University of Padua – cirMYO Interdeparmental Research Center of Myology – Città di Padova

IIM Interuniversity Institute of Myology

2008Spring PaduaMuscleDays

Functional Recovery of Muscle Tissue

Terme Euganee & Palazzo Bo, Padova (Italy), April 13 - 15, 2008

Summaries

Adami Nicoletta, Poster 6
Adami N (1), Kern H (2, 3), Biral D (4), Zampieri S (1, 5), Mayr W (6), Carraro U (1, 4). (1) Laboratory of Translational Myology, Interdepartmental Research Institute of Myology, Department of Biomedical Science, University of Padova, Italy; (2) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, and (3) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (4) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, Padova, Italy; (5) Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Padova, Italy; (6) Center of Biomedical Engineering and Physics, Medical University of Vienna, Austria. E-mail: nicolettaadami@libero.it

Long-term survival (in tens of months) of rat muscle fibers after bilateral sciatectomy

Permanent lack of motor innervation results in streaking alterations of functional and structural properties of muscle. In spinal cord injury (SCI), direct Functional Electrical Stimulation of denervated degenerated muscles (FES of DDM) delays/reverts muscle atrophy. FES is, however, believed to be effective when started early after SCI, since long-term (in months) denervated human muscle do not contract by using commercially available surface electrical stimulation (and fully implantable devices have strong limitation related to the need to directly reach millions of muscle fibers). Whether, this is the result of: 1. lack of muscle fibers; 2. their poor excitability, or 3. disarrangement of contractile machinery, is still an issue that deserves detailed time-course studies that are difficult in humans. Thus, we are planning and performing in a rat model several related studies. Here we report on the progression of denervation-induced long-term changes of Tibialis Anterior (TA). We elicited muscle contractions by external electrode up to 12 months after bilateral sciatectomy, a good model of human *Conus Cauda* Syndrome. Chronaxie analysis and histological structural findings showed that the denervated TD undergoes: 1. rapid atrophy during the first month post sciatectomy, accompanied by progressive increases of chronaxie that reached a stable increase during second month (early stage of fast progressive atrophy); 2. A second phase of slow-progressive atrophy follow s up to the 4^{th} denervation month, during which minor changes were observed; 3. A third phase, during which the gross muscle bulk is stable, while chronaxie either increased to infinite value, since no contractions were palpable (severe atrophy stage, from 4 to at least 12 months) or returned to the very low value of reinnervated muscle. Histochemistry showed early disappearance of the type I (slow) fibers, followed by disappearance of the ATPase and of the SDH activities. On the other hand, the long-term denervated muscle presents numerous "severely atrophic" muscle fibers, that is, long fibers empty of any myofibrillar apparatuses and with peculiar groupings of 3 to ten grouped myonuclei ("morulae"). Furthermore, Francini et al. previously showed that the severely atrophic myofibers have resting membrane potential and action potentials [1, 2]. Our conclusion is that the lack of contractility of the long-term denervated rat muscle is due to complete loss of contractile proteins arranged in sarcomeric structures long before muscle fibers decreases in number, a process that requires much more than 12 months in rat fast-twitch muscles.

[1] Squecco R, Kern H, Biral D, Rossini K, Francini F. Mechano-sensitivity of normal and long term denervated soleus muscle of the rat. Neurol Res 2008; 30(2): 155-159.
[2] Squecco R, Francini F, Kern H, Biral D, Rossini K, Carraro U. L-type Ca2+ channel mechano-sensitivity in long-term denervated Soleus muscle of the rat. Basic Appl Myol 2005; 15: 187-190.

Biral Donatella, Poster 5
Kern H (1, 2), Hofer C (1, 2), Biral D (3), Adami N (4), Boncompagni S (5), Zampieri S (4, 6), Jakubiec-Puka A (7), Mayr W (8), Protasi F (5), Carraro U (3, 4). (1) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, and (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, Padova, Italy; (4) Laboratory of Translational Myology, Interdepartmental Research Institute of Myology, Department of Biomedical Science, University of Padova, Italy; (5) IIM - Interuniversitary Institute of Myology, CeSI - Centro Scienze dell'Invecchiamento, Università degli Studi G. d'Annunzio, Chieti, Italy; (6) Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Padova, Italy; (7) Nencki Institute of Experimental Biology, Warsaw, Poland; (8) Center for Biomedical Engineering and Physics, Medical University Vienna, Austria. E-mail: dbiral@bio.unipd.it

FES training improves structure and function of tight muscles in upper motor neuron thoracic level paralysis

To investigate the structural and functional relationships and the progression or functional electrical stimulation (FES)-induced regression of muscle atrophy up to 20 years of spastic paraplegia, clinical follow-up was performed in Vienna, Austria, and muscle biopsies were analyzed by light microscopy in Padua and by electron microscopy (EM) in Chieti, Italy. Force was measured as knee extension torque; trophism by computer tomography scan; tissue composition and fiber morphology by histopathology and EM. Main results are that: 1. In the long-term group of patients (17.0+/-2.6 years), force and size of thigh muscles were only slightly different from those of mid-term subjects (2.2+/-0.5 years): 2. Histology and ultrastructure confirm that the difference in average size of muscle fibers between long-term and mid-term paralyzed leg muscles is actually very small. In addition, muscle fibers maintain the striated appearance characteristic of normal skeletal fibers even after 14-20 years of paralysis; 3. Ultrastructural alterations of the activating and metabolic machineries, and the presence of fibers with lower motor neuron denervation features, may explain the low-force output and the reduced endurance of paretic muscles; 4. Two-year FES-training substantially reverses the process and takes the muscle fibers to almost normal values for sedentary adults. Indeed, the mean fiber diameter after FES increases more than 50% in comparison to the pre-FES values. The stable muscle atrophy that characterizes long-lasting spastic paraplegia, and the fact that extent of FES-training results do not correlate with time from SCI, strongly suggest that there are no upper-time limits to begin a FES training program.

[1] 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(2): 137-140.

[2] Boncompagni S, Kern H, Rossini K, Hofer C, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibers in absence of innervation in humans. Proc Natl Acad Sci U S A. 2007 Dec 4;104(49):19339-44. Epub 2007 Nov 27.

[3] Kern H, Hofer C, Modlin M, Mayr W, Vindigni V, Zampieri S, Boncompagni S, Protasi F, Carraro U. Stable muscle atrophy in long term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord (2007). ISSN: 1362-4393. Spinal Cord. 2007 Oct 23; [Epub ahead of print]

Breen Paul, Poster 3
Breen PP (1, 2), Nene A (3, 4), Hermens H (3), Schaake L (3), ÓLaighin G (1, 2). (1) Department of Electronic Engineering, and (2) National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Galway, Ireland; (3) Roessingh Research & Development, and (4) Het Roessingh Rehabilitation Centre, Enschede, The Netherlands. E-mail: paul.breen@nuigalway.ie

An exploration of M-wave response to electrical stimulation in the triceps surae

In the present study, surface and fine-wire electromyography (SEMG and FWEMG) signals were recorded from the lateral gastrocnemius (LG), medial gastrocnemius (MG) and soleus (SOL) muscles. The purpose of this study was to quantify the M-wave response of the LG, MG and SOL muscles to neuromuscular electrical stimulation (NMES) at the stimulation sites recommended in the literature and establish what effect the application of NMES to the predominately slow-twitch component of the triceps surae (SOL) has on the fast-twitch gastrocnemius (GAS) components and vice versa. Electrode stimulation was used to elicit individual

contraction of the GAS, SOL and of the GAS and SOL combined (GSOL). Our study established that selective surface stimulation of the GAS or SOL muscles at the recommended sites results in highly selective activation of these muscles and that the electrode position used for GAS stimulation produces matched levels of M-wave response in the LG and MG. Stimulation frequency has been shown to have little effect during selective stimulation of the GAS or SOL. Stimulation of the GSOL combination produced lower M-wave peak-to-peak amplitudes and higher levels of variance than using individual stimulation channels for each muscle, but could be used as an effective compromise if two stimulation channels are not available to stimulate the GAS and SOL individually. Finally, we have shown, using the technique described, that SEMG is a suitable method for the measurement of M-waves during NMES activation of the LG, MG and SOL muscles.

