University of Padova & Interuniversitary Institute of Myology
Interdepartmental Research Center of Myology & C.N.R. Institute of Neuroscience, Padova, Italy

2007Spring Padua Muscle Days

2007 E. Gutmann Heritage, 30-year after

The Long Lasting Denervated Muscle

Vrbova G and Carraro U, *Organizers*
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Summaries

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MUSK-positive patients: A paticular bulbar atrophic phenotipe. Clinical features and therapeutic response
A small proportion of Myasthenia Gravis (MG) patients has antibodies against MuSK kinase.
In our study we evaluate clinical aspects and treatment of MuSK-positive patients. Five MuSK-positive were selected in our MG database..F/M ratio in MuSK-positive were 3:2. According to MGFA score, at the onset 40% of patients were in class II (a or b) and 60% in a higher class. At the last follow-up visit all of them improved. Three were thymectomised, 2 improved after thymectomy. Corticosteroids and anticholinesterases were sufficient only in one patient; the other needed additional immunosuppressive treatment. All patients were treated with IVIG and had clinical improvement. MuSK positive patients seem to be more severe than SNMG, requiring more aggressive treatment. Cyclosporine showed a good effect in our MuSK-positive group.

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Mitochondria, cyclosporin and collagen VI disease: an update
Ullrich congenital muscular dystrophy (UCMD) is a severe muscle disorder linked to collagen VI deficiency. We identified a latent mitochondrial dysfunction in myoblasts from patients with different genetic defects of collagen VI and variable clinical presentations of UCMD. This event matched an increased occurrence of spontaneous apoptosis that could be normalized by treatment with cyclosporin A or with the specific cyclophilin inhibitor MeAla3EtVal4-cyclosporin, which does not affect calcineurin activity. This study represents an essential step towards a pharmacological therapy of UCMD with cyclosporin A and MeAla3EtVal4-cyclosporin. Based on these results a pilot clinical study is currently under way.

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Reinnervation events in long-term denervated rat muscles
Following permanent sciatectomy, the distal-leg muscles undergo a severe atrophy during the first 4-6 months of denervation, which ends in tissue degeneration after additional 3-5 months. The ATPases demonstrate expression of a fast-like type of myosin, while slow myosin disappears. Histochemical SDH, a marker of mitochondria of oxidative myofibers, decreases and finally disappears in both slow and fast myofibers, togheter with all sarcomeric proteins. On the other hand, some regenerative myogenetic events randomly appear as demonstrated by anti-MHCemb monoclonals. N-CAM, an adhesion molecule restricted to the synapse in innervated myofibers, is expressed along all atrophic fibers

early after denervation. It also marks myotubes and early regenerated myofibers. It seems to be lost in the very atrophic myofibers at 4-6 months of denervation. In spite of the surgical approaches utilized to prevent reinnervation from the sciatic proximal stump, some reinnervation of the distal leg muscles occurs, resulting in minimal or substantial recovery of muscle mass and function. These reinnervation events are recognizable at the light microscopy level by size and morphology of the reinnervated myofibers and by the recovery of the fascicle muscle structure. A clear type grouping (clustering of muscle fibers with the same contractile and metabolic profile) is the golden standard of muscle reinnervation. The innervated fibers neither express embryonic myosin nor NCAM (outside the synapse). How long rat or human myofibers re-express N-CAM during permanent denervation remains to be analyzed in details.

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Myositis: an heterogeneous inflammatory pathology of skeletal muscle
In seven polymyositis, 2 dermatomyositis, one focal myositis due to *Sphingomonas paucimobilis* infection and one viral myositis (HIV) diagnosis was on the base of clinical, laboratory and histopathological features. All patients presented progressive muscle weakness, myalgia and high creatine kinase levels. Muscle biopsy showed different diagnostic patterns. In one case, DNAs extraction from skeletal muscle could identificate gene sequences of gram negative bacillus (*Sphingomonas paucimobilis)*. Muscle MRI in different myositis revealed different muscle inflammation distribution: a more severe intra-fascicular oedema with slight inter-fascicular and no subcutaneous involvement in polymyositis; a strong involvement of inter-fascicular and subcutaneous spaces in dermatomyositis. HIV-related myositis showed a slight diffuse intra- and inter-fascicular oedema. Sphingomonas related myositis was focal. Radiological differences could reflect different pathogenetic mechanisms involving different inflammatory myopathies.

