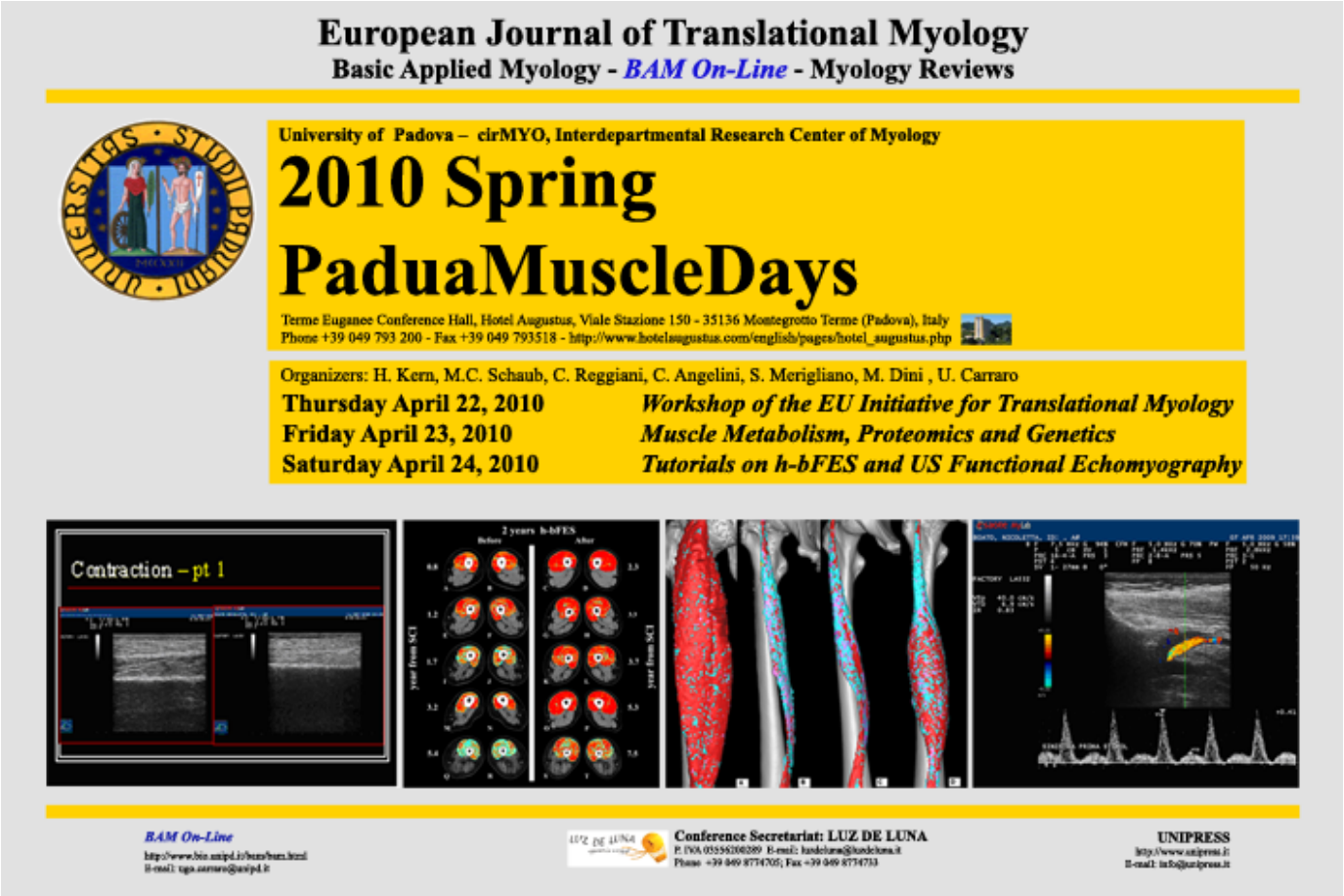
European Journal of Translational Myology

Basic Applied Myology - *BAM On-Line* - Myology Reviews



The 2010Spring PaduaMuscleDays, held in Terme Euganee, Padova (Italy) next April 22 to 24 2010, are organized by the Interdepartmental Research Center of Myology (cirMYO), University of Padova. Main goal is an EU Initiative for Translational Myology on Myology Genetics&Proteomics, Malignancy&Myopathies and Monitoring&Treatments. The Workshop of the Eu Initiative will open the meeting April 22, 2010.

Two tutorials on h-bFES (home-based Functional Electrical Stimulation) and Functional Echomyography, co-organized by the cirMYO of the University of Padova and the Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Wilhelminenspital Vienna, Austria are offered to all participants, in particular to those registered for the "Master in Exercise Testing and Research in Rehabilitation Medicine" organized by the Neurorehabilitation Unit of the University of Pisa, Italy.

To down-load general information and program of the 2010Spring PaduaMuscleDays please link to BAM On-Line at: http://www.bio.unipd.it/bam/bam.html. You will also find the Information for Authors to submit manuscripts for both the PaduaMuscleDays and the Journal.

Looking forward to your contributions to the European Journal of Translational Myology, I send best regards.

Ugo Carraro

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Topics

EU Initiative for Translational Myology - Muscle Genetics&Proteomics&Metabolomics - Malignancy&Myopathies – Monitoring&Treatments

Organizers: H. Kern, M.C. Schaub, C. Reggiani, C. Angelini, S. Merigliano, M. Dini and U. Carraro

Thursday April 22, 2010

Terme Euganee Conference Hall, Hotel Augustus, Viale Stazione 150 - 35136 Montegrotto Terme (Padova), Italy

14.00 – 20.00 Registration

Workshop of the EU Initiative for Translational Myology, H. Kern, U. Carraro, *Organizers*

14.45 Ugo Carraro, Padova, Italy: *Wp-HEALTH.2011, two promising calls for Translational Myology*

15.00 Winfried Mayr, Vienna, Austria: *Robotics versus FES, Competition or Synergy?*

15.30 Thordur Helgason, Reykjavik, Iceland: *Application of ultrasound current source density imaging on denervated muscle, some theoretical considerations*

15.50 Jonathan C Jarvis, Liverpool, UK: *A systems approach to muscle transformation - first steps*

16.10 Helmut Kern, Vienna, Austria: *Are denervated muscles a relevant model of aging?*

16.30 Feliciano Protasi, Chieti, Italy: *Progressive un-coupling of mitochondria from calcium release sites in aging*

17.00 *Break*

17.20 Fabio Francini, Roberta Squecco, Florence, Italy: *Electrophysiological and mechanical changes in long-term denervated rat muscles*

17.40 Ahmet Bozkurt, Aachen, Germany: *A new/sophisticated sciatic nerve sleeve model in the rat*

17.55 Ahmet Bozkurt, Aachen, Germany: *Use of the CatWalk-system for functional assessment after sciatic nerve injuries in the rat*

18.10 Barbara Zavan, Padova, Italy: *Scaffolds screening for the improvement of the neuronal differentiation of neurospheres derived from adipose tissue and skin*

18.30 **cirMYO Lecture**: 40 years European Society for Muscle Research, *Marcus C. Schaub, Zurich, Switzerland*

RESTORE, Invited*: winfried.mayr@meduniwien.ac.at, christian.hofer@wienkav.at, christian.hofer@ottobock.com, oskar.aszmann@meduniwien.ac.at, manfred.frey@meduniwien.ac.at, gregor.kasprian@meduniwien.ac.at, harald.kubiena@meduniwien.ac.at, tatjana.paternostro-sluga@meduniwien.ac.at, hans.stoehr@meduniwien.ac.at, wil.pys.kern-forschung@wienkav.at, stefan.loefler@wienkav.at, martina.grim-stieger@wienkav.at, nejc.sarabon@fsp.ini-lj.si, rupert.koller@wienkav.at, s.salmons@liverpool.ac.uk, jcj@liverpool.ac.uk, ugo.carraro@unipd.it, corrado.angelini@unipd.it, rosario.rizzuto@unipd.it, marco.sandri@unipd.it, roberto.stramare@unipd.it, vincenzo.vindigni@unipd.it, stef.masiero@unipd.it, aba@mail.bio.unipd.it, romeo.martini@sanita.padova.it, pietro.traldi@adr.pd.cnr.it, fabio.francini@unifi.it, vbianchi@libero.it, cesarell@unina.it, pabifulc@unina.it, fprotasi@phobos.unich.it, thordur@landspitali.is, gisliein@landspitali.is, paologar@landspitali.is, palling@landspitali.is, stefanyn@landspitali.is, antonio.musaro@uniroma1.it, abozkurt77@gmx.de, npallua@ukaachen.d, annajakubiec@o2.pl, lars.dahlin@med.lu.se, goran.lundborg@med.lu.se, vogt.peter@mh-hannover.de, gutenbrunner.christoph@mh-hannover.de, guy.vanderstraeten@ugent.be, guenter.fuhr@ibmt.fraunhofer.de, klaus-peter.hoffmann@ibmt.fraunhofer.de, wigand.poppendieck@ibmt.fraunhofer.de, siegfried.steltenkamp@ibmt.fraunhofer.de, df@hst.aau.dk, hans.dietl@ottobock.com, michael.russold@ottobock.com, feldbacher@schuhfried.at, wolfgang.grisold@wienkav.at, hans.lassmann@meduniwien.ac.at*

20.00 *cirMYO Dinner at Hotel Augustus and Evening discussion of the EU Initiative for Translational Myology*

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Friday April 23, 2010

Terme Euganee Conference Hall, Hotel Augustus, Viale Stazione 150 - 35136 Montegrotto Terme (Padova), Italy

Session 1. **Muscle protein balance in health and disease**, Carlo Reggiani, *Organizer*

08.50 **cirMYO Lecture** Effects of feeding and physical activity on muscle protein metabolism and associated signalling mechanisms - the confounding effects of age and inactivity, *Michael Rennie, Derby, UK*

09.30 Marco Sandri, Padova, Italy: *Signaling pathways that control protein breakdown and muscle loss*

10.00 Gianni Biolo, Trieste, Italy: *Regulation of muscle mass by nutrition and activity in health and disease*

10.30 Feliciano Protasi, Chieti, Italy: *Progressive un-coupling of mitochondria from calcium release sites in aging: a possible explanation for the age-related decline of skeletal muscle performance.*

Session 2. **Malignancy&Myopathies**, Stefano Merigliano, Ugo Carraro, *Organizers*

11.00 Sandra Zampieri, Padova, Italy: *Signs of myopathy in patients affected with newly diagnosed colorectal cancer*

11.30 Olof Danielsson, Istvan Gati, Björn Lindvall, Jan Ernerudh, Linköping University, Sweden: *Classification and pathologic characterization of 99 consecutive patients with morphological findings of idiopathic inflammatory myopathy*

11.50 Donatella Biral et al., Padova, Italy: *Acquired dystrophinopathies of human muscle: Contrasting effects of early and late denervation atrophy*

12.00 **cirMYO Lecture** Role of skeletal muscle damage & regeneration in cancer cachexia, *Dario Coletti, Rome, Italy*

13.00 *Lunch*

Session 3. **Muscle Genetics&Proteomics**, Marcus C. Schaub, Corrado Angelini, *Organizers*