Caplan Arnold I, Invited Lecture, Aula Magna, Palazzo Bo, April 14, 2008
Skeletal Research Center, Department of Biology, Case Western Reserve University , Cleveland, Ohio, USA. E-mail: "Arnold I. Caplan" <aic@case.edu>

Adult mesenchymal stem cells, muscle and regenerative medicine

The dynamics of embryonic muscle development with regard to various cell contributions is now at an understandable level. The dynamics of adult muscle repair and turnover is not described in detail. It is clear that in certain neuromuscular diseases that muscle cells die at high rates and that this loss of cells is not sufficiently matched by stem cell-mediated replacement events. Moreover, large muscle defects are often replaced by scar tissue or fatty degeneration. Adult Mesenchymal Stem Cells (MSCs) from marrow and other sources are capable of differentiating into skeletal muscle. These MSCs appear to decrease in number with age and this decrease seems to reflect the tissues' ability to regenerate versus repair itself. New information indicates that MSCs do more than differentiate into discrete phenotypic compartments. These progenitor cells have the capacity to secrete a large array and large quantities of bioactive factors. These factors are strongly immuno-regulatory and trophic. The trophic capacity sets-up a local regenerative micro-environment capable of allowing tissue intrinsic progenitors to regenerate limited tissue segments. These effects are particularly well studied in the case of acute myocardial infarcts and under-studied in skeletal muscle trauma and disease models. The basic information available on MSCs suggests to me that all MSCs are pericytes, i.e. mesenchymal cells that reside on the abluminal side of all blood vessels (large and small). These pericytes are released at sites of tissue damage and they, then, divide. As such, these dividing cells appear to be similar to isolated MSCs that are expanding in cell culture. Both MSC/pericytes would be expected to be immuno-regulative and, thus, to protect tissue-damage sites from immuno-surveillance and, therefore, inhibit the establishment of auto-immunity. The trophic activity would encourage the tissue intrinsic progenitor cells to regenerate the damage tissue. Importantly, the decrease in tissue vascular density with age may be highly correlated with the decreased ability of tissues to regenerate themselves, increased tissue atrophy and decreased titers of isolatable MSCs. Experimental evidence for the above will be provided from work in our labs and others. A detailed understanding of the natural tissue turnover, regeneration and repair events in adults and as a function of age provides a new opportunity for cell-based therapies and regenerative medicine. Supported in part by funds from NIH and the Baldwin Foundation.

[1] Sorrell JM, Baber MA, Caplan AI. Human dermal fibroblast subpopulations; differential interactions with vascular endothelial cells in coculture: Nonsoluble factors in the extracellular matrix influence interactions. Wound Repair Regen. 2008 Mar-Apr;16(2):300-9.
[2] Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol. 2007 Nov;213(2):341-7. Review.
[3] Sorrell JM, Baber MA, Caplan AI. A self-assembled fibroblast-endothelial cell co-culture system that supports in vitro vasculogenesis by both human umbilical vein endothelial cells and human dermal microvascular endothelial cells. Cells Tissues Organs. 2007;186(3):157-68. Epub 2007 Jul 26.
[4] Sorrell JM, Baber MA, Caplan AI. Clonal characterization of fibroblasts in the superficial layer of the adult human dermis. Cell Tissue Res. 2007 Mar;327(3):499-510. Epub 2006 Nov 9.

Carraro Ugo
Kern H (1, 2), Musarò A (3), Protasi F (4), Sandri M (5, 6, 7), Carraro U (8, 9). (1) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, and (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) Department of Histology and Medical Embryology, Sapienza University of Rome, Italy; (4) IIM - Interuniversitary Institute of Myology, CeSI - Centro Scienze dell'Invecchiamento, Università degli Studi G. d'Annunzio, Chieti, Italy; (5) Venetian Institute of Molecular Medicine, Padova, and (6) Dulbecco Telethon Institute, and (7) Department of Biomedical Sciences, University of Padova, Italy; (8) Laboratory of Translational Myology, Interdepartmental Research Institute of Myology, and (9) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, University of Padua, Italy. E-mail: ugo.carraro@unipd.it

INTERREG III A – Functional & structural results of the Vienna-Bratislava Project

The project concerns the potential of FES training in rehabilitation of elderly after hip or knee replacement. Our preliminary goal is to study new hypertrophy-oriented training protocols in subjects that had before training their maximal muscle mass/force related to their life style, and thus we selected the very difficult task to improve muscle mass in young sportsmen during their active seasonal activities. Twelve young sportsmen were randomly divided in two groups. One performing usual muscle hypertrophy training by isokinetic exercises ("isokinetic group"), and the second performing in addition a new "swinging" protocol ("vibration group") that obliged the muscles to perform series of eccentric tetanic contraction at high frequency. Our goals were, first to test feasibility and safety of the new training protocol, and then to adapt it by lowering its intensity for elderly training, and preceding/combining it with Functional Electrical Stimulation (FES) training. Biopsies were harvested before and after the end of the training period from the vastus lateralis of the two groups of young sportsmen. Morphometric analyses showed that: 1. No differences in tissue distribution (myofibers, adipocytes, scar collagen, and loose connective tissues) have been observed comparing before and after training series, while 2. The distribution of myofibers diameters presented a shift toward higher values in the "vibration group". The new protocol seems to be able to increase muscle bulk over values young sportsmen performing "force training" usually achieve. No signs of muscle damage/repair were identified. Ultra structural studies and molecular analyses of muscle atrophy/hypertrophy master genes are in progress to confirm safety of the new training strategy and to understand the mechanisms of the contractile protein accumulation i.e., whether molecular or cellular processes - or both - are responsible for the increased muscle bulk/performance. Furthermore, by adding these biopsies from young sportsmen, we reached completeness of our data bank of human muscle biopsies harvested before and after FES and different mechanical trainings from normal and paraplegic adults. A long-term goal, now an excellent result, is granted: "Our unique world-class databank of human biopsies from FES-trained muscles".

Carraro Ugo, Poster 7
Kern H (1, 2), Hofer C (1, 2), Adami N (3), Biral D (4), Zampieri S (3, 5), Jakubiec-Puka A (6), Carraro U (3, 4). (1) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, and (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) Laboratory of Translational Myology, Interdepartmental Research Institute of Myology of the University of Padua, and (4) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, Padova, Italy; (5) Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Padova, Italy; (6) Nencki Institute of Experimental Biology, Warsaw, Poland. E-mail: ugo.carraro@unipd.it

FES training improvements of tight muscles structure in lower motor neuron paralysis: Histochemical and immunohistochemical evidence

We performed several histochemical and immunohistochemical analyses on muscle biopsies from Spinal Cord Injured (SCI) persons to establish if large muscle fibers that are present after two-year Functional Electrical Stimulation (FES) training are trivial results of sparse residual innervation/reinnervation or one of the several not expected observations made possible by our unique databank of long-term denervated human muscle biopsies. Indeed, the community of physiatrists and neurologists do not take for granted some pioneering observations of Ernest Gutmann in the Sixties (and of Ugo Carraro in the Eighties) of the last Century. Present results are that: 1. NCAN immunolabelling of the sarcolemma, a sound marker of early

muscle denervation, disappears more than one-year after SCI, while a new late-denervation marker appears, that is, the differential labeling of the sarcolemmal dystrophin molecule by anti-C (absent) and anti-N (present) terminals antibodies. These features are time-correlated with the disappearance of type I (slow) ATPase activity and to the dramatic decrease of both type II (fast) ATPase and of SDH activity, a marker of mitochondrial content. After FES training, the majority of myofibers are "big fibers" that partly recovered, but not up to the level of muscle fibers, we described in long-term "spastic paraplegia". Thus, they are "denervated" myofibers that recovered from long-term severe atrophy.

[1] Graupe D, Cerrel-Bazo H, Kern H, Carraro U. Walking performance, medical outcomes and patient training in FES of denervated muscles for ambulation by thoracic-level complete paraplegics. Neurol Res 2008; 30(2): 123-130.

[2] Boncompagni S, Kern H, Rossini K, Hofer C, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibers in absence of innervation in humans. Proc Natl Acad Sci U S A. 2007 Dec 4;104(49):19339-44. Epub 2007 Nov 27.

[3] Kern H, Hofer C, Modlin M, Mayr W, Vindigni V, Zampieri S, Boncompagni S, Protasi F, Carraro U. Stable muscle atrophy in long term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord (2007). ISSN: 1362-4393. Spinal Cord. 2007 Oct 23; [Epub ahead of print]

Coletti Dario

Coletti D, Berardi E, Aulino P, Moresi V, Pristerà A, Molinaro M, Adamo S. Department of Histology and Medical Embryology, "La Sapienza" University of Rome, Italy. E-mail: dario.coletti@uniroma1.it

Hampered muscle homeostasis and functional recovery in the presence of cytokines

Cachexia, a debilitating muscle wasting syndrome, increases mortality and morbidity of a significant fraction of chronically hill as well as ICU patients. We use tumor (C26)-bearing mice and TNF expressing mice as models of cachexia. In the presence of elevated levels of cytokines several populations of cells with myogenic potential are stable or increased in muscle, including satellite (Pax7-expressing) cells, hematopoietic stem (Sca1- CD45-expressing) cells and muscle interstitial stem (Sca1- CD34-expressing) cells also characterized by PW1expression. PW1 is involved in myogenic cell differentiation, fiber size control and p53-mediated pathways. The increase in myogenic cells in cachectic muscle suggests an attempt to cope with wasting by activation of a myogenic response. Nonetheless, we observe that muscle regenerative capacity is reduced by cytokines. TNF negatively affects the onset of regenerating fibers, without exacerbating fiber death following focal injury. Several interstitial cells, expressing Sca1, CD34 and PW1, show caspase activity during regeneration and the number of caspase activated cells is markedly increased by TNF, concomitant with an inhibition in regeneration. Block of caspase activity improves muscle regeneration either in the absence or presence of TNF. Muscle treatment with the myogenic factor vasopressin (AVP) counteracts TNF effects on regeneration. Regeneration extent affects muscle performance and AVP-mediated rescue of TNF effects results in functional recovery of the regenerated muscle similar to controls. Our data show that cytokine negative effects on muscle are counteracted by hormonal or pharmacological approaches. They also highlight the importance of proper muscle regeneration for functional recovery of muscle tissue.

