Severely atrophic muscle morulae, markers of long-term denervation, are 3 to 5 years denervated human myofibers

N. Adami (1), S. Zampieri (1), D. Biral (2), U. Carraro (1,2), C. Hofer, S. Loeffer, M. Vogelauer, H. Kern (3)

(1) Laboratory of Translational Myology, Interdepartmental Research Center of Myology of the University of Padua; (2) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, Padova, Italy; (3) Department of Physical Medicine and Rehabilitation, Wilhelminenspital, Wien, Austria. E-mail: nicoletta.adami@unipd.it

The long-term denervated human muscle fibers in complete Conus Cauda Syndrome present the peculiar features of the severe atrophy, that is, they completely lose the myofibrillar apparatus and present nuclear groupings, which produce myofiber cross-section a feature described as “morulae” [1,2,4]. This very late stage of denervation atrophy appears 3 years after the spinal cord injury (SCI) and lasts at least up to 9 years, when fibrosis takes over to neuro-missing severe atrophy. Meantime, adipocytes fill some of the empty spaces of the muscle (lipodystrophy). After 2 or more years of FES-training for denervated muscle (with a five days per week stimulation program), these aspects are very rare between 3 and 6 years from SCI, while the majority of myofibers are large myofibers, that recovered the structure and the mass, up to the level of the muscle fibers we described in long-term spastic paraplegia (15-20 years from the SCI) [3]. These results provide the rational to plan researches aimed to recover these severe atrophic muscle fibers, when FES of denervated muscle ought to be started several years after SCI. Furthermore, our observations strongly suggest that the “morulae” present in muscles of Amyotrophic Later Sclerosis, and of other neurodegenerative muscle atrophies, are from 3 to 5 years denervated muscle fibers, i.e., the consequences of spinal motor neuron lesions that long-precede the clinical onset of the disease.


[4] Kern H, Carraro U, Adam N, Biral D, Hofer C, Stefan Loeffer S, Vogelauer M, Mayr W, Rupp R, Zampieri S. One Year of Home-based Functional Electrical Stimulation (FES) in Complete Lower Motor Neuron Paraplegia: Recovery of Upper Motor Neuron lesion on human muscle by studying before and after FES training morphological and biochemical parameters in quadriceps muscle of 1 to 20 years after spinal cord lesion (SCI). The mean diameter of all types of muscle fibers in the upper motor neuron denervated patients before-FES is 34.4+/-19.1, while the mean fiber diameter of post-FES patients is 53,0 +/- 24.6 (+54% increase). Histo-chemical Myosin ATPase properties of the fibers allow to distinguish three types of fibers, i.e. type I, type IIA, type IIB. In normal human muscle around 40% are of type I, 10% of type IIA, and 50% of type IIB. After 1 year of SCI the biopsies from UML patient shows that about 30% of fibers are of type I, 15% are of type IIA, and 55% of type II B. The percentages decrease to 15% (type I) and 8% (type IIA), while type IIB increase to 76% in the 3 years after SCI muscle biopsies. In the very long-term UML patients (more than 5 and up to 20 years from SCI) only 5% of the fibers are of type I, the remaining fibers (95%) being of type IIB. The same amount of type I fibers is found in these patients after 2 years of training. Since the diameter of the slow type I muscle fibers do not change with the progression of SCI time or after FES-training, the size increase is restricted to fast type muscle fibers (in particular IIB fibers). Accordingly, staining for the succinic acid dehydrogenase, an enzyme of the tricarboxylic acid cycle of the mitocondria, decreases with SCI time, but do not disappeared, being also present in the long-term upper motor neuron lesion (more than 15 years). The results here reported on ATPase staining are in agreement with our previous ultrastructural analyses [4], which showed that the ultrastructure of the myofibrillar apparatus is well preserved in these paraplegic patients.


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Structural rescue of long-term denervated skeletal fibers by functional electrical stimulation (FES)


(1) IIM - Interuniversity Institute of Myology, CeSI - Centro Scienze dell’Invecchiamento, Università degli Studi G. d’Annunzio, Chieti, Italy; (2) Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital, A-1171 Vienna, Austria; (3) C.N.R. Institute of Neuroscience, Laboratorio di Miologia Applicata, Dipartimento di Scienze Biomediche, Università di Padova, Italy; (4) Department of Biomedical Engineering and Physics, University of Vienna, Austria. E-mail: fprotasi@unich.it

An essential question, important especially for the treatment of SCI patients, is whether it is possible to reverse muscle wasting after long-term denervation in the absence of nerves. Up to date, it is generally accepted that no effective treatment is available for rescuing human muscles that have undergone severe atrophy as a result of a long-standing complete denervation. Biopsies from patients enrolled in the RISE project offered the unique opportunity of studying structural recovery of human muscle fibers from severe atrophy induced by FES in the total absence of motor and sensory innervation. FES treatment induced surprising recovery of muscle structure mass and force in the patients that were effectively stimulated, even after prolonged denervation. Analyzed fibers show a striking recovery of the ultrastructural organization of myofibrils and Ca2+ handling membranes. Thus, despite the almost complete loss of muscle-specific structure, the long-term denervated fibers maintain the capability of a full differentiation program. Interestingly, this almost complete recovery follows a pattern that mimics in many aspects normal muscle differentiation. The effectiveness of this FES treatment augurs well for the future rehabilitation and/or treatment of SCI patients affected by complete lesions of the spinal cord (missing peripheral nerves), who currently do not receive any specific treatment of the denervated extremities.

