*Abstracts of the 2017Spring PaduaMuscleDays, Padua, Italy*
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## *2017Spring PaduaMuscleDays, March 23 – 25*

# *Translational Myology for Impaired Mobility*

***Euganei Hills, Padova (Italy), March 23 - 25, 2017***

**Hotel Augustus, Montegrotto, Euganei Hills (Padova), Italy & Accademia Galileiana di Scienze, Lettere e Arti, Padova, Italy**

**Phone +39 049 793 200 - Fax +39 049 793518 - http://www.hotelaugustus.com/english/pages/hotel_augustus.php - E-mail: info@hotelaugustus.com**

*Organizers: Francesco Ambrosio, Ugo Carraro, Paolo Gargiulo, Helmut Kern, Christiaan Leeuwenburgh, Antonio Musarò, Feliciano Protasi, Marco Sandri*

## *THURSDAY March 23, 2017*

**Hotel Augustus, Montegrotto, Euganei Hills, (Padova), Italy, EU**

*15.00* *Registration*

**15.30** **Workshop: FES in mobility impairments**
*Helmut Kern, Gerta Vrbova and Francesco Piccione, Chairs*

**15.40** ***Lecture*: What can be learned from the time course of changes in low-frequency stimulated muscle?** *Dirk Pette, Konstanz, Germany, EU*

**16.20** **Acute and chronic responses to neuromuscular electrical stimulation in healthy humans: a brief overview, recent advances and futures directions,** *Julien Gondin, Université Lyon 1, France, EU*

**16.40** **Cardiovascular fitness for SCI,** *Emiliana Bizzarini, ASS #4 Friuli centrale, Udine, Italy, EU*

**17.00** **FES of denerveted muscle and beyond,** *Helmut Kern, Physiko- und Rheumatherapie, St. Poelten, Austria, EU*

**17.20** **Presentation of the Stimulette den2x (Dr. Schuhfried Medizintechnik GmbH, Vienna, Austria) for the electrical stimulation of denervated muscles,** *Peter Biowski, Vienna, Austria, EU*

**17.40** **Combined Physiotherapy and FES at the San Camillo Hospital of Venice-Lido,** *Andrea Marcante, IRCCS Fondazione Ospedale San Camillo, Venezia-Lido, Italy, EU*

**18.00** **Home-based FES for denervated muscles: Patients Opinions,** *Roberto Fiorucci, Antonio Piscioneri, Valter Caporello, Christian Cuciniello and more …*

*19.00* *Get-together!*

**Posters on display**

**Poster 1.** **Fighting muscle weakness in premature and advanced age by take-home strategies: Full-body In-Bed Gym and FES for elderly persons,** *Ugo Carraro et al., IRCCS Fondazione Ospedale San Camillo Venezia-Lido, Italy, EU*

**Poster 2.** **Quantitative Computed Tomography and Image Analysis for Advanced Muscle Assessment,** *Ugo Carraro et al., IRCCS Fondazione Ospedale San Camillo Venezia-Lido, Italy, EU*

**Poster 3.** **Testing dexterity and mobility in the elderly, a simplified approach for the older olds,** *Alfonc Baba et al., IRRCS Fondazione Ospedale San Camillo, Venezia, Italy, EU*

**Poster 4.** **Neurorehabilitation in neuromuscular disorders and consequences at microRNAs as circulating biomarkers,** *Laura Giaretta, et al., IRCCS Fondazione Ospedale San Camillo, Venice, Italy, EU*

**Poster 5.** **Inflammmation in the gut and chronic low back pain,** *Sergio Veneziani et al., Adler-Balance, Ortisei, Italy, EU*

**Poster 6.** **Iron homeostasis in iPSC-derived cardiomyocytes from a patient with Friedreich's Ataxia,** *Alessandra Bolotta, University of Bologna, Italy*

**Poster 7.** **Peri-patellar injections of Hyaluronic Acid deeply affect the heart transcriptome: An unexpected hope for the therapy of cardiovascular diseases,** *Provvidenza M. Abruzzo et al., University of Bologna, Italy, EU*

**Poster 8.** **Role of MicroRNA in Amyotrophic Lateral Sclerosis: gender and genetic differences,** *V. Pegoraro et al., IRCCS Fondazione Ospedale San Camillo, Venice, Italy, EU*

**Poster 9.** **Insights and imaging phenotypes of trasportinopathy (limb-girdle muscular dystrophy type 1F),** *C. Angelini et al., IRCCS Fondazione Ospedale San Camillo, Venice, Italy, EU*

**Poster 10.** **PGC-1α overexpression in the skeletal muscle: effects on myogenesis,** *Marc Beltrà, et al., Turin University, Italy, EU*

**Poster 11.** **Cancer and chemotherapy-induced cachexia in mice: effects of moderate exercise training,** *Riccardo Ballarò, et al., Turin University, Italy*

**Poster 12.** **FES training protocols for the functional recovery of permanent complete denervated human muscles,** *Christian Hofer, et al., Vienna, Austria*

**Poster 13.** **The nutraceutical potential of Flaxseed bioactive compounds to treat muscular dystrophy,** *Felicia Caroteneuto, et al., Rome, Italy, EU*

*19.30* *Dinner in Hotel Augustus*

*21.00* *Brain Storming on Organizing International Research Activities of Translational Myology*

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## *FRIDAY March 24, 2017*

**Accademia Galileiana di Scienze, Lettere, Arti,** *Via Accademia 7, Padova, Italy, EU*

***08.00*** ***Shuttle BUS to Padova (Be strictly on time, please!)***

**9.00** **Openings of the 2017Spring PaduaMuscleDays**
**Annalena Venneri**, IRCCS Fondazione Ospedale San Camillo, Venice, Italy and University of Sheffield, UK, EU
**Rosario Rizzuto**, Rector of the University of Padova, Italy, EU

**9.10** **Workshop: Molecular Approaches on Interactions of Pain & Mobility in Elderly**
*Helmut Kern, Christiaan Leeuwenburgh and Francesco Ambrosio, Chairs*

**9.15** ***Lecture*: The underlying molecular mechanisms of muscle atrophy and sarcopenia,** *Marco Sandri, University of Padova, Italy, EU*

**10.00** **Low-back pain: Molecular approaches to test effects of cayenne pepper cataplasm on skin and muscle,** *Sandra Zampieri et al., University of Padova Italy, LBI, Vienna, Austria, EU*

**10.20** **Pain management: Expression of the endocannabinoid receptors in human fascial tissue,** *Giovanna Albertin, et al., University of Padova, Italy, EU*

**10.40** **Pain management: Laser-induced biological effects on muscle mitochondria,** *Giulia Ottaviani, University of Trieste, Italy, EU*

*11.00* *Coffee break: Open bar*

**11.20** **Mitochondrial turnover in muscle: effect of exercise and age,** *David Hood, York University, Toronto, Canada*

**11.50** **Interventions to reduce low grade chronic inflammation and improve geriatric outcomes,** *Stephen Anton, University of Florida, Gainesville, FL, USA*

**12.20** **Muscle, systemic iron deregulation and aging: therapies to prevent disability and mobility impairments,** *Christiaan Leeuwenburgh, University of Florida, Gainesville, FL, USA*

***13.00*** *Lunch in Piazza Duomo, Padova*

**14.00** **Workshop: Muscle & Imaging, from basics to clinical evidence**
*Paolo Gargiulo, Feliciano Protasi and Ugo Carraro, Chairs*

**14.00** **Discovery of new SR/TT junctions that mediate $Ca^{2+}$ Entry in skeletal muscle,** *Felicano Protasi, University of Chieti, Italy, EU*

**14.30** **Localization of the ERG1 potassium channel in mammalian skeletal muscle,** *Amber L. Pond, J Cheatwood , LB Anderson, BA Cobb , CD Latour , SIU, Carbondale, IL; U Carraro, Venice-Lido; M Sandri , S Zampieri, UniPd, Italy, EU*

**14.50** **Dysfunctional accumulation of STIM1 and Orai1 in Tubular Aggregates results in impaired $Ca^{2+}$ entry in ageing muscle,** *Simona Boncompagni, Univeristy of Chieti, Italy, EU*

**16.10** **Exercise-induced alterations of M-line organization in obscurin k-o mice: Implications for human pathology,** *Pierantozzi E et al. Siena, Italy, EU*

**16.30** **S6K1 Is required for increasing skeletal muscle force during hypertrophy,** *Bert Blaauw, et al. University of Padova, Italy, EU*

*16.50* *Coffee break: Open bar*

**17.00** **Advanced muscle assessment by mean of 3-D modeling and profiling,** *Paolo Gargiulo, Clinical Engineering and Information Technology, Landspitali - University Hospital, Reykjavik, Iceland*

**17.30** **QMC-CT, a quantitative muscle assessment that oldest patients understand and that can get them to take-home full-body in-bed gym strategies,** *Ugo Carraro, et al., IRCCS Fondazione Ospedale San Camillo Venezia-Lido, Italy, EU*

**17.50** **MicroRNAs expression in muscles and serum in DM1,** *Corrado Angelini, et al., IRCCS Fondazione Ospedale San Camillo Venezia-Lido, Italy, EU*

***18.10*** ***Lecture*: Physical exercise as a preventative strategy to slow down cognitive decline in aging. Evidence for the value of aerobic physical activity,** *Annalena Venneri, Matteo De Marco, University of Sheffield, UK; IRCCS Fondazione Ospedale San Camillo, Venezia-Lido, Italy, EU*

***18.50*** ***Shuttle Bus to Hotel Augustus, Montegrotto, Euganei Hills (Padova)***

*20.00* *Dinner in Hotel Augustus*

*21.30* *Brain Storming: Organizing the International League of Myology Centers (ILMC)*

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## *SATURDAY March 25, 2017* Hotel Augustus, Montegrotto, Euganei Hills (Padova), Italy, EU

**09.00** **Workshop: Functional Rejuvenation in Aging** - *han Jarvis, Chairs*

**09.00** ***Lecture*: Experimental activation of damaged nerves and** onathan Jarvis, Liverpool John Moores University, Liverpool, UK, Mike Willa Morten Schmoll, Hermann Lanmüller, Vienna, Austria

**09.40** **Chronic electrical stimulation for reversing signs of laryngeal muscle ageing,** *Markus Gugatschka, Michael Karbiner, Phoniatry, Graz, Austria, EU*

**10.00** **3D reconstruction of the thyroarytenoid muscle combined with phonation analysis in an ex-vivo animal study on aged sheep larynges,** *Claus Gerstenberger, Phoniatry, Graz, Austria, EU*

10.20 *Coffee break*

**11.00** **Workshop: ES in Neuromuscular Disorders** - *Winfried Mayr, Lukas Kneisz, Chairs*

**11.00** **Clinical Results of ES in Unilateral Vocal Fold Paralysis (uVFP),** *Berit Schneider-Stickler, Matthias Leonhard, Department of Otorhinolarangology/Division of Phoniatrics-Logopedics Vienna; Gerd Fabian Volk, Orlando Guntinas-Lichius, ENT Jena, Germany; Winfried Mayr, Medical, Center for Medical Physics and Biomedical Engineering, University Vienna, Austria, EU*

**11.30** **Clinical results of ES in facial nerve paralysis as a model of ES for muscles with a mixed lesion pattern: chronic denervation and misdirected reinnervation,** *Fabian Volk, Orlando Guntinas-Lichius, ENT, Jena, Germany, Winfried Mayr, Vienna, Austria, EU*

**12.00** **Poster Session**

13.00 *Lunch in Hotel Augustus*

**14.00** **Workshop: Rehabilitation strategies for severe muscle atrophy and dystrophy**
*Ugo Carraro, Corrado Angelini, Chairs*

**14.00** ***Lecture*: Physical, pharmacological and nutritional interventions against muscle wasting and dystrophy,** *Dario Coletti, Rome&Paris*, EU

**14.30** **Cancer and chemotherapy-induced cachexia in mice: effects of moderate exercise training,** *Ballarò Riccardo et al. Turin University, Italy, EU*

**14.50** **Generation of iPSC lines from NLSDM patients carrying different PNPLA2 gene mutations: a novel frontier for the study of neutral lipid metabolism disorders,** *Daniela Tavian et al. Milan, Italy, EU*

**15.10** **SpillOver stimulation - preliminary results for a novel hypertrophy model in rats,** *Martin Schmoll et al. Liverpool/Vienna, EU*

**15.30** ***In vitro* surface characterization of adapted platinum neural stimulating electrodes,** *Polona Pečlin, Janez Rozman, University of Ljubljana, Slovenia, EU*

**15.50** **Comparing somatosensory evoked potentials resulting from transcutaneous spinal cord stimulation (tSCS) and tibial nerve stimulation in healthy and CP subjects,** *Thordur Helgason, Bragi Arnason, Vilborg Gudmundsdottir, Gigja Magnusdottir, Gudbjorg Kristin Ludvigsdottir, Reykjavik, Icelad; Karlsruhe, Germany, EU*

16.10 *Coffee break*

**16.45** **Testing dexterity and mobility in the elderly, a simplified approach for the older olds,** *Alfonc Baba et al., IRCCS Fondazione Ospedale San Camillo, Venezia-Lido, Italy, EU*

**17.00** **Neurorehabilitation in Neuromuscular disorders and consenquences at microRNAs as circulating biomarkers,** *Laura Giaretta, et al., IRCCS Fondazione Ospedale San Camillo, Venezia-Lido, Italy, EU*

**17.15** **Inflammation in the gut and CLBP: Intestinal permeability and microbiota,** *Sergio Veneziani, Adler-Balance, Ortisei, Italy, EU*

**17.30** **Peri-patellar injections of Hyaluronic Acid deeply affect the heart transcriptome: An unexpected hope for the therapy of cardiovascular diseases:** *Provvidenza M. Abruzzo et al., Dept. DIMES, University of Bologna, Italy, EU*

**17.45** **Iron homeostasis in iPSC-derived cardiomyocytes from a patient with Friedreich's Ataxia and from a healthy subject**, *Alessandra Bolotta et al. , Department of DIMES, University of Bologna, Italy, EU*

**18.00** **The nutraceutical potential of Flaxseed bioactive compounds to treat muscular dystrophy,** *Felicia Carotenuto et al., Rome, Italy, EU*

*18.30* *Ugo Carraro: Arrivederci, Auf Wiedersehen, Kveðja þín, See You to the 2018Spring PaduaMuscleDays*

*20.00* *Dinner in Hotel Augustus*

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**What can be learned from the time course of changes in low-frequency stimulated muscle?**

**Dirk Pette**

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Chronic low-frequency stimulation (CLFS) was originally used to study the influence of neural impulse activity on the establishment of phenotypic properties of skeletal muscle.[1] Since then it has been applied as an experimental model for the study of muscle plasticity, specifically the transformation of fast, fatigable muscle fibers towards slower, fatigue-resistant ones. Qualitative and quantitative changes of major elements (e.g., myofibrillar proteins, membrane-bound and soluble proteins involved in $Ca^{2+}$-dynamics, and mitochondrial and cytosolic enzymes of energy metabolism) follow a specific time course and are reflected in physiological changes of contractile and fatigue resistance properties.[2] These transitions correspond to pronounced shifts in the patterns of multiple protein isoforms with specific time courses of different components, e.g. myosin light and heavy chains. The present review re-evaluates data from previous studies focusing on whether and to what extent functional changes are preceded or accompanied by alterations at the levels of molecular and/or metabolic organization. CLFS-induced resistance to fatigue is correlated with sequential alterations both at the levels of metabolic and cellular organization (e.g., increases in glucose uptake and phosphorylation capacities, activation of glycogenolysis and glycolysis, changes in fiber volume, capillary density and mitochondrial content). Fast to slower fiber type transitions correlate with sequential transitions in myosin isoforms and partially synchronous exchanges of fast with slow isoforms of $Ca^{2+}$-regulatory proteins both of thin filament and sarcoplasmic reticulum. The changes in energy-rich phosphates that cause an almost immediate drop in energy charge after the onset of stimulation and its persistence, are the most likely trigger of $Ca^{2+}$-dependent signaling pathways involved in fast-to-slow fiber transitions. Time course studies therefore, not only disclose correlations between various stimulation-induced changes but also provide insights into mechanisms regulating the expression of fiber type-specific properties.