Francini Fabio

Squecco R (1), Luciani G (1), Formigli L (2), Tani A (2), Nosi D (2), Zecchi Orlandini S (2), Francini F (1).Departments of (1) Physiological Sciences and (2) Anatomy, Histology, and Forensic Medicine, University of Florence, Italy. E-mail: fabio.francini@unifi.it

Muscle stretch injury may be reduced by sphingosine 1-phosphate (S1P) treatment

Injuries of skeletal muscle can lead to significant pain and disability. Traumatic muscle injuries including crush, contusion, laceration, or freezing can have dramatic and prolonged effects on muscle functional capacity. Contraction-induced muscle injuries resulting from severe muscular works or exercises happen more often. Damages of injured muscle, include degeneration, inflammation, regeneration and remodelling. E-C coupling failure occurs after eccentric contraction-induced injury, so we attempted to determine its damage by electrophysiological methods and to evaluate if sphingosine 1-phosphate (S1P) could reduce the muscle damage. S1P is a bioactive lipid that exerts multiple biological effects in a large variety of cell

types, acting either as an intracellular messenger or an extracellular ligand operating as important mediators of fundamental biological processes, including proliferation, differentiation and survival. Mouse EDL muscle injury was induced by stretching EDL during K+-contractures. EDL was stretched to 120% of its resting length. 10 contractures lasting 1 min were repeated every 4 min interval. Ionic currents, membrane potential and passive properties of the fibres were evaluated in single fibres by intracellular microeledrodes in voltage or current-clamp condition. Injured fibres were characterized by sarcolemnic membrane depolarization, reduced sarcolemnic resistance and Na+ and Ca2+ currents. All these alterations were reversed when stretches and K+-contractures were made in the presence of S1P in the bath solution. Membrane depolarization and reduction of sarcolemnic resistance suggested that muscle stretch during contracture induced plasma-membrane damage. Moreover S1P prevented such damages probably by increasing the cytoskeleton activity that, in turn, reduced the intracellular muscle damage.

Gargiulo Paolo
Gargiulo P (1, 2), Vatnsdal B (1), Ingvarsson P (3), Knútsdóttir S (3), Gudmundsdóttir V (3), Yngvason S (3), Helgason T (1, 2). (1) Department of Research and Development, HTS, Landspitali-University Hospital, Reykjavik; (2) Department of Biomedical Engineering, Reykjavik University School of Science and Engineering; (3) Department of Rehabilitation Medicine, Landspitali-University Hospital, Reykjavik, Iceland. E-mail: "Paolo Gargiulo" <paologar@landspitali.is>

Restoration in denervated muscle treated with FES: Quantitative Measurements of volume, density and shape

This work shows in novel way restoration process induced in denervated and degenerated muscles through FES. The growth in denervated muscles involves and causes changing of different parameters. Indeed muscle volume, density and geometry vary remarkably during the electrical stimulation treatment and a correlation between these parameters can be measured quantitatively. To this purpose, Spiral CT and special image processing tools are used to isolate muscle bellies and measure volume, density and geometry changes very accurately. The muscle belly most influenced by the electrical stimulation treatment and therefore the most interesting for this study is Rectus Femoris. During 4 years of monitoring, 3-dimensional reconstructions of the Rectus Femoris muscles from patients with long-term flaccid paraplegia were made at different points of time. The growth of the muscle and its changes through the time period are seen in the 3-dimensional representation and are measured quantitatively. Furthermore, changes in shape are measured and compared with respect to healthy muscles in order to estimate the degree of restoration. The results clearly show a slow but continuing muscle growth induced by electrical stimulation. Increase of volume and density is accompanied with change of muscle shape suggesting a correlation between local density distribution and change of shape. Finally, this work allows a unique way of monitoring, providing qualitative and quantitative information on the denervated degenerate muscle behaviour otherwise hidden.

Gáti István
Gáti I (1), Danielsson O (1), Betmark T (1), Ernerudh J (2), Öllinger K (3), Dizdar N (1). (1) Neuromuscular Unit, Department of Neurology, (2) Division of Clinical Immunology, Department of Molecular and Clinical Medicine, (3) Division of Experimental Pathology, Department of Neurosciences and Locomotion, Linköping University, Linköping, Sweden. E-mail: "Gati Istvan" <Istvan.Gati@lio.se>

In vitro formation and morphological characteristics of spheroid-like structures in cultures of muscle biopsies

Material and method: Primary muscle tissue cultures were established from diagnostic muscle biopsies of different neuromuscular disorders. In series of experiments, tissue pieces with 1-2 mm diameters were prevented from monolayer outgrow, by continuous agitation. In vitro attachment capability and morphology of the samples were investigated. After one month culture period, histological studies were carried out on paraffin -, frozen sections, and with electron microscopy. The differences between cultures of „normal muscle" and „dystrophic muscle" were compared. Results: The tissue pieces were unshaped in the beginning of the cultures, but they formed spheroid-like structures, during the culture period. The attachment capability of the „dystrophic muscle" was strong at the beginning of the culture period, but decreased with time, however the weak attachment capability of the „normal muscle" continuously

increased. The histological investigations of the "dystrophic muscle" demonstrated bigger myofiber destruction and a much weaker satellite cell activation and myofiber regeneration as compared to the „normal muscle". Conclusion: The cellular elements of the muscle tissue can survive and proliferate in a spheroid-like, primary culture system, where the degeneration of the original fibers, and the activation and the proliferation of the satellite cells can be studied in a culture system, what reflects a better physiological condition, then others. This system can be useful to study the effects of denervation and physical damage for the muscle tissue regeneration in different disorders, and also to investigate drug effects.

Helgason Thordur
Helgason T (1, 2), Gargiulo P (1, 2), Halldorsdottir G (3), Ingvarsson P (3), Knútsdóttir S (3), Gudmundsdóttir V (3),Yngvason S (3). (1) Department of Research and Development, HTS, Landspitali-University Hospital, Reykjavik; (2) Department of Biomedical Engineering, Reykjavik University School of Science and Engineering; (3) Department of Rehabilitation Medicine, Landspitali-University Hospital, Reykjavik, Iceland. E-mail: Þórður Helgason <thordur@landspitali.is>

Comparing muscle and bone density changes in denervated and degenerated muscle treated with electrical stimulation

Three patients have bee treated with electrical stimulation since autumn 2003 and until now, 2008. They all have a conus cauda lesion as a result of different accidents. Consequences of this lesion are damage to the lower motor neuron resulting in denervation and total inactivity of the muscles. The muscles degenerate in the course of time and would end with no contractile elements being mostly connective tissue and fat. If electrical stimulation therapy is started within several years form the accident, preferably less than three to four years, this degeneration process can bee stopped and reversed. By stimulation once a day for a half to two hours a day six days a week the patients can build up the muscle volume and to a great extent the force again. The three patients have been monitored with a quantitative spiral computer tomography (QCT) taken three times a year, i.e. every four moths. From this data the muscle rectus femoris, the femur bone and the patella have been segmented and volume, density and form analysed. In this work we compare the changes in muscle density to those of bone density. Results are a clear shift of muscle density towards higher Hounsfield values with an increase in muscle volume whereas the bone density changes are not as clear. Both decrease and increase of density can be detected in the same bone in the same period.

Jakubiec-Puka Anna
Nencki Institute of Experimental Biology, Warsaw, Poland. E-mail: annajakubiec@o2.pl; ajpe@nencki.gov.pl

Effect of passive movement on the denervated rat leg muscles depends on the training intensity and time of its beginning

As recently found, passive movement seems to attenuate some of the pathological processes within the denervated soleus and extensor digitorum longus (EDL) rat muscles when compared with the untrained denervated muscles [1]. However, training can also cause a damage of muscle fibers. Fundamental difference between these two muscles was observed in the degree of damage and regeneration. While in soleus the number of severely damaged muscle fibres was reduced, in EDL it was evidently increased. Similar difference concerned the amount of regenerating fibers [2]. To explain reasons of these differences, rats were subjected to locomotor training at different time after denervation and with variable intensity. The muscles were examined by morphological, immunohistochemical and biochemical methods. Surprisingly, it was found that both the less intensive training and delay in training were disadvantageous for the soleus muscle, while did not significantly influence EDL muscle. In conclusion, passive movement, in order to be beneficial for denervated muscle, should be carefully controlled concerning the time and intensity in each individual case.

[1] Jakubiec-Puka A, Sławińska U, Rędowicz MJ, Biral D, Łapińska I, Chomontowska H, Karczewska E, Krawczyk K, Bilski E Pliszka B. Influence of locomotor training on the structure and myosin heavy chains of the denervated rat soleus muscle. Neurol Res 2008; 30 (2): 170-178.

[2] Jakubiec-Puka A. Effect of passive movement on the 2-month-denervated fast and slow rat muscles. Basic Appl Myol 2007, 17, 208.