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Epidemiology of Cauda Equina syndrome in the Spinal Unit of the Vicenza General Hospital

A. Borghero, R. Duca, M. Leucci, F. Cortese

Unità Spinele /Unità Gravi Cerebrolesioni, Dipartimento Funzionale di Riabilitazione. Azienda ULSS 6 Vicenza, Italy E-mail: feliciana.cortese@ulssvicenza.it

The Spinal Unit of the General Hospital of Vicenza (VGH) hospitalizes patients with Spinal Cord Injury (SCI) after discharge from Neurosurgery and Reanimation Divisions of Veneto Hospitals. The Spinal Unit is one of the two Regiono Veneto High Specialization Rehabilitation Units, whose standards are those of The Istituto Superiore di Sanità (ISS), ISS is the leading technical and scientific public body of the Italian National Health Service, whose activities include research, control, training and consultation in the interest of public health protection. The Cauda Equina Syndrome is characterized by bladder and intestines areflexia, legs flaccid paralysis and pelvic anesthesia. It is a "rare disease": incidence per year is 3.4 per million people, and prevalence 8.9 per 100.000 Italians. It affects adults, tough any-age patients are possible. The most common etiology is lumbar slipped disc. We revisited 159 stories of SCI patients at their first admission to the Spinal Unit of VGH from 01/01/05 to 30/06/08. Demographic data, etiology, the need of surgery, duration of hospitalization, bladder managements, and mobility at admission and discharge were collected. The mean age was 43 years (range 17-73); 13 males e 6 females. From 2005 to 2008 we observed an increase of the syndrome. In our sample the etiology was traumatic in 12 patients, non-traumatic in 7 patients. Road accident was the main traumatic cause (6 pt); among non-traumatic the most representative was post surgery (4). 16 patients were workers or students. 4 and 15 patients were classified complete and incomplete lesion respectively (ASIA Scale). At the beginning all 4 inpatients with complete lesion had permanent bladder catheter and all of them passed through intermittent catheterism becoming outpatients. About the 15 patients with incomplete lesion, at the discharge 1 had permanent bladder catheter, 6 intermittent catheterism and 8 normal function. Walking: of the 4 patients with complete lesion, at discharge 2 used only wheelchair for self transfer, 1 used common leg braces with walking aid (e.g. crutches) and 1 just one crutch. Of the 15 incomplete-lesions, at the discharge 10 did not need wheelchair for transfer: among them 2 used walker (1 with leg braces); 4 used walking aids (2 with leg brace); 3 used just one crutch, 1 used AFO. All patients have been treated with conventional rehabilitation strategies for enhancing lower limbs function. At the same time these patients had active exercise for upper limbs and training for daily mobility tasks (transferring, bed mobility and sitting). This is a pretty opportunity to engage a long and wide road that leaded us to be partners of the 2008Rise2-Italy Project. We have not long
experience with electrical stimulation, but we believe that we have to consider this approach to give more chance to our patients in their recovery and possibly in less time.

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Skeletal muscle and inflammation: the heterogeneous immunological synapse

C. Borsato, C. D’Ascenzo, L. Bello, V. Codemo, C. Semplicini, V. Cima, M. Volpe, K. Koutsikos, V. Romeo, C. Ferrari, R. Dal Borgo (1), R. Stramare (1), M. Fanin, G. Sorarù, E. Pegoraro, C. Angelini

Department of Neurosciences and (1) Department of Radiology, University of Padova, Italy.