14.30 **cirMYO Lecture** Primer for sarcomeric muscle genetics, *Marcus C. Schaub, Zurich, Switzerland*

15.15 Marie-Louise Bang, Milan, Italy: *The functional role of nebulin's SH3 domain in the sarcomeric Z-line*

15.45 Lauren Fisher, Jonathan C Jarvis , Liverpool, UK: *Time- and frequency-dependent transcriptional changes in stimulated rat muscle*

16.15 Andrew Fisher, Jonathan C Jarvis, Liverpool, UK: *Study of intra- and inter-individual variability in muscle transcripts across a rat population*

16.45 *Break*

17.15 Stefano Cagnin, Padova, Italy: *Characterization of altered processes and protein modifications in patients affected by inflammatory myopathies*

17.45 Sarah Pigozo, Libero Vitiello, Padova, Italy: *Efficiency of antisense-mediated exon skipping in normal and mutated dmd genes*

18.15 Scaramozza Annarita, Bologna, Italy: *Satellite cells dysfunction may contributes to impaired skeletal muscle regeneration in sporadic amyotrophic lateral sclerosis (ALS)*

18.30 Marco Spinazzi, Padova, Italy: *Assessment of respiratory chain and antioxidant enzymes in skeletal muscle biopsies*

18.45 Enza Abruzzo et al., Bologna, Italy: *Oxidative stress in denervated rat muscle*

19.00 Carlo Borsato, Padova, Italy: *Evolution in dysferlinopathies*

19.15 Angelica Anichini, Padova, Italy: *Novel mutations and genotype-phenotype correlations in a large cohort of muscular-type cpt-2 deficient patients*

19.30 Chiara Ferrati, Padova, Italy: *Are brain and muscle involvement related in dm1?*

20.00 *cirMYO Dinner at Hotel Augustus and Evening discussion of the EU Initiative for Translational Myology*

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Saturday April 24, 2010

Terme Euganee Conference Hall, Hotel Augustus, Viale Stazione 150 - 35136 Montegrotto Terme (Padova), Italy

Session 4. **Monitoring myopathy progression and recovery in SCI and beyond** – 11 ECM Credits

H. Kern, M. Dini and U. Carraro, *Organizers*

08.40 Daniel Graupe, Chicago, USA: *Recognition and prediction of individual and combined muscular activations modes via surface EMG analysis*

09.10 Jon Cheetham, Ithaca, NY, USA: *Determination of rheobase and chronaxie in denervated laryngeal muscle in conscious horses*

09.40 Paolo Gargiulo, Reykjavic, Iceland: *3D-ColorComputer Tomography image assessment for skeletal muscle volume, composition and density evaluation in SCI*

10.10 Humberto Cerrel-Bazo, Milan, Italy: *FES in thoracic level SCI: 20-year experience in USA and Italy*

10.40 Emiliana Bizzarrini, Udine, Italy: *FES in thoracic level SCI: Experience of Udine Gervasutta Hospital*

11.10 **cirMYO Lecture**: Ultrasound-based investigations of changes in myotendinous properties with disuse and ageing, *Marco V. Narici, Manchester, UK*

11.50 Sigrid Pillen, Nijmegen, The Netherlands: *Detecting neuromuscular disorders and spontaneous muscle activity using muscle ultrasound.*

12.20 Vera Nussgruber, Vienna, Austria: *Ultrasound of nerve and muscle in clinical neurology*

13.00 *Lunch*

Tutorials on h-bFES and US Functional Echomyography – 11 ECM Credits

H. Kern, R. Stramare, U. Carraro, *Organizers*

14.20 Ugo Carraro, Padova, Italy: *Translational myology: from hope to facts*

14.30 **cirMYO Lecture** Home-base FES of LMN denervated muscles, *Helmut Kern, Vienna, Austria*

15.10 **Practice** Devices and protocols of h-bFES, *Helmut Kern, Vienna, Austria*

16.40 *Break*

17.00 **cirMYO Lecture** Echogenicity, contraction and perfusion properties of skeletal muscle by US Functional Echomyography, *Roberto Stramare, Leonora Martino, Padova, Italy*

17.40 **Practice** Devices and protocols of US Functional Echomyography, *Leonora Martino, Riccardo Zanato, Padova, Italy*

18.40 **Practice** Devices and protocols of US-based investigation of myotendinous apparatus, *Marco V. Narici, Manchester, UK*

19.40 *ECM tests to gain the 11 Credits*

19.50 *Ugo Carraro* Auf Wiedersehen, Sjámst, Aurevoir, Arrivederci, See You to the 2010Autum PaduaMuscleDays

20.00 *cirMYO Dinner at Hotel Augustus and Evening discussion of the EU Initiative for Translational Myology*

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Abstracts

Oxidative stress in denervated rat muscle

Provvidenza M. Abruzzo (1), Simona Di Tullio (1), Cosetta Marchionni (1), Silvia Belia (2), Giorgio Fanò (2,3), Sandra Zampieri (4,5), Ugo Carraro (4,5), Helmut Kern (6,7), Gianluca Sgarbi (6,7), Giorgio Lenaz (8), Marina Marini (1,3)

(1) Dept. Histology, Embryology, and Applied Biology, University of Bologna, Italy; (2) Dept. Basic and Applied Medical Sciences, University G. d'Annunzio, Chieti, Italy; (3) Interuniversity Institute of Myology, Italy; (4) Laboratory of Translational Myology, Interdepartmental Research Institute of Myology, Padova University, Italy; (5) Italian C.N.R. Institute of Neuroscience, c/o Dept. Biomedical Sciences, University of Padua, Italy; (6) Dept. Physical Medicine and Rehabilitation, Wilhelminenspital, Wien, Austria; (7) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Wien, Austria; (8) Dept. of Biochemistry, University of Bologna, Italy
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Partial or total denervation of skeletal muscles occurs in a number of congenital or acquired diseases and conditions, including ageing and traumas. In an experimental model of surgical hind limb denervation in rats, we demonstrate here that oxidative stress takes place. In fact: i) ROS are formed; ii) oxidation of membrane lipids occurs; iii) ion channels and pumps display a loss of activity, likely owing to oxidative damage; iv) all the above mentioned events increase with denervation time; v) mRNA abundance of cytoprotective and anti-oxidant proteins (Hsp70, Hsp27, Sod1, Catalase, Gpx1, Gpx4, Gstm1) is increased in 15-day and, to a lesser extent, in 3-month denervated muscle; vi) SOD1 enzymatic activity and HSP70i protein increased in the denervated muscle; vii) increased cPLA2α expression (mRNA) and activation (increased [Ca2+]_i) may lead to increased hydroperoxides release. Further data, obtained from mRNA and protein analysis, suggest that the mitochondria are the most likely candidate as cellular source of ROS, since denervation induces an unbalance in the amount of mitochondrial enzymes involved in the respiration process and electron transport, particularly a decrease in Complex I components. In conclusion, an anti-oxidant therapeutical strategy seems to be advisable in the many medical conditions where the nerve-muscle connection is impaired, to aid healing and recovery processes.

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Novel mutations and genotype-phenotype correlations in a large cohort of muscular-type cpt-2 deficient patients

Angelica Anichini (1), Marina Fanin (1), Corrado Angelini (1), Claudio Bruno (2)

(1) Dept. Neurosciences, University of Padova, Italy; (2) IRCCS Stella Maris Found., Pisa, Italy
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Carnitine-palmitoyltransferase-2 (CPT2) deficiency is the most common disorder of mitochondrial β-oxidation, and the second most common cause of genetically determined myoglobinuria. The adult or "muscular" form of the disease is the most frequent type, with onset between 6 and 20 years, consisting of recurrent rhabdomyolytic episodes with cramps, myalgias, exercise intolerance, and myoglobinuria, most frequently triggered by prolonged exercise, or sometimes by fasting, mild infection, fever, exposure to cold. Our muscle tissue bank was surveyed for patients showing attacks of rhabdomyolysis and myoglobinuria. After exclusion of cases with toxic myoglobinuria, McArdle's disease and Becker muscular dystrophy, over 100 patients were selected for a determination of CPT-2 enzyme activity in muscle and/or in platelets. A defective enzyme activity was found in 32 cases, which were studied in order to offer biochemical-genotype-phenotype correlations. In 5 patients the characteristic acyl-carnitines profile in CPT defect was investigated, confirming its value as a screening method for further diagnostic investigations. We identified novel mutations, and the recurrence of the rare p.R631C mutation in a genetic isolate in the southern Italy. The identification of several symptomatic heterozygous carriers might suggest that additional epigenetic or environmental factors may contribute to determine the phenotype.

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The functional role of nebulin's SH3 domain in the sarcomeric Z-line

Marie-Louise Bang

Dulbecco Telethon Institute, Istituto Tecnologie Biomediche (ITB) at Consiglio Nazionale delle Ricerche (CNR), Segrate, Milan, Italy and I.R.C.C.S Multimedica, Scientific and Technology Pole Milan, Italy
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Nemaline myopathy is a non-dystrophic neuromuscular disorder, caused by mutations in genes encoding thin filament proteins, including the nebulin gene. Nebulin is a giant skeletal muscle-specific protein, which spans the length of the thin filament in the sarcomere and plays important roles in thin filament length regulation, force generation, and maintenance of sarcomeric integrity during skeletal muscle contraction. We have previously generated and characterized nebulin knockout mice, which develop misaligned and widened Z-lines, resembling "nemaline" rod bodies, characteristic for nemaline myopathy. However, due to early postnatal death, we were unable to further study nebulin's role in the Z-line. An SH3 domain in nebulin's extreme C-terminal end is thought to be involved in signaling at the Z-line and its importance has been demonstrated by the identification of nemaline myopathy patients with a truncated form of nebulin missing the SH3 domain. To study the role

of nebulin's SH3 domain in vivo, we have generated mutant mice with a premature stop codon, resulting in deletion of nebulin's SH3 domain. Surprisingly, the mice have no obvious basal phenotype based on histological and biophysical analyses. However, following eccentric contractions a larger decline in isometric stress production is observed in nebulin SH3 deleted muscle compared to wildtype, suggesting that nebulin SH3 deleted mice are more susceptible to eccentric contraction-induced injury. This is currently being studied in more detail. Furthermore, to identify novel binding partners of nebulin's SH3 domain, we have performed microarray studies as well as in vitro pulldown experiments. The effect of deletion of nebulin's SH3 domain on the localization and expression levels of its interactors is being evaluated.

[1] Bang ML, Li X, Littlefield R, Bremner S, Thor A, Knowlton K, Lieber R, Chen J (2006). Nebulin-deficient mice exhibit shorter thin filament lengths and reduced contractile function in skeletal muscle. J. Cell Biol 2006; 173: 905-916.