Jarvis Jonathan
Jarvis JC (1), Coulson JM (2), Moore LM (1), Fisher AG (1), Salmons S (1). (1) Department of Human Anatomy and Cell Biology and (2) Physiology, School of Biomedical Sciences, The University of Liverpool, UK. E-mail: "Jarvis, Jonathan" <jcj@liverpool.ac.uk>

Transcription in stimulated muscle

We have designed QPCR primer pairs to monitor the expression of key transcripts involved in the adaptive response of rat muscle to changes in activity. These include primers for the myosin heavy chains 1, 2A and 2B, IGF-1, MGF, FOXO1A and myostatin. In control fast and slow muscle, the average levels of transcript for the myosin isoforms correspond well with the known fibre type distribution. We measured transcript levels in rats in which the left tibialis anterior muscle had been stimulated for 3 hours under hypnorm/valium anaesthesia. The right muscles were used
as internal unstimulated controls. While QPCR gave excellent consistency between triplicate analysis of samples from each batch of cDNA, the variability between the measured transcript level for individual control samples is as high, in our first experimental cohort of 18 animals, as the difference between control and stimulated muscles. This is a surprising result: we will analyse further the natural variability in transcript level for these genes in control tissue before we can use the data for a systems biology approach to stimulated muscle.

Kern Helmut, Invited Lecture, Aula Magna, Palazzo Bo, April 14, 2008
Kern H (1 ,2), Löfler S (2), Vogelauer M (1), Mödlin M (1), Mayr W (3), Hofer C (2). (1) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Vienna, Austria; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) Center for Biomedical Engineering and Physics, Medical University Vienna, Austria.
E-mail: helmut.kern@wienkav.at

RISE project and beyond, the clinical value of FES

In the course of the European research project RISE we could demonstrate a significant structural and functional regeneration of the long-term denervated, degenerated muscle (DDM) by applying highly intensive transcutaneous electrical stimulation training (FES Training of DDM). Using an increased number of stimuli per day, thus increasing the amount of the muscle activity, we were able to improve muscle bulk, force, perfusion and excitability of thigh muscles in patients with permanent flaccid paralysis, i.e., with complete irreversible lesion of the lumbar-sacral spinal motor neurons. This FES training was not only capable of increasing the size of the denervated muscles as measured by CT-scans and morphometry of muscle biopsy, it could also restore the capacity of the muscle to generate tetanic contractions, a precondition for inducing recovery of muscle mass. Measuring the muscle cross sectional area with computed tomography (CT) the m. quadriceps area increased by of 35%. The electrically induced contraction force was improved depending on the denervation time. This is also confirmed by the perfusion, which was increased by 100% - 480%. FES Training by direct electrical stimulation over a long period of time first induced knee extension, later allowed the patients to perform stand up and walking exercises. In patients with long term leg denervation the above mentioned improvements provided an important contribution to prevention of secondary diseases, like decubitus ulcers. Since functional and structural characteristics of muscles in elderly (75-85 years) are similar to those we found in 1-2 year complete DDM (and we were able to revert to a much better conditions by means of FES training) our future clinical research is pointed to the transfer of the knowledge acquired training paralysed muscle to ageing delay and physiotherapy of elderly.

[1] Graupe D, Cerrel-Bazo H, Kern H, Carraro U. Walking performance, medical outcomes and patient training in FES of denervated muscles for ambulation by thoracic-level complete paraplegics. Neurol Res 2008; 30(2): 123-130.

[2] 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(2): 137-144.

[3] Boncompagni S, Kern H, Rossini K, Hofer C, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibers in absence of innervation in humans. Proc Natl Acad Sci U S A. 2007 Dec 4;104(49):19339-44. Epub 2007 Nov 27.

[4] Kern H, Hofer C, Modlin M, Mayr W, Vindigni V, Zampieri S, Boncompagni S, Protasi F, Carraro U. Stable muscle atrophy in long term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord (2007). ISSN: 1362-4393. Spinal Cord. 2007 Oct 23; [Epub ahead of print]

[5] Kern H, Rossini K, Boncompagni S, Mayr W, Fanò G, Zanin ME, Podhorska-Okolow M, Protasi F, Carraro U. Long-term denervation in humans causes degeneration of both contractile and excitation-contraction coupling apparatus that can be reversed by functional electrical stimulation (FES). A role for myofiber regeneration? J Neuropathol Exp Neurol, 2004; 63 (9), 919-931.

Kern Helmut, April 15, 2008
Kern H (1, 2), Adami N (3), Boncompagni S (4), Zampieri S (3, 5), Biral D (6), Jakubiec-Puka A (7), Mayr W (8), Protasi F (4), Carraro U (3, 6). (1) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, and (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) Laboratory of Translational Myology, Interdepartmental Research Institute of Myology, Department of Biomedical Science, University of Padova, Italy; (4) IIM - Interuniversitary Institute of Myology, CeSI - Centro Scienze dell'Invecchiamento, Università degli Studi G. d'Annunzio, Chieti, Italy; (5) Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Padova, Italy; (6) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, Padova, Italy; (7) Nencki Institute of Experimental Biology, Warsaw, Poland; (8) Center for Biomedical Engineering and Physics, Medical University Vienna, Austria.
E-mail: ugo.carraro@unipd.it

Long-term survival of muscle fibers after peripheral denervation

Human muscle fibers undergo severe atrophy that ends, from 10 to 20 years after Spinal Cord Injury (SCI), in lipofibrotic substitution of the muscle fibres as the result of long-term lower motor neuron denervation. However the majority of biopsies harvested from 4 to 8 years from SCI of the EU Rise Project, that is, from paraplegics suffering flaccid paraplegia (complete Conus Cauda Syndrome) display a small or a rather large percentage of muscle fibers with very large fibers diameter ("big fibers") among the thousands of severely atrophic muscle fibers. These severely atrophic muscle fibers have diameter in the range of 10-15 μm, absence of contractile proteins patterned in myofibrils, and central groupings of three to ten myonuclei every 100 μm in longitudinal patterns, which fill what looks as loose connective intramuscular space at low magnification. In transverse muscle sections these features are described as "morulae" in persons suffering of Spinal Lateral Atrophy (SLA). The goal of the several analyses we performed on "big fibers" by immunohistochemistry and electro microscopy (EM) was to establish if they are the trivial results of sparse residual innervation/reinnervation or one of the several not expected observation that our unique data-bank of long-term denervated human vastus lateralis m. biopsies provided to us and to the community of astonished neurologists and physiatrists, who took not for granted the pioneering observations of Ernest Gutmann in the Sixties (and of Ugo Carraro in the Eighties) of the last Century. The overall results are that: 1. NCAN immunostaining, the soundiest marker of early muscle denervation, disappears more than one-year after denervation, while a new late-denervation marker appears, that is, the differential labeling of the sarcolemmal dystrophin molecule by anti-C (absent) and anti-N (present) terminals antibodies. These features are correlated to the disappearance of the type I (slow, acid-resistant)) ATPase activity and the dramatic decrease of both type II (fast, alkali-resistant) ATPase activity and of SDH activity, a marker of mitochondrial function/content. Furthermore, the EM analyses provide the final evidence of ultra structural disarrangements of contractile and sarcoplasmic reticulum membrane systems that are characteristic of the "peripheral denervation" of the "big fibers", which never presented the orderly organization of myofibrils that are present in both normal adult muscle fibers and in those we studied after long-term (in tens of years) disused muscles, e.g., those that , in "spastic paraplegia", are in contact with spinal motor neurons, but isolated from their central motor neurons. Interestingly, after Functional Electrical Stimulation training, many of the denervated "big fibers" recover at least in part the features of normal muscle tissue.

[1] 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(2): 137-144.
[2] Boncompagni S, Kern H, Rossini K, Hofer C, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibers in absence of innervation in humans. Proc Natl Acad Sci U S A. 2007 Dec 4;104(49):19339-44. Epub 2007 Nov 27.
[3] Kern H, Hofer C, Modlin M, Mayr W, Vindigni V, Zampieri S, Boncompagni S, Protasi F, Carraro U. Stable muscle atrophy in long term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord (2007). ISSN: 1362-4393. Spinal Cord. 2007 Oct 23; [Epub ahead of print]

Martinek Johannes, Poster 2
Martinek J (1, 2), Stickler Y (1), Mayr W (2), Rattay F (1). (1) Institute for Analysis and Scientific Computing, Vienna University of Technology; (2) Center of Biomedical Engineering and Physics, Medical University of Vienna, Austria. E-mail: "DI Johannes Martinek" <johannes.martinek@technikum-wien.at>

Influence of the electrode position on the potential distribution and excitation of a denervated muscle fiber

Analyzing the data gained from the EU project RISE, where patients with long term denervation were stimulated with Functional Electrical Stimulation (FES), there were many new questions concerning the correlation between the generated electrical field and the development of action potentials. To enlighten characteristic excitation effects a Hodgkin-Huxley like model was implemented into the finite-element tool Comsol Multiphysics. The major improvement of the model compared to previous models is the coupling, the simultaneous calculation of the intracellular potential of the fiber and the potential of the surrounding tissue. This offers the possibility to simulate the interaction and the influence between (activated) fibers and the electrical field in the adjacent tissue. The aim of the study was to analyze the effects of different electrode configurations on the electrical field on the one hand and on the activating process of muscle fibers on the other hand. Starting with a two surface electrode configuration the activation process was analyzed and compared with “real-life” data. In a second step implanted electrodes were added to the model to analyze different electrode configurations (e.g. one active surface electrode and one active implanted electrode). First investigations using the coupled finite element model show comparable results for a simulated stimulation using 2 surface electrodes both with experimental animal and human data. Up to now the simulated implanted electrode configurations did not show substantial improvements concerning threshold currents in comparison with the currently used surface electrodes.