*1. Vrbová G. The effect of motoneurone activity on the speed of contraction of striated muscle. J Physiol 1963;169:513-26.*

*2. Pette D, Vrbová G. The contribution of neuromuscular stimulation in elucidating muscle plasticity revisited. Eur J Transl Myol 2017;27:33-9.*

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**Acute and chronic responses to neuromuscular electrical stimulation in healthy humans: a brief overview, recent advances and futures directions**

**Julien Gondin**

Institut NeuroMyoGène (INMG), UMR CNRS 5310 – INSERM U1217, Université Claude Bernard Lyon 1, Villeurbanne, France
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Neuromuscular electrical stimulation (NMES) usually involves the application of intermittent stimuli over the muscle with the aim to produce strong contractions through the activation of intramuscular nerve branches. The main physiological uniqueness of these electrically-evoked contractions is that motor unit recruitment is different from a voluntary action, as it has been shown to be spatially fixed, temporally synchronous, mainly superficial and non-selective.[1] Indeed, NMES leads to the activation of both slow and fast motor units even at relatively low force levels. This specific motor units activation pattern has been associated with an exaggerated metabolic demand and a greater muscle fatigue as compared with voluntary exercise performed at the same intensity,[1] thereby limiting the widespread utilization of NMES in clinical settings. It has been recently highlighted that NMES can also induce significant muscle damage as illustrated by major histological alterations such as z-lines disruption and macrophage infiltration as well as by the prolonged decrease in voluntary force production capacities.[2] In the first part of the presentation, we will provide an overview of the main physiological consequences of the peculiar motor unit recruitment associated with NMES and provide some recommendations for limiting or preventing the corresponding "adverse" effects of NMES. Over the last two decades, chronic NMES application has been used as an effective way of improving muscle strength in both healthy humans and athletes. The magnitude of the strength gains has been related to the level of electrically evoked force. Given that the subject's tolerance of the electric current determines the force evoked by NMES, there is a large inter-individual variability in NMES response. Of interest, the time course of neuromuscular adaptations to NMES training appears similar to that taking place in response to voluntary strength training programs. Indeed, adaptations within the central nervous system occurred in the early phase of NMES training as illustrated by the increased electromyographic activity and neural activation,[3] enhanced V-wave amplitudes,[3] and significant cross-education effects.[4] These findings clearly indicated that NMES does not actually bypass the central nervous system due to the activation of both muscle and cutaneous afferent fibers. In addition, long-term NMES training programs (i.e., >6-8 weeks) may further induce muscle hypertrophy, improve muscle oxidative capacity and result in a fast-to-slow muscle fiber type transition.[5] Surprisingly, the relevance of such phenotypic adaptations for the translation to endurance performance that is particularly important for sport and daily activities remains to be demonstrated. The second part of the presentation will address how and to what extent NMES-induced neural and muscle adaptations might be relevant in a clinical context. We will also suggest potential directions for future implementation of NMES in inactive patients with advanced disease.

1. *Gregory CM, Bickel CS. Recruitment patterns in human skeletal muscle during electrical stimulation. Phys Ther 2005;85:358–64.*
2. *Crameri RM, Aagaard P, Qvortrup K, et al. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. J Physiol 2007;583:365–80.*
3. *Gondin J, Duclay J, Martin A. Soleus- and gastrocnemii-evoked V-wave responses increase after neuromuscular*

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*electrical stimulation training. J Neurophysiol 2006;95:3328–35.*
4. *Hortobágyi T, Maffiuletti NA. Neural adaptations to electrical stimulation strength training. Eur J Appl Physiol. 2011;111:2439–49.*
5. *Gondin J, Brocca L, Bellinzona E, et al. Neuromuscular electrical stimulation training induces atypical adaptations of the human skeletal muscle phenotype: a functional and proteomic analysis. J Appl Physiol 2011;110:433–50.*

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**Cardiovascular fitness in SCI**

**Emiliana Bizzarini**

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In people with spinal cord injuries (SCI) autonomic dysfunction is related with several conditions which increase cardiovascular risk: abnormalities in blood pressure, heart rate variability, arrhythmias and an altered cardiovascular response to exercise. If all these factors limit the performance in physical activity in the SCI population, several evidences in literature show that physical inactivity is the main independent risk factor for the development of cardiovascular diseases.[1-3] Aims of the study was the monitoring of cardiovascular performance parameters, respiratory parameters and muscular working capacity of a population of disabled athletes with complete spinal cord injury in chronic phase. 29 athletes, performing agonist sport were evaluated. The characteristics of the population are: a complete spinal cord injury classified as ASIA A (13 persons had a neurological level above Th6 and 16 a neurological level below Th6), 25 males and 4 females; age 42.24 ± 12.40 years; BMI 23.20 ± 3.26; time to the lesion (the spinal cord injury) 17.14 ± 12.30 years. Assessments (clinical evaluation, blood tests, spirometric test, incremental test at the crank ergometer with monitoring of cardio-respiratory parameters) were carried out in 2008 (t0) and after 6 year in 2014 (t1). By multiple regression we analyzed at t0 and t1 the contribution on maximum oxygen consumption parameters (VO2max) of variables as age, Body Mas Index (BMI), lesional level, years to the injury and weekly hours of training. At t0 the contribution on VO2max parameters of the other variables taken into account was statistically significant ($p = 0.0075$) for the lesional level. The correlation between VO2max and the lesional level was confirmed by analysis of variance (ANOVA) ($p = 0.096$). This means that the lower the lesional level the higher the VO2max in subjects who practice sports. At t1 we achieved a statistically significant correlation between VO2max parameters and weekly training hours ($p = 0.0091$), therefore in the long term in our subjects an increase in VO2max is related to the increase in weekly training hours. We also checked at t1 a statistically significant correlation between VO2max and BMI, with an increase in VO2 max correlated with a reduction in BMI ($p = 0.005$) of our athletes. The continued practice of physical activity is critical in improving cardiovascular performance in people with spinal cord injuries, especially in most affected persons. In the SCI population in chronic phase, hours of practice in sports activities and maintaining an adequate BMI are extremely important for saving cardiovascular fitness.

1. *Valent L, Dallmeijer A, Houdijk H, et al. The effects of upper body exercise on the physical capacity of people with a spinal cord injury: a systematic review. Clinical Rehabilitation 2007;21:315–30.*
2. *Ravensbergen HR, de Groot S, Post M, et al. Cardiovascular function after spinal cord injury: prevalence and progressione of dysfunction during inpatient rehabilitation and 5 years following discharge. Neurorehabil Neural Repair 2014;28:219-29*
3. *Groah SL, Weitzenkamp D, Sett P, et al. The relationship between neurological level of injury and symptomatic cardiovascular disease risk in the aging spinal injured. Spinal Cord 2001;39:310-17.*

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**FES of denervated muscle and beyond**

**Helmut Kern**

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Long standing lower motor neuron denervation of skeletal muscle is known to end in fibrotic degeneration of muscle tissue.[1] However, long term survival of a subset of skeletal myofibers also occurs.[2,3] We performed transverse and longitudinal studies of SCI patients suffering with complete *Conus* and *Cauda Equina* Syndrome and of sedentary and active seniors which included analyses of muscle biopsies from the *quadriceps* muscle. Surprisingly, we discovered that human denervated myofibers survive years of denervation after full and irreversible disconnection from their motor neurons.[1] Open is, however, the extent of contribution of muscle fiber regeneration to these observations.[4] We found that atrophic myofibers could be rescued by home-based Functional Electrical Stimulation (h-bFES), using purpose developed stimulators and electrodes.[5,6] Although denervated myofibers quickly lose the ability to sustain high-frequency contractions, they continue to respond to single, very long impulses (up to 200 millisec) that are able to recover enough muscle excitability to allow for re-emergence of tetanic contractions. A description of the very early changes in humans are hampered by a paucity of patients suffering complete *Conus* and *Cauda Equina* Syndrome, but the cohort enrolled in the EU RISE Project has shown that even five years after SCI, severe atrophic myofibers, with a peculiar cluster reorganization of myonuclei,[3] are present in human muscles and respond to h-bFES.[5,6] Thus, human myofibers survive permanent denervation much longer than generally accepted and they maintain the capacity to respond to h-bFES beyond the stage of simple atrophy. Furthermore, long-term denervation/reinnervation events occur in elderly,[7] and is part of the mechanisms responsible for muscle aging and again h-bFES was beneficial in delaying aging decay.[8,9] Indeed, physical exercise is known to have beneficial effects on muscle trophism and force production modulating signaling pathways involved in fiber type plasticity, muscle growth and mitochondria respiratory efficiency. It has been shown that the decrease of muscle mass and strength observed in aging is linked to intracellular and extracellular abnormalities, that is, sarcoplasmic reticulum-to-mitochondria malfunctions and extracellular matrix metabolism, respectively. When healthy seniors are exposed to regular neuromuscular ES training for a period of 9 weeks

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outcomes are an increase in muscle strength and muscle fibers and, most importantly, an increase of fast fibers, the more powerful of skeletal muscle motor units.[8,9] Electron microscopy analyses show remodelling of mitochondrial apparatus as a consequence of fusion phenomena that are consistent with adaptation to physical exercise. Altogether the results indicate that the ES-dependent beneficial effects on muscle mass and force are associated with changes in mitochondrial-related proteins involved in $Ca^{2+}$ homeostasis, providing new targets to develop therapeutic strategies to promote healthy aging.


1. *Kern H, Carraro U. Home-based Functional Electrical Stimulation (h-b FES) for long- term denervated human muscle: History, basics, results and perspectives of the Vienna Rehabilitation Strategy. Eur J Transl Myol 2014;24:27-40.*
2. *Squecco R, Carraro U, Kern H, et al. A subpopulation of rat muscle fibers maintains an assessable excitation-contraction coupling mechanism after long-standing denervation despite lost contractility. J Neuropathol Exp Neurol 2009;68:1256-68. doi: 10.1097/NEN.0b013e 3181c18416.*
3. *Carraro U, Kern H. Severely Atrophic Human Muscle Fibers With Nuclear Misplacement Survive Many Years of Permanent Denervation. Eur J Transl Myol 2016;26:5894. doi: 10.4081/ejtm.2016.5894.*
4. *Carraro U, Boncompagni S, Gobbo V, et al. Persistent muscle fiber regeneration in long term denervation. Past, present, future. Eur J Transl Myol 2015;25:77-92*
5. *Kern H, Carraro U, Adami N, et al. One year of home-based Functional Electrical Stimulation (FES) in complete lower motor neuron paraplegia: Recovery of tetanic contractility drives the structural improvements of denervated muscle. Neurol Res 2010;32:5-12.*
6. *Kern H, Carraro U, Adami N, et al. Home-based functional electrical stimulation rescues permanently denervated muscles in paraplegic patients with complete lower motor neuron lesion. Neurorehabil Neural Repair 2010;24:709-21. doi: 10.1177/ 1545968310366129. Epub 2010 May 11.*
7. *Mosole S, Carraro U, Kern H, et al. Use it or Lose It: Tonic Activity of Slow Motoneurons Promotes Their Survival and Preferentially Increases Slow Fiber-Type Groupings in Muscles of Old Lifelong Recreational Sportsmen. Eur J Transl Myol 2016;26:5972.*
8. *Kern H, Barberi L, Löfler S. et al. Electrical stimulation counteracts muscle decline in seniors. Front Aging Neurosci. 2014; 6:189.*
9. *Barberi L, Scicchitano BM, Musaro A. Molecular and cellular mechanisms of muscle aging and sarcopenia and effects of electrical stimulation in seniors. Eur J Transl Myol 2015;25:231-6.*
10. *Zampieri S, Mosole S, Löfler S, et al. Physical exercise in Aging: Nine weeks of leg press or electrical stimulation training in 70 years old sedentary elderly people. Eur J Transl Myol 2015;25:237-42.*


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**FES training protocols for the functional recovery of permanent complete denervated human muscles**

Christian Hofer (1), Stefan Löfler (1), Winfried Mayr (2), Michaela Mödlin (3), Samantha Urban (4), Helmut Kern (1,4)

(1) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, (2) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, (3) Institute of Physical Medicine and Rehabilitation, Wilhelminenspital, Vienna, (4) Institute of Physical Medicine and Rehabilitation, Physiko- und Rheumatherapie, St. Pölten, Austria

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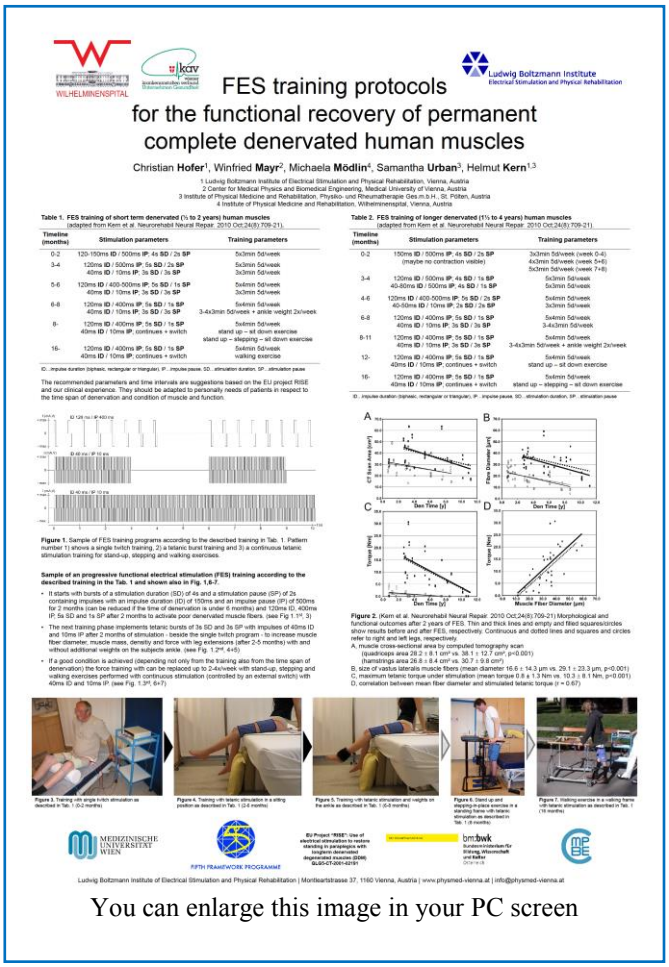
Spinal cord injury causes paralysis and subsequent muscle wasting and loss of muscle function, which are especially severe after complete and permanent damage to lower motor neurons. However, long term survival of a subset of skeletal myofibers also occurs.[1] We performed transverse and longitudinal studies of SCI patients suffering with complete *Conus* and *Cauda Equina* Syndrome[2] and found that atrophic myofibers could be rescued by home-based Functional Electrical Stimulation (h-bFES), using purpose developed stimulators and electrodes.[3] The recommended parameters and time intervals are suggestions based on the EU project RISE and our clinical experience.[2,3] They should be adapted to personal needs of patients in respect to time span of

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denervation, and condition of muscle and function. Patient training should start with single twitch stimulation with an impulse duration (ID) of 150ms and an impulse pause (IP) of 500ms for the first 2 months (can be reduced if the time of denervation is under 6 months) and 120ms ID, 400ms IP, after 2 months to excite denervated muscle fibers still hard to activate. After eliciting sufficient muscle reaction the next training phase implements tetanic bursts of a stimulation duration (SD) of 3s and a stimulation pause (SP) of 3s with impulses of 40ms ID and 10ms IP after 2 months of stimulation – in addition to the single twitch program - to increase muscle fiber diameter, muscle mass, density and force with leg extensions (after 2-5 months) with and without additional weights on the subjects ankle. If a good condition is achieved (depending not only from the training also from the time span of denervation) the strength training can be replaced with stand-up, stepping and walking exercises in parallel bars performed with continuous stimulation controlled by an external switch. In conclusion, human myofibers survive permanent denervation much longer than generally accepted[1-5], and they maintain the capacity to respond to h-bFES beyond the stage of simple atrophy[2,3].


