Mobility disorders and pain, interrelations that need new research concepts and advanced clinical commitments

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Abstract

This Perspective will discuss topics recently suggested by Prof. Helmut Kern, Vienna, Austria, to advance the research activities of his team, that is: Topic A, 10 years post RISE; Topic B, New research for new solutions on old research questions; Topic C, Working groups on nerve regeneration, training-parameters of seniors in different ages, muscle adaptation; and studies of connective tissue and cartilage. This *Perspective* summarizes some of the basic concepts and of the evidence-based tools for developing further translational research activities. Clinically relevant results will ask for continuous interests of Basic and Applied Myologists and for the support during the next five to ten years of public and private granting agencies. All together, they will end in protocols, devices and multidisciplinary managements for persons suffering with muscle denervation, neuromuscular-related or non-related pain and for the increasing population of old, older and oldest senior citizens in Europe and beyond.

Key Words: muscle and pain, proof of concept, muscle wasting, stem cells and muscle regeneration, *ex-vivo* and *in-vivo* studies, translational myology, clinical trials

Eur J Transl Myol 27 (4): 225-233

In a recent Commentary in Biology, Engineering, Medicine (BEM), titled "*From BAM to BEM, a personal journey through EJTM and Padua Muscle Days*",[1] Prof. Ugo Carraro tried to explain how a local community of basic and applied myologists, since long organized as the Interdepartmental Myology Center of the University of Padova, Italy (CIR Myo), was able in recent years to maintain a long term tradition of skeletal muscles studies. In an *Editorial* recently published in the European Journal of Translational Myology (EJTM),[2] he stressed that important articles were published in international high-impact journals by Padua scientists and clinicians and that contributing factors were both a series of dedicated International Conferences held in Euganei Hills and Padua (PaduaMuscleDays - PMD) during the last 20 years and the publication by an International Community of Myologists of more than 100 articles in EJTM during the last four years.[3-41] Based upon the strong evidence produced by Home-based Functional Electrical Stimulation (h-bFES) of denervated degenerating muscles (DDM), as validated by the successful European Union (EU) Program: RISE [Use of electrical stimulation to restore standing in paraplegics with long-term DDMs (QLG5-CT-2001-02191)],[43-60] the Project RISE-2_Italy was established in Padua University and then extended to the IRCCS Fondazione Ospedale di Venezia-Lido, Italy with the essential support and expert supervision of Prof. Helmut Kern and Colleagues of Vienna, Austria. The opinions of compliant patients were heartening,[56] and the extent to which they recovered ability to rise with support and to stand enduringly was indeed remarkable. Thus, Prof. Helmut Kern and Prof. Ugo Carraro hope to be able to convince the attendees of the incoming 2018 Spring PaduaMuscleDays that their bottled messages floating on the ocean of time will not contain the maps to the hidden treasure of Peter Pan's Captain Hook or to the entrance to the Alibaba's Cave, but that they will be steps toward the approval of research projects and clinical trials by ethical committees with the support of public and private granting agencies. This was true in the past.[43-44] They hope it will be true in the future.

With those goals in mind, this *Perspective* will discuss the following topics recently suggested by Prof. Helmut Kern for the future research activities of his team: Topic A, 10 years post RISE. Subtopics: A1, Training parameters on denervated degenerating muscles (DDM); A2, Improvement by cell therapy of exhausted muscle fibers in DDM; A3, Stimulation parameter depending on time from SCI and age; A4, Magnetic stimulation; A5, Stimulation during rest or sleeping to prolong the daily stimulation time. Topic B, New research for new solutions on old questions. Subtopics:

B1. Common new research projects as ageing is; B2, Translational research of new topics; B3, New research on biomarkers, but only from blood samples, saliva, skin, hairs; Topic C, Working groups on: C1, nerve regeneration; C2, muscle adaptation; C3, studies of connective tissues, including cartilage.

Training parameters of h-bFES for DDM, optimized for age and denervation time: perspective for muscle stimulation during period of resting, in particular, night time

The stimulation parameters applied for eliciting muscle contractions are depending on physiological conditions of the muscle. Of particular importance for electrical stimulation (ES) is whether the connection between the muscle and its innervating nerve is preserved or the muscle remains long-term denervated due to Spinal Cord Injury (SCI) or peripheral nerve lesions. In the latter cases the denervated muscle within few months becomes not responsive to commercial stimulators and undergoes ultrastructural disorganization, while severe atrophy with fibro-fatty degeneration and nuclear clumping appears within 3 and 6 years from nerve discontinuation.[43-48] Information on these changes are essential for developing stimulation protocols, since functional activation of denervated muscles requires electrical stimulation with long impulse duration in the range of 20 – 150 (up to 300) msec. Moreover, contrary to innervated muscle where the nerve distributes the action potential, in the denervated muscle an electrical field distribution capable of depolarizing the fibers in almost every part of the muscle has to be achieved. Therefore, to counteract the deterioration of the denervated muscle a therapy concept for home-based electrical stimulation was developed. To carry out the training a stimulator suited to deliver the necessary high-intensity and long duration impulses by new large electrodes was designed.[49,50] Specific clinical assessments to monitor the condition of the patient's muscles and guidelines for training were developed at the Wilhelminenspital Wien, Austria.[51,52] The novel therapy concept, together with newly designed devices, was evaluated in the RISE clinical study. After completing a 2-year home-based therapy program the subjects showed a significant increase of muscle mass and myofiber size, improvements of the ultrastructural organization and recovery of tetanic contractility with significant increase in developed muscle force output during electrical stimulation.[43,44] EU-approved products (Stimulette den2x) and purpose developed large safety electrodes are now commercially available (Schuhfried, Vienna, Austria).[43,53-57]