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Role of skeletal muscle damage and regeneration in cancer cachexia

Emanuele Berardi, Paola Aulino, Veronica Cardilllo, Viviana Moresi, Alessandro Pristerà, Sergio Adamo, Dario Coletti

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Cachexia is a debilitating muscle wasting syndrome that increases mortality and morbidity of a significant fraction of chronically hill as well as ICU patients. Cachexia is characterized by degradation of muscle bulk proteins, including myosin and dystrophin. The latter exposes the musculature to the risk of mechanically-induced damage. The effects of enhanced physical activity (endurance exercise) on skeletal muscle damage will be discussed. In tumor (C26)-bearing mice, we observe sarcolemmal damage and muscle fiber necrosis. Muscles respond to these events by activating a regenerative response. The cachectic musculature is enriched in activated satellite (Pax7-expressing) cells, hematopoietic stem (Sca1- CD45-expressing) cells and muscle interstitial stem (Sca1- CD34- PW1-expressing) cells. However, a morphometry evaluation of the muscle regenerative potential indicates that cachectic muscles lack the ability to fully regenerate. While in cachexia we observe a low grade of systemic inflammation, the latter could explain the impairment of muscle regeneration following acute, focal injury. In this condition TNF injection significantly decreases regeneration, an effect at least in part mediated by interstitial cells expressing stem cell markers in the absence of apoptotic phenomena. We conclude that both increased damage and decreased regeneration contribute to muscle wasting in cachexia, suggesting to counteract cytokine effects on muscle regeneration by pharmacological and gene therapy approaches.

Acquired dystrophinopathies of human muscle: Contrasting effects of early and late denervation

Donatella Biral (1), Nicoletta Adami (2), Helmut Kern (3), Ugo Carraro (1,2), Sandra Zampieri

(1) C.N.R. Institute of Neuroscience, c/o Dept. Biomedical Science, University of Padova, Italy; (2) Laboratory of Translational Myology of the Interdepartmental Research Center of Myology, Dept. Biomedical Science, University of Padova, Italy; (3) Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Dept. Physical Medicine, Wilhelminenspital. Vienna, Austria.
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Force produced by individual muscle fibers is transmitted to the extracellular matrix and to the neighboring muscle fibers via dystrophin and its glycoprotein complex that links cytoskeletal proteins to the extracellular matrix protein laminin. While exercise is the well known co-factor of muscle damage in genetic disorders of the dystrophin - glycoprotein complex, much less is known about the role of dystrophinopathies in acquired myopathies. Studying the effects of disuse on dystrophin content of muscle fibers in normal animals, an increase was observed after early denervation (in muscles harvested not later than 21 days from denervation) [1], while dystrophin changes in normal muscles were never observed after volunatary exercise. On the other hands, some dystrophinopathies were observed in rat muscles acutely electrostimulated in extention (conceivably, an animal model of excentric contractions) [2]. By immuno-histochemistry we are studying dystrophin of human muscle fibers in biopsies harvested from Spinal Cord Injury (SCI) persons suffering with complete denervation of the upper or lower motor neurons (UMN vs LMN, spastic vs flaccid paraplegia, respectively) [3]. Some of these subjects were also studied after 2 years of home-based Functional Electrical Stimulation (h-bFES) that induced a substantial recovery of the atrophic muscles [4,5]. Biopsies harvested from UMN SCI patients presented 50% stable muscle atrophy, whatever the time from SCI. In these biopsies the myofiber profiles were labeled by both anti C-terminus epitopes (anti C-DYS) and anti N-terminus epitopes (anti N-DYS) antibodies up to 5 years after high-level thoracic SCI. After more than 20 years after UMN denervation the reactivity with the antibody anti C-DYS disappeared. Patients suffering with complete lesion of the LMN pool demonstrated a remarkable 50% atrophy up to one year from SCI. Then, denervation myopathy progressed during the following two years to severe atrophy. However, some biopsies presented also atrophy-resistant large fibers lacking

cross striations. These "swollen big fibers" of long term LMN denervated human muscles, although positive after anti N-terminus epitopes (N-DYS) antibodies, were negative after staining with the anti C-terminus epitopes (C-DYS) monoclonals. After two years of h-bFES, and thus of longer lasting LMN denervation, the myofibers reacted with both anti C-DYS and anti N-DYS antibodies. In conclusion, two contrasting processes occur in the denervated myofibers: (i) early after denervation there is an up-regulation of dystrophin expression (related to extra-synaptic aceytilcholine super-sensitivity), while (ii) in long-lasting denervation, the selective changes in anti C-DYS vs N-DYS stainability, possibly related to activation of specific proteolytic enzymes, may precipitate molecular mechanisms leading to final sarcolemmal dysfunction and myofiber apoptosis. Interestingly, similar processes were recently described in severe atrophy occurring in patent cachectic muscles of tumor-bearing mice [6], but not in myopathic patients affected with newly diagnosed colorectal cancer [7].

[1] Biral D., Senter L., Salviati G. Increased expression of dystrophin, β-dystroglycan and adhalin in denervated rat muscles. Journal of Muscle Research and Cell Motility 1996; 17: 523-532.

[2] Biral D, Jakubiec-Puka A, Ciechomska I, Sandri M, Rossini K, Carraro U, Betto R. Loss of dystrophin and some dystrophin-associated proteins with concomitant signs of apoptosis in rat leg muscle overworked in extension. Acta Neuropathol. 2000; 100: 618-626.

[3] Biral D, Kern H, Adami N, Boncompagni S, Protasi F, Carraro U. Atrophy-resistant fibers in permanent peripheral denervation of human skeletal muscle. Neurol Res 2008; 30: 137-144.

[4] Kern H, Carraro U, Adami N, Biral D Hofer C, Forstner C, Mödlin M, Vogelauer M, Boncompagni S, Paolini C, Mayr W, Protasi F, Zampieri S. Home-based Functional Electrical Stimulation (h-bFES) recovers permanently denervated muscles in paraplegic patients with complete lower motor neuron lesion. Neurorehab Neur Rep 2010, accepted Jan 22, 2010.

[5] Kern H, Carraro U, Adami N, Biral D, Hofer C, Loefler S, Vogelauer M, Mayr W, Rupp R, Zampieri S. One Year of Home-based Functional Electrical Stimulation (FES) in Complete Lower Motor Neuron Paraplegia: Recovery of Tetanic Contractility Drives the Structural Improvements of Denervated Muscle. Neurol Res, 2010; 32: 5-12.

[6] Acharyya S, Butchbach ME, Sahenk Z, Wang H, Saji M, Carathers M, Ringel MD, Skipworth RJ, Fearon KC, Hollingsworth MA, Muscarella P, Burghes AH, Rafael-Fortney JA, Guttridge DC. Dystrophin glycoprotein complex dysfunction: a regulatory link between muscular dystrophy and cancer cachexia. Cancer Cell 2005; 8: 421-432.

[7] Zampieri S, Doria A, Adami N, Biral D, Vecchiato M, Savastano S, Corbianco S, Carraro U, Merigliano S. Subclinical myopathy in patients affected with newly diagnosed colorectal cancer at clinical onset of disease: evidence from skeletal muscle biopsies. Neurol Res. 2010; 32: 20-25. Epub Haead of print 2009 Nov 26.

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FES in thoracic level SCI: Experience of Udine

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A rehabilitation method for recovering stand-up and walking in Spinal Cord Injury (SCI) patients is to compensate the function of paralyzed muscles with Functional Electrical Stimulation (FES). We know by Literature the effects of FES on muscles, heart, lung, bone mass and spasticity. On the other hand, an evidence-based validated program to prepare FES walking is not still defined in Literature. FES locomotion requests a high energetic cost for gait: it is, thus, necessary to prepare a person with complete SCI to this kind of locomotion. We proposed a specific program to prepare complete SCI patients for walking with FES, monitoring every steps of the training with outcome measures. We tested our training program in six thoracic level chronic SCI patients with an average age of 33.56±1.14 years. All the patients use for daily activity the wheelchair. We evaluated all subject with the Asia Impairment Scale and for all of them we confirmed a score of A. To verified spasticity we use the Ashworth Scale: the six patients analyzed had a score between 1-3. For the FES walking program we used three different types of electrical stimulators: 1. Parastep System, intensity 0-300 mA, frequency 24 Hz, pulse width 0-300 μsec; 2. PO22 Fequa System, intensity 0-150 mA, frequency 0-50 Hz, pulse width 300-700 μsec; 3. BiacMed System, intensity 0-150 mA, frequency 1-170 Hz, pulse width 0-1000 μsec. The training was developed in four consecutive steps. The first step was based on Patterned Electrical Stimulation (PES) assisted isometric exercises for the quadriceps muscles. We realized five day sessions for week, with duration for a single session of 30 minutes. This step was extended for three weeks, the time necessary to lift a weight at the ankle of 500 gr. The second step was based on FES cycling, 60 minutes for session, realized 3 times a week for 3 weeks. At the cycle the subjects switched electrical stimulation from quadriceps to hamstrings. This step is necessary also to increase the cardiovascular performance of the patients. At the third step we realized FES walking at body weight supported treadmill (TR Spacetrainer) beginning

2010Spring PaduaMuscleDays

Terme Euganee, Padova (Italy), April 22 - 24, 2010

Abstracts

with a cargo of 30% of body weight (BW) with the aims to reach at the end of the three weeks of training a cargo of 70% of (BW). Time for a single session was 30 minutes. The sessions were realized 3 times a week for 3 weeks. The last step was over-ground FES walking training, 3 times a week, session duration of 30 minutes for 4 weeks. We monitored the muscle's efficiency parameters and of the energetic cost of gait. Outcome Measures were tested at every step through 1) plicometer to calculate the thigh muscle area; 2) isokinetic dynamometer to test quadriceps isometric torque during PES; 3) respiratory gases analyzer (VO2000, Medgraphics-USA) to measure O2 maximal consumption and the energetic cost of gait. At the end of the training we verified an increase of the thigh muscle area (from 114.21 to 120.33 cm2), an increase of quadriceps thigh torque (from 0.360 to 0.502 Nm/kg), an increase of the aerobic performance and a decrease of the energetic cost of gait (from 25.76 to 19.94 J/Kg/m). We believe that the monitoring of muscle's efficiency parameters and of the energetic cost of gait could permit the definition of a specific training to prepare complete SCI patients walking with FES. We believe that even complete SCI could have a chance to restore walking, but it is necessary to prepare their muscles and their cardiovascular system to face the challenge.

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Dysferlinopathies: natural history and effects of physical activity

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Dysferlin is involved in the repair of sarcolemma. Dysferlinopathy, due to 2p13 mutations in the Dysferlin gene, presents either as LGMD2B or Miyoshi myopathy. Progression of disease is highly variable and unpredictable, implying epigenetic or environmental factors can modify the clinical presentation and development. We evaluated the natural history of disease and the role of physical activity on the clinical progression. We have collected 17 patients with biochemical and molecular diagnosis of LGMD2B/MM. We conducted a retrospective study of the natural history, using the Gardner-Medwin-Walton and GSGCA scales for the disability. We also focused on physical activity, performed before the onset of disease, to split all the patients into two groups, sporty and non sporty, using a 1000 hours cut-off. All the patients have a typical progression of disease and clinical disability. The group of sporty patients presents a more rapid clinical progression than non sporty patients, in terms of number of years of disease needed to reach grade 4 of Gardner-Medwin-Walton scale (Gowers sign). Patients affected with dysferlinopathy present different clinical phenotypes and different clinical progression. Physical activity produces a regular and important muscle stress, which could determine a more severe disability and should be considered a negative prognostic factor which might anticipate muscle weakness.