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**Presentation of the *Stimulette den2x* (Dr. Schuhfried Medizintechnik GmbH, Vienna, Austria) for the electrical stimulation of denervated muscles**

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The *Stimulette den2x* is a high performance 2-channel electrotherapy stimulator, specialized to be used for activating flaccid paralyzed denervated muscles. Damages of the lower motor neuron in *conus-cauda*-lesion or peripheral nerve injury cause dramatic changes in the affected muscle.


With adequate stimulation parameters those changes can be stopped or even reversed.[1,2] This bridges the time gap until reinnervation occurs in nerve injury. In *conus-cauda*-lesion, where we usually see severe muscle atrophy, it preserves/recovers muscles mass improving its trophic state, thus helping to prevent pressure sores. In this workshop the changes due to denervation, and the constrains for results of adequate electrical stimulation will be discussed. Furthermore the practical application of the stimulation device *Stimulette den2x*, now commercially available, will be fully demonstrated with the help of voluntary persons and patients. The EU Project RISE demonstrated that home based FES of denervated muscles is a secure and effective home therapy. Benefits of stimulating denervated muscles are: 1. Recovery of tetanic contractility; 2. Restoration of muscle fibre structure; 3. Recovery of fibre size and muscle mass; 4. Better skin condition; 5. Reduced risk of pressure sores; 6. Improved cosmetic appearance of lower extremities; 7. Increased self-esteem. Furthermore, if standing upright is accomplished: 8. Improved cardiovascular fitness; 9. Unloading of seating surface. The conclusion of the RISE project was that a commercial electrotherapy device for home based FES was a priority. The *Stimulette den2x* by Dr. Schuhfried is the first device that delivers the needed power and technical requirements to fullfil the clinical requests. The following parameters are programmable: Impulse amplitude: max +/- 300 mA; Impulse waveform: rectangular / ramp shaped (3 different waveforms); Impulse duration ID: 10 ms—200 ms; Impulse pause IP: 1 ms-2 s; Surge duration: 100 ms—11 s; Rise: 5 % - 100 % surge duration; Decay: 5 % - 100 % surge duration; Surge interval: 0 ms—11 s; Treatment duration: 1—59 min; all currents are biphasic. The Switchbox: The Switchbox has been developed to enable flaccid paraplegic patients to practice standing, stepping and a type of „walking“ at the parallel bars. Functional Electrical Stimulation of denervated muscles — a novel therapeutic option after peripheral nerve lesion is a realistic option. In conclusion, the *Stimulette den2x* represents a major breakthrough in FES.


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## Combined physiotherapy and FES at the San Camillo Hospital of Venice: Muscle imaging for monitoring ES effects in rehabilitation

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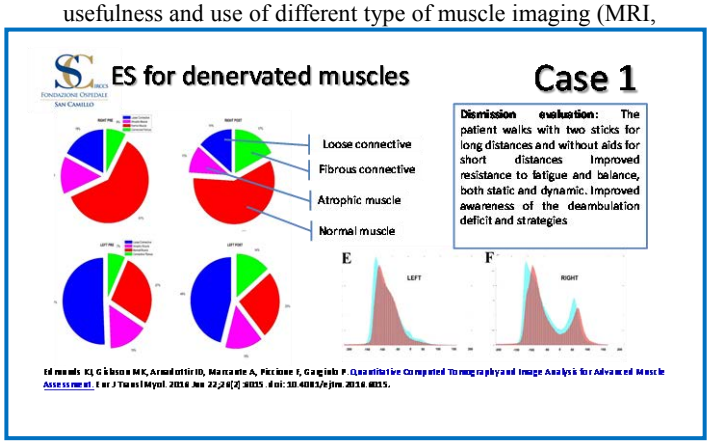
Rehabilitation treatment is still a challenge for clinicians in patient suffering from muscle atrophy following spinal cord Injury and/or peripheral neuropathies. Electrical Stimulation (ES) is a discussed option, but it plays in our opinion an important a role at least to maintain muscle trophism of denervated muscles and recover from atrophic innervated muscles.[1-3] In our hospital, functional and electrical stimulation tests are part of the standard evaluation in patients treated with electrical stimulation for denervated muscle after peripheral nerve injury. However, to better explain the effects of ES and verify the efficacy of the treatment, muscle imaging could help clinician for the follow up of this kind of patients. In this presentation we discuss the usefulness and use of different type of muscle imaging (MRI, CT, dynamic echomyography) to assess muscle tissue health in clinical rehabilitation perspectives.[4-6] We will present case reports to offer the opportunity to discuss rehabilitative pathwaies for diagnostics and rehabilitation of patients suffering of peripheral denervation, a condition that is still a challenge for clinicians. In particular we would like to evaluate the opportunities of the Quantitative Muscle Color Computed Tomography (QMC-CT), a quantitative imaging analysis introduced by our group to monitor skeletal muscle. Validation of QMC-CT will provide physicians an improved quantitative tool to diagnose the condition of skeletal muscle during rehabilitation of mobility-impaired persons, so that managements can be better prescribed, evaluated and altered where needed.

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## The underlying molecular mecahnism of muscle atrophy and sarcopenia

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The cellular basis of age-related tissue deterioration remains largely obscure. The ability to activate compensatory mechanisms in response to environmental stress is an important factor for survival and maintenance of cellular functions. Autophagy is activated both under short and prolonged stress and is required to clear the cell of dysfunctional organelles and altered proteins. We report that autophagy in muscles declines with ageing and its inhibition

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correlates with age-dependent muscle loss and weakness. Specific autophagy inhibition in muscle has a major impact on neuromuscular synaptic function and, consequently, on muscle strength, ultimately affecting the lifespan of animals. Inhibition of autophagy also exacerbates aging phenotypes in muscle, such as mitochondrial dysfunction, oxidative stress, and profound weakness. Mitochondrial dysfunction and oxidative stress directly affect acto-myosin interaction and force generation but show a limited effect on stability of neuromuscular synapses. Mitochondria shape is also a critical factor for sarcopenia and for systemic ageing. Mechanistically, mitochondria control a cascade of signalling events that induce muscle secretion of myokines that cause systemic ageing and premature death.[1-4]


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**Low-back pain: biological approaches to test cayenne pepper cataplasm effects on skin and muscle**

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Physical medicine therapies are first line of intervention, with pharmacologic prior to surgical treatments for several musculoskeletal diseases, such as low back pain. Herbal cataplasms containing a *rubefacient substance*, (Cayenne pepper, CP) are directly applied to the skin at the site of the painful areas provoking a hyperemic response, that involves both epidermis and muscle tissue nociceptor fibers, with beneficial analgesic effects. Capsaicin is the most abundant capsaicinoid present in the Cayenne pepper and it is an agonist of Transient Receptor Potential Vanilloid 1 (TRPV1). This treatment is generally well tolerated, but data on its possible side effects and secondary targets are missing. We tested 20-min application of 5% Cayenne pepper cataplasm (CPC) on healthy subjects, monitoring its effects on serum levels (before and 0.5, 1, 3, 6, 24 hrs after application) of general laboratory parameters (hemogram, CRP, sedimentation, CK, albumin, cortisol), pro- and anti-inflammatory cytokines (TNF-alpha, IL-1β, IL-6, TGF-β1) biomarkers specific for blood vessels damage (leukotriene B4, E-selectin, P-selectin, VCAM-1), and a panel of selected miRNAs possibly implicated in the cellular processes modulated by Caspaicin topical treatment.[1-3] Specifically, we analysed miRNA regulating TRPV1 transcription (miR-199a, and miR-199b), those mediators of inflammation (miR-155, miR-21, miR-146a), intracellular $Ca^{2+}$ homeostasis (miR-25), endothelial cell damage (miR-126), cardiac and skeletal muscle homeostasis (miR-1, miR-133, and miR-206). No significant changes in the serum levels of tested cyokines or laboratory parameters have been observed over the analysed time period. Interestingly, changes of the plasma levels of c-miRNA regulating Th1>Th2 inflammatory response and TRPV1 (specific pharmacologic target of Capsaicin) were detected. These results suggest that 5% Munari cataplasm seems to be a safe treatment targeting specific receptor responsible for pain sensation. In addition, circulating miRNAs are novel good candidate biomarkers for testing and monitoring treatment's effects in patients affected with Low Back Pain. Further studies are needed to investigate the immediate and long-term effects of repeated CPC applications as well as to understand the intersecting underlying mechanisms activated by Capsaicin and other identified factors, in order to further validate them for physical medicine therapies.


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**Expression of the endocannabinoid receptors in human fascial tissue**

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Endocannabinoids are endogenous lipid mediators with wide range of biological effects similar to those of marijuana. They exert their biological effects via two main G-protein-coupled cannabinoid receptors, the CB1 (cannabinoid receptor 1) and CB2 (cannabinoid receptor 2). Cannabinoid receptors have been localized in the central and peripheral nervous system as well as on cells of the immune system, but recent studies gave evidence for the presence of cannabinoid receptors in different types of tissues.[1,2] Their presence was supposed in myofascial tissue, suggesting that the endocannabinoid system may help resolve myofascial trigger points, suppressing proinflammatory cytokines such as IL-1beta e TNF-alpha and increasing anti-inflammatory cytokines.[3,4] However, until now the expression of CB1 and CB2 in fasciae and in fascial fibroblasts has not yet been

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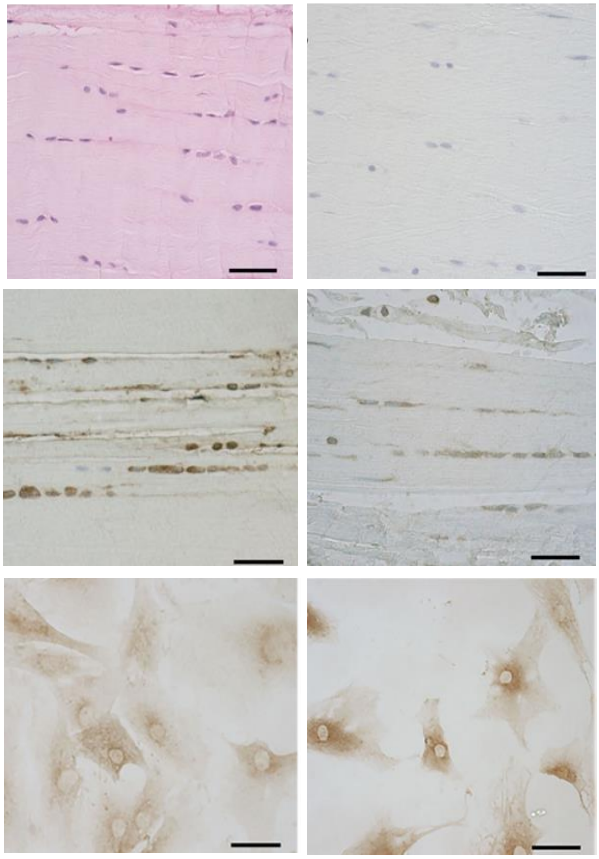

established. In this work small samples of fascia were collected from volunteers patients: for each sample were done a fibroblast cell isolation, immunohistochemical investigation (CB1 and CB2 antibodies) and real time RT-PCR to detect the expression of CB1 and CB2 and evaluation of gene expression of CB1 and CB2 receptors after fibroblasts mechanical stimulation. The immunostaining results demonstrate the expression of CB1 and CB2 on fascial fibroblasts and fascial tissue. In the tissue not all the fibroblasts are positive, whereas the isolated and expanded cells are homogeneous. These results are confirmed by the real time PCR where the specificity of the reaction on fibroblasts and fascial tissue is the same, but the amount of expression in the tissue is lower, for both CB1 and CB2. The mechanical stimulation has shown that there is an increase of CB2 expression on fibroblasts. This is the first demonstration that the fibroblasts of the muscular fasciae express CB1 and CB2. These results could represent a new target for drugs to care fascial fibrosis and inflammation. The presence of the endocannabinoid system in the fascial fibroblasts can also explain the efficacy of cannabis to care myofascial pain and the observation that a mechanical stimulation has given an increase of receptor gene expression could explain the possible stimulation during manipulative treatments and exercises.[5] More studies about the interactions between fibroblasts, extracellular matrix and CB1 and CB2 receptors could help to understand the role of these receptors on myofascial pain.