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Muscle tissue engineering: the in vivo axial-vessel approach
Tissue engineers have sought to develop more robust methods of generating engineered contractile muscle tissues. Such constructs would be valuable to patients and reconstructive surgeons seeking to restore lost form or function. Our group has developed novel methods of producing engineered muscle in vitro and in vivo. In this presentation I discuss multiple methods used to generate the constructs. Our group has been developing interfaces between the engineered constructs and the circulatory and nervous systems. I discuss their evaluation by histology and force production. Progressive enhancements may allow the generation of clinically useful contractile muscle constructs.

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Botulinum Neurotoxin A promotes slow myosin heavy chain expression in mouse skeletal muscle
On the basis of the specific myosin heavy chain isoform that they express, mammalian skeletal muscle fibers are classified as Slow (type I) or Fast (type 2) fibers. Slow fibers show low velocity of shortening and high fatigue resistance, whereas fast fibers show high velocity but lesser resistance. Muscle fibers composition is strongly influenced by nerve activity in adult skeletal muscle; in fact, a reduced neuromuscular activity (for example, spinal cord injury) promotes a slow-to-fast fiber transformation in muscle, whereas an increase of muscle activity cause a shift in the opposite direction (fast-to-slow).
We observed that a blockade of nerve activity, induced by Botulinum Neurotoxin A (BoNT A), promotes the expression of the slow isoform of myosin heavy chain (MyHC) in contrast with other neuromuscular inactivity models.

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"The Denervated Muscle" - 45 Years Later
Forty five years after its publication, Ernest Gutmann's book, "The Denervated Muscle," still stands as a landmark publication. It summarized the state of knowledge of the time and introduced much new research that was ongoing at the Institute of Physiology in Prague. At the time, the response of a muscle to denervation was viewed primarily

through the lens of the neurotrophic trophic theory. Advances in our understanding of neurotrophic effects and mechanisms would now call into question some of the hypotheses and interpretations presented in the book, but many of the research findings have stood the test of time. This talk will review some of the questions asked and data presented in this book and will place them into the context of contemporary muscle biology.

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Satellite cells seeded onto 3D scaffolds: a powerful tool for cell therapy protocols as well as basic myo-sciences
Here we present a novel strategy to restore dystrophin expression in mdx mice. Single-fiber derived satellite cells were delivered into the muscles of aged mdx recipient mice, either through the implant of cellularized 3D collagen scaffold or via direct intramuscular injection. Muscles implanted with 3D collagen scaffold showed many more clusters of dystrophin-positive fibers compared to injected muscles. Cellularized scaffolds provide also a way to study myogenic cells in different environments and conditions. We are presently using hydrogel scaffolds, in which cells grown in ordered structures are electrically stimulated in order to study their transcriptional changes via microarray analysis.

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Human myofiber permanent denervation: Long lasting atrophy, and recovery by Functional Electrical Stimulation
Human lower-motoneuron-denervated muscle undergoes a long-lasting severe atrophy stage: adipocytes and fibrotic tissue (denervated degenerated muscle, DDM) prevail only from two-three years after spinal cord injury (SCI). Monoclonals against embryonic myosin show that regenerative events are present from 1- to 37-year post-SCI. In 2-year FES-trained muscles regenerative events are present, but at a lower rate than in DDM. After FES the muscles present large round myofibers. The average diameter went from 15.4 to 27.0, a 76% increase after two years of FES. We conclude that the Vienna FES-training of paraplegic muscles is safe and effective.

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The contemporary role of electron microscopy muscle pathology
Electron microscopy has a strategic position improving the diagnostic accuracy of numerous muscular diseases, giving insight into pathophysiological mechanisms, and guiding molecular analysis. Myopathies can be divided into three groups. In the first, of muscle interstitial tissue diseases, histological and immuno-histochemical techniques are more effective than electron microscopy. The second group refers to diseases associated with defects of sarcolemma components, extracellular matrix and nuclear membrane: with these diseases electron microscopy can be considered a valuable tool confirming diagnosis suggested by immunohistochemical and molecular analysis. Finally, ultrastructural study provides the best results for sarcoplasmic abnormalities including vacuoles, inclusion bodies, myofibrillar disorganization with or without abnormal inclusion material.

