Myokines in Home-Based Functional Electrical Stimulation-Induced Recovery of Skeletal Muscle in Elderly and Permanent Denervation

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Abstract

Neuromuscular disorders, disuse, inadequate nutrition, metabolic diseases, cancer and aging produce muscle atrophy and this implies that there are different types of molecular triggers and signaling pathways for muscle wasting. Exercise and muscle contractions may counteract muscle atrophy by releasing a group of peptides, termed myokines, to protect the functionality and to enhance the exercise capacity of skeletal muscle. In this review, we are looking at the role of myokines in the recovery of permanent denervated and elderly skeletal muscle tissue. Since sub-clinical denervation events contribute to both atrophy and the decreased contractile speed of aged muscle, we saw a parallel to spinal cord injury and decided to look at both groups together. The muscle from lifelong active seniors has more muscle bulk and more slow fiber-type groupings than those of sedentary seniors, demonstrating that physical activity maintains slow motoneurons that reinnervate the transiently denervated muscle fibers. Furthermore, we summarized the evidence that muscle degeneration occur with irreversible *Conus* and *Cauda Equina* syndrome, a spinal cord injury in which the human leg muscles may be permanently disconnected from the peripheral nervous system. In these patients, suffering with an estreme case of muscle disuse, a complete loss of muscle fibers occurs within five to ten years after injury. Their recovered tetanic contractility, induced by home-based Functional Electrical Stimulation, can restore the muscle size and function in compliant Spinal Cord Injury patients, allowing them to perform electrical stimulation-supported stand-up training. Myokines are produced and released by muscle fibers under contraction and exert both local and systemic effects. Changes in patterns of myokine secretion, particularly of IGF-1 isoforms, occur in long-term Spinal Cord Injury persons and also in very aged people. Their modulation in Spinal Cord Injury and late aging are also key factors of home-based Functional Electrical Stimulation - mediated muscle recovery. Thus, Functional Electrical Stimulation should be prescribed in critical care units and nursing facilities, if persons are unable or reluctant to exercise. This will result in less frequent hospitalizations and a reduced burden on patients' families and public health services.

Key Words: Human denervated muscle; SCI; Elderly, Recovery of external-work contractility; Home Based Functional Electrical Stimulation, Myokines, IGF-1.

Eur J Transl Myol 28 (4): 337-345, 2018

During aging, several morpho-functional changes occur in skeletal muscle, i.e., the generalized loss of muscle mass, reduced myofiber size, and the progressive reduction in muscle strength, leading to a pathologic condition known as sarcopenia and dynapenia.[1-3] This progressive age-related muscle wasting process is associated with an increased prevalence of falls, a greater incidence of diseases and the loss of functional independence.[4,5] Inadequate nutrition, disuse, neuromuscular disorders, and metabolic diseases are among the many possible causes for the age-related decline in skeletal muscle mass. Several interventions have been suggested to counteract sarcopenia, that imply different types of molecular triggers and signaling

pathways for muscle wasting.[6,7] In particular, exercise and muscle contractions, whether volitional or induced by Electrical Stimulation (ES), may counteract muscle atrophy by releasing a group of peptides, termed myokines, to protect the functionality and to enhance the exercise capacity of skeletal muscle.[8-10] This review summarizes the potential positive effects of exercise-induced myokines looking at the role of myokines and of other molecular mechanisms in the recovery of denervated and elderly muscle tissue.