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**Laser-induced biological effects on muscle mitochondria**

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Skeletal muscle repair goes through a modulation of several stages, which are mainly accomplished through changes in the activation profile of macrophages. This process results in changes in the phenotype and function of involved cells and macrophages, which play a key role in this progression and are considered the targets for therapeutic intervention.[1,2] Mitochondria also exert a crucial modulatory effect on inflammatory macrophages pathways, leading to the production of cytokines (Mitogen Activated Protein Kinases and Nuclear Factor-Kappa β) pathways. When an inflammatory stimulus triggers macrophage activation, the mitochondria amplify these pathways, resulting in increased production of cytokines and inflammatory mediators. Over the last ten years, many studies demonstrate that the employment of the laser therapy modulates many biochemical processes, especially the decrease of muscle injures, the increase in mitochondrial respiration and ATP synthesis, crucial to accelerate the healing process. However, nowadays there is no consensus over the best laser protocol to employ in the clinical practice in order to obtain the most efficient biological response. For this reason, many in vitro studies focus their attention on the highest effect on mitochondria by laser light. Among the most clinical employed wavelengths, it is already known that red and infrared laser lights stimulate photochemical and photophysical events in mitochondria, thus resulting in increased mitochondrial membrane potential and higher enzyme activity in the respiratory chain. It is possible to observe structural changes, such as the formation of giant mitochondria through the merging of membranes of smaller and neighbouring mitochondria, which lead to higher levels of respiration and ATP to cells. It has also been demonstrated that laser therapy improves enzyme activity of the complex IV (cytochrome c oxidase) in skeletal muscle mitochondria. This effect is crucial since the oxidative capacity of muscle fibres is related to the density of mitochondria, able to oxidize glucose, fatty acids and proteins for ATP synthesis during muscle contraction. To optimize muscle recovery, when adding laser therapy to low intensity exercises, it is possible to foster this mechanism working on mitochondrial

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biogenesis, both to favour aerobic metabolism and to reduce muscle fatigue from metabolic origin.[3-6] MTT assay on myocytes assesses an increased mitochondrial activity and cell activation after laser treatment. In addition, it is possible to observe a clear reduction in Tumor Necrosis Factor-α production 24 hours after the irradiation of activated macrophages. So, thanks to laser therapy muscle performance could be increased reducing its fatigue; the most accredited and studied mechanisms to this specific behaviour are: i) enhance mitochondrial activity, ii) phosphocreatine re-synthesis and iii) mitochondria lactate oxidation. Although in vitro studies offer the possibility to standardize the obtained results, thanks to their cellular and molecular highly reproducible models, the results of such studies cannot be directly correlated with clinical outcomes. Nevertheless, the knowledge of the effect of laser therapy on the mitochondria contained in different muscle cell types is of paramount importance for the design of *in vivo* protocols that can exert more effective modulation of the muscle repair process.

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**Inflammation in the gut and chronic low back pain (CLBP). Analysis of intestinal permeability, digestive performance and microbiota. Effectivness of a cleansing and recolonization protocol**

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Chronic low back pain (CLBP) is a disabling condition affecting a majority of people of the western countries. It deeply affects the quality of life as it is often linked to multidimensional disturbances such as poor sleep, mood disorders, chronic fatigue and joint pain. There is no other condition with higher social and economic costs. It has been reported that only a minority of patients with gut inflammation suffers from intestinal symptoms. In a previous paper it was proposed that gastrointestinal disturbances, beyond mechanical issues, could be overlooked in the management of these patients. Dietary changes were successful in the positive resolution of the described clinical case. In this paper we further test this hypothesis. We measured on 5 subjects specific parameters related to gastrointestinal and digestive physiology that have been associated with metabolic and immune related pathological conditions. Specifically we tested the levels of zonuline (related to intestinal permeability) the presence of undigested substances, ph and the colonization of specific bacteria (symbiotic vs pathoghenous). Inflammation in the gut can lead to altered mucosa permeability indeed. The entrance in the blood stream of abnormal molecules activates the immune system in a cascade of events affecting remote systems and possibly the integrity of structures like the neuromuscolar junction or the pathways of energy production. Conditions that are currently managed by orthopaedists, reumatologists or neurologists could benefit from a screening of the gastrointestinal functionality.

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**Mitochondrial turnover in muscle: effect of exercise and age**

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It is well known that repeated bouts of exercise (i.e. exercise training) lead to an elevated content of mitochondria within muscle. This adaptation confers metabolic advantages during exercise, such as an increase in the aerobic metabolism of lipids, reduced glycogen usage, and diminished lactate production. The molecular basis for this increase in organelle content involves the activation of PGC-1α along with numerous transcription factors which increase the expression of nuclear genes encoding mitochondrial proteins. Among these are Tfam, the transcription factor which mediates mtDNA replication and transcription, in an effort to coordinate the nuclear and mitochondrial genomic responses to the exercise signals. These organelle synthesis processes (termed biogenesis) have been well-studied, and reviewed recently.[1] On the other hand, it is also recognized that the steady state mitochondrial content of muscle is determined not only by rates of synthesis, but rather by organelle turnover, represented by a balance between synthesis and degradation. The degradation process is termed mitophagy. In contrast to biogenesis, our understanding of mitophagy in muscle is in its infancy. Mitophagy involves the activation of the general autophagy pathway within the cell, where the ultimate target for degradation is the dysfunctional mitochondrion. Targeting mitochondria involves tagging the organelle for degradation by ubiquitination, followed by its engulfment within an autophagosome for fusion to a

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lysosome, and subsequent proteolysis. We have previously shown that a single bout of exercise initiates mitophagy flux signaling, measured as the activation of kinases which trigger autophagy, along with localization of LC3-II and p62 on the surface of the organelle. We found that the degree of mitophagy flux enhanced by exercise was PGC-1α-dependent, such that the absence of the coactivator led to reduced mitophagic responses to exercise. Thus, PGC-1α is involved not only in organelle biogenesis, but also in its degradation.[2] In contrast to the enhanced mitochondrial content in muscle in response to exercise, aging is a progressive condition in which mitochondrial content and function, along with the level of PGC-1α, are reduced in muscle, contributing to altered metabolism and decrements in muscle mass.[3] In addition, while muscle adaptations are certainly possible in response to exercise, the biogenesis adaptations to standardized workloads is not as robust with age, as it is in younger subjects.[4] Thus, while previous work has documented blunted stages of biogenesis in aged muscle, no research has documented the degree of change in mitophagy. The prevailing dogma suggests that mitophagy is decreased in aging muscle, however limitations in methodologies preclude this conclusion. Furthermore, how chronic exercise may affect mitophagy in aged muscle remains unexplored. Thus, we have examined the effect of aging and chronic exercise on mitophagy flux using 6 and 36 month old Fisher 344 Brown Norway rats that serve as an excellent model of aging skeletal muscle. To invoke comparable levels of chronic exercise, the animals were implanted with a stimulator to activate the peroneal nerve which innervates the tibialis anterior muscle to induce chronic contractile activity (CCA; 3hrs/day, 9 days). The contralateral limb served as control. Colchicine is a microtubule inhibitor which interferes with the transport of the autophagosome to the lysosome for degradation. Thus, administering this drug for 3 days (0.4 mg/kg/day) allowed us to measure mitophagic flux when levels of p62 and LC3-II are compared to vehicle-treated animals. To evaluate mitophagy, intermyofibrillar mitochondria were isolated from the TA muscle and protein localization was assessed by immunoblotting. As expected, aged animals exhibited reduced mitochondrial content and an attenuated adaptation to CCA in agreement with previous work.[4] Colchicine successfully inhibited autophagy in our model and allowed for the quantification of mitophagy flux. In young animals following the mitochondrial adaptations to 9 days of CCA we observed decreased mitophagy,[5] consistent with the idea that improved mitochondrial content or function after CCA obligates lower organelle degradation rates. In contract, mitophagy flux was higher in muscle of aged animals,[5] in contrast to suggestions from the literature, and the attenuation of mitophagy as a result of CCA was less pronounced. These high rates of mitophagy may contribute to the age-related loss of mitochondrial content, but when combined with a reduced capacity for biogenesis, this pattern of organelle turnover within aged muscle is insufficient to maintain the high quality of mitochondria compared to muscle from younger animals. Our data also fortify the concept that exercise is a useful therapy to modify mitochondrial turnover rates, in an effort to sustain, or enhance, the healthiest mitochondrial pool within skeletal muscle.

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**Interventions to reduce low grade chronic inflammation and improve geriatric outcomes**

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Preserving mobility is central to maintaining a high quality of life and participation in activities to be fully independent in the community.[1] Unfortunately, aging is associated with a progressive decline in mobility, as well as cognitive and physical function, leading to a loss of independence. As diverse as the etiologies of physical disability are, a growing body of evidence strongly implicates *chronic low-grade systemic inflammation* as playing a significant role in contributing to sarcopenia and associated functional decline.[2;3] A variety of endogenous factors (e.g., adiposity) and exogenous factors (e.g., lifestyle habits) appear to contribute to the rise in systemic levels of inflammation seen with aging.[4] To date, few therapeutic approaches have been specifically identified to reduce chronic systemic inflammation with the goal of reducing pain levels and improving functional performance in seniors. There are, however, a number of promising approaches that have emerged during the past decade that appear capable of targeting chronic systemic inflammation. Given the increasing number of older adults with elevated levels of systemic inflammation who are at risk for functional decline, new therapies are urgently needed to reduce systemic inflammation levels and improve or maintain functional ability in this high risk population. Thus, the purpose of this presentation is to provide an overview of promising therapeutic approaches, including lifestyle interventions, hormonal replacement, natural compounds, and pharmaceutical agents, to avert levels of chronic systemic inflammation during aging and preserve function in older adults.

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## Muscle, systemic iron deregulation and aging: therapies to prevent disability and mobility impairments

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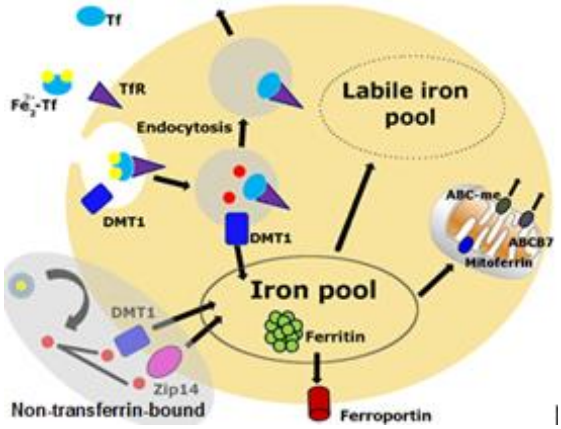
Iron dyshomeostasis (high cellular and low systemic levels) are strong risk factors in the development of disease, disability and premature death. Systemic iron deficiency (anemia with old age) impairs oxygen carrying capacity, while in contrast increased cellular levels can increase DNA lesions. Disturbances of iron metabolism including uptake, export, and storage have shown to play a causal role in cellular and mitochondrial dysfunctions with age and disease.




Iron is found in several forms: heme iron (i.e., haemoglobin, myoglobin) and non-heme iron (i.e., Ferritin). A distinct fraction of chelatable non-heme iron is referred to as the labile iron pool, which comprises less than 5% of total cellular iron. Labile iron consists of $Fe^{2+}$ and $Fe^{3+}$ ions associated with a variety of small molecules, including organic anions, polypeptides, and phospholipids. Labile iron can participate in Fenton reactions, producing highly destructive hydroxyl radicals, which are thought to be a major contributor to the formation of DNA mutatons. Cellular iron acquisition occurs through iron import proteins such as transferrin receptor (TfR1), divalent metal transporter-1 (DMT1), and Zip14, whereas cellular iron export is mediated by ferroportin (FPN), the only known iron exporter in mammals. The mitochondria contain mitoferrin (Mt iron importer), iron storage proteins such as frataxin and Mt ferritin (MtF) (which binds with iron), and ABCB7 (a heme export protein), all known to play an important role in the storage and regulation of Mt iron. We and others have found that in animals and humans, labile iron and non-heme iron increases with age and is associated with elevated expression of ferritin. In contrast, transferrin receptor 1 (TfR1; cellular iron import protein) showed a dramatic down regulation with age. In addition, mitochondrial iron levels effect Mt permeability transition pore opening susceptibility (i.e., $Ca^{2+}$ retention capacity) in mitochondria from old animals. Further studies to better understand iron metabolism with aging are warranted to design interventions to reduce DNA lesions.

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## Discovery of new SR/TT junctions that mediate $Ca^{2+}$ Entry in skeletal muscle

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Depletion of calcium ($Ca^{2+}$) from intracellular stores triggers store-operated $Ca^{2+}$ entry (SOCE), a ubiquitous mechanism that allows recovery of $Ca^{2+}$ ions from the extracellular space. To date, the subcellular location for SOCE in skeletal muscle fibers has not been unequivocally identified. Here we show by electron microscopy (EM) that 1 hour of incremental treadmill running of mice (from 5 m/min to 25 m/min) drives a striking remodeling of the existing sarcotubular system in skeletal fibers leading to formation of previously unidentified junctions between sarcoplasmic reticulum (SR) and transverse-tubules (TTs). In addition, using immunohistochemistry, immunogold labeling for EM, and western blot analyses we demonstrate that these new SR-TT junctions contain the molecular machinery that mediate SOCE: a) stromal interaction molecule-1 (STIM1), which functions as $Ca^{2+}$ sensor in the SR, and b) $Ca^{2+}$ permeable Orai1 channels in TTs. Finally, we used a stimulation protocol (30 x 1s-60Hz pulses every 5 seconds) to compare susceptibility to *in vitro* muscle fatigue of EDL muscles from either control or exercised mice. EDL muscles from exercised mice exhibited an increased capability of maintaining contractile force in presence of 2.5 mM extracellular $Ca^{2+}$, that was abolished by either the presence of SOCE inhibitors (BTP-2 and 2-APB) or by equimolar replacement of extracellular $Ca^{2+}$ with $Mg^{2+}$. We propose that exercised-induced formation of newly formed SR-TT junctions containing STIM1 and Orai1 proteins function as *$Ca^{2+}$ Entry Units (CEUs)*, structures that provide a pathway to rapidly recover $Ca^{2+}$ ions from the extracellular space during repetitive muscle activity..

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**The ERG1 Potassium Channel is detected in human skeletal muscle with greater abundance in cachectic patients**

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The ERG1 potassium channel is known to participate in repolarization of the cardiac action potential.[1] However, we reported detection of this protein in the *Gastrocnemius* muscle of mice experiencing atrophy as a result of both disuse (i.e., unweighting) and cancer cachexia while it was not detected in the *Gastrocnemius* muscles of appropriate control animals.[2] In subsequent studies, we showed that ERG1 participates in muscle degradation by enhancing ubiquitin proteolysis through increased abundance of the E3 ligase, MuRF1.[3,4] However, to our knowledge, ERG1 has not been reported in human skeletal muscle. Here we have used immunohistochemistry and confocal microscopy to image ERG1 protein with a fluorescent marker and report detection of ERG1 immunofluorescence in the *Rectus abdominis* (RA) muscle of adult humans. Interestingly, we detect statistically greater immunofluorescence (67.0%; $p \leq 0.01$) in the RA muscle of people having cancer cachexia (n=6) than in the same muscle of age-matched healthy adults (n=7). We detect ERG1 immunofluorescence at low levels only in the RA muscle of young adults (n=4); however, our results show that the signal trends toward greater fluorescence (11.0%) in the RA muscle of healthy aged adults than in that of the younger ones. Although the difference in ERG1 immunofluorescence in the healthy aged and young adult RA muscle is not statistically significant, Power analysis of the data demonstrates that an increase in sample size to 46 (23 each group) from the current size of 11 people would produce a significant difference in the data. Indeed, our data suggest that ERG1 may be related to the skeletal muscle loss that occurs with cachexia and aging in humans.