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The Epineural Sheath Tube (EST)-technique: A sophisticated sciatic nerve sleeve model in the rat

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Peripheral nerve injury (PNI), occurring in approximately 2.8% of trauma patients, typically leads to life-lasting loss and/or disturbances in functions mediated by the injured nerve. Moreover, development of neuropathic pain is frequently observed following nerve injuries. The functional outcome of PNI depends on the severity of the injury, i.e. neurapraxia, axonotmesis or neurotmesis. Silicones have been used in numerous medical applications because its properties include biocompatibility, biodurability, hydrophobicity, elastic properties, chemical and thermal stability. Silicone tubulization of nerve gaps has been the standard experimental model for many years. However, silicone conduits are prone to becoming encapsulated with fibrous tissue, resulting in the eventual constriction of the nerve. Modern bioengineering and tissue engineering approaches are being used to develop orientated growth promoting substrates that can match those of the autologous peripheral nerve graft by generating an "intelligent" substrate that is capable of reproducing several of the important physical and molecular characteristics of nerves undergoing Wallerian degeneration. Materials scientists and protein chemists have been able to develop numerous forms of scaffold or conduit design including variations in compositional and physical parameters including geometry, orientation and dimensions of micro-pores and mechanical properties such as the elastic or shear modulus. So far, the rat sciatic nerve model is the most widely used animal model for PNI. Here we present the Epineural Sheath Tube (EST)-technique, a new microsurgical nerve model to extend the application of the rat sciatic nerve model, which can be adapted to other nerve models like median or facial nerve models. The EST-technique provides a cavity or chamber, consisting of an outer epineural sleeve freed from nerve fascicles, but continuous with proximal and distal nerve stumps and providing the original vascularization. Since this cavity has no intrinsic regenerative capacity, this cavity could potentially be used for testing a variety of applications as outlined above. It provides hollow natural chamber without the need of a foreign body like the silicon conduits, which are prone to becoming encapsulated with fibrous tissue. Furthermore, it makes independent form commercially available nerve conduits, which are not inert and are expensive. We tested the practicability to use the epineural sleeve as the EST-technique regarding feasibility, reproducibility, mechanical stability, openness of the lumen

2010Spring PaduaMuscleDays

Terme Euganee, Padova (Italy), April 22 - 24, 2010

Abstracts

and closeability. We, therefore, implanted cylinders of fibrin without cells or growth factors into a 2cm- sciatic nerve gap within the epineural sleeve over a period of 6 weeks. We used fibrin cylinders, because on the one hand they provided openness of the lumen after suturing and on the other as a test model due to their inert and biocompatible character lacking of cells or growth factors. We compared the EST-technique with the autologous nerve transplantation (neurotmesis) and the crush lesion (axonotmesis).

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Use of the CatWalk-system for functional assessment after sciatic nerve injuries in the rat: Divergency in functionality of axon regeneration

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Repair of peripheral nerve gaps still represents a clinical challenge. Although gaps smaller than about a centimeter mostly allow suture repair, larger gaps require bridging therapies. An immense number of investigations have been performed to find suitable and effective bridging materials. After decades of research in this arena, autologous nerve grafting is still regarded as the preferred bridging material in clinical practice. The cellular and molecular composition of an autologous nerve is of vital importance to axon regeneration and accounts for the therapeutic effect of autologous nerve grafting. Nevertheless, issues like limited amounts of donor tissue and comorbidity at the site where the donor tissue is obtained cast a shadow over the grateful use of autologous nerves in peripheral nerve gap repair. For this reason, it is still a critical goal in regenerative medicine to find an alternative bridging therapy which is at least equal in efficacy and can replace autologous nerve grafting in peripheral nerve gap repair. A vast amount of nerve guides composed of biological or synthetic materials have been tested in animal models for their effect on regeneration of severed axons and/or functional recovery. The search of an effective alternative to autologous nerves is hampered by a number of issues. First, the issue of selective target reinnervation is still not solved, which means that regenerating axons remain non-functional after reinnervating the wrong end-organ. Second, a wide variety of functional tests is available to assess the functionality of axon regeneration. These tests range from electrophysiology to behavioral tests for analysis of static parameters such as the static sciatic index (SSI) or gait parameters. For gait analysis, the walking track is used most frequently to obtain the sciatic function index (SFI), the stance factor, ankle kinematics and/or toe out angle (TOA). Recently, we reported the use of the CatWalk gait analysis to assess deficits and subsequent recovery of both dynamic and static gait parameters after sciatic nerve injury. Nevertheless, not a single one of these tests can measure the various and particular components of sensorimotor function which are impaired after sciatic nerve injury. Obviously, practical and logistic issues put a limitation to the number of behavioral tests which can be included in individual investigations. At present, there is not a clear preference of behavioral tests to assess the functionality of axon regeneration after sciatic nerve injury, and a “combination of tests” is recommended to examine the overall sciatic nerve regeneration. In the present study we made an attempt to find differences in the suitability of behavioral tests to assess functionality of axon regeneration after sciatic nerve injury. To this end we compared a test for assessment of static parameters (SSI) with a test for assessment of both static and dynamic gait parameters (CatWalk gait analysis). Both tests were used to assess functional recovery after autolgous nerve grafting to bridge 20-mm-long sciatic nerve gaps.

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Characterization of altered processes and protein modifications in patients affected by inflammatory myopathies

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Inflammatory myopathies (IM) are autoimmune disorders that affect skeletal muscles. Causes and antigens that activate the inflammatory response in the IM are not clarified. We combined transcriptome and proteome studies in skeletal muscle biopsies of IM patients to elucidate the molecular cascades associated with the degeneration of muscle tissues and to discover specific inflammatory processes. RNA and proteins were extracted from biopsies obtained from 8 polymyositis (PM), 6 dermatomyositis (DM) patients and 7 healthy individuals as controls. 50µg of pooled proteins from biopsies of each pathology were analyzed by 2D-DIGE and mass spectroscopy. RNA was used for microarray gene expression analysis and qRT-PCR experiments. Results were confirmed by microarray meta-analysis, ELISA and high-throughput immunostaining experiments. Results: 233

protein spots were changed between control subjects and IM patients. PM and DM patients showed a reduction of key enzymes involved in glycolysis and an increment in proteins involved in the antigen presentation and the stress response. This conclusion was in agreement with microarray results, but it is not discriminant between different IM. We performed a modified gene set enrichment analysis with a meta-analysis approach. We evidenced the discriminant role between DM and PM of interferon-related genes. qRT-PCR analysis on 178 IFN related genes revealed the prevalence of IFNα/β response in DM and IFNγ response in PM. Paradigmatic of this is the up-regulation of ISG15 (1000 fold more abundant in DM than in PM). We confirmed the presence of ISG15 in the sera patients and identified ISG15 linked proteins (ISGylation) in the muscle of DM and PM. No data were available about ISGylated proteins in skeletal muscle affected by IM. To relate the effect of the ISGylation with the pathology, we identified ISGylated proteins, evidencing the importance of this modification in the sustainment of mitochondrion function under an IFN response.

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Wp-HEALTH.2011, two promising calls for Translational Myology

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The RESTORE application (Translational strategies to improve functional outcomes after peripheral nerve lesions) we submitted last year to Health.2010 is a sound, focused research project that we may revise and resubmit to Health.2011 Calls. Two chances are offered in the confidential draft of the FP7-Health-2011, namely: 1.4 Innovative therapeutic approaches and interventions. - The main focus is on regenerative medicine and to provide opportunities to SMEs active in this sector. Regenerative medicine offers hope for sufferers of diseases which are currently untratable, where life is at stake and for regenerating diseased, damaged or defectives tissues and organs. It also offers possibilities for addressing complex problems of an ageing population and has potential for combatting rising healthcare costs. It is a high-value new technology offering Europe competitiveness and this opportunità is henhanced by the recent adoption of a European Regulation on advanced therapy medicinal products. To meet the challanges and promise of regenerative medicine and to promote its translation to the clinic, two topics for medium-sized Collaborative Projects are presented. The first concerns the therapy itself and aims to drive translation of promising therapeutic approaches along the pathway to practical use. The second topic focuses on tools and technologies required to progress regenerative medicine and its practice in the clinic.
Health.2011.1.4-1: Regenerative medicine clinical trials. FP7-Health-2011-two-stage. Research should aim to develop regenerative therapies. Involve SMEs and test promising products or techniques in clinic. Since it is intended to encourage regenerative medicine as an approach, proposals may address any justified disease or condition.
2. Translating research for human health - This activity aims at increasing knowledge of biological processes and mechanisms involved in normal health and in specific disease situations, to transpose this knowledge into clinical applications including disease control and treatment, and to ensure that clinical (including epidemiological) data guide further research.
Health.2011.2.2.2-1 Investigator-driven clinical trials for therapeutic interventions in the elderly populations. FP7-Health-2011-two-stage. Elderly people are susceptible to a wide range of medical conditions, including …, muscular diseases … etc.
Once chance is to work around the concept that the denervated muscle could be a relevant experimental model of aged muscle. Other unifying topics are welcomed. It will be a hard work to construct a winning Consortium around the many new solutions the managements of denervated/reinnervating muscles ask for, but alltogether we have the expertise to meet the target.

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Determination of rheobase and chronaxie in denervated laryngeal muscle in conscious horses

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The excitability of muscle is described by the relationship between the duration of a pulse used for stimulation and the amplitude required to elicit a threshold response- the strength-duration curve. We describe a technique for measuring chronaxie and rheobase (derived from the strength-duration relationship) in the dorsal cricoarytenoid (DCA) muscle (is called posterior cricoarytenoid muscle PCA in human) of conscious horses with chronically implanted electrodes. The DCA muscle is the sole arytenoid

2010Spring PaduaMuscleDays

Terme Euganee, Padova (Italy), April 22 - 24, 2010

Abstracts

abductor: Experimental denervation or a common neuropathy induces marked respiratory obstruction. Twelve horses had intramuscular electrodes placed into the medial and lateral neuromuscular compartments of the DCA muscle. Electrodes were connected to a percutaneous 16-pin connector. The baseline thresholds for abduction were recorded using endoscopic visualisation of the vocal cords. Two weeks following implantation the recurrent laryngeal nerve was cut to denervate the DCA muscle. Rheobase and chronaxie were determined at 1,2,4,8 and 10 weeks following denervation using single biphasic square-wave pulses and a five microsecond interphase gap. Concurrent muscle atrophy was determined using transesophageal ultrasound and computed tomography. Results: Instrumentation was well tolerated by all animals. A progressive increase in rheobase was observed to 8 weeks post denervation, after which no further increase in rheobase was observed. This technique will be useful to track excitability changes in horses following functional electrical stimulation for the design of a laryngeal pacemaker.