E-mail: carlo.borsato@unipd.it

The inflammatory myopathies comprise three major and distinct subsets: polymyositis (PM), dermatomyositis (DM) and sporadic inclusion-body myositis (sIBM). Although the presence of muscle weakness and endomysial inflammation are common features in all these conditions, unique clinical, immunopathologic and histologic criteria, along with different prognosis and response to therapies, characterize each subset. DM develops subacutely in young patients and affects skin (heliotrope rash, Gottron papules) and proximal muscles. PM involves adults patients, older than 18 years and has a subacute onset and selectively affects the proximal muscles but spares the skin. sIBM involves adults patients over 50 years and presents a slow onset and progression, affects both the proximal and the distal muscles, and results in significant weakness and atrophy. The muscles of swallowing are also affected and choking episodes are frequent. The diagnosis of these disorders is based on the combination of serum muscle enzymes, electromyography, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy. The CK is elevated in all three subsets, and muscle biopsy.
muscle fibers undergo to chronic inflammatory stress, they can release factors (e.g. NO, μPA, MCP-1 chemokines) that protect themselves from injury, promote repair and activate satellite cells by interaction with macrophages: muscle fibers drive the change of the macrophagic profile from the “early-phagocitic” to the “late-repairing” pattern. At earlier stages, the macrophages recruited by lymphocytes can play an important role in promoting muscle damage by releasing cytokines (e.g. TNF-α), free radicals and by NO-dependent mechanisms. At later stages, macrophages contribute to removal of cellular debris, muscle growth and repair by realising HB-EGF, TNF-α and NO which improve muscle recovery. Recent findings show that the role of some cytokines and molecules (e.g. NO, TNF-α) secreted both by muscle fibers and macrophages, in muscle injury and repair may vary with the type, severity, location and stage of injury. All these events could represent possible new targets for present and future therapy. Available biological agents can be subdivided in three subsets. The first subset represents a selective, antigen-specific immunotherapy which targets the complex of T-cell stimulation, MHC-I and TCR, but application is unrealistic at the present time because the antigen is still unknown. The second subset represents semi-specific therapies using agents and biologicals aimed at various targets of immunopathological network. These targets include intracellular signalling pathways associated with antigen recognition and costimulation; complement activation; cytokines and chemokines; molecules associated with B and T-cell activation, proliferation and transmigration. Agents targeting these molecules are mostly monoclonal antibodies. The last subset comprises conventional, non specific immunosuppressive or anti-inflammatory therapies that include steroids, azathioprine, mycophenolate, methotrexate, cyclophosphamide and cyclosporine.

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Oxidative stress, membrane damage and [Ca2+]i unbalance in longstanding denervated skeletal muscle

G. Bosco (1,2), S. Belia (1,2), H. Kern (3), U. Carraro (1,4), G. Fanò (1,2)

(1) Interuniversity Institute of Myology; (2) BAMS - Department of Basic and Applied Medical Sciences, “G. D’Annunzio” University, Chieti-Pescara, Italy; (3) Department of Physical Medicine and Rehabilitation, Wilhelmenspital, Wien, Austria; (4) Laboratory of Translational Myology, Interdepartmental Research Center of Myology, University of Padua, Italy. E-mail: fano@unich.it

Denervated muscles derived from complete lesion of lower motor neurons have been widely studied in different models e.g. rats, rabbits, frogs and humans [1-4]. However, long-term effects of denervation attracted much less attention considering that, after a relative short time, recovery is not expectable, if spontaneous reinnervation do not occur or FES treatment is not applied [5]. However, it has been suggested that muscle atrophy may be triggered by reactive oxygen species (ROS) which are formed in all tissues including the skeletal muscle by [6]. As a consequence of this, the intra and intercellular membranes of the muscle fibers, may be modi- fied and the Ca2+ transport mechanism altered. Aim of this work is to verify this hypothesis utilizing a cell free approach in chronically denervated muscle of rats. Sarcolemmal fraction was purified essentially as indicated in [6] while SR membranes were prepared by the method of [7]. The activity of intracellular antioxidant apparatus was determined as indicated by [7] Specific binding methodologies were applied to measure the presence of DHPR and RYR-1 Ca-channels and the activity of extrusion Ca-pumps were based on methods of Belia et al 1998 [8]. Results of our experiments show that all the parameters measured are drastically reduced in the samples derived from denervated muscles respect to controls. No time-dependence was evident under our experimental conditions excepted for the activity of sarcolemmal Ca-ATPase, which showed a significant decrease directly correlated to denervation period. In conclusion the data here reported are in accordance with the hypothesis that Ca-homeostasis in the muscle fiber is drastically altered by permanent denervation. This alteration appears very early, and it is maintained at comparable level throughout the investigated period.


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Impact on clinical results of the muscle fiber to non- muscle fiber tissue ratio in muscle biopsies of the European Project Rise

U. Carraro (1,2), N. Adami (1), D. Biral (2) S. Zampieri (1), H. Kern (3)

(1) Laboratory of Translational Myology of the Interdepartmental Research Center of Myology, University of Padova; (2) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, Padova, Italy; (3) Department of Physical Medicine, Wilhelmenspital. A-1171 Vienna, Austria; E-mail: ugo.carraro@unipd.it

Spinal-cord injury causes loss of function and muscle
atrophy, which is especially severe when lower motor neurons (LMN) are involved. A longitudinal study in 25 Europeans suffering of complete Conus Cauda Syndrome from 0.7 to 8.7 years compared thigh muscle properties before and after two years of home-based daily Functional Electrical Stimulation (FES). Muscles were stimulated by large surface electrodes and a custom-designed stimulator. Muscle poor excitability was first improved by twitch contraction training. Tetanic contractions without and, later on, with increasing load were then elicited. Finally, standing-up exercises were daily performed. The bulk of thigh muscles were estimated by transverse CT scan and force measurements. Needle muscle biopsies were harvested before and after two years of FES and analyzed by light microscopy. Two years of home-based daily FES: 1. Induced similar muscle recovery in both legs, as shown by CT scan (+35%), biopsy morphometry (+75% in mean fiber size). Comparison of fiber size profiles shows that this is the result of a shift toward larger-size muscle fibers. Percentage of the cryosection covered by muscle fibers was 30.3 +/- 23.2 in the group of 3-5 years SCI, while it was 52.8 +/- 10.8 (a +74 % increase, p < 0.004) 2 in the group of 3-5 years SCI, who during the last two years were compliant to home-based FES training. Despite the additional two years of denervation none of 20 compliant subjects worsened, while 5 (25%) increased muscle force (+ 900%) up to allow standing-up. The EU Project Rise demonstrates that FES is an effective home-based therapy that may maintain life-long motivation to perform active exercise (electrical stimulation is the only option for denervated muscle), together with other training strategies of leg blood perfusion, as an adjuvant measure to option for denervated muscle), together with other training.