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**Dysfunctional accumulation of STIM1 and Orai1 in Tubular Aggregates results in impaired $Ca^{2+}$ entry in ageing muscle**

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Tubular aggregates (TAs), ordered arrays of sarcoplasmic reticulum (SR) tubes, form in ageing fast twitch fibers of mice, preferentially in males. TAs are also the main morphological alteration in biopsies from patients affected by TA Myopathy (TAM). TAM has been linked to mutations in the genes encoding for STIM1 and Orai1, the two proteins that mediate store-operated Ca2+ entry (SOCE), a mechanism that allows recovery of extracellular Ca2+ when the SR is depleted. We have previously shown that: i) TAs contain SERCA1 and CASQ1, two proteins involved in re-uptake and storage of Ca2+ in the SR; ii) tubes of TAs appear linked by small bridges. Here, we combined different experimental approaches - electron and confocal microscopy (EM and CM), western blots (WB), and ex-vivo stimulation protocol (30 x 1s - 60 Hz pulses every five seconds) performed in inctact EDL muscles - to study localization and function of STIM1 and Orai1 in muscle containing TAs. In EDL muscles from mice of 4 and 24 months of age: i) ageing causes STIM1 and Orai1 to accumulate in TAs; ii) the expression levels of both STIM1 splicing variants increase with age (STIM1S = 0.44±0.03 vs 0.66±0.08 A.U.; STIM1L = 0.38±0.05 vs 0.56±0.05 A.U. respectively for adult and aged mice); iii) EDL muscles from aged mice exhibit a decreased capability to maintain contractile force compared to adult mice (relative force after 10 tetani: 61.6±3.0%, and 52.7±4.3% respectively for adult and aged EDL muscles). Our findings suggest that accumulation of STIM1 and Orai1 in TAs, is dysfunctional as Ca2+ entry during repetitive stimulation is impaired in aged EDL muscles.

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**Reduced performance and loss of sarcomeric M-band organization following heavy exercise in obscurin knockout mice**

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The sarcomere is a highly organized structure that represents the functional unit of the contractile apparatus of striated muscles. The maintenance of both sarcomere integrity and the correct reciprocal arrangement between myofibrils and organelles, like nuclei and sarcoplasmic reticulum, costameres, etc., represent a crucial requirement that striated fibers must fulfill to efficiently accomplish repeated cycles of contraction and relaxation. Obscurin is a giant sarcomeric protein mainly localized at the M-band and, with minor distribution, at the Z-disk. The structural layout of Obscurin, which is based on the presence of different modular binding, adhesion and signaling motifs, allows the simultaneous interaction with sarcomeric and non-sarcomeric proteins, thus placing Obscurin in a key molecular crossroad to contribute to the overall muscle fiber architecture. Indeed, binding of Obscurin to Titin, Myomesin and OBSl1 provides an important structural support to sarcomere integrity and stability at the level of the M-band. In addition, the ability of Obscurin to interact with distinct members of the ankyrin family contributes to establish multiple molecular contacts between the contractile apparatus and sarcoplasmic reticulum, microtubules and costameres.[1] We have recently reported studies with Obscurin KO mice suggesting a role of Obscurin in supporting fiber integrity following heavy exercise.[2] These results will be presented and discussed also in relation to the recent identification of mutations in the Obscurin gene in patients with cardiac and skeletal muscle diseases.[3]

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**S6K1 Is Required for Increasing Skeletal Muscle Force during Hypertrophy**

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Loss of skeletal muscle mass and force aggravates age-related sarcopenia and numerous pathologies, like cancer and diabetes. The AKT-mTORC1 pathway plays a major role in stimulating adult muscle growth, however, the functional role of its downstream mediators in vivo is unknown. Here we show that simultaneous inhibition of mTOR signaling to both S6K1 and 4E-BP1 is sufficient to reduce AKT-induced muscle growth and render it insensitive to the mTORC1-inhibitor rapamycin. Surprisingly, lack of mTOR signaling to 4E-BP1 only, or deletion of S6K1 alone, is not sufficient to reduce muscle hypertrophy or alter its sensitivity to rapamycin. However, while not required for muscle growth, we report that S6K1 is essential for maintaining muscle structure and force production. Hypertrophy in the absence of S6K1 is characterized by a compromised ribosome biogenesis and the formation of p62-positive protein aggregates. These findings identify S6K1 as a crucial player for maintaining muscle function during hypertrophy.

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**Advanced muscle assessment using 3D modeling & soft tissue CT profiling**

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This work outlines the methods and applications of X-ray Computed Tomography imaging to analyze soft tissue and skeletal muscle density and volume in the context of modern challenges in the field of translational myology. The approaches described here use medical imaging processing techniques and computational methods to: quantify muscle morphology, illustrate changes with 3D models, develop numerical profiles specific for each individual, and assess muscle changes due to targeted medical treatment. Applications of these methodologies are employed: to depict subject specific muscle profiling associated with age, to illustrate and quantify muscle degeneration and its partial reversal via Functional Electrical Stimulation (FES), and to highlight recovery following total hip arthroplasty.[1-5]


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**QMC-CT, a quantitative muscle assessment that oldest patients understand and that can get them to take-home full-body in-bed gym strategies**

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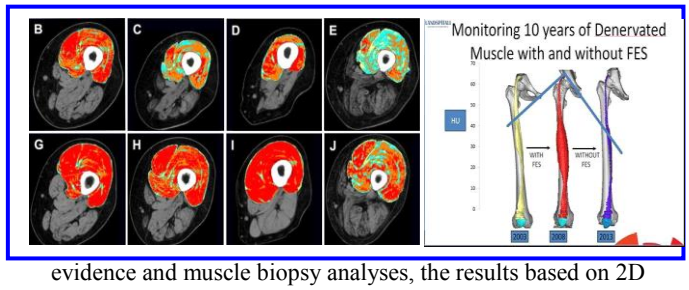
The functional recovery from severe atrophy of long-term denervated muscle by h-bFES of DDM is a fact standing on sound foundations.[1] Among them, a new quantitative muscle color computed tomography (QMC-CT)[2,3] adds to functional




evidence and muscle biopsy analyses, the results based on 2D (left panels) and 3D (right panel) clinical imaging analysis.

We are extending the methods to managements of severe atrophy in oldest persons, which need simplified methods of evaluation, and safe, easy to performe rehabilitations at home.[4] A major problem is to convince subjects to maintain volitional exercise at home. We are confident that strong evidence of structural improvements of muscles could motivate reluctant older persons to take home anti-aging full-body in-bed gym[5] and functional electrical stimulation (FES) for mobility compromised elderly persons.[4]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**MicroRNAs expression in muscles and serum in DM1**

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Myotonic Dystrophy (DM1) is the most common form of adult-onset muscular dystrophy, but is missing circulating biomarkers as well as an effective rehabilitation protocol. In our work we aim to propose a clinical-molecular protocol to monitor rehabilitation therapy versus standard care in this common inherited muscle disorder. For all DM1 patients the maximum standard of care was achieved through special medical attention and locomotor study, cardio-respiratory and nutritional care, interview for psychological problems,

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quality of life, we investigated the role of serum MicroRNAs as biomarkers of the disease in order to correlate their levels with disease severity, multiorgan involvement and possibly the efficacy of physical rehabilitation program. We aimed to explore the cellular action of micro-RNAs that are non-coding-RNAs modulating gene expression, whose expression is dysregulated in DM1. In order to investigate the micro-RNA origin a initial aim was to measure the levels of muscle-specific myo-miRNAs (miR-1, miR-133a/b, miR-206) in muscle of 12 DM1 patients.[1] Muscle fiber morphometry with a new grading of histopathological severity score were used to compare specific myo-miRNA level and fiber atrophy. We found that the levels of miR-1 and miR-133a/b were significantly decreased, while miR-206 was significantly increased as compared to controls. The histopathological score did not significantly correlate with the levels of myo-miRNAs, even if the lowest levels of miRNA-1 and miRNA-133a/b, and the highest levels of miRNA-206 were observed in patients with either severe histopathological scores or long disease duration. The histopathological score was inversely correlated with disease duration. Nowadays DM1 muscle biopsies are scanty, since patients are usually diagnosed by genetic analysis, our study offers a unique opportunity to present miRNA expression profiles in muscle and correlate them to muscle morphology in this rare multisystem disorder. Our molecular and morphologic data suggest a post-transcriptional regulatory action of myo-miRNA in DM1, highlighting their potential role as biomarkers of muscle plasticity.We explored in 10 patients (9 male and 1 female) during our new rehabilitative protocol we developed.[2] Serum microRNAs appeared as biomarkers to monitor DM1 patients while in a protocol of aerobic lower extremity Functional Electrical Stimulation lower aerobic rehabilitation.[2] We observed improvement of our patients during this exercise protocol and all microRNas decreased during rehabilitation (Figure).This study validate clinical use of microRNAs after the first discovery in MD1.[3] In our investigations in muscle and serum, some microRNA (miR-1, miR-133a, miR-133b, miR-206) appeared promising in detecting changes in DM1 in natural history and during rehabilitation to correlate with functional outcomes, we found that reversal of muscle atrophy and onset of muscle regeneration in DM1 might be revealed by decreased microRNA levels. These circulating biomarkers were validated in this study in twelve DM1 cases.

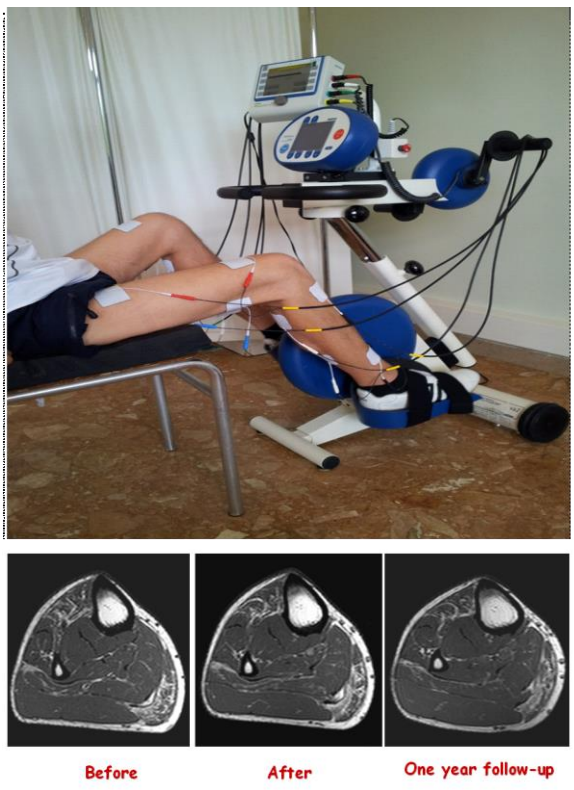


**Fig. 1** Muscle imaging before and after aerobic training FES. T1-weighted axial images of the right leg: he had increased 6MWT and increased muscle mass at MRI. Patient performing cycling FES

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**Physical exercise as a preventative strategy to slow down cognitive decline in ageing. Evidence for the value of aerobic physical activity**

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Several epidemiological studies have repeatedly shown a statistical association between life-long physical exercise and better preserved cognition later in life. This association was based on self-reports coded as variables which do not retain much quantitative variability. Some studies have used metabolic conversion to give a biological flavour to their findings. A few recent experimental studies have identified physical activity as a protective factor for cognitive decline. The role of physical activity as a protective factor has received more attention than other popular ways of stimulating the brain, e.g. cognitive stimulation. Studies have focused on discovering the biological mechanisms behind this effects and attention has been given to mitochondrial activity and the pathways by which ATP is produced, with a specific focus on aerobic exercise. Research studies have also compared the effects of acute vs chronic exercise. Experimental work has been carried out on acute exercise (i.e. single sessions) to explore the mechanisms involved and shed light on the biological underpinning of the beneficial effects of physical activity on cognition. This research has often involved young adults because of the opportunity to implement better manipulation of variables such as intensity and duration of exercise. Brain activity has been measured with Near Infrared Spectroscopy to study how brain function changes during acute exercise in an attempt to infer the mechanisms behind the long term effect of exercise. Because chronic exercise is associated with long term effects, there is a clinical interest to clarify the mechanisms that are involved

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in short and long term benefits due to exercise. Many studies have used exercise in combination with mixed interventions (e.g. diet and exercise, or cognitive stimulation and exercise), however. More recent experimental approaches have put forward possible explanations about the basis of the beneficial effects of exercise and suggested that physical activity triggers an improvement of cardiovascular fitness and improvement in cognition, but it is still unknown whether the two are causally linked. The implication is that cognitive benefits are the indirect outcome of cerebrovascular improvements. Other studies have suggested that physical activity increases neuroplastic mechanisms in humans, by fostering hippocampal neurogenesis, by regulating cortisol and BDNF and by enhancing motor-cortical plasticity as elicited by the TMS-based technique "cerebellar inhibition". A crucial modulating factor appears to be played by individual genetic profiles, such as that for the ApoE gene. There is experimental evidence that suggests that the long term beneficial effects of exercise might be the result of optimisation of prefrontal resources via continuous exercise dependent hypofrontality. Overall, better designed trials with more sophisticated outcome measures are necessary to test experimentally the extent to which physical activity might be an effective form of intervention to prevent cognitive decline in ageing and neurodegeneration. There is, however, some recent evidence that the regular practice of walking improves cognition in Alzheimer's disease, while strength training is particularly more effective for improving postural and motor function, and reducing the risk of developing Alzheimer's disease, since it improves muscle mass and strength, shown to be affected in this disease.

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**Experimental activation of damaged nerves and denervated muscles**

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The neuromuscular system is subject to many kinds of damage, from traumatic nerve injury to slowly progressive neuropathies. The emerging field of electroceuticals aims to intervene by recording, processing and normalising neural activity to enhance the function of failing organ systems. Electrical activation has the potential both to maintain muscle mass and to promote neural growth after peripheral neural trauma.[1,2] But interaction with the musculoskeletal system must take into account the changes in that system that affect the requirements for artificial activation. The most obvious example is that denervated muscles require much greater current to flow in their membranes to activate release of calcium and contraction than do innervated muscles, whose activation is based on the electrochemical generation of action potentials in the muscle fibre membrane beneath the motor end plates. Similarly, if we are to use stimulation therapy to treat diabetes by neuronal stimulation, then we must take into account that diabetes is often associated with altered neuronal function. The need to inject current from implanted electrodes brings its own risks of tissue damage, tissue heating, and electrolysis of electrode materials. A target denervated muscle may be situated among other innervated muscles, or adjacent to sensory structures. Thus the selection of electrode material, shape and size is important to the outcome. This presentation will review theoretical and practical design criteria to achieve safe and efficient activation of musculoskeletal structures, with some examples.[3]

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**Chronic electrical stimulation for reversing signs of laryngeal muscle ageing**

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Age related changes of the muscle and its adjacent structures also affect the larynx.[1] Muscular atrophy leads to an incomplete closure of the vocal folds, leading to a hoarse and breathy voice. The consequences are reduced quality of life

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and reduced working capacity of persons who are depending on their voices professionally (teachers, policemen etc.). Chronic electrical stimulation of the afferent nerve (recurrent laryngeal nerve) is a completely new therapeutic option that has not been tested before. In a preliminary study we could show that electrical stimulation of the recurrent laryngeal nerve led to an increase of mean muscle fiber diameter in aged sheep, even with a very conservative pattern of two minutes tetanic contraction daily over a period of 29 days.[2] Here we present data of an ongoing sheep trial where the electrode was implanted unilaterally adjacent to the terminal branch of the inferior laryngeal nerve. This surgical approach is already close to a clinical setting in humans.