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FES in thoracic level SCI 20-year experience in USA and Italy

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To recognize determinants of failure in generating functional muscle force useful for standing or walking by FES, we are investigating nerve-muscle interactions, excitation-contraction coupling machinery and metabolic response to FES in paralyzed muscles post-SCI. Paraplegics (n. 16) with upper (1st MTN) or lower (2nd MTN) motoneuron SCI were sub grouped according to level and extent of SCI. The 2nd MTN group is a sub-group of the European FES Project "RISE" for the study of long-term denervated-degenerated muscles. Muscle biopsies from vastus lateralis muscle, study of fiber composition, electronic micrographs for ultra structure analysis, CT scan for area and density of thigh measurements, force measurement, 2 years training of FES in denervated muscles were performed. Muscle bulk, muscle fiber size, fiber composition, force generation, ultra structural findings of mitochondria, triads, M lines, Z Lines, T-Tubules, sarcoplasmic reticulum, muscle response to FES, and denervation-anti/NCAM were analyzed. Results may be summarized as follow: 1. Significant ($p < 0.001$) deficit of the EC and metabolic (mitochondria) machinery in lower vs upper motoneuron SCI lesions. 2. Marked muscle atrophy not correlated to years of injury or capacity of generating force in the 1st MTN group, possibly related to a spastic activity, only 45% of fiber size reduction was observed with respect to able-body individuals (AB) at 12 months post injury; 3. Then atrophy level-up at least up to 20 years; 4. Lower motoneuron muscle atrophy was much heavier even at 8 months post injury with a 56% reduction with respect to (AB) and then with a severe progression over the first three years post injury to muscle degeneration; 5. Muscle force was dramatically reduced in both SCI groups: quadriceps strength was recorded in upper motoneuron SCI as around 4% of the normal force in AB. Lower motoneuron force was difficult to be recorded, being lower than 0.5% Nm related to AB; 6. 10-15 μm diameter muscle fibers present anti-NCAM antibody stainability, a marker of denervation, including 17-23% of the muscle fibers in the upper motoneuron group, which may have this characteristic despite a relative short period of SCI (3-5 years) and the presence of spasticity. In conclusion, if surface FES is to be used, determinants for muscle recovery need to be considered. Muscle may recover depending on: 1. type of SCI; 2. extent of excitable and trophic muscle tissue; 3. FES parameters (duration, intensity and frequency) of stimulation. Recovered strength and endurance will depend on the ability of FES to obtain plastic remodeling of the muscle fibers. We know that FES pattern of motor unit recruitment in upper (1st MTN) motoneuron SCI is limited by muscle fiber type and percentage of eventual denervated muscle fibers, but now we know that lower (2nd MTN) motoneuron SCI patients may respond to a new high intensity stimulation parameters, which are opening new perspectives for the all field.

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Progressive un-coupling of mitochondria from calcium release sites in aging: a possible explanation for the age-related decline of skeletal muscle performance

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An impairment of the mechanisms controlling the release of Ca2+ from internal stores (excitation-contraction (EC) coupling) has been proposed to contribute to the age-related decline of muscle performance that accompanies aging (EC un-coupling theory). EC coupling in muscle fibers occurs at specialized intracellular junctions called calcium release units, or triads, which are specifically placed at sarcomere's I-A band transition. In recent publications we have shown that: a) in human muscle, the frequency of triads decreases significantly with age [1]; and b) in mice, triads are tethered to mitochondria placed at the I band (Boncompagni et al., 2009; MBC 20:1059). Here we have studied the frequency,

sarcomeric-localization, ultra-structure, and coupling of triads / mitochondria in EDL from male WT mice using transmission electron microscopy (TEM). Our results indicate that the number of triads/100μm of longitudinal section in aging mice (n=4, 25-35 months of age) decreases compared to the adult mice (n=5, 3-12 months of age): 93±9 vs. 79±8. Also the i) relative volume and ii) number of mitochondria-profiles/100μm2 decrease with age: 8.4±5.1 vs. 7.3±4.7 and 54±7 vs. 43±6 respectively. In addition, we have assessed the positioning of mitochondria in respect to myofibrils and triads: a) the number of mitochondria at the A band (misplaced) slightly increases with age (9.3 vs. 2.5%). These changes cause a significant decrease in the number of CRUs-mitochondria couples: 39±5 vs. 26±5. Our results shows how the age-related partial disarrangement and spatial re-organization of the EC coupling and mitochondrial apparatuses causes a large decrease in the number of mitochondria functionally coupled to Ca2+ release sites. These changes may contribute to the decrease of specific force and endurance of skeletal muscle associated to ageing.

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Classification and pathologic characterization of 99 consecutive patients with morphological findings of idiopathic inflammatory myopathy

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Since the classification of inflammatory myopathies by Bohan and Peter 1975, the need to separately consider disorders such as inclusion body myositis and necrotizing myopathy has become obvious. The 119th ENMC international workshop suggested the introduction of new classification criteria designed by Amato, emphasizing clinical criteria and certain pathologic findings. We compared the outcome using these two classification systems on a consecutive material of patients fulfilling the pathological requirements of an inflammatory myopathy. We searched the register of our laboratory from 1997 through 2002, and found 120 patients satisfying the pathological criteria for an inflammatory myopathy. We collected relevant clinical and pathological data from the patients who consented, and included patients fulfilling the criteria of Bohan and Peter for classification, according to their criteria and those of Amato. Patients with inclusion body myositis were classified according to Griggs. The biopsies which had earlier shown a suspect partial invasion underwent serial sectioning, and the neighboring section was stained with antibodies to MHC class 1, cytotoxic T cells and membrane attack complex to identify fibres meeting strict requirements for partial invasion. More patients with disorders with different pathogenesis were classified as polymyositis according to the criteria of Bohan and Peter than by those designed by Amato. The classification of Bohan and Peter has a greater sensitivity to identify patients with inflammatory changes in muscle, those with as associated neoplasia and a systemic inflammatory disorder, but shows less specificity for isolated polymyositis than the classification of Amato. The frequency of different IIM including those associated with neoplasia and systemic inflammatory disorders will be shown, and the value of certain clinical and pathological criteria will be discussed.

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Are brain and muscle involvement related in DM1?

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We are determining the degree of involvement in brain and skeletal muscle in a series of molecularly-defined DM1 patients in order to highlight possible correlations. Cerebral and muscle impairment in myotonic dystrophy type 1 (DM1) is well documented but there are few reports on their correlations. Twenty four DM1 patients were recruited for the study. Ages at study, at disease onset and disease duration were recorded. Molecular characterization of CTG(n) expansion was done in genomic DNA from blood. Neuromuscular assessment in DM1 patients was performed by MIRS. A morphometry study of muscle biopsies included the quantitative evaluation of fibre atrophy/hypertrophy factor of both fibre types. All patients underwent brain MRI; MRI imaging was classified by the Age Related White Matter Changes (ARWMC) score in order to quantify the pattern of distribution of White Matter Hyperintense Lesions (WMHLs). Morphometry analysis of muscle showed an increased atrophy factor for both fibre types, especially for type 1 fibres in 14/24 cases. 20/24 patients had abnormal MRI imaging, showing scattered supratentorial, bilateral, symmetrical focal or diffuse WMHLs, with a typical temporo-insular diffuse subcortical pattern in most patients. More severe muscle impairment both clinical and

histopathological was observed in patients harboring larger CTG expansion. No significant correlation was found between atrophy factors and MRI total lesion load of WMHL. A greater expansion size is confirmed as risk factor for more extensive cerebral and muscle impairment. Our study indicates that muscle and brain are independently involved.

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Study of intra- and inter-individual variability in muscle transcripts across a rat population

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Previously, we have used quantitative Real-Time PCR (qRT-PCR) to measure changes in transcript levels for Myosin Heavy Chain (MHC) isoforms as well as various other key genes involved in skeletal muscle transformation in rat skeletal muscle following stimulation for 3 hours using an implantable miniature stimulator. Our results showed that the changes detected in the transcript levels of all genes investigated were divergent between rats undergoing the same stimulation pattern. A high degree of inter-individual variability in transcript levels of all genes in control tissue was also observed. We have investigated whether this was primarily attributable to biological variability or to the result of error within our experimental procedures. Using qRT-PCR, we have measured the intra-individual variability in MHC transcript levels between left and right Tibialis anterior muscles as well as across different regions of the same muscle. Furthermore, inter-individual variability was assessed within a population of 10 inbred male Wistar rats. It was found that variability introduced by the experimental techniques accounted for less than half of the total variability observed. Therefore, individual rats show real variation in transcript levels of a number of different genes. In depth analysis of this variability showed that a minimum sample number of 6 will be required in future studies to demonstrate a significant two-fold change in transcript levels following stimulation.

Time- and frequency-dependent transcriptional changes in stimulated rat muscle

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Transcriptional profiling is a powerful tool used to explore the molecular mechanisms underlying skeletal muscle adaptations in response to changed activity patterns. A number of transcriptional pathways have been implicated in the transformation of muscle from the fast, fatigable type to a slower, more fatigue-resistant type that involves changes in the expression of key transcripts linked to fibre-type, metabolism and mitochondrial biogenesis. We have assessed the time- and frequency-dependent response of stimulated skeletal muscle by means of an implantable stimulating device, to produce a transcriptional profile of muscle and to search for thresholds of activity that cause step changes in transcription. Using qRT-PCR we have measured the mRNA levels of key transcripts involved in the transformation of Rat Tibialis anterior muscle. We have investigated the time-course of transcriptional responses within the first week of stimulation at 20Hz to identify the earliest time-point at which a new transcriptional state is achieved. With this information we were then able to perform a comparative study of the effect on transcription of graded activity patterns. Furthermore, using histological analysis of fibre-types we have addressed the issue of whether changes at the molecular level are predictive of changes in proteins and thus the eventual functional outcome of a given pattern of activity. Our first conclusion is that transcript levels for transcription factors related to the energy status of the cell change at a much earlier time point than transcript levels for contractile proteins. After one week of stimulation at 20Hz, when changes in transcript levels for the MHC isoforms are consistent and significant, the transcript levels of transcription factors have returned almost to control levels. Secondly, we found that for some transcripts there are thresholds of activity which bring about changes in transcription whereas for others the changes are more linearly related to activity. Finally, analysis at the level of fibre-type composition showed that mRNA levels are indeed indicative of changes in fibre-types, thus confirming the use of qRT-PCR to look at the response of muscle to changed activity in a more time and cost efficient manner. We have addressed the time and frequency-dependent response of stimulated skeletal muscle at a molecular level and found that different transcripts respond in a different manner. The complexity of these transcriptional responses, even in a limited set of transcripts, suggests considerable complexity in the control of gene expression during muscle adaptation. We have also identified inter-individual variation which suggests that some individuals are required to change their gene expression more than others in order to withstand the changes in functional demand. The signaling pathways that link neuromuscular activity to the gene regulator machinery are not fully understood, but this study has begun to address some of these connections.