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Calsequestrin 1: a new candidate gene for Malignant Hyperthermia (MH) and Exertional/Environmental Heat Stroke (EHS)

M. Dainese (1,2), M. Quarta (2*), A.D. Lyfenko (4), C. Paolini (1,2), M. Canato (1,3), R.T. Dirksen (4), C. Reggiani (1,3), F. Protasi (1,2). E-mail: fprotasi@unich.it

(1) IIM; (2) CeSi - Università G. d’Annunzio, Chieti, Italy. (3) Dept. of Anat. and Phys., Univ. of Padova, Italy; (4) Dept. Pharm. and Phys., Univ. of Rochester, NY., USA

Malignant hyperthermia (MH) and exceptional/environmental heat stroke (EHS) present as life threatening crises triggered by volatile anesthetics and strenuous exercise and/or high temperature, respectively. MH and EHS episodes are characterized by uncontrolled elevations in core body temperature, rhabdomyolysis and are observed to a greater extent in males. Many, but not all, families (70-80%) diagnosed with MH susceptibility (MHS), and a few with EHS, are linked to mutations in the gene that encodes the Ca2+ release channel (RyR1) of muscle. Thus, other MH gene loci remain to be identified. In the present paper, we investigated whether a MH/EHS-like phenotype results from deficiency in skeletal muscle calsequestrin (CASQ1), a SR Ca2+-binding protein that modulates RyR1 function. Strikingly, both halothane and heat challenge trigger lethal MH/EHS-like episodes characterized by elevated core temperature and rhabdomyolysis in male CASQ1-null mice. These episodes are prevented by prior dantrolene administration, the standard treatment for MH episodes in humans. Skeletal muscle from CASQ1-null mice exhibit increased contractile sensitivity to caffeine, temperature-dependent increases in resting Ca2+, and an increase in the magnitude of depolarization-induced Ca2+ release. These findings validate CASQ1 as a candidate gene for linkage analysis in MH and EHS families where mutations in RyR1 are excluded.

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08-Rise2-Italy Trial: How to enroll and start stimulation. A case report

M. Ferraro (1), S. Masiero (1,2), U. Carraro (2), R. Marenzi (1), H. Kern (3)

(1) Rehabilitation Unit of the University-Hospital of Padua; (2) Laboratory of Translational Myology of the Interdepartmental Research Center of Myology, University of Padova, Italy; (3) Department of Physical Medicine, Wilhelminenspital. Vienna, Austria.