Mayr Winfried
Center of Biomedical Engineering and Physics, Medical University of Vienna, Austria.
E-mail: winfried.mayr@meduniwien.ac.at

Stimulation equipment for direct muscle stimulation: available and prospective solutions

Stimulation equipment for functional activation of neural structures is routinely available in a great variety and since many years. Implantable as well as non-invasive solutions are offered for many experimental and clinical applications, many prototype solutions are described in literature. All those have in common to deliver short duration stimuli in a pulse width range below 1 ms. Recent research work, in particular in conjunction with the European project RISE, has opened the field to direct functional reactivation of muscles in absence of the nerve. Equipment for this novel research work is based on much longer impulse durations (up to 200 ms) and important implications for the design of the entire technical system. The most critical part is the electrode tissue interface concerning implantable as well as surface electrodes. Consequent DC-decoupling of electrode outputs to ensure charge balance is mandatory, nevertheless there are further limitations to be respected, above all current density and distribution in the active electrode surface and material dependent charge injection limits. Disregard of these rules leads inevitably to electrochemical electrode corrosion and tissue damage. The necessary increased electrode size causes secondary design problems – big foreign bodies implanted in moving soft tissue or large conductive sheets to be fixed with evenly distributed contact pressure maintained on the skin surface in spite of contracting muscles underneath. A few suitable research solutions are available in the meantime, the first commercial solutions are about to appear, but a lot more of promising concepts wait to be put into practice.

Musarò Antonio
Department of Histology and Medical Embryology, "La Sapienza" University of Rome, Italy.
E-mail: antonio.musaro@uniroma1.it

Role of mIGF-1 and tissue niche on muscle regeneration and repair

One of the most exciting aspirations of current medical science is the regeneration of damaged body parts. The capacity of adult tissues to regenerate in response to injury stimuli represents an important homeostatic process. Regeneration of adult skeletal muscle is a highly coordinated program that partially recapitulates the embryonic developmental program. The major role in growth, remodeling and regeneration is played by satellite cells, a quiescent population of myogenic cells residing between the basal lamina and the plasmalemma. Muscle regeneration is affected by a wide range of environmental signals highly variable not only time-wise but also depending on the physiological or pathological conditions of the musculature. In this context, one of the crucial parameters of tissue regeneration is the microenvironment in which the stem cell populations should operate. Stem cell microenvironment, or niche, provides essential cues that regulates stem cell proliferation and that directs cell fate decisions and survival. Moreover, loss of control over these cell fate decisions might lead to cellular transformation and cancer. Studies on stem cell niche leaded to the identification of critical players and physiological conditions that improve tissue regeneration and repair. The critical role of tissue niche to muscle regeneration and the contribution of specific factors, such as IGF-1, in the modulation of inflammatory response and in the regulation of muscle regeneration and homeostasis will be discussed.

Naro Fabio
Serra F (1), Trotta A (1), Quarta M (1), Nemoz G (3), Reggiani C (2), Naro F (1). (1) Dipartimento di Istologia ed Embriologia Medica, Università degli Studi di Roma "La Sapienza", Italy:; (2) Dipartimento di Anatomia e Fisiologia Umana, Università di Padova, Italy; (3*)* INSERM Unité 870, INSA-Lyon, France.
E-mail: fabio.naro@uniroma1.it

Non steroidal anti-inflammatory therapy in duchenne muscular dystrophy

The only beneficial pharmacological treatment in Duchenne muscular dystrophy (DMD) is the use of glucocorticoids but their mechanism of action is still unknown. These drugs exert anti-inflammatory effects but very few data are available to assess whether their anti-inflammatory activity is the explanation of their efficacy. The aim of this research is to compare the effects of glucocorticoids and non steroidal anti-inflammatory drugs (NSAIDs) in *mdx* mice, to evaluate if the control of inflammation could have a benefical effect on the pathology. Mice were daily treated with: *methylprednisolone*, a*spirin* and *parecoxib* (COX-2 selective inhibitor). Inflammation, necrosis and centronucleated fibers were evaluated in tibialis anterior muscle samples obtained from treated and untreated *mdx* mice (aged 30 days and 11 weeks). Inflammatory cells were conspicuous in *mdx* and extensive areas of infiltration were present. The administration of methylprednisolone and both NSAIDs reduced the inflammation area. All the treatments were also effective to reduce the necrosis. The percentage of regenerating myofibers was not significantly different in untreated and treated *mdx*. Myosin expression in tibialis anterior and diaphragms from *mdx* mice was evaluated by Western blot analysis. Diaphragm strips were dissected to evaluate muscle mechanics and electrophysiology. We are currently evaluating if the suppression of inflammation could modify dystrophy progression by a series of experiments performed with younger *mdx* mice (15 and 20 days). These data suggest that chronic treatment with NSAIDs has a potentially beneficial effect on skeletal muscle morphology of *mdx* mice, comparable with the effects of glucocorticoid treatment.

Nascimbeni Anna Chiara, Poster 4
Nascimbeni AC (1, 2), Fanin M (1, 2), Tasca E (1, 2), Angelini C (1, 2). (1) Neuromuscular Centre, Department of Neurosciences, University of Padova, Italy, and (2) Venetian Institute of Molecular Medicine, Padova, Italy. E-mail: anna.nascimbeni@unipd.it

Molecular pathology and enzyme processing in various phenotypes of acid maltase deficiency

To examine at molecular, biochemical and muscle pathology level the striking clinical heterogeneity resulting from acid α-glucosidase deficiency. We investigated 23 patients with infantile-onset or late-

onset glycogen storage disease type II by enzyme activity, protein expression by immunoblotting, GAA gene mutations, and muscle pathology including immunolabeling for Golgi and sarcolemmal proteins. The enzyme activity was absent or minimal in infantile-onset cases and variably reduced in late-onset patients. Genotype-phenotype correlation (seven novel mutations were found) showed that most of late-onset patients had the heterozygous IVS1 leaky splicing mutation (one patient was homozygous), but the course of the disease was often difficult to predict on the basis of the mutations alone. All patients showed an abnormal pattern of enzyme protein processing, with increased amounts of the inactive forms and very low or absent amounts of the mature forms. The molecular weight of the mature and the intermediate forms appeared higher in patients' samples than in the control muscle. We observed a Golgi proliferation in muscle fibers possibly caused by the retention of inactive forms of enzyme protein that cannot be correctly targeted from Golgi to lysosomes. The vacuolar membranes expressed sarcolemmal proteins in late-onset but not in infantile-onset patients, suggesting an extensive autophagy and vacuolar membrane remodeling in late-onset patients. We attribute the different protein molecular weight between patients and controls to an excessive sialylation of mutant enzyme. This is likely caused by a delayed transport and longer transit of the inactive proteins in the Golgi apparatus.

Pietrangelo Tiziana
Pietrangelo T, Puglielli C, Mancinelli R, Doria C, Bosco G, Toniolo L, Reggiani C, di Tano G, Saggini R, Fanò G, Fulle S. IIM - Interuniversitary Institute of Myology, Department of Basic and Applied Medical Science, Laboratory of Physiology. CeSI - Centro Scienze dell'Invecchiamento, Università degli Studi G. d'Annunzio, Chieti, Italy. E-mail: tiziana@unich.it

Human satellite cells-dependent regeneration of aged skeletal muscle: physiology to molecular biology

Skeletal muscle satellite cells are a distinct myogenic lineage responsible for postnatal growth, repair and maintenance of skeletal muscle. In adult and aged skeletal muscles, satellite cells are normally mitotically quiescent but are activated in response to mechanical activity and/or trauma. The *in vivo* activation of the myogenic pool of cells consist of self-renewal and differentiation/fusion with existing fibers to tissue regeneration (Zammit and Beauchamp, 2001). During ageing, skeletal muscle is subjected to various modifications, defined as sarcopenia. This age-related condition includes progressive loss of mass and strength associated with a decline in fiber functional capacity (Fulle et al., 2004). In this condition, the impairement of satellite cell-dependent regeneration could be essential for defective functional recovery of muscle tissue (Fulle et al, 2005). Unfortunately, conflicting results are documented in the literature concerning the relationship between age and human skeletal muscle regeneration. We collected satellite cells from biopsies of different aged donors and investigated the effects of ageing on undifferentiated and differentiated skeletal muscular cells to characterize them from both the morphological and functional point of view and to analyze their transcriptional profile. Moreover, considering that regular physical activity slow down the sarcopenia progression, we trained elderly people (65-85 years old) with three different training protocols and collected satellite cells from the same subject before and after the specific training period. We analysed the effect of training on functional properties and on gene expression of undifferentiated and differentiated satellite cells at the different conditions. Our data show that during ageing the satellite cells modify their intra and extracellular matrix organization and activate the atrophy pathway resulting in a morphological and functional disorganization of excitation-contraction coupling. Moreover, different training protocols are able to specifically modulate gene expression for metabolic and sarcomeric proteins and increase the muscle strength.