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2010Spring PaduaMuscleDays

Terme Euganee, Padova (Italy), April 22 - 24, 2010

Abstracts

Electrophysiological and mechanical changes in long-term denervated rat muscles

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Denervation of skeletal muscles determines a progressive fibers atrophy and degeneration. In muscles of the rat legs (tibialis anterior, Soleus and gastrocnemius) sciatectomy induces parallel time dependent morphological, electrophysiological and mechanical alterations [1]. These changes were very marked in the first 3 weeks of denervation (short-term) and progressed quite slowly up to 24 weeks (medium-term). From 24 to 44 weeks, the changes were negligible (long-term denervation). The parallel alterations involved a progressive reduction of i) muscle weight-to-body weight ratio; ii) fiber diameter, iii) T-tubule surface area, iv) membrane resistance and resting membrane potential, v) expression and functionality of voltage-dependent Na+ channels, index of a less muscle excitability, vi) expression and functionality of L-type Ca^{2+} channels, index of an affected excitation-contraction, EC, coupling apparatus, vi) loss of the sarcomeric structure and reduced contractility. Notably, in long-term denervated muscles despite the loss of the sarcomeric machinery denervated muscles maintain some fibers able to activate the altered EC coupling process and to generate, albeit in lesser amounts, force. The reduced values of resting membrane resistance and the depolarized resting membrane potential in denervated fibers suggest a leaky sarcolemma and an increase of intracellular Ca^{2+} concentration; this was confirmed by the shift in reversal potential of the L-type Ca^{2+} current toward a more negative potential. This may be a starting mechanism that reduces membrane excitability and the efficacy of electrical stimulation in short-term and, to a greater degree, in long-term-denervated muscles. The depolarized state of the denervated rat fiber is in agreement with the reduced action potential conduction velocity observed in human long-term-denervated muscles. These results are in agreement with the reduced excitability found in muscle of SCI patients as increased values of chronaxie and rheobase. The presence of functional voltage-dependent Na^+ and L-type Ca^{2+} channels may allow transcutaneous muscle electrical stimulation (functional electrical stimulation, FES) to activate Na^+ and L-type Ca^{2+} channels and in turn the affected EC coupling process. The resulting transient increase of intracellular $[Ca^{2+}]$ may be the key that activates Ca^{2+}-dependent signaling pathways to the nucleus, thus inducing the expression of muscle-specific proteins in the sarcoplasma and sarcolemma improving the EC coupling process, rebuilt the sarcomeric structure, the muscle mass and restoring the contractile machinery. Finally, since denervated muscle fibers increase the expression of stretch activated channels continuing to be mechano-sensitive, passive stretching of denervated muscles may by another way to induced intracellular Ca^{2+} transients [2].

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Scaffolds screening for the improvement of the neuronal differentiation of neurospheres derived from adipose and skin tissue

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Recently we have described a simple protocol to obtain an enriched culture of adult stem cells organized in neurospheres from two post natal tissues: skin and adipose tissue. Due to their possible application in neuronal tissue regeneration, here we tested two kind of scaffolds well kwon on tissue engineering application: hyaluronan based membrane and fibrin-glue meshes. Neurospheres form skin and from adipose tissue were seeded into two scaffolds type: hyaluronan based membrane and fibrin-glue meshes. Neurospheres were then induced to acquire a Schwann cells-like phenotype. Proliferative ability, morphological feature and genome stability (with CGH array) were analyzed and compared. Adipose and skin derived neurospheres are able to growth well and to differentiate with a Schwann -like phenotype without any chromosomal imbalance in both scaffolds. In fibrin-glue meshes a bigger quantity of neurospheres are able to attach and then to originate larger clusters of adult neuronal cells. In summary, we have demonstrated that neurospheres isolated from skin and adipose tissues are able to differentiate in Schwann cell-like without any chromosomal imbalance in two scaffolds type

usefully for tissue engineering application: hyaluronan based membrane and fibrin-glue meshes.

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3D-ColorComputer Tomography image assessment for skeletal muscle volume, composition and density evaluation in SCI

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Muscle tissue composition, that is, the relative content of normal muscle fibers, endomisium, perimysium, epimisium and eventual increase of intramuscular adipose and fibrous tissues, can be efficiently analyzeed and quantified using images from Spiral Computer Tomography (S-CT) technology and associated Hounsfield (HU) values distribution. The reliability of this tool though depends from S-CT assessment and calibration. Muscle density distribution especially when including the whole muscle volume, provides remarkable information on the muscle condition. Different physiological scenaries can be depicted using the muscle characterization technique based on HU values. For instance situations like atrophy and degeneration in denervated muscle can be clearly displayed and monitored as well tissue restoration following electrical stimulation treatment [1-3]. To assess imaging quality and use of HU values to display muscle composition three different S-CT devices are compared using Quasar body scanner. Density distribution and volumes of various calibration elements such as lung, polyethylene, water equivalent, trabecular and dense bone are measured with different scanning protocols and at different point of time. The assessment results show that HU distribution from the various calibration elements strongly depend from the S-CT parameters especially from the KV value. The HU distributions are quite different (up to 20%) from time to time even using the same scanning device and protocol. Furthermore, the differences between HU distributions from the same element enhance when this is denser (for instance bone). Finally the use of monitoring technique based on HU distribution must take into account device calibration, S-CT acquisition protocol and image processing methodology. Including these obvous needs of calibration, 3D Color S-CT scanning is a powerful follow-up tool that may complement other analytical methods, driving the choices and timings to validate rehabiliatation approaches and protocols.

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Recognition and prediction of individual and combined muscular activations modes via surface EMG analysis

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The paper discusses how recognition of individual and combined muscular activation modes (functions) and the prediction of intended such modes can be accomplished by identifying parameters of noninvasive surface EMG signals. It outlines the mathematical analysis of surface EMG signal to facilitate such recognition and related prediction, including recognition of intention (in terms of attempts) to activate motor functions from the EMG, without accessing the CNS itself, in cases where a patient, say, a high-level amputee does not have the final-activation muscles and joints. The EMG activity thus allows interpreting and recognizing CNS command from minute variations in the parameters of surface EMG signals that record changes in the firing of motor neurons that trigger contractions in related muscle fibers. We note that although in popular media this is sometimes referred to as detection of “thoughts”, no thoughts are detected, but only motor-outcomes of thoughts as found in the EMG signal. Examples of concrete cases where such recognition or prediction were accomplished in the author’s

lab and of devices that came out of that lab, are given as are references to these in the literature over the last 35 years.

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Application of Ultrasound Current Source Density Imaging (UCSDI) on denervated muscle. Some theoretical considerations

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Denervated and to some extent degenerated muscles are treated with electrical stimulation to stop the degeneration process or to reverse it. Through the treatment muscles have reached normal volume and up to 80% of their former force. In order to electrically stimulate a denervated muscle all muscle fibres in the muscle have to be depolarised over the threshold potential so that an action potential can propagate along the fibre and it can contract. Without contraction a muscle fibre is not trained. Depolarising a whole muscle can be a problem with only two electrodes. This is especially a problem in pennatus muscle structures as is the case f. ex. in the rectus femoris muscle. The therapy result could be a muscle that has grown in the stimulated area but not in the non stimulated areas. Several different methods have been applied to monitor the stimulation therapy. They are of mechanical, neurological, histological and radio graphical nature. They have in common that the result of the therapy is seen retrospectively. Therefore possibilities to change the therapy or adjust it according to the results from these monitoring methods are limited. In this work we present the results from some theoretical considerations about using a so called electro acoustic effect to map electrical current density distribution in a biological tissue. The effect is that an acoustic wave changes the local pressure in the tissue where it passes. This pressure change in turn changes the conductivity of the tissue and therefore the voltage drop over the tissue area. The voltage drop can then be picked up by surface electrodes. Since the voltage is modulated by the frequency of the acoustic wave it can easily by filtered out of other signals. The hypothesis is that the method can be used to map the electrical current density distribution of a pulse from an electrical stimulator and even the current density distribution from the electrical activity of denervated muscle fibres. Results from experimental work [1] suggest that the method can be used to map the electrical activity of a rabbit heart. The result of our theoretical considerations suggest that the method can be used for mapping the current density distribution of a stimulating electrical pulse and the electrical activity of a denervated muscle. In that function voltage resolution and immunity to disturbances of a measurement system play a central role as well as the formation of acoustic wave. Important advantage of the method is the possibility to use it with a normal ultrasound imaging device. The acoustic wave from that device is used to make the electro acoustic signal. In that way the ultrasound image and the current source image correspond exactly to each other.

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Home-base FES of LMN denervated muscles

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After complete Spinal Cord Injury (SCI), causing complete disconnection between the muscle fibers and the nervous system, the denervated muscles become unexcitable with commercial electrical stimulators within several months and undergo severe atrophy and disorganization of contractile apparatus after 1-3 years. Years after the injury the surviving and regenerated myofibers are substituted with adipocytes and collagen. To counteract the progressive changes transforming muscle into an unexcitable tissue, we developed a novel therapy concept for paraplegic patients with complete lower motor neuron (LMN) denervation of the lower extremity. The new stimulators for home-based functional electrical stimulation (h-b FES) have been designed to reverse longstanding and severe atrophy of denervated muscles by delivering high-intensity (up to 2,4 J) and long-duration impulses (up to 150 ms) able to elicit contractions of denervated skeletal muscle fibers in absence of nerves. Concurrent to the development of the stimulation equipment, specific clinical assessments and training strategies were developed at the Wilhelminenspital Wien, Austria, based on sound evidence from animal experiment [5]. Main results of our clinical study on 20 patients which completed a 2 years h-b FES program: 1. significant +33% increase of muscle size and +75% of the mean diameter of muscle fibers, with striking improvements of the ultra-structural organization of contractile material; 2. recovery of the tetanic contractility with significant increase in muscle force output during electrical stimulation; 3. five subjects performed FES-assisted stand-up and stepping-in-place exercises;. 4. data from ultrastructural analyses indicating that the shorter the time span between SCI and the beginning of h-b FES, the larger were the number and the size of recovered fibers [1-4]. The study demonstrates that h-b FES of permanent LMN

denervated muscle is an effective home therapy that results in rescue of muscle mass, function and perfusion. Additional important benefits for the patients are the improved cosmetic appearance of lower extremities and the enhanced cushioning effect for seating.

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Robotics versus FES: Competition or sinergy?

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Restoration of movement functions can mean temporary therapy and support of relearning and restoration, or permanent technical support or substitution. Both Functional Electrical Stimulation (FES) and robot solutions can be and are applied for pure therapy, for temporary functional support with inherent training, and for permanent support or substitution. In any case improvements of quality of life and autonomy in daily living are the main goals. A Robot is by definition a mechanical agent that performs one or more tasks in which it mimics a human or animal agent either through programming or commands. The term “Robot” was introduced in literature by Karel Capek in 1921; Isaac Asimov defined the “3 Laws of Robotics” in 1942 that are still considered relevant for contemporary robot technology. Medical robots play important role in surgery, in laboratory procedures and in rehabilitation. In rehabilitation it can be useful in cardiopulmonary support, restoration of motor control as well as functionality of muscle and skeleton. Rehabilitation robots are mainly applied in the form of exoskeletons that can be passive constructions providing unloading and/or guidance of movements or actively driven devices with electrical, pneumatic or hydraulic actuators. The actuators can substitute or support (boost) the function. A special category is “hybrid orthoses” where the actuators are FES activated muscles. FES has her roots in the 1960s (availability of portable electronic equipment) and is based on eliciting action potentials in nerve (efferent or afferent) or muscle fibres via artificial electrical (implanted or surface electrodes) or magnetic impulse field. Numerous applications for restoration of upper and lower extremity functions were demonstrated and only part of them found their way into clinical practice. From the engineering point of view we face challenging differences in controllability of active exoskeletons and electrically activated muscles. In robotics we have clearly defined and stable technical conditions, closed loop control of position, speed and force is state-of-the-art. In FES closed loop control is impeded by technically undefined excitation and feedback coupling and usual FES applications rely on open loop control and stabilizing overdrive. Contemporary robots show advantages in mimicking physical therapy, repeatability of therapeutic procedures and automated objective documentation. Limitations are given in active muscle training, the need of patient specific adjustments, costs, and need of space and qualified staff for the operation. FES is ahead in providing direct activation of nerves and muscles, easy to organize home based therapy and in comparison low costs. Drawbacks are limited selectivity in recruitment of nerve and muscle fibres, limits in therapy documentation, potential pain sensations and growing handling problems in multi-channel applications. In summary, robotics and FES seem not really in competition. They rather are synergic or complementary, depending on the application. Both have and will keep their justification and significance in rehabilitation medicine. In the foreseeable future an extension to a third strategic component - the upcoming promising biological solution – is to be expected.