E-mail: dott.ferraro@libero.it

The Rise Project opens innovative perspectives about a comprehensive rehabilitation approach to "chronic and complete" SCI (spinal cord injury) patients. Health maintenance, social participation and personal body image values may change during time and aging of these people. Denervated muscles of the spine and lower limbs change their biological properties. Loss of sensibility and muscles changes are well-known causes of pressure ulcers. Loss of muscle cells is important for negative effect of gravity on neuropathic pain, cardiac workload, venous blood pressure, respiratory reserve, personal and social body image. Actually electrical stimulation devices of Italian rehabilitation centers are not useful to stimulate a complete
denervated muscle (warm and hot effect and neuropathic sensation are common effects of those devices on incomplete SCI patients). Social contact and personal values of patients and rehabilitors (including biomedical experts, engineering experts and bioscience experts) can help to start this integrative project. A personal experience is here described. A young male patient, 30 years old was recovered 9 months ago in a Intensive Care Unit for a car-crash injury. Reported injuries were: a mild brain traumatic injury scored 14 by GCS (a closed-head injury), multiple bone fractures: thoracic and lumbar bone fractures, rib fractures, right pneumothorax. Patient was sedated and a tracheal intubation was performed (artificial ventilation was performed for 7 days, anesthesiologic sedation lasted 6 days). First neurologic examination diagnosed a severe spinal cord injury (spinal cord traumatic injury) and a spinal stabilization surgery was performed after 10 days. After 6 months of ICU (intensive care unit) the patient was treated in a rehabilitation unit and evaluated by the rehabilitative team. A L1-complete-SCI (no motor or sensory sensation below L1 level, no anal sensation) without pressure ulcers was diagnosed and a rehabilitative project was to ameliorate functional independence and social participation. Patient learned to use a manual wheelchair, to upper and lower body dressing ability, to self-perform intermittent catheterization for a severe urinary retention (urologic diagnosis was a complete denervated vesical muscle), to use devices for fecal elimination. Sexual complete impotence and endocrinologic syndrome was also diagnosed. The family of the patients was involved in this learning process. After dismissal from the rehabilitation unit, 4 months later, the patient was taught to use technologic devices for independent car driving. Clinical integrative evaluation performed by a translational myologist and physiatrist considered a potential clinical amelioration of reduced gluteal and quadriceps muscles to prevent pressure ulcers and ameliorate respiratory and global resistance to "normal" daily activities workload. Electrical stimulation test performed by a myologist and a global water aerobics exercise program was performed. The RISE Project was explained to the patients and to his family. The technical machine used for the electrical stimulation (a FES device) is not a commercial device used in rehabilitation centers. The first myology evaluation test diagnosed a selective excitationally muscles pattern and a non-muscle-tissue fibrotic syndrome was diagnosed. The myologist and physiatrist decide to ask the patient about a participation to the RISE Project. A physiotherapist planned a treatment for the fibrotic syndrome (hyperflexion of ischiocrural tendons) by using water exercises and stretching programs. A quantitative analysis of muscle dimension was planned. The patient gave the consent to be evaluated in Vienna by dr. Helmut Kern for enrolment in the RISE Project, that include a pre-training and a post-training muscle biopsy. After instruction for the use of the electrical stimulator for denervated muscles, the Informed Consent Form was signed September 12, 2008. Then, both final evaluation for enrolment and pre-FES muscle biopsies were performed in Vienna [1]. The program with the special electrical device for home-based training (not available in Italy) is integrated in a rehabilitative plan and is used to ameliorate denervated muscles properties and to prevent ulcer pressure. Quantitative imaging of thigh muscles will be performed during and at the end of the two-year FES training by Ultra Sound (US), MRI, and/or TO scan.


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Muscle stretch improves external Ca2+ influx in denervated skeletal muscles of the rat

F. Francini, R. Squecco

Department of Physiological Sciences, University of Florence, Italy. E-mail: fabio.francini@unifi.it

Denervated skeletal muscle shows a progressive atrophy and a drastic loss of the sarcromeric proteins. The aim of this work was to evaluate the changes of the mechano-sensitivity in long term denervated soleus muscle of the rat. By intracellular microelectrodes we evaluated the passive properties of the sarcolemma and the presence of functional L-type Ca2+ channels and stretch activated channels (SAC). Our results demonstrate that denervated muscle had a reduced sarcomeric resistance, a depolarized resting membrane potential and an increased mechano-sensitivity. This latter included a greater sensitivity to passive stretch of L-type Ca2+ channels and a larger expression of SACs. The mechano-sensitivity increased progressively with the time elapsed from the muscle denervation, so it is strongly evident in long-term denervated skeletal muscles. Such changes allowed an improved influx of external Ca2+ that may activate Ca2+-dependent signalling pathway (as calcineurin) to the nucleus and therefore to induce the expression of...
muscle specific proteins, to increase the muscle mass and to restore the metabolic and contractile machinery. The observed effects could indicate a role of passive movement in the denervated human muscle limbs in reducing their atrophic state. Moreover, functional electrical stimulation (FES) could be also improved by passive movement for the presence of functional L-type Ca²⁺ channels. The depolarized resting membrane potential observed in denervated skeletal muscle could explain the automatic spontaneous activity (muscle fibrillation) observed in denervated muscles.

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Quantitative Fat and Muscle changes in DDM undergoing Functional electrical stimulation (FES)

P. Gargiulo (1,2), B. Vatnsdal (1,2), P. Ingvarsson (1), V. Gudmundsdottir (1), S. Knutsdottir (1), S. Yngvason (1) ’T. Helgason (1,2),
(1) Landspítal – University Hospital; (2) University of Reykjavik, Iceland.
E-mail: paologar@landspítal.is

FES is applied to severely disabled patients with DDM in lower limbs to restore muscle tissue and function. Very important is to monitor and quantify changes induced by the stimulation treatment in order to understand the restoration process and possibly improve it. For this reason medical imaging from MRI and CT-Scan data combined with image processing tools and 3-Dimensional modelling are used to monitor the muscle growth. The modelling work can provide accurate information in term of volume and density changes on the whole thigh and on defined region of interest. Beside the different tissues on the thigh can be discriminated allowing monitoring of changes in muscle, bone and fat tissue. The degeneration process is characterised with replacement of muscle by fat and fibrous connective tissue, this degeneration can be reversed by using electrical stimulation. The research target in this work is to quantify these changes in muscle and fat tissue. The quantitative measurement made in this study show the dramatic relation between stimulation and non-stimulation with the induced changes of muscle and fat tissue in the thigh. Volume and density in muscle can increase in one year up to 50% and 30% respectively.