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Free-exercise amplifies skeletal muscle damage in cachectic mice
We have reported that cachexia is associated to poor skeletal muscle regeneration following experimentally-induced damage. We are investigating whether cachexia, in combination or not with free-exercise, makes skeletal muscle more susceptible to damage and hampers muscle repair. Evans Blue Dye uptake highlights that either tumor load or free-exercise induce muscle damage, while their combination further increases the number of damaged fibers to about fifty times over the value in control, resting mice. Free-exercise induces oxidative stress in muscle, which may contribute to enhanced muscle susceptibility to damage in cachexia.

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Preliminary approach to study satellite cell in myopathic aging muscle.
Satellite cells, SC, are skeletal muscle progenitor cells located between the basal lamina and the sarcolemma of muscle fibers. They are responsible for muscle growth and repair. In humans, aging results in the depletion of SC population, but not in their function. In fact it is well known that even a reduced SC population in aged animals was sufficient to repair damaged muscle to near control levels. To understand the molecular mechanism involved in human myopathic aging muscle, we isolated satellite cells from myopathic and normal aging muscle biopsies to search after appropriate stimuli for possible differences in growth, morphology, proliferating and differentiative potential.

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Histochemical studies of developing and diseased muscle
My first exposure to Duchenne muscular dystrophy during a residency post in London in 1957 led to a life-time commitment to neuromuscular disorders. In addition to the clinical aspects, I was also keen on basic research, which channelled me into histochemistry, under the guidance of Everson Pearse at the Postgraduate Medical School, a pioneer in the field. Initial studies of dystrophic muscle were followed by normal muscle in human and various animals, the definition of fibre types in muscle on histochemical grounds, and subsequent application to developing human and animal muscle and the effects of denervation, reinnervation and crossed-innervation in muscle.

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Activity and non-activity dependent mechanisms for maintaining functional muscle properties
I will discuss data demonstrating the relationship between the amount and kind of electromechanical stimulation on muscle mass, muscle phenotype and muscle function in rats. These data will be compared with estimates of in vivo activity and mechanical events that occur routinely. These data will also be related to similar types of observations made in human subjects with respect to the importance of specific levels and patterns activity in maintaining muscle mass, phenotype and function. The importance of regulating the population of myonuclei in controlling muscle mass, also, will be discussed.

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Multisystemic lamp-2 defect in Danon disease
Danon disease is an X-linked dominant disorder due to mutations in the LAMP2 (Lysosomal Associated Membrane Protein-2) gene, presenting with hypertrophic cardiomyopathy, skeletal myopathy and mental retardation. We screened LAMP2 gene mutations and LAMP-2 protein deficiency in the skeletal muscle of 9 unrelated patients with hypertrophic cardiomyopathy and vacuolar myopathy, and identified 3 novel families with unreported LAMP2 gene null mutations. LAMP-2 protein deficiency was detectable in various tissues (skeletal and myocardial muscle, leukocytes, fibroblasts) indicating that the biochemical diagnosis can be obtained on leukocytes and might be used for screening in male patients, and that the multiorgan protein deficiency would explain the multisystem clinical involvement. In the female patient, muscle histopathology and LAMP-2 protein analysis was inconclusive, indicating that the diagnosis in females can be obtained only by mutation identification. Muscle immunopathology showed that the mutant protein was not localized in the Golgi complex, the vacuolar membranes express sarcolemmal-specific proteins, and the degree of muscle fibre vacuolisation correlates with clinical muscle involvement.

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The lateral transmission of force in skeletal muscles of dystrophic and wild type mice
Early interpretations of forces generated by contractions of muscles were based on the premise that forces were transmitted longitudinally between proximal and distal tendons. Street (1983), demonstrated in frog muscles that lateral transmission of forces was equally effective. Dystrophin and dystrophin-associated glycoprotein (DAG) complex are positioned to transmit forces laterally in mice. Dystrophin is not expressed in mdx mice and the DAG complex is down-regulated. The hypothesis that compared with longitudinal transmission of equivalent forces, muscles

of control mice transmit forces laterally to the epimysium without decrement, whereas muscles of mdx mice experience a large deficit in forces was supported.

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In vitro engineering of muscle precursor cell niche by mimicking multiple stimuli
Multiple signals, including topological and electrical, control in vivo behavior of muscle precursor cells (MPCs). Engineering of techniques mimicking these in vivo cues is fundamental for any efficient application of stem cells. Micro-contact printing and soft-lithography replica-molding techniques have been applied to recreate 2D structural and topological templates able to direct myofiber formation on soft surface. Aligned single myofibers and highly ordered contractile ensembles were respectively obtained. Physiological-like electrical pulses affected MPC displacement and enhanced differentiation in functional myofibers. 3D-scaffolds can further improve mimicking of stem cell niche and, if cultured in perfusion bioreactor, allow formation of uniform high-density cellularized 3D-construct.