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Abstracts

Ultrasound-based investigations of changes in myotendinous properties with disuse and ageing

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Muscles and tendons show a remarkable plasticity in response to chronic loading, unloading and ageing. Using high-resolution ultrasound imaging in a recent unloading study we showed a significant decrease (-8%) in human vastus lateralis (VL) muscle fibre fascicle length after 23 days of unilateral lower limb suspension (ULLS) (1) and by 9% after 35 days of bed rest. Concomitantly, patellar tendon (PT) stiffness decreased by 29% after 23 days ULLS (1). These changes are similar to those found in ageing since lower limb fibre fascicle length and tendon stiffness are respectively reduced by 10% and 36%, in older humans (2). These changes are expected to have an impact on the VL length-force relation (L-F), since a decrease in tendon stiffness would cause a left shift in the VL fascicles L-F relation, away from the force plateau region(3). However, since in both disuse and ageing, fascicle length was shorter, lesser sarcomere shortening would be expected upon contraction, thus shifting the L-F relation to the right, closer to the optimum region (3). Thus compensation between the fascicular and tendon changes seems to occur. However, this effect may only partly mitigate the force loss, since it cannot compensate the decline in force due to the loss of motor units (MUs) with ageing (4) or to the decrease in single fibre specific tension occurring with ageing and disuse (5). Loading, instead, produces the opposite effects: after 14 weeks of resistance training in older men, VL fascicle length increased by 8-10% (3), while PT tendon stiffness increased by 65%. The rapid muscle remodeling produced by these experimental paradigms, seems regulated by changes in the mechano-sensitive costameric protein focal adhesion kinase (FAK), believed to be an up-stream regulator of protein synthesis. Overloading of avian muscle causes a massive increase in FAK content (+112% after 1 day and 611% after 8 days) and activity (+370% after 1.5 and 13 days) (6). Instead, unloading produces opposite effects, since FAK content and activity decrease by 20 and 30%, respectively, within 10 days of ULLS in humans (7).

Thus the loss of muscle force observed with ageing and unloading likely reflects the combined effect of muscular and tendinous adaptations and, limited to old age, also of neuropathic processes responsible of the loss of MUs. In both ageing and unloading these myotendinous alterations may be largely reversed with loading, though muscle weakness due to MUs loss with age, cannot be prevented. The structural remodeling of skeletal muscle, detectable within few days of loading or unloading, seems mediated by changes in costameric proteins involved in mechanotransduction.

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Ultrasound of nerve and muscle in clinical neurology

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Ultrasound of peripheral nerves and muscles are gaining increasing importance in neuromuscular disease. The method is based on new and advanced technology of ultrasound equipment, and can be seen as a synergistic tool to conventionally available techniques, in particular electroneurodiagnostics. The technique is readily available and can be used in clinical neuromuscular settings. Additional to the morphological aspects, also movement of structures, vascularity and dynamic aspects are added value to this technique. The technique can be used in practically all structures of the peripheral nervous system. Much previous experience has been gained by the use of interventional techniques in anesthesiology, where deep structures as nerve roots, the brachial and lumbar and sacral plexus have been treated with ultrasound techniques for decades. In neurology

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Abstracts

most efforts have been targeted at the diagnosis of mononeuropathies, where constriction of nerves, swelling and also restriction of movement (gliding) have been well described, in particular with commonly examined nerves as median, ulnar, peroneal , tibial, but can also be used in sensory nerves as the lateral cutaneous nerve of the thigh. Prior electrodiagnostic findings as neurapraxia can be visualized. Muscle can be seen from several aspects: atrophy, inflammation, rhabdomyolysis or fatty tissue replacement can be visualized and give a clue to muscle disease. Also local aspects as tears, hematomas or tumors can be visualized. Finally also movement can be seen as fasciculations, or respiratory movements of the diaphragm. Other indication are increasingly found for as some cranial nerves, the nerve plexus and polyneuropathies, and also intervention as pain therapy, botulinus toxin therapy or biopsy guidance are already activated.

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Efficiency of antisense-mediated exon skipping in normal and mutated dmd genes

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Duchenne muscular dystrophy and its allelic form Becker muscular dystrophy (BMD) are caused by mutations in the dystrophin gene. In general, mutations that disrupt the reading frame lead to prematurely aborted dystrophin synthesis and cause DMD; conversely, mutations that do not disrupt the reading frame generally result in the milder BMD phenotype. Thus, in Becker patients the aberrant transcript encodes a dystrophin that, despite being internally truncated and usually present at reduced levels, is at least partially functional. This observation suggested that a therapeutic approach for DMD could be based on the concept of changing the genetic information during processing of primary RNA, in order to restore a viable reading frame. This method, known as exon skipping, depends on the use of sequence-specific antisense oligonucleotides (AO) that interfere with the recognition of intron-exon boundaries. Up to now virtually all AO designing procedures for exon skipping in the human DMD gene have been tested on normal cells. There have been recent indications that the genomic re-arrangements (deletion, duplications, point mutations, etc.) present in patients could affect the efficiency of the desired skipping processes, but this aspect has been barely studied so far. In this work we hence decided to compar the results obtainable in wild type controls and in myoblasts from patients carrying different types of mutations, when using AOs designed from the wild type DMD sequence with the currently available software tools.

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Detecting neuromuscular disorders and spontaneous muscle activity using muscle ultrasound

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Muscle ultrasound is a convenient technique to visualize normal and pathological muscle tissue as it is non-invasive and real-time. Neuromuscular disorders give rise to structural muscle changes that can be visualized with ultrasound: atrophy can be objectified by measuring muscle thickness, while infiltration of fat and fibrous tissue increase muscle echo intensity, i.e. the muscles become whiter on the ultrasound image. Muscle echo intensity need to be quantified to correct for age-related increase in echo intensity and differences between individual muscles. This can be done by gray scale analysis, a method that can be easily applied in daily clinical practice. Using this technique it is possible to detect neuromuscular disorders with predictive values of 90 percent. Only in young children and metabolic myopathies the sensitivity is lower. Ultrasound is a dynamic technique and therefore capable of visualizing normal and pathological muscle movements. Fasciculations can easily be differentiated from other muscle movements. Ultrasound appeared to be even more sensitive in detecting fasciculations compared to EMG and clinical observations, because it can visualize a large muscle area and deeper located muscles. With improving resolution and frame rate it has recently become clear that also smaller scale spontaneous muscle activity such as fibrillations can be detected by ultrasound. This opens the way to a broader use of muscle ultrasound in the diagnosis of peripheral nerve and muscle disorders.

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After 20-year work on food and exercise induced changes in human muscle protein metabolism what gaps remain to be filled?

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The first measurements of the effect of resistance exercise on human muscle protein synthesis (MPS) [1] was made by a collaboration between workers at McMaster and those in my laboratory at the University of Dundee and this collaboration, with different dramatis personae and the active involvement of workers from Marc Francaux lab and that of Michael Kjaer has been responsible for the accretion of a large amount of knowledge chiefly concerning the phenomena surrounding the stimulation of MPS and collagen synthesis in

tendon and muscle and some limited insight into the regulation by signalling pathways and hormones and the superimposed effects of food. It is time to take stock and attempt to see where we go from here. We can summarise the current nutritional understanding as follows: so far as nutrients are concerned, the main driver of muscle protein synthesis is the concentration of essential amino acids, (EAA) particularly leucine, and the effect is independent of any hormonal action, even those of insulin and growth hormone. Growth hormone itself has no effect on MPS but stimulates tendon and muscle collagen synthesis. Amino acids have almost no biologically significant effects on human muscle protein breakdown (MPB). Creatine has no effect on the processes of MPS or MPB. The effects of EAA (and indeed of whole proteins such as whey) is EAA dose dependent and appears to be associated with increases in the activity of well described elements of the mTOR and associated pathways, but what the proximal EAA sensor is remains unknown. This is a major gap in our knowledge, as is the nature of the relationship between changes in phosphorylation of signalling elements and the associated (assumed to be consequent) alterations in MPS and MPB. Although traditionally it was thought that the increases in MPS upon increased availability of EAA were slow and long lasting, recent work in our lab, mainly by Philip Atherton, following on from that carried out by Julian Bohé and myself in Galveston, has shown that this is wrong. In fact after a short latency of about 45 min, anabolic signalling and MPS rise hand in hand, with MPS coming to a peak after ~90-120 min following a whey protein bolus, and then MPS declines despite continued availability of elevated concentrations of EAA and elevated degrees of phosphorylation of a variety of signalling molecules, as expected from the EAA concentrations in plasma and muscle. Thus there is good consonance of signalling and MPS on the rise to maximum, but none thereafter when the muscle appears to be in anabolic state according the signalling patterns but is not according to the MPS rates. Insulin appears as mentioned earlier not to an important regulator of MPS but MPB is exquisitely sensitive to low concentrations of insulin; however here again there is a mismatch between behaviour of signalling molecules and the actual behaviour of MPB [2]. Obviously there are substantial gaps in our understanding of the control processes involved in the effects of nutrition. So far as contractile activity is concerned, whereas we thought many years that resistance exercise caused large increases in MPS, probably not seen with endurance exercise, it now seems that any unaccustomed exercise of any mode will stimulate MPS of all types, but after training in the appropriate mode (by different modes in the two legs of the same person) an acute bout of resistance exercise will only stimulate myofibrillar MPS and an acute bout of endurance exercise will only stimulate mitochondrial MPS [3]. Furthermore how this happens acutely is not known because the signalling changes are identical between legs. In addition, because originally we looked at MPS always in the fed state post exercise, it appeared that the increase due to exercise lasted for up to 48 hours; we can now see that in fact, unless feeding is superimposed , exercise only increases the actual rate of MPS for up to 4 h post exercise, although it seems to increase the sensitivity to food. Exercise and feeding are synergistic in stimulating MPS in the post exercise period an effect which might have to do with the contraction specific effects of membrane derived phosphatidic acid on the mTOR pathway and the separate non-phosphatidic acid dependent effects of leucine on different aspects of the mTOR complex. These ideas however come from animal work and (in our laboratory) tissue culture but the relevance to human muscle protein turnover remains unknown – another gap. There are in fact a great many gaps in the area such as those outlined above. To solve the problems we need to apply more "firepower" at the outstanding problems, collaborating in large groups to make measurements which show the full phenotypic range of responses in young, middle aged and elderly men and women, apply more closely techniques for analysis of micron, epigenetics, and look at other methods of signal transduction such as ubiquitinylation - not just as a signal for proteolysis but for control of metabolism in a wider sense. And we need to figure out how changes in stem cell metabolism can influence whole muscles phenotypes in response to plane of nutrition and levels of physical activity. The future beckons, but it will only be rosy if we are clear sighted and challenge all of our current assumptions.