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Patella bone density by denervated and degenerated thigh muscles

T. Helgason (1,2), P. Gargiulo (1,2), B. Vatnsdal (1,2), P. Ingvarsson (1), V. Gudmundsdottir (1), S. Knutsdottir (1), S. Yngvason (1)
(1) Landspítal – University Hospital; (2) University of Reykjavik, Iceland.
E-mail: thordur@landspítal.is

During the degeneration process of thigh muscles after a conus cauda or cauda equina lesion resulting in a total denervation of the muscles the bone density is also diminishing. During the process the patients never stand in their feeds loading the leg bones. The muscles below the lesion are never contracting and thus never apply a force on the bones. The only forces on the bones are the gravitational forces on them and the thigh muscles. During an electrical stimulation therapy (EST) of the denervated and degenerated muscles (DDM) their mass and to a great extent their force can be restored. This has been shown by several groups and is beyond any reasonable doubt. A therapy of this kind was applied to three patients over a period of five years (2003 - 2008). Electrodes where placed on the skin above the quadriceps muscle group and it stimulated for 20 to 60 minutes, once a day, six days a week. This did build up the rectus femoris muscle, which pulls the patella bone. In this work we look at the density of the patella bone. The patella is different from the femur or the other leg bones in that it is only pulled and not pushed. Another difference is that it is not inside the current path between the stimulating electrodes, therefore the electrical current should not be influencing its density changes. Observations from CT slices indicate a correlation between break down and build up of the patella bone density on one side with the size of the rectus femoris muscle on the other side.

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Membrane action potential of human long-term denervated muscle

C. Hofer (1,2), W. Mayr (2), C. Forstner (3), M. Mödlin(3), H. Kern (1,3)
(1) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (2) Center for Biomedical Engineering and Physics, Medical University Vienna, Austria; (3) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Vienna, Austria.
E-mail: christian.hofer@wienkav.at

Long-term denervation of a muscle after conus cauda or cauda equina lesion causes structural, electrophysiological and biomechanical changes of the muscle. To assess changes of the electrophysiological properties of long-term denervated muscle fibres, conduction velocity and shortest inter-stimulus interval were studied using needle stimulation (single and double pulses) and needle EMG recording. In the examined patients a markedly decreased muscle fibre conduction velocity (MFCV, 1.0 - 2.1 m/s) compared to healthy muscle (3.5 m/s) was measured before start of electrical stimulation training. After two years of training with functional electrical stimulation MFCV was increased but not reaching standard values (2.2 – 2.4 m/s). The shortest inter-stimulus interval (ISI) showed a decrease (from 4.0 - 8.0 ms to 3.3 - 3.0 ms). This indicates that the muscle fibre membrane recovers faster after depolarisation, when having been stimulated for a prolonged period of time. The findings give evidence that therapy of denervated muscle with electrical stimulation is able to reverse denervation related changes in the electrophysiological parameters MFCV and shortest ISI after denervation to near normal values.

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denervated fibers can maintain membrane excitability longer time, in vitro electrophysiological recordings show that population of very small, but vital myofibers. At the same myofiber death/regeneration processes maintain a decreasing undergoes a dramatic weight loss; during this process, months from severe atrophy to a dystrophic stage and In adult rats, the sciatectomized muscle progresses in 4-6 motoneuron denervated myofibers may survive several years. shown that after complete Conus Cauda lesion, the lower motorneuron denervated muscle the expression of 12 selected genes was differentially regulated at 3- and 9-month denervation. At both time points, indexes of muscle activity/inactivity and tissue re-modeling (protein synthesis, energy usage, angiogenic factors) were down-regulated, while indexes of regenerative myogenesis (Myogenin, MyoD, Mr4, MHCemb) were up-regulated. Immunohistochemistry with anti-MHCemb and anti-N-CAM monoclonal antibodies show that such regeneration events were focally distributed. We conclude that myofiber regeneration is a non-compensatory mechanism, which prolongs the chance of re-innervation during long lasting denervation. It may also contribute to muscle recovery in paraplegic patients, even when rehabilitation strategies based on Functional Electrical Stimulation (FES) start late after Spinal Cord Injury (SCI).

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New devices for muscle stimulation and testing
W. Mayr (1), C. Hofer (1,2), H. Kern (2), M. Bijak(1), H. Lannmüller (1), D. Rafolt (1), E. Unger (1), H. Stöhr (3)
(1) Center for Biomedical Engineering and Physics, Medical University Vienna, Austria; (2) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Vienna, Austria; (3) Center for Biomedical Research, Medical University Vienna, Austria. E-mail: winfried.mayr@meduniwien.ac.at