Muscle plasticity after Aging or Spinal Cord Injury and home based Functional Electrical Stimulation

In recent years, we have performed basic research and clinical trials on the use of electrostimulation to provoke positive muscle plasticity adaptations after Spinal Cord Injury (SCI). These studies investigated the use of long impulse electrical stimulation as a treatment for long-term permanently denervated human muscles, i.e., home-based Functional Electrical Stimulation (hbFES).[11,12] Purpose developed electrical stimulators and very large electrodes that are now both commercially available (https://www.schuhfried.com/umbraco/Surface/AuthenticationSurface/Login?returnUrl=%2Fportal) were designed and implemented to achieve our goals.[13,14] Those studies demonstrated a tremendous improvement in muscle trophism and an increase in external muscle power. [13-19] Muscle strength was indeed improved to the point that individuals could stand up with assistance from direct electrical stimulation of the denervated muscles as well as perform in-place stepping and walk 100-200 m. The hbFES rehabilitation program was initiated relatively late after denervation (more than 8 month after SCI) because of clinical constraints and/or the belief that electrical stimulation of denervated muscles is ineffective and may actually interfere with eventual myofiber reinnervation. These ideas are still maintained by some experts despite recent evidence that electrostimulation may indeed enhance nerve growth and appropriate muscle reinnervation. [20-22] Alternative options will hopefully be realized with the future stem cells approaches.[23] Management based on *in vivo* protocols, such as the induction of muscle regeneration by injection of anesthetics in local anoxic conditions or ex-vivo techniques such as the proliferation of autologous myoblasts derived from patient's muscle biopsies, is still to be fully explored by pre-clinical and clinical research.[24-28] Although myokines will certainly have a main role, we leave to the experts in the field discussion of these approaches. The aim of the present review is to contribute the experience of muscle specialists (biologists, physiologists, and rehabilitation experts) to first the impact of denervation-reinnervation processes on shaping aging muscle fibers and then to the role of myokines in the overall process of muscle aging and in its effective countermeasures. Ten years of experience in hbFES for SCI patients[13,14] were translated with positive results in trials for rejuvenation of skeletal muscles of elderly persons.[29-38] Myokines are produced and released by muscle fibers during contraction exercise and exert both local and systemic effects.[10] Changes in patterns of myokines secretions during hbFES-induced muscle recovery in both long-term SCI persons and old people were observed, particularly with Insulin-like Growth factor 1 (IGF-1), suggesting that they are key factors of muscle recovery mediated by hbFES.

Role of myokines in volitional physical activity and hbFES to counteract muscle aging decay

The scientific literature on the aging nervous system is immense, in particular, the works on the decay of cognition and mobility. However, the impact of muscle denervation on aging skeletal muscle fibers is a relatively orphan topic. [39-42] This is mainly related to the undetermined molecular nature of the trophic factors released by motor neurons to the muscle fibers of the different types of motor units.[43] It is well known that such mechanisms contribute to neuromuscular junction development and maintenance; however, if and what chemical trophic factors influence the synchronized expression of the hundreds of nuclei belonging to a single muscle fiber remains a subject of hypotheses, while the synchronized spread of muscle action potential seems to be a more rational mechanism.[43] Two reviews provide an excellent summary of what is known or hypothesized about this subject.[1,44] It is well established that physical exercise and dietary proteins, as well as the intake of specific amino acids, are able to counteract the processes related to the progression of muscle mass loss. This may have beneficial effects on improving the anabolic response of muscle in the elderly.[45] However, the world record series of Master athletes shows an almost linear decay in muscle power that starts at age 30 years and declines to zero, theoretically at approximate 110 years of age. It is worth stressing that this occurs despite the fact that these record-holding sportsmen are the best that the human species can provide at any time point in terms of both genetic sport talents and the capacity and opportunity to train with the best possible advice.[46] A series of investigations on counteracting muscle decay involving active or sedentary seniors and SCI patients can be found in which myokines play the main role in explaining the results after Electrical Stimulation. The studies show that volitional exercise and h-bFES are crucial for maintaining both motor neurons and muscle fibers,[20-,22,47] despite some doubts present in the literature.[48-50] In addition to a progressive loss of muscle mass, aging presents also a conspicuous reduction in myofiber plasticity with alterations in muscle-specific transcriptional mechanisms.[1,30,31] During the aging process, protein synthetic rates decrease and an increase in protein degradation follows, affecting characteristics