Protasi Feliciano, Invited Lecture, Aula Magna, Palazzo Bo, April 14, 2008
Boncompagni S, Protasi F. IIM - Interuniversitary Institute of Myology, CeSI - Centro Scienze dell'Invecchiamento, Università degli Studi G. d'Annunzio, Chieti, Italy.
E-mail: fprotasi@unich.it

Denervation-induced disarrangement of muscle fibers and effectivness of fes in restoring their ultra-structure: The experience gained in four year of collaboration within the EU Rise project

Skeletal muscles fibers are characterized by a highly ordered internal organization of contractile elements, metabolic machinery, and excitation-contraction (EC) coupling apparatus. However, the maintenance of the internal ultrastructure is strictly dependent upon innervation and muscle activity: in fact, when muscle fibers

loose innervation as a result of traumatic events (spinal cord injury, SCI or peripheral nerve damages) skeletal muscles undergo loss of mass, or atrophy, and striking disarrangement of internal organization. Within the RISE project we have studied: a) the denervation-induced alterations in human biopsies and in animal models (rabbits and rats); and b) the effectiveness of FES protocols in restoring muscle structure in humans and rabbit muscles. Our results show that: A) Denervation induces similar alterations in muscle from humans and animals models. One of the early events following denervation - and preceding the loss of contractile elements - is the misalignment of EC coupling and metabolic machineries. B) Contrary to common belief, functional electrical stimulation (FES) is extremely effective in reversing muscle atrophy and restoring muscle fiber ultrastructure, both in complete absence of innervation (humans and rabbit), and also in upper motor neuron patients. These studies support the use of animal models to develop treatment strategies for SCI patients and indicate that long-term denervated muscle can be rescued if appropriately stimulated.

[1] Boncompagni S, Kern H, Rossini K, Hofer C, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibers in absence of innervation in humans. Proc Natl Acad Sci U S A. 2007 Dec 4;104(49):19339-44. Epub 2007 Nov 27.
[2] Kern H, Hofer C, Modlin M, Mayr W, Vindigni V, Zampieri S, Boncompagni S, Protasi F, Carraro U. Stable muscle atrophy in long term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord (2007). ISSN: 1362-4393. Spinal Cord. 2007 Oct 23; [Epub ahead of print].
[4] Kern H, Rossini K, Boncompagni S, Mayr W, Fanò G, Zanin ME, Podhorska-Okolow M, Protasi F, Carraro U. Long-term denervation in humans causes degeneration of both contractile and excitation-contraction coupling apparatus that can be reversed by functional electrical stimulation (FES). A role for myofiber regeneration? J Neuropathol Exp Neurol, 2004; 63 (9), 919-931.

Protasi Feliciano, April 15
Boncompagni S, Protasi F. IIM - Interuniversitary Institute of Myology, CeSI - Centro Scienze dell'Invecchiamento, Università degli Studi G. d'Annunzio, Chieti, Italy.
E-mail: fprotasi@unich.it

Spatial relationships between calcium release sites and mitochondria in developing and adult muscle

In muscle fibers, an extremely well organized system of tubules and vesicles, collectively named sarcotubular system, is able to finely control the cytoplasmic Ca2+ concentration. The sarcotubular system is formed by exterior membranes (sarcolemma and the transverse-tubules, T-tubules) and internal membranes (the sarcoplasmic reticulum, SR), which form specialized intracellular junctions called calcium release units (CRUs), or triads. CRUs in adult muscle fibers are precisely positioned next to mitochondria, at the edge of the A band. However, mitochondrial localization drastically changes during post-natal development. At 15 days mitochondria are mostly found in longitudinally oriented clusters under the sarcolemma and between myofibrils. This disposition progressively changes up to a point in which, at 2-4 months after birth, most mitochondria are precisely targeted next to triads, between the edge of the A band and the Z line. Using electron tomography (ET), we have reconstructed the tri-dimensional architecture of triad/mitochondria interface providing additional information on the structural relationship between these two myoplasmic organelles. In addition, using electron microscopy (EM), we have identified short strands (or tethers, ~10 nm long) connecting specifically the outer membrane of mitochondria to the SR, on the opposite side to the T-tubules. The molecular nature of this physical linkage is not yet identified. For now we can only speculate as to the possible structural importance that these small bridges may have in holding SR and mitochondria together. However, it is also appealing to dream about the possibility that these structures may represent a "tunneling" protein allowing a preferential route for Ca2+ to reach the inner mitochondrial space.

Rossi Carlo Alberto
Rossi CA (1), Ditadi A (1), Malerba A (1), Franzin C (2), Sanna M (2), Pozzobon M (2), Vettor R (2), De Coppi P (2). (1) Pediatric Oncohematology, Stem Cell Transplantation Unit, Department of Pediatrics, and (2) Endocrine-metabolic laboratory, Department of Medical and Surgical Sciences, University of Padua, Italy. E-mail: carloalberto.rossi@unipd.it

Are satellite cells a homogenous population? Adipogenic potential is linked to their proliferative capacity

Cell therapy represents a valid tool for tissue replacement, in particular in the contest of muscle dystrophies or structural defects. Satellite cells (SCs) have been frequently used as source of cells for skeletal muscle replacement, because they represent in vivo the pool of myogenic precursors. SCs are located between the basal lamina and the plasma membrane of skeletal myofibers. They offer the possibility of in vitro expansion and autologous transplantation. According to recent studies, they seem to be divided into two subpopulations, one of committed muscle precursors and one of cells with more stem-like properties. This distinction correlates to both diversity in markers expression and differentiation potential. In this study, for the first time, two subpopulations of SCs obtained from rat flexor digitorum brevis muscle through single fiber selection and disgregation, were distinguished based on both different proliferative and differentiative capacities. Quantitative analyses showed that a fixed proportion of SCs possess a great proliferative potential. These SCs spontaneously give rise to adipocytes in culture. Immunofluorescence and PCR analyses showed that while initially SCs are homogenously positive for early myogenic markers such as Pax7 and Myf5, pluripotent clones lose in culture myogenic markers and form lipid droplets in cytoplasm, becoming adipocytes. These observations could be relevant in muscle regeneration therapies. Selection of satellite cells by their proliferative ability could have implication in their in vivo regeneration potential.

Sacchetto Roberta
Sacchetto R (1), Testoni S (2), Damiani E (3), Gentile A (4), Mascarello F (1)

SERCA1 activity in chianina cattle congenital pseudo-myotonia

(1) Departments of Experimental Veterinary Sciences; (2) Veterinary Clinical Sciences; (3) Experimental Biomedical Sciences, Padova University; and (4) Veterinary Clinical Department, Bologna University, Italy.
Roberta.sacchetto@unipd.it
Recently a congenital pseudo-myotonia was described in Chianina cattle. Chianina is one of the most important Italian breed for meat quality and leather. Symptoms affect the musculature of the limbs and are exercise-induced stiffness and impaired skeletal muscle relaxation. Since the clinical aspect remembers myotonia but electromyography investigations resulted negative, symptoms could be due to an abnormal cytosolic Ca2+ concentration. In skeletal muscle Ca2+ is stored into the sarcoplasmic reticulum (SR). The RyR releases Ca2+ in the citosol and the SR Ca2+-ATPase (SERCA) pumps Ca2+ back into the SR, resulting in relaxation. RyR plays a crucial role in malignant hyperthermia (MH) which was described in human and porcine. Our clinical data excluded MH in Chianina breed, so we investigated SERCA. From fast twitch semimembranosus muscle of diseased animals, we prepared a crude microsomes membrane fraction (TM), selectively enriched in the main markers of junctional (RyR) and non junctional SR (SERCA). TM fractions were used for determination of SERCA ATPase activity, assayed by a spectrophotometer method. By comparison with control bovine muscle of the same species, ATPase activity in pathological muscles resulted reduced of about 50% in two specimens and almost absent in other subjects. Immunoblotting data with antibodies against SERCA1 isoform in the same fractions, confirmed the absence or reduction of expression of Ca2+-pump in pathological animals. Since the relation between the bovine skeletal muscle disorder found in our study and the human Brody's disease (a rare inherited disease associated to a mutation in the ATP2A1 gene encoding SERCA1 isoform) bovine muscle might be used as a suitable animal model.