The technical outcome of the European research and development project RISE includes novel equipment for stimulation and functional assessment of denervated muscles which had been missing for both efficient therapy after peripheral lesion and associated research initiatives. Traditional functional electrical stimulation (FES) targets neural structures and indirect control of muscle contraction. The recent EU FP5 project RISE has demonstrated the feasibility and effectiveness of direct FES of denervated muscles. The main difference between nerve and direct muscle stimulation is the required impulse width of the applied stimuli. Nerves are stimulated with less than 1 ms, muscles with 30 to 200 ms at comparable intensity levels. The resulting charge per impulse and average electrical power levels are critical in applications of both surface electrode based and implantable stimulation systems. In non-invasive systems up to 30 mC of impulse charge and 25 W of power may occur. To minimize the risk of skin damage a large contact surface and holohedral current distribution are essential in design and handling of the electrodes. Stimulators must guarantee charge balance and zero DC at the outputs and must include all thinkable safety monitoring, operation and handling features to avoid failures in daily home based routine treatment. A suitable dual-channel system was developed in RISE and successfully tested in a patient study for a period of more than 2 years. It is currently in transfer to industry. The Austrian medical company Schuhfried has licensed a patent (WO2007131248, Winfried Mayr) on
special electrodes, that reduce the risk of skin burns, and the developed stimulator. The company aims to make the electrodes and a dual-channel stimulator available on the European market within the year 2008. A 4-channel version is planned for 2009. Implanted systems are not yet developed to a level that justifies clinical application, but a single-channel battery powered implant for animal research has been tested within RISE and is available on request. FES of denervated muscles requires not only novel stimulation equipment but also alternative measurement solutions. A new oscillation tonometry method, where the oscillation frequency and the decay curve of the freely swinging lower leg are assessed provides sensitive parameters for even minimal muscle reactions. A second biomechanical method measures the contraction twitch of quadriceps femoris via a sensor at the patella sensor quantifying the contraction dynamics (time to peak, half relaxation time). Not at least single fibre M-wave recordings provide sound data on conduction velocity and recovery of the muscle fiber membrane. All mentioned technical solutions are presented in detail and in relation to older and actual industrial and research equipment.

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**Rise2-Italy Trial: Muscle FES after peripheral nerve lesions. Our approach**

E. Rossato, S. Pegoraro, A. Marziali, G. Dri, D. Carniel (1), R. Stramare (2), S. Masiero

Physical Medicine and Rehabilitation Unit, and Orthopedics Unit (1) of the Department of Medical Specialties, (2) Department of Medical-Diagnostic Science and Special Therapies, University of Padua, Italy. E-mail: stef.masiero@unipd.it

The first goal of the 08Rise2-Italy Project is to identify among the clinical cases of the Physical Medicine and Rehabilitation Unit of the University of Padua a group of subjects with permanent injuries (complete or incomplete) of arm/leg skeletal muscles aimed at extending the results of the European Project RISE [1-4]. Demonstration that a high-power electrical stimulator associated with large surface electrodes induces single (twitch) or sustained (tetanus) contractions in long-term denervated human muscles of paraplegics, open the opportunity to obtain trophic effect and some functional recovery of denervated muscle in patients with severe muscle atrophy secondary to peripheral nerve lesions. The eligible patients suffered traumatic injuries to plexus or single nerve (e.g., circumflexus or femoral nerve). At enlistment, shoulder or leg muscles of the patients do not respond to the clinical stimulation protocols for innervated muscles (twitch stimulation with 0.5 msec long impulse at 5-20 V/mAmp, or tetanising “Kotz currents”). Using an electrical stimulator for denervated muscle (i.e., with an adequate power) that discharges triangular waves 150-200 msec long at 20-80 mAmp to large surface wet electrodes (20x40cm, in Lap case) the denervated muscle produces repeatedly single contractions (twitch training). Patient will be reevaluated monthly to verify if the denervated muscle partially recovers its excitability to a sufficiently short stimulations (duration <50msec), to be able to respond to short trains of impulse at 10-20 Hz frequency to perform tetanic contractions 2-3 sec long. This electrical stimulation protocol (5 times a week) will not replace, but complement the standard University of Padua Rehabilitation Unit protocol that includes passive and active functional rehabilitation. To monitor changes in thickness and tissue composition of trained muscles ultrasound scan will be performed before and every three months during the 12 months of programmed treatment. Extent of innervation/reinnervation will be checked with periodic EMG. Our first observations suggest that this pilot study of the Padua Rehabilitation Unit could be extended with clinically significant results.


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**Ultrasound (US) in muscle pathology**

R. Stramare

Department of Medical-Diagnostic Science and Special Therapies, University of Padova, Italy. E-mail: roberto.stramare@unipd.it