However, even these new tools are effective in preventing or recovering denervated degenerating muscle only if h-bFES starts early than ten years from SCI, with the best results achievable up to 6 years from permanent nerve lesions.[43-60]

Induced muscle fiber regeneration in permanent denervation of skeletal muscle: Implication for FES of long-term denervated muscles

The differentiation of muscle fibers regenerating in the absence of the nerve is well documented in animal experiments and in muscle biopsies harvested from human patients suffering with permanent long-term denervation.[43,44,58,59] During the last twenty years, clinical studies have employed long impulse biphasic electrical stimulation as a first step treatment for humans living with long-term denervated muscles subsequent to SCI. Trophic and functional recovery, from severe atrophy/degeneration due to lower motoneuron damage, occurred in long-term denervated degenerating muscles treated with two years of h-bFES beginning from 1 to 5 years from SCI using purpose-developed muscle stimulator and large electrodes that recently were made commercially available.[43,44] This fact has sound foundations on muscle biopsy analyses,[43,44-60] and on Quantitative Muscle Color Computed Tomography (QMC-CT) of treated muscles.[58-68] On the other hand, the extent of recovery decreases with time elapsed from SCI with poor results after 7 to 10 years from lower motoneuron damage.[43,44] If induced-myogenesis,[5,69-81] could be modulated in patients during the many months needed to recover tetanic contractility of denervated muscles, the period to achieve functional recovery of long term denervated human muscle by h-bFES will be shortened and possibly obtained also when h-bFES will be started later than ten years from SCI.

Recent advances on conversion (i.e. transdifferentiation, see https://stemcells.nih.gov/info/basics/4.htm)[82] of adult differentiated somatic human cells, e.g., surface epithelia and fibroblasts, to fully potential stem cells able to induce and maintain high-level myogenesis, may provide personalized treatments that may open new hope for the vast majority of people in need, i.e., those patients at more than 8 years from SCI.[43,44] For more detailed discussion of stem cells potentials, see at: https://stemcells.nih.gov/info.htm a NIH's Stem Cell Reports.[83,84]

Histopathological analyses of skin in DDM: New options for h-bFES and beyond

The skin is the body's heaviest sensory organ, accounting for approximately 16% of the body's weight. Other functions are protection from chemical, physical and biological insults and maintenance of the internal environment.[85] Several pathologies are associated to skin changes affecting skin cells and other structural proteins, thickness of the various epidermal layers, inflammatory cells, and amount of water.[86] Qualitative and quantitative analysis of several components and properties of the skin are necessary to understand these disorders and to follow-up eventual managements. Previous studies have shown that denervated

Quadriceps muscles of patients suffering with complete *conus* and *cauda equina* lesions were rescued by two years of home-based functional electrical stimulation (h-bFES) using a new electrical stimulator and very large skin electrodes.[43,44] Muscle mass, force, and structure of the stimulated Quadriceps muscles were studied before and after 2 years of h-bFES, using: Computed Tomography (CT), measurements of knee torque during stimulation, and muscle biopsies which were analyzed by light and electron microscopy. To harvest muscle biopsies the overlying skin was also collected and evaluated by histological morphometry of hematoxylin eosin (H-E) and immuno-stained on paraffin-embedded sections. Analysis of the structural characteristics of epidermis, i.e., thickness, morphology of the papillae and content of hairs together with some neural and inflammatory molecular markers were organized. Preliminary results are interesting and stimulate additional analyses to better describe skin adaptation to this peculiar type of electrical stimulation by surface electrodes. Those approaches will offer also new opportunities to study adaptation of the skin to other physical and pharmacological therapies, e.g., based on application of rehabilitation managements through the skin,[87] in particular for pain relief.