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Sodium and L-type Ca2+ current in skeletal muscle from normal and long-term denervated rat
The most important features of the denervated muscle is a progressive reduced excitability, excitation-contraction coupling and force generation. The block of motoneurons activity and the consequently muscle inactivity determine a reduced molecular and mechanical signals to the nucleus that in turn impair the expression of new molecules and determined a progressive atrophic state of the muscle fibres. The anomalous cytoskeleton organization determines leaky plasma-membrane, reduced the excitability and the excitation-contraction coupling. We have studied these properties by electrophysiological methods in long-term, 4 months, denervated soleus fibres of the rat. Results showed that denervated fibres had a reduced sarcolemnic resistence and large leak current that determined membrane depolarization. Such depolarization increased the inactivation state of the Na+ channels and consequently the ability of the fibres to elicited action potentials and in turn the excitation-contraction coupling. Moreover, denervation induced a slow kinetics of Na+ channel activation as well as of the action potential size that may be, together with the reduced diameter of the fibres, the cause of the slow conduction velocity observed in denervated muscle fibres.

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An evolutionary look at the architecture of protein interactions in the control of calcium movements in muscle cells
Muscle cells are regulated by large movements of calcium ions and these in turn are controlled by complex systems of membranes. All muscles, from the most primitive to the most evolved organisms share a common set of organelles and proteins that are involved in calcium homeostasis. A calcium pump protein of the sarcoplasmic reticulum and an homologous pump in the plasmalemma are the major contributor to the maintenance of the very low resting concentrations of cytoplasmic calcium. A major SR calcium release channel, has been detected from worms up. In worms as well as in all other animals RyRs are located at specific sites (calcium release units) mostly associated with either the plasmalemma or its invaginations the T tubules.

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Ventilatory support with NPPV-Helmet in 2 patients with acute respiratory failure secondary to Guillain-Barrè syndrome
Non-invasive positive pressure ventilation (NPPV) is a method of delivering ventilatory support via a facial or nasal mask or through a helmet (NPPV-helmet). Compared with invasive ventilation, NPPV is associated with lower mortality in other causes of respiratory failure. Few and discordant data are available on feasibility and safety in patients with acute respiratory failure (ARF) secondary to Guillan-Barré syndrome (GBS). A 72-yr-old male with a 4-

day history (patient 1) and a 66-yr-old male with a 2-day history (patient 2) of gradually increasing weakness, both treated with I.V. immunoglobulin therapy, were admitted to intensive care unit (ICU) for ARF secondary to GBS. Ventilatory support was delivered through an NPPV-helmet. Patient 1 required tracheal intubation after 24 hours of NPPV-helmet due to clinical deterioration. Patient 2 was treated with NPPV-helmet for 12 days and discharged to a specialist respiratory ward. Good recovery from ARF secondary to GBS can be achieved with NPPV-helmet, with the following recommendations: early admission to ICU and regular neurological assessment (consciousness and bulbar muscle function maintenance).

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Segmentation of muscle bellies: a novel technique to monitor growth of denervated degenerated muscle treated with functional electrical stimulation
Advanced 3D image processing and segmentation patterns are developed to segment single muscle bellies for patients with long-term flaccid paraplegia. The goals are: 1. Monitor selectively muscle growth influenced by Functional Electrical Stimulation. 2. Measure and map muscle volume and density during the RISE therapy . To allow muscle segmentation CT/MRI referenced data are processed using a special computer program. Automatic and semiautomatic editing tools are used to isolate from the scanner data single muscle bellies in the region of interest. The muscles bellies are then measured and compared through the therapy time. Monitor of volume changes, and muscle density are made on the region of interest through the therapy time. The technique shows, in quantitative and qualitative way, stimulation effects on DDM providing information otherwise hidden.

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Molecular Mechanisms for the Protein Loss in Atrophying Muscle
The atrophy of specific muscles upon denervation or disuse and the systemic muscle wasting seen in fasting and many systemic diseases (e.g. cancer, sepsis) result primarily from accelerated protein degradation through activation of the ubiquitin-proteasome pathway and involve similar changes in the expression of a group of genes we've termed "atrogenes". Activation of FoxO family of transcription factors leads to the induction of many atrogenes including the ubiquitin ligases, atrogin-1 and MuRF1 and also stimulates protein breakdown in lysosomes by enhanced autophagy. Contractile activity not only stimulates the production of IGF-1 and thus the PI3K/Akt pathway, but also enhances production of the transcriptional coactivator, PGC-1, which inhibits these FoxO3-dependent responses and atrophy.