Skeletal muscles are subject to a range of pathological processes that can alter or limit their function. These pathologies can lead to changes that may be observed on ultrasound as abnormality of echo texture, disruption of the muscle architecture, change in muscle size or the presence of focal mass lesions. Alterations in muscle vascularization may be observed on Doppler ultrasound. The capability of ultrasound to study muscle tissue through dynamic evaluation, to compare normal structures in the contra lateral limb and follow lesions through time, make it a very versatile tool in clinical practice. MRI represents the main alternative imaging modality for study of muscle pathology. Traumatic
Injuries are one of the most common indications for ultrasound examination. Muscle hematomas and ruptures are easily recognized and muscle healing can be determined. The size, location and, occasionally, the nature of muscle masses and tumors may be demonstrated. Ultrasound can provide useful complementary information to MRI staging examinations, and is often utilized in protocols for sarcoma follow-up. Ultrasound may also be used in evaluation of infective lesions and diffuse muscle disease, and provides a means to perform image guided drainage and biopsy procedures. Broadband high frequency linear array probes (5–10 MHz) are most desirable for the study of muscle tissues and allow optimum imaging resolution. One of the major disadvantages of ultrasound imaging compared with MRI can be limitation of the field of view (FOV), which in linear transducers is determined by probe width. Recently the introduction of extended FOV imaging has provided the ability to acquire high quality panoramic images in real time. The potential advantages of this technique include better demonstration of large masses, improved accuracy of measurements and clearer representation of spatial relationships. Ultrasound is commonly employed for assessment of many sports injuries. In the evaluation of muscle trauma ultrasound may be performed in both the acute setting and during rehabilitation. Ultrasound can be used to grade the injury, identify the muscle groups involved, to help predict rehabilitation time, to monitor the healing response and to evaluate scar tissue formation and other complications of muscle rupture. Muscle neoplasms are relatively rare, and malignant muscle tumors represent less than 1% of all muscle rupture. Muscle neoplasms are relatively rare, and malignant muscle tumors represent less than 1% of all muscle ruptures. 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Expression of regeneration markers in muscle biopsies from patients affected with autoimmune myositis

S. Zampieri (1,3), N. Adami (1), A. Ghirardello (1), N. Bassi (1), M. Rampudda (1), U. Carraro (1,2), A. Doria (1,3)

(1) Laboratory of Translational Myology of the Interdepartmental Research Center of Myology and (2) C.N.R. Institute of Neuroscience c/o Department of Biomedical Science, University of Padova, I-35121 Padova, Italy; (3) Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Padova, Italy.
E-mail: sanzamp@unipd.it

Muscle biopsies from patients affected with polydermatomyositis (PDM) are characterized by infiltrating inflammatory cells, muscle fiber death and regeneration, indicating that in response to injury, damaged fibers are actively replaced by newly formed myofibers. It has been recently demonstrated that myositis specific autoantigens are expressed at high levels during regenerative myogenesis, with possible implications for the induction and/or amplification of the autoimmune response in these patients. In order to screen the affected muscles of PDM patients for the presence of regenerating fibers, we analyzed frozen sections from muscle biopsies of 8 PDM patients, including 3 paraneoplastic forms of PDM, using mouse monoclonal antibody to the embryonic isoform of myosin heavy chain (MHCemb) and rabbit polyclonal antibody to neural cell adhesion molecule (N-CAM), as markers of early and late stage of muscle regeneration and denervation, respectively. As controls we included skeletal muscle biopsies from patients affected with colorectal cancer (3) and from healthy subjects (3). We observed early regenerating (MHCemb positive) as well as late regenerating myofibers (N-CAM positive) in our muscle biopsies. N-CAM positive fibers (which represent also fibers before reinnervation) were significantly more frequent than MHCemb positive ones in PDM compared to the other conditions. N-CAM expressing myofibers strongly displayed positive distribution of the protein within the cytoplasm of small developing myofibers or on the plasma membrane of the bigger developing ones. Noteworthy, N-CAM positive fibers were predominantly and significantly detected in patients affected with cancer associated myositis (CAM) compared to patients affected with pure myositis. Our observations indicate that in PDM patients the presence of N-CAM positive fibers may be the result of prolongation of the aneural phase of muscle fiber regeneration. Moreover, in patients affected with CAM, N-CAM positive muscle fibers may also represent denervation events associated to a tumor induced immune reaction against muscle fibers.

Neural potential of a stem cell population in the adipose tissues

B. Zavan (1), L. Lancerotto (2), V. Vindigni (1,2,3)

(1) Department of Histology, Microbiology and Medical Biotechnology; (2) Unit of Plastic and Reconstructive Surgery; (3) Interdepartmental Research Center of Myology of the University of Padova, Italy.
E-mail: vincenzo.vindigni@unipd.it

A significant amount of recent interest has been focused on the possibility that adult human stem cells are a realistic therapeutic alternative to embryonic stem cells. Multipotent stem cells that have characteristics reminiscent of embryonic Neural Crest (NC) stem cells have been isolated from several postnatal tissues, including skin, gut, dental pulp, and the heart and are potentially useful for research and therapeutic purposes. However, their neurogenic potential, including their ability to produce electrophysiologically active neurons, is largely unexplored. In the present work, we investigated this issue with regard to skin-derived precursors (SKPs) and Adipose derived stem cells (ADSc). Adult stem cells isolated from skin and from adipose tissue derived from the same adult donor, were treated with EGF and FGF2. Neurospheres obtained were firstly expanded and evaluated in term of proliferative ability, and then their neuronal differentiation potential was analyzed. With this protocols the spheres has been able to proliferate and to origin Schwann and glial like cells. In summary, we have demonstrated here that multipotent adult precursor cell can be isolated and expanded from two accessible adult tissue sources: skin and adipose tissue. The work described here provides the framework for our attempts to use SKPs or ADSc as autologous adult stem cell population for cell replacement and discovery research.