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**3D reconstruction of the thyroarytenoid muscle combined with phonation analysis in an *ex-vivo* animal study on aged sheep larynges**

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The access to different structures in the larynx - especially to the intrinsic muscles *in vivo* - is limited. Additionally the volumetric quantification is problematic due to their covering with mucosa. Nevertheless it is necessary to generate accurate models of these structures for the purpose of answering muscle-specific issues. Nowadays this is possible with modern imaging procedures such as micro-CT scanning. This technology has advantages over MRI in terms of better resolution and the samples are not destroyed during the imaging process as in histologic sampling. To differentiate the muscles from soft tissue and cartilage, the samples are fixed and preserved in neutral buffered formalin (NBF) and stained with iodine potassium iodide ($I_2KI$) to enhance contrast in the CT-scan.[1] The purpose of this study is to generate 3D-models of the laryngeal frameworks and the intrinsic laryngeal muscles by segmentation and finite-element generation using the 3D-analysis-software Avizo®. This modeling technique will be used in ongoing experiments in the field of muscle stimulation for analysis of the results, especially muscle volumes, surfaces and structure. Additionally, phonation experiments on the same subjects were performed to find out correlations between functional parameters and morphometric measurement parameters.[2] Phonation analysis included aerodynamic parameters such as the subglottal pressure or the laryngeal flow resistance and acoustic parameters such as the sound pressure level or the fundamental frequencies. Furthermore, high-speed recordings have been performed to visually assess the vocal fold vibrations.[3,4]

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**Clinical Results of ES in Unilateral Vocal Fold Paralysis (uVFP)**

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Vocal fold paralysis is a pathological motion impairment of the vocal fold, mostly caused by damage of the N. vagus or the N. laryngeus. If the vocal fold does not reinnervate, paralysis occurs due to denervation of the *M. posticus*[1] Patients with unilateral vocal cord paralysis suffer from hoarseness due to additional atrophy of the *M. vocalis* with glottal closure insufficiency during phonation. Today's standard treatment of unilateral paralysis includes surgical medialization through either injection augmentation or laryngeal framework surgery.[2] In combination with voice therapy also electrical stimulation of laryngeal muscles has already been used in order to achieve muscle hypertrophy.[3] Furthermore research with functional electrical stimulation of patients with long-term denervated limb muscles showed very promising results.[4] The selective stimulation of denervated muscles has been investigated in rabbits with unilateral paresis of the recurrent laryngeal nerve. It could be shown that with triangular ramping and very long pulses (> 200ms) afferent and efferent nerve fibers where not reacting at intensity level that already stimulated denervated muscle,

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with change in muscle fibers confirmed through histology. [5,6] Combining these facts led to the following investigations: Investigating a screening possibility using surface electrodes onto the neck to selectively stimulate the denervated muscle fibers of the vocalis avoiding pain or excitation of sensory nerve fibers or the activation of innervated muscles was the goal of several test stimulations. First results applying long triangular ramping pulses (>200ms) using surface electrodes are surprising. The position and size of electrodes used in the trials were improved continuously. Success could be reported only in the non-awake patient, whereas reasons have to be identified.

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**Clinical results of ES in facial nerve paralysis as a model of ES for muscles with a mixed lesion pattern: chronic denervation and misdirected reinnervation**

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Facial nerve paralysis as a peripheral nerve injury results in neuromuscular atrophy or in a combination of muscle atrophy and false reinnervation of facial muscles. The symptoms include significant aesthetic, functional and often life-altering consequences. Several procedures such as nerve grafting, facial reanimation by muscle transfer and rehabilitation physiotherapy have been developed to treat functional and cosmetic aspects of this disease.[1] Nerve grafting is a sophisticated surgery, that requires experience but offers promising results. Although cable grafting is state of the art, the method suffers the disadvantage of long nerve regrowth time.[2] Facial pacing systems show promising results to treat facial paralysis.[3,4] Former research showed good results stimulating denervated extremity muscles using functional electrical stimulation (FES).[5] Nevertheless this field of research has been neglected so far for facial muscles and is lacking optimal stimulation settings to selectively recruit denervated atrophic or simply age-related atrophic facial muscles under non painful conditions. To analyze first optimal FES setting will be the prerequisite to establish FES as a screening tool to select patients for facial pacing. Several ES devices were considered to investigate optimal stimulation settings in patients with chronic facial palsy. To encourage noninvasive screening methods for facial pacing, surface electrodes were used to estimate the optimal settings for stimulations. The use of surface electrodes need for optimized electrode positioning, which was also investigated. Martin et al.[6] showed that recruitment of denervated muscles requires exponentially shaped pulses with long phase durations (>200ms). The outcome of our investigation confirmed these findings as well, showing best performance when recruiting paralyzed facial human muscles with biphasic long-duration impulses. It is crucial to position the surface electrodes appropriately in order to avoid stimulation of neighboring muscles not affected by facial palsy, for instance the masseter muscle. Surface electrodes, combined with the optimal stimulation settings, offer a screening possibility for facial pacing but also a therapeutic option to prevent atrophy. Since muscles affected by age-related atrophy could be recruited too, further research is necessary to show effectiveness of training using the determined exponential patterns.

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**Physical, pharmacological and nutritional interventions against muscle wasting and dystrophy**

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Recent studies have correlated physical activity with a better prognosis in cachectic patients, although the underlying mechanisms are not yet understood. In addition, diets enriched with n-3 polyunsaturated fatty acids (n-3 PUFAs) have been shown to exert a positive effect on diseased muscle. Muscle diseases as different as cachexia and dystrophy are characterized but reduced or absence of dystrophin expression, latent or overt muscle damage and impaired regeneration, thus sharing several pato-

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physiological features, such as muscle wasting, loss of muscle mass and function. With the aim to test in preclinical models and in human patients the efficacy of physical, pharmacological and nutritional interventions against muscle wasting and disease, we exploited two different rodent models of cachexia and muscular dystrophy and validated part of these findings in human patients.

Part 1. Cancer cachexia. Since we previously found that satellite cells (SC) impairment, due to Pax7 over-expression, contributes to cachexia,[1] we studied the effects of voluntary exercise on these cell in colon carcinoma (C26)-bearing mice. We found that endurance exercise rescues Pax7 expression to physiological levels, suggesting that this could be a mechanism underlying its beneficial effects in this condition.[2] Moderate exercise training protocols induced muscle adaptation in both control and C26-bearing mice, which are mediated by PPARgamma in a Hsp60-dependent way.[3] Indeed, voluntary exercise prevented loss of muscle mass and function, ultimately increasing survival of C26-bearing mice. We found that the exercise mimetic AICAR, rapamycin and exercise equally affect the autophagic system and counteract cachexia.[4] We believe autophagy-triggering drugs may be exploited to treat cachexia, especially in conditions in which exercise cannot be prescribed, since cancer patients show abnormal expression of autophagy markers, suggesting that the autophagic flux is blocked in cachexia, thus contributing to muscle wasting.

Part 2. Muscle dystrophy. Since flaxseed is one of the richest sources of the n-3 PUFA acid α-linolenic acid (ALA), we assessed the effects of flaxseed and ALA in models of skeletal muscle degeneration characterized by high levels of Tumor Necrosis Factor-α (TNF) and exhaustion of SC myogenic potential. Our study was carried out on dystrophic hamsters and differentiating C2C12 myoblasts treated with TNF, both in the absence or presence of flaxseed diet or ALA treatment, respectively.[5] The flaxseed-enriched diet protected the dystrophic muscle from apoptosis and preserved muscle myogenesis both *in vivo* and *in vitro*, indicating that flaxseed may exert potent beneficial effects by preserving skeletal muscle regeneration and homeostasis partly through an ALA-mediated action.

In conclusion, physical activity, pharmacological treatment (exercise mimetics such as AICAR) and nutritional supplementation (such as ALA) are beneficial for muscle mass preservation and life span increase in the presence of cancer cachexia or muscle dystrophy and should be considered when planning multimodal therapies for muscle diseases.

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**Cancer and chemotherapy-induced cachexia in mice: effects of moderate exercise training**

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Cachexia is a multifactorial syndrome characterized by body weight loss, muscle wasting, and metabolic abnormalities, that occurs in 50 to 80% of cancer patients and is considered as a predictor of reduced survival accounting for more than 20% of cancer-related deaths.[1] Cachexia was defined also as an energy-wasting syndrome, in which mitochondria play a central role as the main energy source. Indeed, mitochondrial alterations and an upregulation of mitophagy markers have been found in the skeletal muscle of cachectic animals.[2] In addition to the effects exerted by the tumor, also anti-cancer treatment may contribute to muscle wasting.[3] Some years ago, exercise has been proposed as a therapeutic tool to counteract cachexia and the related metabolic alterations,[4] including autophagy dysregulation, mitochondrial dysfunction and oxidative capacity reduction.[2,5] The present study aimed at evaluating the effects of moderate exercise training on muscle wasting in C26-bearing mice treated with chemotherapy (oxaliplatin+5-fluorouracil; OXFU), focusing on both alterations of muscle autophagy/mitophagy and mitochondrial function. OXFU administration was able to extend the lifespan of the C26-bearing mice (100% survival at 28 days after tumor implantation), but also resulted in exacerbated cachexia. In C26 OXFU mice, exercise partially protected from muscle mass loss and associated with an improvement of muscle function. Chemotherapy further dysregulated cancer-induced autophagy, increasing the levels of Beclin-1 and LC3I. Exercised C26 OXFU mice showed a lower content of Beclin-1 and of both LC3B isoforms compared to sedentary mice. Focusing on mitochondria, the levels of cytochrome c, used as a measure of mitochondrial content, decreased in sedentary C26 OXFU mice, associated with a reduction of SDH protein levels and enzymatic activity. Sedentary C26 OXFU mice showed also increased levels of Bnip-3 and PINK-1, two proteins involved in mitophagy. In C26 OXFU mice, exercise increased the levels of cytochrome c, PGC1α and both SDH content and activity, decreasing also the levels of PINK-1. The alterations seen in C26 OXFU animals were associated with a strong reduction in protein synthesis, that was not improved by exercise. In conclusion, chemotherapy exacerbated tumor-associated muscle wasting and metabolic alterations. Moderate exercise training was able to partially counteract muscle loss and recover muscle function, increasing mitochondrial content, autophagy and damaged-mitochondria clearance, and rescuing muscle oxidative capacity. Therefore, exercise

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exerts beneficial effects potentially exploitable in the management of cancer patients receiving chemotherapy.

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**Generation of iPSC lines from NLSDM patients carrying different PNPLA2 gene mutations: a novel frontier for the study of neutral lipid metabolism disorders**

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Neutral Lipid Storage Disease with Myopathy (NLSDM) is a very rare disorder characterized by a defect in the degradation of cytoplasmic neutral lipids and their accumulation in the lipid droplets (LDs). This neutral lipid metabolism deficiency is associated with mutations of PNPLA2 gene, which encodes adipose triglyceride lipase (ATGL).[1-2] ATGL leads to the breakdown of triacylglycerols (TAGs), releasing free fatty acids. NLSDM patients may develop progressive myopathy (100%), cardiomyopathy (44%), diabetes (24%), hepatomegaly (20%), chronic pancreatitis (14%) and short stature (15%). No specific therapy is available today.[3-4] Fibroblasts cell lines from two patients and one healthy subject have been reprogrammed into induced pluripotent stem cells (iPSCs). iPSCs are a new technology which can provide an unlimited number of human disease-affected stem cells from different somatic cell lines.[5] The first NLSDM patient was homozygous for the c.541_542delAC PNPLA2 mutation that causes the production of a truncated protein lacking the LD-binding domain.[3] The second patient was homozygous for the c.662G>C PNPLA2 mutation, determining the p.R221P amino-acid change; this mutation leads to the production of ATGL protein with decreased lipase activity, but able to bind to LDs.[2] After about 4 weeks from the Senday infection, karyogram showed a normal karyotype of controls and NLSDM-iPSCs; moreover genomic sequencing analysis confirmed that NLSDM-iPSC lines still contained the disease-specific mutations of PNPLA2 gene. We tested the pluripotency properties of NLSDM-iPSCs evaluating the expression of TRA-1-81, SSEA4 and OCT4 by immunostaining and of SOX2, NANOG, ZFP42, OCT4, hTERT, LIN28, DPPA2 and TDGF1 by qRT-PCR analysis. NLSDM-iPSCs were also able to differentiate into three-germ layers, as revealed by β-III tubulin (ectoderm), α-smooth muscle actin (mesoderm), and FOXA2 (endoderm) expression. Finally, we demonstrated that NLSDM-iPSCs showed an higher storage of TAGs in comparison with control iPSCs, exactly as it could be observed in NLSDM original fibroblasts when compared with control fibroblasts. Indeed, after 3 days in culture, cells were stained with Nile Red and the LD number and dimension were analysed by immunofluorescence analysis; compared to control cells, the NLSDM-iPSCs had 20 times more LDs and almost 5 larger LDs, similar to fibroblasts obtained from the patients. Moreover, oleic acid pulse-chase experiments were performed to confirm that lipase activity was impaired in NLSDM-iPSCs compared to control cells. Collectively, data from this study consistently show that NLSDM-iPSCs recapitulate the disease phenotype of interest. The perspective to differentiate iPSCs into striatum/cardiac muscle lineages will allow us to define a disease model to investigate the pathogenetic mechanisms and to evaluate specific approaches for new pharmacological treatments.

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**Seeking economy of charge injection in activation of motor nerves**

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How does one choose a pattern of electrical stimulation for therapeutic effect? Often there is a useful guide from normal physiology, and many therapeutic strategies try to mimic or replace a natural activation pattern. Another strategy is to try to generate a numerical model of the excitable tissue to be stimulated so that trials can be achieved in silico.[1] Many optimised activation strategies are based on such simulations. We have tested some of the conclusions of studies that have investigated the charge efficiency of activation.[2-7] We have used the simple experimental model of a single motor nerve trunk activated by two electrodes placed near to the nerve (common peroneal in rats). The degree of activation has been monitored indirectly by measuring the isometric force of the edl muscle because it has discrete proximal and distal tendons and can thus be mechanically isolated between a proximal clamp and a distal load sensor. We are in a process of critically analysing this data because some of our initial results appeared surprising. We will present results that compare the actual electrode current against the anticipated current based on the use of a voltage-to-current converter. We will also present further analysis of the linearization method that we used to select optimal parameters for the various pulse shapes that we tested. We find that the opportunities to improve energy efficiency are more relevant to monopolar stimulation with one remote electrode far from the nerve than to bipolar stimulation, in which the current field is created between two electrodes both near to the nerve. Such fine differences are important when designing low energy implanted stimulators such as may be used in retinal stimulation or brain stimulation or activation of fine autonomic nerves.