of muscle fibers.[51] Recently, it was demonstrated that age-associated loss of OPA1 in muscle impacts muscle mass, metabolic homeostasis, systemic inflammation, and epithelial senescence.[52] It is generally accepted that the failure to repair damage is a contributory cause of functional impairment with aging,[8,9,53,54] and also promotes the detrimental replacement of functional contractile muscle with fibrous tissue.[51] Volitional physical exercise can reverse these damaging processes in both aging and cancer cachexia.[53,57-60] Interestingly, it has been shown that both acute and prolonged resistance exercise stimulates the proliferation of satellite cells in healthy sedentary subjects, though blunted in elderly people.[53,57-60] The increased levels of myostatin,[61] a negative regulator of muscle mass, may explain this fact.[62,63] An increase in autophagy in the muscle of athletic people has been reported,[64-67] suggesting that exercise may activate an important system that detoxifies muscle cells. Another major factor that is associated with physical exercise is IGF-1.[68] Its production by muscle increases after 5–10 min of moderate to high-intensity exercise.[69,70] The evidence suggests that training and regular exercise modulate expression of myokines (e.g., IL-6) and IGF-1, thereby regulating functional autophagy, and thus increasing muscle strength and attenuating sarcopenia.[71,72] There is strong evidence that IGF-1 secretion by muscle fibers also influences synaptogenesis in the brain,[73] and at the neuromuscular junctions.[74,75] Further, agrin (along with other proteins such as MuSK, rapsyn, ChAT, Hb9, munc18, etc.) is important to denervation/reinnervation processes.[76,77] Because elderly people are often reluctant or unable to participate adequately in physical exercise,[78] an alternative approach with Electrical Stimulation was designed and implemented. Krenn et al.,[79] designed a stimulator that especially addresses the requirements of elderly people. Subjects in the study were exposed to regular neuromuscular ES training for a period of 9 weeks. The outcome was an increase in muscle strength, associated with an increase in abundance of fast fibers, which indeed are the first to respond to ES and are related to the power of skeletal muscle.[30] In addition, with ES an increase in expression of IGF-1 factors as well as markers of both satellite cell proliferation and extracellular matrix remodeling was detected along with downregulation in the expression of proteases just as occurs during volitional physical exercise.[31] Furthermore, Mosole et al.,[80] recently demonstrated that ES modulate also the Calcium (Ca^{2+})-handling proteins, NFAT, and related proteases. The aims were to study the molecular mechanisms that support functional muscle improvement by ES. Indeed, Ca^{2+} cycling and activation of specific molecular pathways are essential in contraction-induced muscle adaptation. This study attains human muscle sections and total homogenates prepared from biopsies obtained before (control) and after 9 weeks of training by ES on a group of elderly volunteers. Evidence for activation of kinase/ phosphatase pathways after ES was obtained. Moreover, expression of Sarcalumenin, Calsequestrin, and sarco/endoplasmic reticulum Ca^{2+}-ATPase (Serca) isoforms was regulated by training. The conclusions were that neuromuscular ES applied to *Vastus lateralis* muscle of sedentary seniors combines fiber remodeling with activation of Ca^{2+}-Calmodulin molecular pathways and modulation of the key Ca^{2+}-handling proteins.[80] Collagen expression was also reported to be remodelled during both volitional physical exercise and ES; indeed, expression of three different forms of collagen was upregulated in electrically stimulated muscle.[30] However, the increase in collagen expression seems not to stimulate fibrosis as is shown by both morphological evidence and at the level of important fibrosis modulators, namely the increase in expression of miR29.[77] Several longitudinal studies have shown that regular exercise is beneficial to the aged population.[1,82] The Interreg IVa project recruited therapy-stable sedentary seniors with a normal lifestyle who were trained for 9 weeks with either volitional exercise by leg press,[83] or Electrical Stimulation.[29-31,77] Functional tests of trained subjects showed that LP and ES induced improvements in both leg muscle force and mobility tests.[84,85] ES significantly increased the size of fast type muscle fibers, together with a significant increase of Pax7 and NCAM positive satellite cells. Furthermore, muscle biopsies did not present evidence of muscle damage and/or inflammation.[31] Altogether, the results demonstrated that physical exercise, either voluntary or induced by ES, improves functional performance and the structural properties of aged muscles