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Abstracts

Signaling pathways that control protein breakdown and muscle loss

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Skeletal muscle is the most abundant tissue in the human body accounting for almost 50% of the total body mass and it is a major site of metabolic activity. Since muscle is the largest protein reservoir, it serves as a source of amino acids to be utilized for energy production by various organs during catabolic periods. For instance, amino acids generated from muscle protein breakdown are utilized by the liver to produce glucose and to support acute phase protein synthesis during fasting. A number of catabolic disease states, including sepsis, burn injury, cancer, AIDS, diabetes, and renal failure, neurological disorders are characterized by muscle wasting, mainly reflecting increased breakdown of myofibrillar proteins and loss of organelle including mitochondria. The recently discovery of two muscle specific ubiquitin ligases, atrogin-1/Mafbx and Murf1 that are necessary for muscle loss, has begun to define the individual Ubiquitin-Proteasome components critical to the atrophy process. The expression of these Ub-ligases increases 8-40 fold in all types of atrophy studied and precedes the onset of muscle weight loss. We and others have recently defined that these ubiquitin ligases and protein breakdown in general are blocked by the growth-promoting IGF1/PI3K/AKT pathway. The FoxO family, a downstream target of AKT, was identified as the main transcription factors regulating not only atrogin-1 expression but also an atrophy program which leads to a loss of muscle mass. FoxO family members have been shown to regulate various cellular functions including apoptotic cell death. Here we investigated the possible role of FoxO in inducing mitochondria damage and activation of autophagy pathways. Overexpression of constitutive active Foxo3 in adult muscle fibers causes a reduction in mitochondrial content, apparently due to their destruction in autophagic vacuoles. The fact that FoxO controls mitochondria and muscle atrophy prompted us to investigate the role of autophagy during muscle loss. Muscle-specific autophagy knockout mice showed different features of muscle degeneration including alteration in mitochondrial content, accumulation of protein aggregates and reduction in myofiber size and force. Mitochondrial removal depende from Bnip3 upregulation and mitochondrial dysfunction trigger a positive feedback loop on FoxO3 activity. Thus mitochondria damage/loss and energy balance become critical steps in muscle wasting.

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Satellite cells dysfunction may contributes to impaired skeletal muscle regeneration in sporadic amyotrophic lateral sclerosis (ALS)

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ALS is a progressive neurodegenerative disorder characterized by selective degeneration and death of both upper and lower motor neurons, resulting in paralysis due to muscle weakness and atrophy. Generally skeletal myofibers are able to regenerate after injury, thanks to Satellite Cells (SCs), myogenic precursor cells located between the basal lamina and the sarcolemma of mature myofibers. In ALS patients the regenerative capacity of skeletal muscle is compromised. In order to study in more detail the cellular and molecular mechanism of SCs, we isolated SCs from ALS aging human muscle biopsies and analyzed their morphology by TEM and ICC. We performed End-Point RT-PCR to evaluate the expression of MRFs during both proliferation and differentiation phases. In addition we studied the effect of Conditioned Media (CM) of CTR SCs on ALS SCs and vice versa in order to understand the influence of some soluble factors on the regulation of myogenic program. ALS SCs showed a high ability to proliferate, but their capacity to proceed through the myogenic program and actively form myotubes seems to be altered compared to the aging control samples. In addition we observed in vitro that differentiating ALS SCs display an altered morphology which could be linked to their impaired regenerative potential.

Forty years of European Society for Muscle Research

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The European Muscle Club was founded 1971 and since 1972 yearly muscle conferences were held in turn in different European countries. Regular scientific meetings in the field of muscle research did not exist before. The name of the Muscle Club was 1988 changed to European Society for Muscle Research (ESMR). The yearly meetings usually attract 200-300 participants. The 39th meeting will be held 11-15 Sept. 2010 in Abano Terme near Padova, and the 40th meeting 14-18 Sept. 2011 in Berlin. Since 1980 the meeting

reports and abstracts are regularly published in the Journal of Muscle Research and Cell Motility (JMRCM). The history of the society and the muscle scientists involved with it will be outlined.

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Primer for sarcomeric muscle genetics

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Over the past 150 years average human lifespan has doubled from 40 to 80 years in Western countries. Mutations causing late-onset diseases have accumulated in the genome over millions of years. Today's medicine is confronted with these genetic diseases which in earlier times had no impact because of premature death for other reasons. ~2% of the 3 billion base pairs (bp) code for ~20'500 genes giving rise to over 200'000 proteins by different splicing and posttranslational modifications. DNA sequence variations comprise ~15 million SNPs (single nucleotide polymorphisms), over 4000 structural variants (deletions, insertions, inversions, copy number variation, segmental duplications), and chromosomal translocations. SNPs are inherited in groups called haplotypes which may extend beyond coding sequences. Up to 2000 monogenetic (Mendelian inheritance) diseases have surfaced. But most common diseases are multigenic with individual gene defects contributing only little to the disease phenotype. This renders disease-specific attribution of gene defects difficult. Beside the DNA sequence variations in protein-coding genes, over 94% of the entire genome (earlier considered as evolutionary accumulation of "junk" DNA) are expressed as various non-protein-coding RNA species (~70% of all genes). Beside the well-known transfer RNAs and ribosomal RNAs many of these are involved in genomic regulation including the microRNAs (mostly 22 bp long), and interference RNAs. ~1000 microRNAs are expressed in a cell-specific and typical temporal pattern affecting development, maturation, aging, and disease phenotype. A specific microRNA may affect expression of up to hundreds of genes, and one specific gene may be affected by many microRNAs. Over 7000 genes may be regulated by microRNAs. A number of microRNAs are specifically expressed in skeletal and cardiac muscle. Genetics for hypertrophic and dilated cardiomyopathies as well as for the main cardiac regulatory adrenergis system will be discussed. Microarrays for identification and resequencing of disease-related groups of genes are commercially available for diagnostics, as well as to follow treatment response and disease development.

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Assessment of respiratory chain and antioxidant enzymes in skeletal muscle biopsies

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The diagnosis of mitochondrial disorders is a difficult task due to the clinical heterogeneity, the extreme range of mutation types both in the nuclear DNA and mtDNA, and heteroplasmy of different tissues. In this settings, biochemical measurement of respiratory chain enzymes is still a crucial screening step in the diagnosis of these devastating conditions, in order to restrict the molecular investigations. The skeletal muscle biopsy is often the most suitable tissue for biochemical studies due to easy accessibility and the high metabolic rate. Moreover, antioxidant enzymes dysregulation often follow mitochondrial dysfunction and their assessment could be useful in the study of the pathogenesis of mitochondrial disorders. However, these biochemical tests have not been uniformely standardized, leading to a huge variety of results and reliability of the biochemical diagnosis within different diagnostic centers. We investigated the effect of different parameters on the yield of the enzymatic results on bovine skeletal muscle. Surprisingly, many factors, both pre-analytic (i.e. modality of homogenization, type of homogenization buffer) and analytic (temperature, PH, concentration of substrates, presence of adjuvants or inhibitors, modality of mitochondrial membrane disruption), were found to greatly influence the rates of the enzymes. These results clearly indicate the requirement of strictly standardized methods to improve the diagnostic accuracy.

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Echogenicity, contraction and perfusion properties of skeletal muscle by US Functional Echomyography

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Permanent denervated muscles were evaluated by ultrasound to monitor changes in morphology, thickness, contraction-relaxation kinetics and perfusion due to the electrical stimulation program of the Rise2-Italy project [3-5]. In a case of monolateral lesion, morphology and ultrasonographic

structure of the denervated muscles changed during the period of stimulation from a pattern typical of complete denervation-induced muscle atrophy to a pattern which might be considered "normal" when detected in an old patient. Thickness improved significantly more in the middle third of the denervated muscle, reaching the same value as the contralateral innervated muscle. Contraction-relaxation kinetics, measured by recording the muscle movements during electrical stimulation, showed an abnormal behavior of the chronically denervated muscle during the relaxation phase, which resulted to be significantly longer than in normal muscle. The long-term denervated muscles analyzed with Echo Doppler showed at rest a low resistance arterial flow that became pulsed during and after electrical stimulation. As expected, the ultra sound measured electrical stimulation-induced hyperemia lasted longer than the stimulation period. The higher than normal energy of the delivered electrical stimuli of the Vienna home-based Functional Electrical Stimulation strategy (h-b FES) demonstrate that the explored muscles were still almost completely denervated during the one-year of training. In conclusion, this pilot study confirms the usefulness of US Functional Echomyography in the follow-up [3-5] and the positive effects of h-b FES of denervated muscles [1,2].

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Early signs of a tumor associated myopathy in patients affected with newly diagnosed colorectal cancer

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The main objective of this study was to verify whether it is possible to detect myopathic features which may be indicative of an early stage of tumor associated myopathy in patients affected with a newly diagnosed cancer. Muscle biopsies from the rectus abdominis of 22 patients (9 male, 13 female), with a new diagnosis of primary cancer (16 colorectal, 1 stomach, 1 esophagus, 1 anus, 1 liposarcoma, 2 high grade displasia) were collected during the surgical removal of the tumor. All biopsies were analyzed by muscle morphometry, histochemical and immunohistochemical analyses. Muscle biopsies from rectus abdominis of 14 subjects affected with non neoplastic gastrointestinal diseases were collected as controls. In cancer patients we surprisingly observed a high percentage of internally nucleated myofibers, that was significantly higher compared to controls (mean 15.01±14.33 % vs 2.73±2.32%, $p<0.03$). Notably, the percentage of myofibers with centrally located nuclei correlated with that of myofibers with nuclei placed more closer to the sarcolemma, but still inside the myofibers ($R2=0.5$). Electron microscopy analyses of transverse sections of muscle biopsies showed that chromatin was typically packed inside the abnormally located nuclei, similarly to the chromatin organization observed in myonuclei normally placed at the periphery of myofibers. No differences of mean myofibers diameters and muscle fiber type distribution were noted in muscle biopsies from cancer patients compared to controls. Regenerating NCAM (0.3±0.7 positive fibers/mm2) and MHCemb (0.05±0.2 positive fibers/mm2) expressing myofibers were detected only in muscle biopsies from cancer patients, whereas control biopsies were negative. Preliminary results using anti-dystrophin monoclonal antibody (COOH terminus) showed

that the protein is normally expressed on the sarcolemma of all myofibers. The pathologic features observed in muscle biopsies from cancer patients are early sign of an asymptomatic myopathy, probably induced by factors released by the tumor microenvironment. Further analyses investigating the molecular mechanism underlying this association, described for the first time at the very early stage of cancer diagnosis, will hopefully provide prognostic markers and therapeutic targets for progression of the disease to tumor cachexia.

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Abstracts

Index