Salmons Stanley, Invited Lecture, Aula Magna, Palazzo Bo, April 14, 2008
Department of Human Anatomy and Cell Biology, The Sherrington Buildings, Ashton Street, University of Liverpool, Liverpool L69 3GE, Merseyside, U.K. E-mail: "Stanley Salmons" <s.salmons@liverpool.ac.uk>

Understanding some of the influences on the response of muscles to long-term electrical stimulation

Functional adaptation [1, 2] is the key to clinical applications that involve long-term activation of skeletal muscle by electrical stimulation. In most cases, the stimulation program is used to bring about an increase in fatigue resistance. However, it can also induce changes that are not appropriate to the application, such as a decrease in force-generating capacity and slow contractile characteristics. In cardiomyoplasty, for example, a pedicled graft of latissimus dorsi muscle was wrapped around the heart [3]. The application called for fast, fatigue-resistant muscle [4, 5] but the stimulation protocol yielded slow, fatigue-resistant muscle. This reduced the power available and posed problems of synchronization: during systole the wrap was slower-contracting than the myocardium and could not reinforce the ventricular wall; during diastole it relaxed too

slowly, interfering with filling. Thus a poor choice of stimulation regime resulted in clinical outcomes that were not as good as they could have been. For this and other reasons the technique has fallen into disrepute [6]. How can mistakes like this be avoided? How do we generate a functional profile for the muscle that is appropriate to the application? The answer lies in understanding the influences of different patterns of stimulation and their variation in different animal models. The principles will be illustrated with clinical applications, current and potential.

[1] Salmons S: Functional adaptation in skeletal muscle. Trends Neurosc 1980; 3: 134-137.
[2] Salmons S, Henriksson J: The adaptive response of skeletal muscle to increased use. Muscle Nerve 1981; 4: 94-105.
[3] Grandjean PA, Lori Austin RN, Chan S, Terpestra B, Bourgeois IM: Dynamic cardiomyoplasty: clinical follow-up results. J Card Surg 1991; 6: 80–88.
[4] Jarvis JC, Mayne CN, Salmons S: Basic studies on skeletal muscle for cardiac assistance. J Card Surg 1991; 6 (Suppl.): 204-209.
[5] Salmons S: Optimizing the benefits of cardiomyoplasty. Br J Hosp Med 1992; 49: 137.
[6] Salmons S, Jarvis JC: Cardiomyoplasty reviewed: lessons from the past, prospects for the future. Basic Appl Myol 2008; in press.

Salmons Stanley, April 15, 2008
Salmons S, Jarvis JC. Department of Human Anatomy and Cell Biology, The Sherrington Buildings, Ashton Street, University of Liverpool, Liverpool L69 3GE, Merseyside, U.K. E-mail: s.salmons@liverpool.ac.uk

Therapeutic effects of stimulation on denervated muscle: taking the holistic view

Techniques for electrically stimulating skeletal muscle create opportunities for studying the molecular regulation of phenomena of clinical importance, such as the atrophy that results from bed rest, weightlessness, and other forms of disuse. There is intense interest in the cell signalling pathways involved, but it is important not to lose sight of events taking place at the level of tissues and organs. This will be illustrated by reference to the results of an extensive basic study of electrical stimulation in established denervation atrophy [1-5]. The choice of animal model is important: rat differs from other species in its response to denervation. Stimulation-induced increases in mass, cross-sectional area, and force probably all stem from upregulation of protein synthesis, yet differ in extent. The kinetics of isometric contraction seem refractory to stimulation, yet shortening velocity increases; the reasons may be sought in the ultrastructural organization of myofibrils and sarcotubular membrane systems. Increases in mitochondrial volume are, paradoxically, not associated with enhanced fatigue resistance, for reasons that remain unclear. Changes in muscle excitability in human patients are not reproduced in the animal studies; the explanation appears to lie in changes at the level of the whole limb. There are therefore many layers to such phenomena, and these need to be taken into account if we are to interpret the signalling events responsible.

[1] Ashley Z, Salmons S, Boncompagni S, Protasi F, Russold MF, Lanmuller H, Mayr W, Sutherland H, Jarvis JC: Effects of chronic electrical stimulation on long-term denervated muscles of the rabbit hind limb. J Muscle Res Cell Motil 2007; 28: 203-217.
[2] Ashley Z, Sutherland H, Lanmuller H, Russold MF, Unger E, Bijak M, Mayr W, Boncompagni S, Protasi F, Salmons S, Jarvis JC: Atrophy, but not necrosis, in rabbit skeletal muscle denervated for periods up to one year Am J Physiol Cell Physiol 2007; 292: C440-51.
[3] Ashley Z, Sutherland H, Russold MF, Lanmuller H, Mayr W, Jarvis JC, Salmons S: Therapeutic stimulation of denervated muscles: the influence of pattern. Muscle Nerve 2008; in press.
[4] Lanmüller H, Ashley Z, Unger E, Sutherland H, Reichel M, Russold M, Jarvis J, Mayr W, Salmons S: Implantable device for long-term electrical stimulation of denervated muscles in rabbits. Medical & Biological Engineering & Computing 2005; 43: 535-540.
[5] Salmons S, Ashley Z, Sutherland H, Russold MF, Li F, Jarvis JC: Functional electrical stimulation of denervated muscles: basic issues. Artif Organs 2005; 29: 199-202.

Sampaolesi Maurilio
Coppiello G, Crippa S, Dellavalle A, Salvadè A, Cassano M, Sampaolesi M. Stamcelinstituut, Katholieke Universiteit Leuven, Belgium & Sezione di Anatomia Umana, CIT Università di Pavia, Italy.
E-mail: maurilio.sampaolesi@unipv.it

Role of sarcoglycan proteins in proliferation and differentiation of satellite cells

Sarcoglycans are dystrophin-associated proteins (DAPs), forming a tight complex necessary for the physiological function of skeletal and cardiac muscles. Mutations in genes coding for sarcoglycans produce cardiomyopathy and/or muscular dystrophy in animal models and humans (LGMD). Alpha sarcoglycan null mice develop muscular dystrophy, much more severe than that observed in mdx mice which is caused by a point mutation in dystrophin gene. We recently demonstrated that intra-arterial delivery of wild type or dystrophic, genetically corrected (by lentiviral gene transduction) mesoangioblasts (vessel-associated stem cells) corrects morphologically and functionally the dystrophic phenotype of virtually all downstream muscles in adult, immunocompetent alpha sarcoglycan null mice. When we challenge the same experiments in mdx mice we couldn't get similar results. Considering a possible competitive role of resident dystrophic stem cells we investigated the regenerative capacities of satellite cells. Preliminary results showed that satellite cells from αSG null mice produced a similar number of clones than wt or mdx cells, but with a dramatically reduced number of cells for each clone. However their ability to differentiate into myotubes was similar to the wild type satellite cells, as well as the expression of the major transcriptional factors involved in myogenesis. In order to understand a possible interaction between αSG and growth factors involved in myogenesis, we isolated and cloned satellite cells from dystrophic and control muscle and tested them for responses to growth factors (FGFb, PDGF, SCF, HGF). Our results suggest a possible involvement of α-sarcoglycan with FGF signalling, in addition α-SG co-immunoprecipitate with FGFR1 in wilde type satellite cells.

Sandri Marco
Mammucari C (1, 3), Romanello V (1, 2), Coletto L (1), Masiero E (1), Milan G (1, 2), Sandri M (1, 2, 3). (1) Venetian Institute of Molecular Medicine, Padova, Italy, (2) Dulbecco Telethon Institute, (3) Department of Biomedical Sciences, University of Padua, Italy.
E-mail: marco.sandri@unipd.it

Signaling during muscle wasting

The size of skeletal muscle is determined by a balance between protein synthesis and protein degradation. In mammalian cells half life of proteins is controlled by two proteolytic systems the ubiquitin-proteasome and the autophagy-lysosome. Autophagy is an evolutionarily conserved mechanism that allows cell survival during starvation through the bulk degradation of proteins and organelles by lysosomal enzymes. However, the mechanisms responsible for the induction and regulation of the autophagy program are poorly understood. Here we show that FoxO transcription factors are required for the induction of autophagy in skeletal muscle. Suppression of FoxO3 activity prevents autophagosome formation induced by starvation. Akt/PKB activation blocks FoxO activation and autophagy, and this effect is not prevented by TORC1 inhibition. FoxO3 is able to induce autophagosome formation and up-regulation of the autophagy genes and Bnip3. Bnip3 appears to be a major mediator of FoxO3, as FoxO3-dependent autophagy is markedly reduced by knockdown of Bnip3. FoxO3 activates the proteasomal system by inducing the ubiquitin ligases atrogin-1 and MuRF1, however FoxO3-dependent autophagy is not affected by loss of atrogin-1 and MuRF1 or by blockade of proteasome. We further identified the substrates of autophagy which resulted to be mitochondria. Foxo3 in adult muscle fibers causes a reduction in mitochondrial content, and mitochondrial fragmentation induced by Bnip3 pathway is sufficient to induce time-dependent muscle loss. Conversely inhibition of the fission machinery suppressed mitochondrial fragmentation and Foxo3-mediated muscle atrophy. Thus, FoxO3 controls independently the two major proteolytic systems in skeletal muscle, the ubiquitin-proteasome and autophagy-lysosome.