Conclusive Remarks

Functional electrical stimulation using long biphasic impulses and large surface electrodes is able to restore muscle mass, force production and movement in humans even after years of complete irreversible denervation. Patients suffering with flaccid paraplegia (complete and permanent denervation of lower extremity muscles, e.g., *conus* and *cauda equina* syndrome) are especially good candidates for these approaches if hbFES managements start from 1 to 6 years after SCI.[13,14] Electrical stimulation-induced up-regulation of myokines is an essential mechanism to achieve this goal. Furthermore, we recently demonstrated that two-years of hbFES also improve the thickness of the stimulated skin in *conus* and *cauda equina* syndrome patients, providing a valuable anti-decubitus effect.[86,87] Thus, pilot human studies and the application of existing experimental knowledge support the use of h-bFES to improve the condition of long-term denervated muscles.[88] Taking the idea 2 steps further, in the future, we may thereby prepare the muscle in SCI patients for bridging and reconnecting it to the central motor control unit. Neither volitional training nor hbFES may stop the aging process as we have learned from the Master Athletes.[46] However, detrained elderly

persons may significantly improve their muscle structure and function to a level that allows safer and increased mobility. Our results show that ES is a safe home-based method able to counteract atrophy of fast type muscle fibers, a biomarker of skeletal muscle aging in sedentary seniors.[30,31] If seniors are unable or reluctant to perform a volitional physical activity, the electrical stimulation-induced up-regulation of myokines is an important contributing mechanism. Adding h-bFES to good nutrition and exercise where possible (e.g., volitional Full Body In-Bed Gym)[12,89,90] will allow the elderly and SCI patients (even those that will be stimulated by the recently developed soft subdural implants for the delivery of electrochemical neuromodulation therapies to the spinal cord) [91-103] to look forward to increased autonomy and quality of life, and the prospect of better professional and social integration. In case of severe muscle atrophy in the older elderly, commercially available neuromuscular electrical stimulators may improve mobility with substantial reductions of fall risk and of the severity of secondary medical problems. Thus, hbFES should be prescribed in critical care units and nursing facilities, if persons are unable or reluctant to exercise. This will result in less frequent hospitalizations and a reduced burden on patients' families and public health services.

List of acronyms

ES - Electrical Stimulation
hbFES – acute respiratory distress syndrome
IGF-1 - Insulin-like Growth factor 1
SCI - Spinal Cord Injury

Author's contributions

SS, GSG, and BMS conceived the review, analyzed the data and wrote the paper.

Acknowledgments

We thank all the persons that take part to the research activities, in particular the volunteers, which trained by hbFES
Funding: Supported by A&CM-C Foundation for Translational Myology; The Italian Ministero per l'Università e la Ricerca Scientifica e Tecnologica (M.U.R.S.T.); The EU Commission Shared Cost Project RISE (Contract no. QLG5-CT-2001-02191), Austrian Research Promotion Agency, Federal Ministry for Transport, Innovation and Technology (bmvit) and the Ludwig Boltzmann Society, Vienna, Austria; European Regional Development Fund - Cross Border Cooperation Program Slovakia -.Austria 2007–2013 (Interreg-IVa), project Mobilität im Alter, MOBIL, N_00033 (partners: Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Austria, and Faculty of Physical Education and Sports, Comenius University in Bratislava, Slovakia); Austrian national cofinancing of the Austrian Federal Ministry of Science and Research. Progetto di ricerca d'interesse di Ateneo, Università Cattolica del Sacro Cuore, Linea D.3.2 2015 to BMS. The contents of this article are solely the responsibility of the authors.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Received for publication 31/8/2018
Revision received: 06/11/2018
Accepted for publication: 08/11/2018