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Selective muscle denervation and adaptive changes in ALS
In the SODG93A transgenic ALS mouse model, we have resolved an early, selective and progressive loss of the largest and fastest motor units with corresponding denervation of the type IIB muscle fibers. Concurrent with progressive denervation, the remaining innervated muscle fibers demonstrate adaptive activity-dependent fiber-type conversions toward type IIA fibers. This accounted for slowing of the progressive motor unit loss in the late stages of disease and predicted that early rapid motor unit loss could be slowed by increasing neuromuscular activity. Using partial denervation as our experimental model, we demonstrated that muscle denervation was slowed and motor units saved by increasing their neuromuscular activity.

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Review of Performance and Patient Training in Walking with FES by Thoracic-Level Paraplegics
We review performance and patient training related to the Parastep functional electrical stimulation (FES) transcutaneous system, which allows patients with traumatic complete/near-complete thoracic-level spinal-cord-lesion (SCI) to stand and walk independently. It is the first (and only) such system to have received US FDA approval (1994)

and approval for reimbursement by US Medicare /Medicaid and most US insurers. Performance by users depends on age, physical condition, injury level, commitment and patient's training. At end of training, patients may average 450 m/walk in one training method while 100m/walk in another. We shall discuss criteria for patient acceptance into training and review training methodology and procedures. We shall summaries findings on Medical outcomes in Parastep users, as published over the years. Of great significance is the improvement (over 50%)in blood flow to the lower extremities. Effects on decubitus ulcers and spasticity, bone density and psychological test outcomes will be discussed.

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Monitoring bone mineral density of femur during electrical stimulation therapy of denervated degenerated thigh muscles.
To monitor electrical stimulation therapy of denervated degenerated thigh muscles (DDM) spiral computer tomography (CT) is taken every 4 months. Thereby the thigh is scanned from the hip joint to the knee joint with 1,25 mm thick slices. This enables monitoring of tissue changes in the whole area, that is volume, shape and density changes of muscle, bone and other tissue. In this work femur bone tissue is segmented according to four Houndsfield unit (HU) intervals. Each interval is compared to its predecessor. Results suggest that the electrical stimulation therapy is contributing to remodelling of the femur bone.

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Do changes in the vascular bed contribute to the development of denervation atrophy in skeletal muscle?
In one of his early papers, Ernest Gutmann described that muscle ischemia impaired denervation atrophy. However, neither blood flow nor transcapillary transport decreased in muscles for several weeks after denervation, possibly due to the vasodilator action of various metabolites released by degenerating muscle fibres. Velocity of flow and thus possibly shear stress enables the maintenance of the capillary bed in these earlier stages, but with long lasting atrophy (several months) capillaries degenerate and the increased amount of extracellular collagen impairs the diffusion of substrates towards the muscle fibres. Electrical stimulation and /or long-term increase in blood flow stimulate capillary growth in normal muscles but until now it is not known whether these procedures could have the same effect in denervated muscles.

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Beneficial effect of locomotor training on the structure of the denervated rat soleus muscle
In order to study mechanisms of muscle denervation atrophy, a locomotor training on a treadmill was applied to denervated rat leg muscles. Two months after denervation the soleus muscle was investigated by morphological methods. It has been found that in the soleus of trained rats the pathological changes caused by denervation were less pronounced than in the soleus of untrained animals. For exemple, mithochondria were less damaged, accumulation of collagen fibrils was reduced, ''degenerated'' myofibres were hardly seen and the contractile structure was better preserved and more regular. Thus, locomotor training seems to attenuate some pathological processes within denervated muscle.

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Restoration of the longterm denervated human muscle by FES
Denervation atrophy in human skeletal muscle can be reversed by adapted FES protocols. 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 the patients thigh muscles. 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 by 828% in patients which were paralyzed up to 2 years. This is also confirmed by the perfusion, which was increased

by 100% - 480%. Regarding the excitability, the mean MFCV was increased from 1.6 up to 2.4 m/s and the refractory period decreased from 5.3 to 3.1 ms. The above mentioned improvements are also an important contribution for preventing secondary diseases like decubital ulcers in patients with longterm denervation of the lower extremity.