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***In vitro* surface characterization of adapted platinum neural stimulating electrodes**

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In the development of implantable prosthetic devices, much effort has been put into finding optimal anatomical targets for different nerve stimulation techniques. Little work however, has been done to improve the efficiency of nerve stimulation by using analytically driven designs and configurations of the stimulating electrodes. Namely, an electrode geometry can affect the effective impedance, spatial distribution of the electric field in tissue, and consequently the pattern of neural excitation. One approach to enhance the efficiency of neural stimulation is to increase the irregularity of the surface current profile. In this relation, it has been shown, that adequately optimized electrode geometries and surfaces that increase the variation of current density on the electrode surface enable also an increase of the efficiency of neural stimulation. In this relation, a variety of mechanical adaptations, such as geometry and surface roughness of the electrodes, have been investigated and implemented. The purpose of the study was therefore to assess "*in vitro*" the electrochemical performance of two stimulating electrodes (WEs) with different surface structures obtained by treating the surface with smooth and rough sand paper. To craft the stimulating electrodes, 0.03-mm-thick cold-rolled platinum foil strips with 99.99 wt.% purity and dynamic annealing in an argon atmosphere were used. The obtained final dimensions of the electrodes exposed to the physiological solution were: width 0.66 mm, length 3 mm and surface area 2 $mm^2$. For adaptations of two investigated WEs via increase their real surface, two differently grained sand papers (Waterproof Silica Carbide Paper FEPA P#500 and FEPA 4000, Struers ApS, Pederstrupvej 84, 2750 Ballerup, Denmark) were used. A surface of the WE1 was enlarged using rough sand paper FEPA P#500 while WE2 was enlarged using fine-grained sand paper FEPA P#4000. For the purpose of spot welding of the stainless-steel wire and the platinum foil, a custom-designed, capacitive-discharge, research-spot-welding device, providing a standard single pulse, was developed. The welding energy for both electrodes is defined experimentally. To analyse any failure and to reveal the microstructure of the weld, and consequently to set up optimum welding conditions, scanning electron microscopy was used. The results provide evidence that the welds between the stainless-steel wire and the platinum foil do not show any typical welding defects, such as oxide films, oxide inclusions, gas bubbles or shrinkage porosity. Obtained results also show that an impedance of WE1 is lower than impedance of WE2. Accordingly, the WE1 is more suitable for safe stimulation than WE2.

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**Comparing somatosensory evoked potentials resulting from transcutaneous spinal cord stimulation (tSCS) and tibial nerve stimulation in healthy and CP subjects**

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Transcutaneous spinal cord stimulation (tSCS) has been shown to abbreviate spasticity in lower limbs in people with incomplete spinal cord injury (SCI) people.[1,2] Therefore tSCS is a therapy of choice for SCI in our clinic. It is also known that SCI modulates the organisation of the brain in the way that it decreases the areas allocated for the control of the not connected extremity part.[3] Therefore we hypothesize that the tSCS treatment can influence the plasticity of the brain as well. In this work the footprint of the tSCS in the EEG is sought in order to verify that the stimulating signals are transmitted to the brain. In this first approach one healthy subject for control and one Cerebral palsy (CP) patient participated. Cortical somatosensory evoked potentials (SEP) where recorded during tibial nerve stimulation and during tSCS. The recording of SEP during tibial nerve is well documented so it serves as a proof of method. Then SEP was also recorded during voluntary ankle dorsiflexion and analyzed for event-related (de-)synchronization (ERD/ERS).[4] SEP is clearly to be seen in the sensorimotor cortex during tSCS. It is though different in form from the SEP during tibial nerve stimulation. As expected the ERD/ERS were focused over the Cz electrode as documented in the literature.[4] After movement by the CP subject the synchronisation was limited and therefore different to a healthy subject. But no significant changes where found after treatment. As the tSCS modifies the SEP the hypothesis that the treatment could influence the brains plasticity is supported. The difference in SEP between tSCS and tibial nerve stimulation suggests that different fibres in the spinal cord are stimulated. ERD/ERS patterns are changed in CP compared to a healthy subject.

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**Testing dexterity and mobility in the elderly, a simplified approach for the older olds**

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Aging of the human skeletal muscles results from decline of both muscles strength and power.[1] The athletic world records of the Master athletes at ages ranging between 35 to 100 years are an excellent proof of such decline in all competitions. The world record performances can be transformed into dimensionless parameters proportional to the power developed in the trials. Such parameters range from 1 for the Senior world record (i.e. the maximum human performance) through medium values for the Master athletes to reach 0 for a null performance.[1] Therefore, the decline of the power parameter with relation to human aging can be analysed and compared as follow: the trend-lines start to decline very close to the age of 30 years and arrive to 0 around the age of 110 years for each athletic discipline. There are no reasons, for each one of us, to decline differently from the world record-men, provided that each of us remains in a stable fitness condition without disabling pathologies. On the other hand, the methods to evaluate decline in the older olds need to be adapted to the extent of decay (as it is very commonly done in pathology). This is particularly important after 70 years of age and according to sex difference in power.We have adapted clinical methods,[2,3] to evaluate dexterity and mobility in normal older olds introducing 5 simplified Tests. Patients are assessed with the Timed Up and Go Test (TUGT), Five Chair Rise Test (5xCRT), and Jug Test (JT).[3,4] The Timed Up and Go Test has been validated as a useful indicator of leg muscle performance in numerous populations, including patients with neuromuscular diseases. Additionally, maximal isometric torque of quadriceps muscle on a force measurement chair is determined as [Nm/s] and the time which a subject needs to rise from a chair with arms folded across the chest 5 times (i.e., Five Chair Rise Test, 5xCRT) is measured.[3] The "jug test" (floor-to-table jug test, JT) provides information on the behaviour of arm, shoulder and trunk muscles. Specifically, participants move five 1-gallon jugs (≈3.9 kg) from the floor to a normal 75 cm high table level - as quickly as possible.[4] This action is quite like the everyday activity of lifting a shopping bag from ground to

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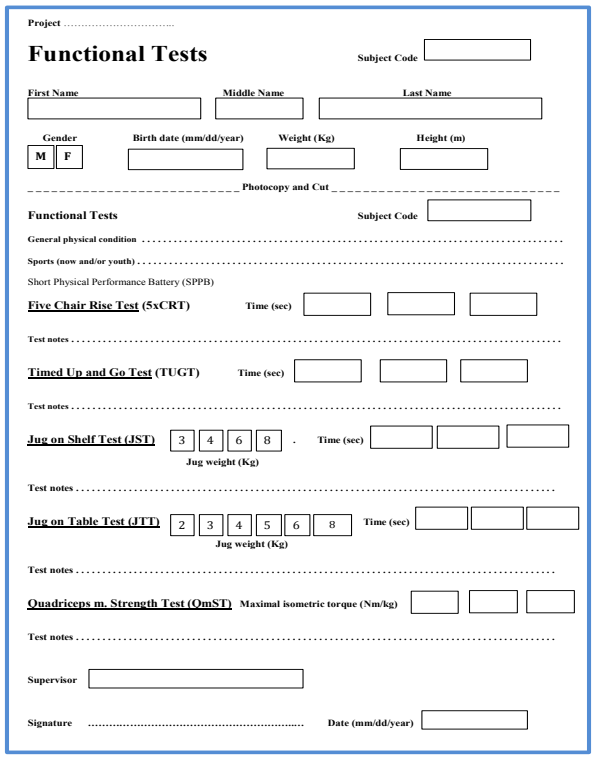
table. The weight of the jug varies according to age and gender of subjects as indicated in the following template of Functional Test Report.



Project .....

**Functional Tests** Subject Code

First Name | Middle Name | Last Name

Gender (M F) | Birth date (mm/dd/year) | Weight (Kg) | Height (m)

_____ Photocopy and Cut _____

**Functional Tests** Subject Code

General physical condition .....

Sports (now and/or youth) .....

Short Physical Performance Battery (SPPB)

Five Chair Rise Test (5xCRT) Time (sec)

Test notes .....

Timed Up and Go Test (TUGT) Time (sec)

Test notes .....

Jug on Shelf Test (JST) 3 4 6 8 . Time (sec)

Jug weight (Kg)

Test notes .....

Jug on Table Test (JTT) 2 3 4 5 6 8 Time (sec)

Jug weight (Kg)

Test notes .....

Quadriceps m. Strength Test (QmST) Maximal isometric torque (Nm/kg)

Test notes .....

Supervisor

Signature ..... Date (mm/dd/year)

Further, every day mobility is assessed by providing a pedometer (Nakosite, USA). The participants hold it 24 hours a day, for two weeks with break periods of three months. All functional results are correlated to 3D false color computed tomography of skeletal muscles.[5]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## Neurorehabilitation in neuromuscular disorders and consequences at microRNAs as circulating biomarkers

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MicroRNAs (miRNAs) are small non-coding RNAs that have been shown to modulate a wide range of biological functions under various pathophysiological conditions. miRNAs are 17-27 nucleotides long molecules that regulate post-transcriptional mRNA expression, typically by binding to the 3'-untranslated region of the complementary mRNA sequence, and resulting in translational repression and gene silencing. Therefore, an increase in a specific miRNA results in a decreased expression of the corresponding protein product. Several studies have shown that there are thousands of different human miRNA sequences that control the expression of 20-30% of protein-coding genes, indicating that miRNAs are "master regulators" of many important biological processes. MiRNAs are known to be secreted by various cell types and, unlike most mRNAs, they are markedly stable in circulating body fluids due to proteic protection from ribonucleases. Because of these properties, miRNAs have recently gained attention for their potential as minimally invasive and cost-effective disease biomarkers. Because of their stability in plasma and serum, they can be reliably detected even at low concentration and used not only as markers of disease, but also of disease staging, and possibly to quantitatively measure the effectiveness of novel drug therapies. These miRNAs (miR-206, miR-133a, miR-133b, miR-1) are called "myo-miRNA" and are considered as markers of muscle regeneration, myogenesis, fiber type differentiation, degeneration, injury and might represent indicators of residual muscle mass consequent to a chronic atrophy of muscle. Myo-miRNAs are variably expressed in several muscle processes, including myogenesis, and muscle regeneration.[1-3] We explored their function beside in several conditions with severe muscular atrophy, including Amyotrophic Lateral Sclerosis (ALS). ALS is a rare, progressive, neurodegenerative disorder caused by degeneration of upper and lower motoneurons. The effects of exercise and rehabilitation in patients with ALS are still debated. A moderate and regular exercise is supported in the treatment of many neuromuscular diseases. We previously conducted microRNAs studies in ALS patients and we observed differences in myomiRNAs levels in spinal versus bulbar onset (4). In this study we analysed the role of circulating myomiRNAs after physical rehabilitation. We measured muscle specific microRNAs (miR-1,miR-206,miR-133a,miR-133b) by Real Time PCR in 19 ALS patients (12 male,7 female). We analysed the levels of these microRNAs in serum collected before (T0) and after (T1) a period of 6-8 weeks of rehabilitation. We observed a general down-regulation of all miRNAs studied after rehabilitation. In our population myomiRNAs decreased in a similar manner in male and female patients, therefore no gender effect was found. On the contrary the age of patients under study was found to be relevant: patients under 55 years old have a more marked decrease in myomiRNAs levels than patients with older age. We have found that microRNAs are an important tool to monitor rehabilitation in ALS patients and suggests a positive effect of the treatment. There seems to be a more pronounced decrease in myomiRNA levels in patients with

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younger age in this motoneuron disease after physical rehabilitation. Further studies are needed to correlate circulating microRNAs with muscle atrophy and to confirm age differences.


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**Peri-patellar injections of Hyaluronic Acid deeply affect the heart transcriptome: An unexpected hope for the therapy of cardiovascular diseases**

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Within a study which eventually demonstrated the efficacy of peri-patellar injections of high molecular weight Hyaluronic Acid (HA) in the maintenance of the tendon structure during detraining in the rats[1,2,] a transcriptomic study using Next Generation Sequencing was carried out in rat hearts in order to evaluate training- and detraining-associated adaptations in gene expression. While the comparison between trained and untrained hearts yielded 593 differentially expressed ($p \leq 0.05$) genes, as many as 762 genes were found to be differentially expressed in the comparison between the hearts of detrained rats receiving either HA or saline peri-patellar injections. Differentially expressed genes were assigned to functional categories and to KEGG pathways by using the FatiGO software. By and large, gene expression analysis suggested that HA injections at a distant site appear to support the ability of the heart to repair injuries and to enforce differentiative pathways. HA has a well-known role in cardiac differentiation, by activating the ERK 1/2 and NFκB pathways[3] and modulating the WNT/β-catenin and Smad signaling.[4,5] The experimental use of HA in in vivo recovery from ischemia/reperfusion injuries has been so far limited to animal studies, owing to the concept that, in order to be effective, HA-containing hydrogels should be applied on the site of injury, a very delicate and potentially harmful procedure. Should the present transcriptomic study be validated by ongoing proteomic studies, these serendipitous results may pave the way for the validation of HA administration at distant sites and even orally, in the therapy of infarcted patients and even in the prevention of cardiovascular diseases in subjects at risk and in the elderly.

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**Iron homeostasis in iPSC-derived cardiomyocytes from a patient with Friedreich's Ataxia and from a healthy subject**

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Friedreich's Ataxia (FRDA, OMIM #229300) is a severe neurodegenerative disease due to an autosomal recessive mutation and characterized by progressive impairment of voluntary movements. In most patients, FRDA is associated with hypertrophic dilated cardiomyopathy, which is the more frequent cause of death. The underlying mutation in FRDA causes a marked reduction of a small protein, frataxin, which is involved in iron handling, mostly, but not exclusively, in mitochondria; its main role is the assistance in the formation of iron-sulphur containing protein complexes. Patients affected by FRDA show iron inclusions in cardiomyocytes and iron aggregates in the cardiac tissue1. We therefore devised to study the iron homeostasis in iPSC-derived cardiomyocytes obtained from a patient affected by FRDA, which were compared to iPSC-derived cardiomyocytes obtained from a healthy subject. Induced Pluripotent Stem Cells were obtained from skin fibroblasts according to the Yamanaka procedure, differentiated following the GiWi protocol2, and thoroughly characterized. The gene expression of Hepcidin, Ferroportin, Transferrin Receptor 1 and Ferritin was studied in basal conditions; their change following an iron load is the object of a study presently being carried out in our lab. Messenger RNA levels for Hepcidin were found to be increased in cardiomyocytes from the FRDA patient, while the amounts of Ferroportin and Transferrin Receptor 1 mRNAs were decreased with respect to cardiomyocytes from

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a control subject. These data will be discussed in the light of the role played by the proteins coded by the above mentioned genes in iron homeostasis and of their expression in different experimental models.