Candidate biomarkers for testing Cayenne pepper cataplasm (CPC) treatment for low back pain

Herbal cataplasms containing rubefacient substances, such as Cayenne pepper, are commonly used as natural medications to treat painful areas. In this perspective we summarize the effects of a 20-min application of a mixture of Cayenne pepper and kaolin powder cataplasm on healthy subjects. Treatments were evaluated by: cold/hot feeling, blood pressure, body temperature, skin light touch sensation, two-point discrimination, pressure algometry, before and 0/15/30 min after different concentrations of Cayenne pepper. We tested for its safety by measuring changes in circulating levels of inflammatory-related biomarkers. Results confirmed that 5% concentration CPC did not induced a significant increase of circulating inflammatory–related biomarkers (Sarabon N et al., submitted).[88-92] Further studies are mandatory to confirm evidence-based efficacy of CPC and of the involved mechanisms of pain relief.

Mitochondrial dynamics, molecular pathways activation and muscle remodeling after Electrical Stimulation in aged human muscle

Age-related sarcopenia is characterized by a progressive loss of muscle mass with decline in specific force. The etiology of sarcopenia is multifactorial and underlying mechanisms are currently not fully elucidated. Physical exercise, including electrical stimulation assisted muscle contraction,[93] is known to have beneficial effects on muscle mass and force production.[94,95] Alterations of mitochondrial Ca^{2+} homeostasis regulated by mitochondrial calcium uniporter (MCU) have been recently shown to affect mice muscle trophism *in vivo*.[96] To understand the relevance of MCU-dependent mitochondrial Ca^{2+} uptake in aging and to investigate the effect of physical exercise on MCU expression and mitochondria dynamics, we analyzed skeletal muscle biopsies from 70-year-old subjects 9 weeks trained with either neuromuscular electrical stimulation (NMES) or leg press. We demonstrated that improved muscle function and structure induced by both trainings are linked to increased protein levels of MCU.[97] Ultrastructural analyses by electron microscopy showed remodeling of mitochondrial apparatus in ES-trained muscles that is consistent with an adaptation to physical exercise, a response likely mediated by an increased expression of mitochondrial fusion protein OPA1.[98] Altogether these results indicate that the ES-dependent physiological effects on skeletal muscle size and force are associated with changes in mitochondrial-related proteins involved in Ca^{2+} homeostasis and mitochondrial shape. Indeed, calcium cycling and activation of specific molecular pathways are essential in contraction-induced muscle adaptation, being important regulators of metabolic and Excitation – Transcription Coupling (ETC). An experimental protocol was set-up starting from muscle biopsy sections that can be obtained before and after a period of electrical stimulation on volunteers. By this approach, nuclear localization of specific transcription factors such as NFAT or PGC1α and phosphorylation level of regulative kinases can be compared in a muscle before and after training to study activation of related pathways. Moreover, it was shown that down-stream targets of muscle contraction induced pathways such as Ca^{2+} handling proteins of the Sarcoplasmic Reticulum are modulated by ES (Mosole et al., submitted manuscript), supporting the conclusion that ES is able to promote fiber remodeling in sedentary seniors.[98-102] These results support the clinical findings related to physical activity in elderly and validate ES when seniors can't or wouldn't perform volitional exercises.[103]

Perspectives

In conclusion, the present information summarizes some of the basic concepts and of the evidence-based tools for developing further translational muscle research activities.[104-113] Clinically relevant results will ask for continuous interests of Basic and Applied Myologists and for the support during the next five to ten years of granting agencies. We are confident that we will proceed steps by steps toward the approval of our research projects and clinical trials. This was true in the past.[43-60] It will be hopefully true in the future. All together our efforts would end in protocols, devices and personalized managements for persons suffering with muscle denervation, neuromuscular–related or non–related pain and for the increasing number of old, older and oldest citizens of Europe and beyond.

List of acronyms

BAM – Basic Applied Myology
BEM – Biology, Engineering, Medicine
CIR Myo – Interdepartmental Myology Center of the University of Padova, Italy
CPC – Cayenne pepper cataplasm
CT – Computed Tomography
DDM – denervated degenerating muscles
EJTM – European Journal of Translational Myology
ES – electrical stimulation
ETC – Excitation–Transcription Coupling
EU – European Union
FES – Functional Electrical Stimulation
h-bFES – home-based Functional Electrical Stimulation
MCU – mitochondrial calcium uniporter
NMES – neuromuscular electrical stimulation
PMD – PaduaMuscleDays
QMC-CT – Quantitative Muscle Color Computed Tomography
RISE – Use of electrical stimulation to restore standing in paraplegics with long-term DDMs (QLG5-CT-2001-02191)]
SCI – Spinal Cord Injury

Author's contributions

Sascha Sajer designed and implemented the typescript.

Acknowledgments

The author thanks Helmut Kern, Christian Hofer, Stefan Loefler and Sandra Zampieri for comments and critical readings of the typescript. The typescript was sponsored by Physiko- und Rheumatherapie, St. Poelten, Austria and by the A&C M-C Foundation for Translational Myology, Padova, Italy.

Conflict of Interest

The author declares no conflicts of interests.

Ethical Publication Statement

Author confirms that he has read the Journal's position on issues involved in ethical publication and affirms that this report is consistent with the guidelines of the EJTM.

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