, RyR/CRC 'answer' with a retrograde regulation that facilitate the movement of the qγ and qh charges and increases the Ca^{2+} channel activity of the L-CaC [44] causing an enhancement of I_{Ca}. Nifedipine reduced the amount of qγ and qh moved by ~90 % and ~55 % respectively, whereas 1-alkanols completely abolished them. Ryanodine and Ruthenim Red did not affect the amount of qγ and qh moved, but shifted their voltage dependence and that of I_{Ca} activation more positively by ~4-9 mV. Conversely, 1-alkanols, Ryanodine and RR spared qβ. The effect of ryanodine together with those of nifedipine and 1-alkanols, are a further demonstration that qγ and qh both reside in the DHPR/L-CaC and represent separate independent processes from qβ. Moreover, these results demonstrate that, in the absence of RyR/CRC blockers, the opening of the RyR/CRC facilitates qγ and qh movement as well as the opening of L-CaC by a retrograde signal and, in turn, the ECC process [60,61]. Conversely, it may be supposed that any reduction of RyR1/CRC functionality or expression could affect the ECC.

Again, increases the number of uncoupled RYR/CRC supporting the concept that DHPR-RYR1 uncoupling results in alterations in the voltage-gated sarcoplasmic reticulum Ca^{2+} release mechanism, decreases in myoplasmic Ca^{2+} elevation in response to sarcolemmal depolarization, reduced Ca^{2+} supply to contractile proteins and reduced the force of contraction with aging [48,69].

8. Excitation-contraction coupling in rat denervated skeletal muscles

Denervated rat leg muscles (soleus, tibialis anterior, gastrocnemius, EDL) showed a progressive decrease in muscle weight-to-body weight ratio. This decrease was very marked in the first 3 weeks, progressed slower up to 26 weeks whereas later the decrease progressed quite slowly indicating that the late loss of muscle mass is negligible [3,62].

The muscle weight-to-body weight ratio changes are paralleled by alterations involving a progressive reduction of i) fiber diameter, iii) T-tubule surface area, iv) membrane resistance and resting membrane potential, v) expression and functionality of voltage-dependent Na^{+} channels, index of a less muscle excitability, vi) expression and voltage dependence of L-CaC, index of an affected ECC apparatus, vi) loss of the sarcomeric structure and reduced contractility. Because of this similar time dependence of all the above parameters it is hard to evaluate the trigger signal of atrophy.

Notably, in long-term denervated muscles despite the loss of the sarcomeric machinery denervated muscles maintain some fibers able to activate the altered ECC process and to generate force, albeit in lesser amounts. The reduced values of resting membrane resistance and the depolarized resting membrane potential in denervated fibers suggest a leaky sarcolemma [55] and an increase of intracellular Ca^{2+} concentration; this is confirmed by the shift in reversal potential of the L-

CaC current toward a more negative potential. This may be a starting mechanism that reduces membrane excitability and the efficacy of electrical stimulation in short-term and, to a greater degree, in long-term-denervated muscles.

The reduced fiber diameter and the depolarized state of the denervated rat fiber are in agreement with the reduced action potential conduction velocity observed in long-term-denervated human muscles. In denervated muscle the voltage-dependence for I_{Ca} activation is shifted towards more positive potentials and consequently stronger depolarization mast be applied to activate L-CaC. These findings are in agreement with the reduced excitability found in muscle of SCI patients as increased values of chronaxie.

The reduced T-tubule surface area is an index of a reduced T-tubule volume. So, the total T-tubule Ca^{2+} content could be reduced and this could be a factor that enhances the L-CaC inactivation due to T-tubule Ca^{2+} depletion. Moreover, the increased number of RyR1 uncoupled with L-CaC may be another factor of the altered ECC since the reduced potentiating action of the retrograde action of RyR1 on L-CaC [4,60,61]. However, the presence of residual functional voltage-dependent Na^{+} and L-type Ca^{2+} channels may allow FES to activate Na^{+} and L-CaC and in turn the affected EC coupling process. The resulting transient increase of intracellular [Ca^{2+}] may be the trigger event that activates Ca^{2+}-dependent signaling pathways to the nucleus, thus inducing the expression of muscle-specific proteins in the sarcoplasma and sarcolemma improving the EC coupling process, rebuilt the sarcomeric structure, the muscle mass and restoring the contractile machinery.

Moreover, in denervated muscle fibers the expression of stretch activated channels (SACs) increases [64] and the L-CaC are more sensitive to stretch [63]. Thus, due to this increased mechano-sensitivity, passive stretching of denevated muscles may ameliorate the Ca^{2+} entry from the external medium [17]. Denervated skeletal muscle appears as stabilized in a less differentiated state for its increased SAC expression and sensibility resembling myoblast-myotube. Finally, from these results we may suppose that the effects of FES could be improved in stretched muscles respect to muscles at resting length. The leaky sarcolemma may be an index of the affected structure of the extra- and intra-myofibrillar myofibrillar cytoskeleton that decreases the transport of new proteins toward the sarcolemma and the sarcolemmal stiffness [55].

9. Conclusion

Permanently denervated muscles undergo 3 stages of alteration. An early phase with a rapid atrophy. The following medium phase is a period of slow progressive atrophy and the last phase (long term denervated muscle) the fiber sizes and ECC function of the severely atrophic muscle fibers remain stable [2, 3, 62]. Meanwhile, myofibers decrease in number and fibrosis increases, the residual surviving fibers conserve some functionality as excitability and ECC function with an increased mechano-sensitivity. Moreover, despite the disassembled sarcomeric apparatus, fibers are able to maintain residual contractile activity.

Altogether, these findings support the hypothesis that electrical stimulation-induced changes in intracellular [Ca^{2+}] may mimic the lost nerve influence and may play a key role in modifying denervated muscle gene expression. Hence, these observations provide a potential molecular explanation of the muscle recovery that occurs in response to the rehabilitation strategy of FES in mammals and humans [3,5,38], which was developed as a result of empirical clinical observations [37,38]. In SCI patients after months of twitch training [37], and thus of intracellular [Ca^{2+}] transients, the restored muscle morphology and tetanic contractility agree with an improvement of the synthesis/degradation balance of sarcolemmal, ECC, cytoskeleton and myofibrillar proteins. The increased mechano-sensitivity of denervated muscle agrees with the recovery effects of passive stretch [31].

List of non-standard abbreviations:

CRC: Ca^{2+} release channel; CaMKIV: calcium/calmodulin-dependent protein kinase IV; DHPR: dihydropyridine receptor; ECC: excitation-contraction coupling; FES: functional electrical stimulation; I_{Ca}: L-type Ca^{2+} current; IGF-1: insulin-like growth factor 1; IL-1 and IL-6: interleukin 1 and 6; L-CaC: L-type Ca^{2+} channel; LMN: lower motoneuron; MGF: mechano-growth factor; MyHC: myosin heavy chain; NF–α transcription factor complex; RYR: ryanodine receptor; SAC: stretch activated channel; SCI: spinal cord injury; S1P: Sphingosine 1-phosphate; TNF-α: tumour necrosis factor-α.

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