This work has been partly supported by AISA ONLUS (Associazione Italiana per le Sinromi Atassiche)

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## Fighting muscle weakness in premature and advanced aging by take-home strategies: Full-body In-Bed Gym and h-bFES for elderly persons

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All progressive muscle contractile impairments need permanent managements, including aging-related muscle-strength decline . Frail elderly persons due to advanced age or associated diseases are often hospitalized for long periods of time. There, their already modest amount of daily physical activity is reduced, contributing to limit their independence up to force them to the bed. Immobility is associated with neuromuscular weakness, functional limitations, thromboembolism and high costs.[1-3] Beside the eventual pharmacology therapy, a home-based physical exercise approach is helpful. Awaiting development of electroceuticals, as effective as pace-makers or cochlear implants, education of hospitalized patients to take-home physical exercise managements is an effective low cost alternative. Inspired by the proven capability to recover skeletal muscle strength by home-based Functional Electrical Stimulation even in the worse cases of neuromuscular traumatic injuries,[3-4] but, guided by common sense, we suggest a brief (15-20 minutes) daily routine of twelve easy-to-be-done physical exercises that are performed in bed (*Full-body In-Bed Gym*).[5] *Full-body In-bed Gym* is an extension to all body muscles of well-established physiotherapy approaches of in-bed cardio-circulation-ventilation workouts. If sedentary borderline persons challenge, without stress, them-self, in hospital *Full-body In-Bed Gym* may increase muscle strength, fatigue resistance and independence in daily life activities. In surgical units this will grant standing of patients soon after operation, a mandatory measure to prevent risk of thromboembolism. *Full-body In-Bed Gym* helps also to mitigate the bad mood that accompanies mobility limitations, strengthening patients' confidence in recovering partial or total independence. *Full-body In-Bed Gym* may also mitigate eventual arterial hypertension, a major risk factor in elderly persons. Continued regularly, *Full-body In-Bed Gym* may help to maintain the independence of frail older people and to reduce the risks of the possible serious consequences of accidental falls. Simplified Functional Tests may be used to follow-up the suggested approaches.

Take home messages: It is never too early, it is never too late to start anti-aging *Full-body In-Bed Gym* and FES to help older olds and change lazy, depressed person into active seniors. There are no needs of personal trainers or demanding devices. Secure to your self, please, a better life-style watching the video of ***Full-body In-Bed Gym***.[5] http://www.bio.unipd.it/bam/video/InterviewCarraro-tutorial.mp4


**Figure 1. Full-body In-Bed Gym for elderly or mobility impaired persons.**

**A, B, Flexion and extension of ankles; C, Cycling movements; D, E, Arms up and down; F, Deep breathing; G and H, Forward bending; I, To raise your pelvis; J, K, L, Neck torsions; M, Lift the body; N, O, Lift the legs; P, Stand up from bed; Q, Get up on his toes; R, Push up (Active person).**

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**Role of MicroRNA in Amyotrophic Lateral Sclerosis: gender and genetic differences**

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MicroRNAs are small non coding RNAs that are associated to stress granules, mitochondria and other subcellular organelles in muscle. Few studies have explored microRNAs role in muscle atrophy in Amyotrophic lateral sclerosis(ALS).We previously observed that there is different serum microRNA profile in spinal versus bulbar ALS. We have investigated muscle biopsies in a series of ALS cases both sporadic and genetic. We studied, in EI Escorial proven ALS cases muscle biopsies obtained for diagnostic reasons, myomicroRNAs (MiR-1;MiR-206; MiR-133a; MiR-133b; MiR-27a) and inflammatory microRNAs (MiR-155; MiR-146a; MiR-221; MiR-149*) by qRT-PCR.ALS cases were divided according to gender and age of onset. Atrophy factors were calculated in muscle fibers according to Dubowitz. Two cases had mutation of SOD and c9orf. Morphometric analysis of muscle fiber size was done to correlate muscle atrophy with molecular parameters. All microRNAs studied were strongly up-regulated in muscle biopsies of ALS patients versus controls with the exception of miR-149*. Significant overexpression of miRNAs was present in genetic versus sporadic and in male versus female gender.Morphometric analysis confirmed a muscle fibre atrophy in ALS patients compared to controls. Two genetic ALS (SOD, C9ORF) were atrophic with high fiber CSA variability in agreement with the up-regulation we found of myomiRNAs that directly correlates with the degree of atrophy.In conclusion, these results provide evidence on molecular role of microRNAs in correlation to muscle atrophy. In addiction we observed an increased expression of microRNAs in genetic ALS and dysregulation of inflammatory microRNA.

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**Insights and imaging phenotypes of trasportinopathy (limb-girdle muscular dystrophy type 1F)**

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We report muscle histopathological, ultrastructural and radiological features of a large Italian-Spanish family with autosomal dominant LGMD, previously mapped to 7q32.2-32.2 (LGMD1F). We collected the DNA, clinical history, muscle biopsies histopathology of one LGMD1F kindship. Biopsy of two affected patients mother and daughter was studied (in the daughter two consecutive biopsies at 9 and 28 years and in the mother at 48 years). In LGMD1F patients the age of onset varied from 2 to 35 years, weakness occurred either in upper or in lower girdle; in 14 cases there was hypotropy both in proximal upper and lower extremities in calf muscles. Muscles MRI showed hyperintensity in proximal limb muscles. The daughter has a severe clinical course and the fiber atrophy was more prominent in the second biopsy at 28 years. The mother has a relatively compromised histopathology and many small muscle fibers, and autophagic changes by acid-phosphates stain. Immunofluorescence against desmin, myotilin, p62 and LC3 showed accumulation of myofibrils, ubiquitin binding proteins aggregates and autophagosomes. Ultrastructural analysis revealed myofibrillar disarray, vacuolar changes, granular material and dense subsarcolemmal bodies deriving from cytoskeleton-myofibrillar proteins. We hypotize that the pathogenetic mechanism in LGMD1F might lead to disarrangement of desmin-associated cytoskeletal network. Transportin-3 (TPNO3), which was found by NGS to be the causative gene in LGMD1F, is suggested to mediate the nuclear inport-export. The non-stop mutation identified in this family encodes for a longer protein which is expected to be unable to move to the nucleus. Clinical phenotype penetrance in this family correlates at 92% with mutation presence. MRI imaging is a powerful tool for the follow up in the evolution of this dominant LGMD and demonstrated atrophy of lower girdle.

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## PGC-1α overexpression in the skeletal muscle: effects on myogenesis

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Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a master regulator of mitochondrial biogenesis. In skeletal muscle, PGC-1α expression is induced by exercise.[1] Along this line, mice overexpressing PGC-1α specifically in the skeletal muscle are characterized by enhanced exercise performance in comparison with wild-type animals; this is mainly due to increased myofiber mitochondrial content that results in markedly improved energy metabolism. In addition to an increased proportion of oxidative fibers *vs* glycolytic ones,[2] the histological analysis of muscle overexpressing PGC-1α revealed a high number of fibers with centrally located nuclei, which is indicative of muscle regeneration. Starting from this unexpected observation, the aim of the study was to investigate the effects on myogenesis exerted by PGC-1α overexpression. Myogenic stem cells are more abundant in transgenic mice compared to wild-type animals. When cultured in differentiating medium, cells isolated from PGC-1α mice form myotubes larger than those generated by cells derived from wild-type animals. To understand if such improved *in vitro* myogenic capacity also occurs *in vivo*, both wild-type and PGC-1α transgenic mice received an intramuscular injection of $BaCl_2$ in order to induce muscle regeneration. While 14 days after muscle injury myofiber cross sectional area was not different in wild-type and transgenic mice, at day 8 from $BaCl_2$ injection the number of central nuclei was higher in the latter than in the former. On the whole, these results suggest that overexpression of PGC-1α might favor both myogenic differentiation and regeneration when mild damage occurs, such as during exercise, but it is not able to accelerate muscle recovery when acute damage is inflicted, despite the high propension to myogenesis shown *in vitro*.

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## Quantitative Computed Tomography (QMC-CT) and Image Analysis for Advanced Muscle Assessment

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Skeletal muscle atrophy is the loss of muscle size and strength which occurs with neural and skeletal muscle injuries, prolonged bed rest, space flight, normal aging, and diseases such as sepsis cachexia, diabetes, etc. If unabated, skeletal muscle atrophy can be extremely debilitating, increasing mortality and morbidity in affected people. Current strategies for diagnosis and evaluation of skeletal muscle are not adequate to evaluate fully the condition of this tissue. Thus, proper diagnosis and treatment are often delayed, resulting in unnecessary human discomfort and down time. Quantitative Muscle Color Computed Tomography (QMC-CT) is a highly sensitive quantitative imaging analysis recently introduced by our group to monitor skeletal muscle condition. Despite its powerful potential, this technique is not widely known. Therefore, the objective of this project is to validate QMC-CT as a superior Muscle Imaging technique for evaluating skeletal muscle. This project addresses the "Barriers to Successful Therapy Outcomes" option within the Rehabilitation Focus Area of the DOD Peer Reviewed Orthopaedic Research Program because it will explore the sensitivity of QMC-CT and thus validate its use as an improved method for monitoring skeletal muscle health and recovery. Validation of QMC-CT will provide physicians an improved tool to quantitate skeletal muscle before and during rehabilitation so that therapy for mobility-impaired persons can be better prescribed, evaluated and altered where needed. Benefit to Military Service Members and Veterans: A recent report from the U.S. Army describes injuries as an "epidemic" which has become the "number one health threat" to the U.S. military.[1] This document reports that the majority of injuries occurring at Army garrisons were musculoskeletal injuries to the ankle, knee, lower back or shoulders. Further, it has been reported that non-combat injuries have resulted in more medical air evacuations from Iraq and Afghanistan than combat injuries.[2] These injuries result in physical discomfort and potential mental duress in addition to some degree of personnel down time. The more serious injuries can result in life long issues. QMC-CT will provide medical personnel with a superior technique for imaging skeletal muscle and surrounding tissues. In the short term, the use of QMC-CT will enhance the speed and accuracy of patient evaluation, thus improving diagnosis, treatment and patient morale. In the long term, the improved initial treatments will reduce

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patient treatment time, personnel down time and enduring negative injury-related issues. Because the technology has the potential to improve medical treatment in both military and non-military facilities, the method has the potential to improve health care for soldiers, veterans and the population at large.

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**The nutraceutical potential of Flaxseed bioactive compounds to treat muscular dystrophy**

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The most severe forms of muscular dystrophies (MD) occur due to mutations in the components of the dystrophin-glycoprotein complex (DGC), a molecular scaffold which is localized to sarcolemma and provides mechanical stability to striated muscle. Studies have shown that loss of DGC proteins results in the activation of several pathological cascades[1]. Dystrophic muscle is characterized by chronic inflammation, fibrosis and progressive myofiber loss. No effective treatment is currently able to counteract MD pathological cascades. Plant-derived nutritional compounds exhibit ability to modulate several pathological pathways in various degenerative diseases[2]. Our studies have been demonstrated that a Plant-based diet enriched of flaxseed (FS-diet), is able to stimulate multiple protective and regenerative mechanisms on skeletal muscles of dystrophic hamster, affected by a deletion in the δ-sarcoglycan gene. The FS-diet modulates lipid membrane composition preserving expression of key-role signaling proteins, such as caveolin-3, α-dystroglycan, and sarcoglycans, therefore repairing the sarcolemma damage, which is the primary consequence of gene mutation. The FS-diet prevents inflammation, fibrosis and skeletal muscle degeneration in dystrophic hamster, extending the animals' lifespan[3]. The mechanisms involved include modulation of various pathways such as the TNF, PI3K/Akt, TGF-β, and Bax/Bcl-2 signaling pathways. Because flaxseed is one of the richest sources of omega-3 fatty acid, α-linolenic acid (ALA) a further step of "in vitro" experiments were performed on ALA-treated differentiating myoblasts[3,4]. ALA prevents the TNF-induced inhibition of myogenesis and reduces apoptosis in C2C12 cells by regulating key proteins involved in balancing survival/death in skeletal muscle such as caveolin-3, caspase-3 and Bcl-2. These findings indicate that flaxseed may exert pleiotropic beneficial effects on the dystrophic skeletal muscle partly through an ALA-mediated action. As a nutraceutical that exerts multifaceted effects, the omega-3 fatty acid ALA, as well as others compounds contained in flaxseed, should be clinically developed further for use in the prevention and treatment of the muscular dystrophies.

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**SpillOver stimulation - preliminary results for a novel hypertrophy model in rats**

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Understanding the underlying mechanisms involved in maintenance, increase and loss of muscle mass remains an interesting and challenging field with potential applications not only in bodybuilding, but also with respect to counteract the decline in muscular function caused by disease or ageing. Muscular activity and loading are essential parameters controlling the equilibrium between protein synthesis and degradation. Various animal models have been used in the past to investigate hypertrophy in rodents by increasing the average loading of particular muscles.[1-9] Some models demonstrated the effect of compensatory hypertrophy by removal or denervation of antagonists producing a constant overload.[1-3] Others established training modalities including squats,[3] weight lifting,[4,5] jumping for a food reward, and treadmill or ladder climbing,[6] sometimes with added weights,[7] to increase the muscular effort. Our recent study tests the effect of programmed resistance training of the tibialis anterior (TA) muscle by means of electrical stimulation, on muscular hypertrophy. In the rat hind limb, the dorsiflexor muscles that lift the foot are supplied by the common peroneal nerve (CPN) whereas the plantarflexor muscles are supplied by the tibial nerve. In preliminary force

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measurements we investigated the loading experienced by the TA muscle for unloaded concentric contractions and isometric contractions for the fully recruited CPN. Further measurements were performed in which part of the antagonistic plantarflexors was simultaneously activated with the fully recruited TA muscle. This was achieved with a single channel pulse generator by placing the cathode under the CPN and the anode under the tibial nerve, further referred to as "SpillOver" stimulation of the plantarflexors, because the amount of activation of the tibial nerve can be controlled by adjusting stimulus amplitude above the level that produces supramaximal activation of the CPN. The results of these force measurements suggest that unloaded contractions, even with full activation of the CPN might not provide a sufficient stimulus to induce muscular hypertrophy. To test this hypothesis we performed experimental trials on 10 animals comparing the hypertrophic response of unloaded concentrations elicited by stimulation of the CPN (n=5) versus antagonistic co-contraction using the proposed SpillOver stimulation (n=5). A stimulation pattern of one session per day consisting of 5 sets of 10 repetitions at 100Hz (2s ON 2s OFF) and 2.5 minutes between sets, was applied for a duration of 4 weeks by small implantable pulse generators (MiniVStim 12B, Center of Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria). After the experiments the TA muscles were harvested, weighed and snap-frozen for further histometric analysis. The wet weight of the TA muscle showed an increase of +5.4 % ± 2.5 % (MEAN ± SEM) for unloaded contractions while antagonistic co-contraction revealed an increase +13.9 % ± 1.3 %. The average differences of the median fibre cross-sectional-area were +12.8 % ± 6.4 % and +33.3 % ± 16.5 % for unloaded contractions and co-contractions, respectively. We will use this model to investigate further the sensitivity to hypertrophy of the various fibre types and the cellular pathways that are activated in this response.


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