Sferrazza Rossella
Sferrazza R (1), Vindigni V (1), Adami N (2), Carraro U (2), Bassetto F (1), Mazzoleni F (1). (1) Unit of Plastic Surgery and (2) Laboratory of Translational Myology of the Interdepartmental Research Centre of Myology, University of Padua, Italy E-mail: rosella.sferrazza@unipd.it

Myofiber regeneration and spontaneous reinnervation in post traumatic free flap reconstruction

In plastic surgery free flaps are used to reconstruct lost tissues, in particular post traumatic lesions of skin, muscle and other soft tissues. Different kinds of free flap may be used, according to extent of damage and

residual function in the traumatic area. Here we report two cases of post traumatic free flap reconstruction. A lower limb trauma with conspicuous loss of soft tissue had been covered with a vastus lateralis fasciacutaneous free flap. The receiving area was biopsied 3.5 years after surgery. Morphometry of the cryosection of the specimens shows some atrophic muscle and a prevailing adipose tissue area presenting patent vessels. Among very small myofibers MHCemb-positive fibers are demonstrated by the specific monoclonal antibody, thus demonstrating that myofiber regeneration is still active. Due to surgical denervation, in free muscle flap fibrosis takes usually place several years after intervention. Seldom free muscular transfer has functional aims: if a nervous anasthomosis is made (free neurovascular flaps). In a few cases, even in absence of nervous anasthomosis, spontaneous reinnervation of transplanted muscles is reported, as it is shown in our second case. A muscle free flap has been harvested from latissimus dorsi and transferred to a foot, without nerve anasthomosis. Eight years after surgery, in a harvested biopsy myofibers appear of normal muscle size, suggesting that local reinnervation occurred. Histology, histochemistry and immunohistochemistry confirm, myofiber type grouping (reinnervation), and regenerative events. Since functional recovery never happened, while the foot appears to be over sized, the histology suggests decreasing foot size by chemical denervation with botulinum toxin.

Stickler Yvonne, Poster 1
Stickler Y (1), Martinek J(1, 2), Mayr W (2), Rattay F (1). (1) Institute for Analysis and Scientific Computing, Vienna University of Technology and (2) Center of Biomedical Engineering and Physics, Medical University of Vienna, Austria.
E-mail: "Yvonne Stickler" <ystickler@osiris.tuwien.ac.at>

Modeling the denervated human thigh: effects of functional electrical stimulation (FES) and characteristics of the fiber excitation process

The complete denervation of muscles leads to changes in the muscle fibers as well as in the surrounding tissue. Concerning excitability the most important changes are reductions in fiber diameter, in muscle cross sectional area and in electrical conductivity of the muscle tissue. These changes can be partially reversed by intensive electrical stimulation. The effects of these alterations are examined with the coupling of a 3D finite element model of the human thigh and a muscle fiber compartment model. Muscle fiber compartment models usually assume fibers to be homogeneous in terms of channel distribution and diameter and to be perfectly sealed at the end. However, biopsies have often shown a tapering in muscle fiber diameter towards the tendon. Also, the channel distribution is known to be highly inhomogeneous. In denervated like in innervated fibers there is a sharp decrease in voltage-dependent sodium channels towards the end of the fiber. Additionally a current leakage at the fiber end might influence the excitation process. Intensive electrical stimulation training leads in all patients to lower threshold values. Model analysis demonstrates that the increase in fiber diameter is the main factor for this improvement. A sealed end boundary condition makes the fiber end region likely to produce action potentials. Altering this condition by assuming either a tapering in fiber diameter towards the end or a decrease in sodium channel density or current leakage at the fiber end also leads to excitation, but with significantly higher thresholds especially in case of lower sodium channel density. In mid fiber regions thresholds are comparatively higher and excitation is likely to be initiated near inhomogeneities in the extracellular field.

Vincenzo Vindigni
Zavan B (1), Tonello C (1), Michelotto L (1), Nalesso E (1), Abatangelo G (1), Cortivo R (1), Vindigni V (2). (1) Department of Histology, Microbiology and Medical Biotechnology, and (2) Clinic of Plastic and Reconstructive Surgery, University of Padova

Dermis as a source of multipotent stem cells

A significant amount of recent interest has focused on the possibility that adult human stem cells are a realistic therapeutic alternative to embryonic stem cells [1, 2]. This interest has arisen largely as a consequence of recent work demonstrating that several adult stem cells show a surprisingly diverse differentiation repertoire. Multi-potent cells that have characteristics reminiscent of embryonic Neural Crest (NC) stem cells have been isolated from several postnatal tissues, including skin, gut, dental pulp, and the heart and are potentially useful for research and therapeutic purposes. However, their neurogenic potential,

including their ability to produce electrophysiologically active neurons, is largely unexplored. The persistence of NC-related precursors within accessible postnatal tissues raises the possibility of their use for a variety of research and therapeutic purposes. We investigated this issue with regard to skin-derived precursors (SKPs), multipotent NC-related precursors isolated from the dermis of skin. SKP cultures follow an appropriate pattern and time-course of neuronal differentiation. These SKP-derived neuron-like cells survive and maintain their peripheral phenotype for at least 5 weeks. Human SKPs grew in suspension as spheres in the presence of the mitogens fibroblast growth factor 2 and epidermal growth factor and expressed nestin, fibronectin, vimentin, and characteristic embryonic transcription factors. Human SKPs could be maintained in culture for long periods of time and would still differentiate into Schwann cells.

Vitiello Libero

Pigozzo S (1), Repele A (1), Reggiani C (2), Ausoni S (3), Zaglia T (3), Baroni MD (1), Ambegia E (4), McLachlan I (4), Vitiello L (1). (1) Department of Biology, (2) Department of Anatomy and Physiology, (3) Department of Biomedical Sciences, University of Padova, Padova, Italy; (4) Protiva Biotherapeutics Inc., Burnaby, BC, Canada.
E-mail: libero.vitiello@unipd.it

In vivo delivery of naked and lipid-complexed antisense oligos in mdx mice: effects on skeletal and cardiac muscle

Antisense-mediated exon skipping holds great potential for the treatment of DMD. In mdx mice, functional recovery of skeletal muscle has been reported upon systemic delivery of "naked" oligonucleotides or viral vectors encoding for antisense snRNAs. However, only one study achieved dystrophin restoration in cardiac muscle (using an adeno-associated vector). Here we report the in vivo delivery of morpholino oligos in aged mdx mice, both in skeletal muscle, via intra-arterial injection, and in cardiac muscle, via intramuscular injection. Intra-arterial delivery yielded levels of dystrophin restoration comparable to those reported in the literature with the intra-venous approach, but with smaller amounts of oligonucleotides. Intra-cardiac injections, on the other hand, showed that the level and duration of the skipping effect found in cardiac muscle were greatly decreased compared to skeletal muscle. This latter finding provides the first direct evidence that antisense-mediated dystrophin restoration in cardiac muscle might suffers from limitations that do not exist in skeletal muscle. All data published so far have indicated that systemic delivery via the vasculature requires large amount of naked oligos to achieve therapeutically significant results. Here we also report that the use of lipid carriers has the potential to greatly improve the delivery efficiency; in particular, we found that the use of lipid-encapsulated oligo RNA allowed to detect dystrophin re-expression with a single dose of ~40 μg of oligos per adult mdx mouse. Importantly, dystrophin restoration could be seen not only in skeletal and but also (albeit to a smaller extent) in cardiac muscle.

Zampieri Sandra
Zampieri S (1, 2), Adami N (1), Carraro U (1, 3), Vindigni V (4), Bassetto F (4), Mazzoleni F (4), Doria A (2). (1) Laboratory of Translational Myology, Interdepartmental Research Institute of Myology of the University of Padua; (2) Division of Rheumatology, Department of Clinical and Experimental Medicine; (3) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, Padua; (4) Clinic of Plastic and Reconstructive Surgery, University of Padua, Italy.
E-mail: sanzamp@unipd.it

New concepts for the role of immune-inflammatory markers in the pathogenesis of autoimmune myositis.

The pathogenesis of autoimmune myositis is still unknown, but it seems to involve several features including genetic predisposition and environmental factors [1, 2]. Environmental factors comprise virus infections, which are known for their muscle tropism. As consequence of infection, in genetically predisposed individuals a prolonged inflammatory response may occur leading to chronic inflammation and localized tissue damage. The autoimmune origin of the pathologic process is suggested by the presence of circulating autoantibodies which are specific markers of disease, and our recent data indicate that autoantigens targeted by autoantibodies are overexpressed by regenerating muscle fibers, concomitantly with the flogistic reaction within the affected muscle. These data suggest that the overexpression of myositis specific autoantigens during regenerative myogenesis may induce and/or amplificate the immune response in patients affected

with autoimmune myositis. Our aim is to identify specific markers involved in the pathogenesis of the myositis immune-inflammatory response by immunoistochemical analyses of muscle biopsies from patients affected with autoimmune myositis, in order to shed more light on the biologic network which regulate muscle injury, regeneration and disease outcome.

[1] Atzeni F, Doria A, Ghirardello A, Villalta D, Zampieri S, Carrabba M, Sarzi-Puttini P. Organ-specific autoantibodies in patients with rheumatoid arthritis treated with adalimumab: a prospective long-term follow-up. Autoimmunity. 2008 Feb;41(1):87-91.

[2] Zampieri S, Ghirardello A, Rossini K, Iaccarino L, Bassi N, Atzeni F, Sarzi-Puttini P, Doria A. Antigen preparation for immunological studies in systemic autoimmune diseases. Ann N Y Acad Sci. 2007 Aug;1109:193-202. Review.