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Ernest Gutmann in his own words

I shall use a selection of excerpts from my fathers diary to demonstrate some aspects of his life, especially in the early 70s, the years of the so called normalisation process. What did it mean for him? How did he deal with it? What kept him going? In addition, I shall read a short passage from Prof. Zdenek Lodins memoirs that highlights his innocent and disarming appeal. Prof Lodin was his friend and colleague and the book is called A contribution to a study of a Czech doctor-researchers life.

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Signalling to actin cytoskeleton in mast cells
Activation of mast cells and the resultant secretion of inflammatory mediators is associated with a dramatic reorganisation of the actin cytoskeleton. Calcium- and GTP- binding proteins are crucial regulators of both actin and exocytosis. Their function was studied using intact and permeabilised mast cells. Cortical actin disassembles in the early steps of exocytosis. In the cell interior, newly formed tubular actin structures become closely associated with the secretory granules. Calmodulin-dependent, myosin II-based contraction of the actin cortex is a prerequisite for its disassembly, while de novo actin polymerisation requires the activity of the small GTPase Rho.

Malerba Alberto
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Macrophage-secreted factors allow to selectively stimulate a sub-population of putative myogenic stem cells and allow in vivo muscle reconstruction after subtotal ablation
We showed that macrophagic factors allow to selectively expand a sub-population of desmin- and MyoD-positive primary cells derived from adult rat muscle. They have properties already described in murine muscle stem cells and could represent a new type of stem-like stimulated by macrophages, which could play an important role in muscle repair. Consistently, in rat the supply of macrophagic factors in vivo increased by ten folds the amount of newly formed muscle after extensive muscle ablation. Altogether, our in vitro and in vivo data suggest that macrophages can activate and preserve a subpopulation of myogenic stem cells during muscle regeneration.

Marini Marina
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Gene expression variations in long term denervated rat muscle
The possible occurrence of oxidative stress in short-, medium-, or long-term denervated rat Tibialis Anterior was examined by a variety of methods (RT-PCR of selected genes, colorimetric evaluation of SOD enzymatic activity, WB for SOD protein amount evaluation, fluorescence development with DCF probe). Results suggest that denervation induces oxidative stress, that increases with time. The primary cause of such oxidative stress is still an unresolved question, since no sign of inflammation is evident, at least in medium- and long-term denervated muscles, and mitochondrial morphology is apparently preserved. Nevertheless, this finding suggests that antioxidant supplementation may be helpful for denervated patients.

Mayr Winfried
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Technical equipment for FES of denervated muscles: demands and limitations
The European FP5 project RISE has demonstrated recently in a multidisciplinary effort that Functional Electrical Stimulation (FES) of denervated muscles is an efficient method for restoring and maintaining long-term denervated muscles. As a major technical difference to traditional neural stimulation (0,5 to 1 ms) direct muscle stimulation requires stimuli with 30 to 200 ms. Consequently both non-invasive and implantable stimulation equipments have to meet special critical safety demands. Further novel biomechanical electrophysiological measurement techniques are required to gain valid data even from severely atrophied and degenerated muscles. A set of developed technical solutions for therapy of the lower extremity in flaccid paraplegia is presented and concepts for technological support of further related research are discussed.

Messina Sonia
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Effect of vascular endothelial growth factor gene transfer on muscle regeneration in mdx mice
Vascular endothelial growth factor (VEGF) is a major regulator of angiogenesis. Several studies support its role in myogenesis. We tested the effect of VEGF delivered by adeno-associated-virus (AAV) vectors on functional and morphological parameters in *mdx* mice. Treated *mdx* mice showed higher strength and strength normalised to weight. VEGF-treated muscles showed a reduction of necrotic area and an increase of small centrally nucleated fibers area and of cells positive for regeneration markers. We report the novel observation of a beneficial effect of VEGF in *mdx* mice. Further studies are needed to better clarify the VEGF mechanisms and possible therapeutic implications.

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Nuclear Factor of Activated T cells (NFAT) family members control activity-dependent specification of skeletal muscle fiber types
The Ca 2+ -dependent phosphatase calcineurin has an essential role in the control of the slow muscle gene program by dephosphorylating Nuclear Factor of Activated T cells (NFAT), a family of five transcription factors. However, the role of the calcineurin/NFAT pathway in the induction and maintenance of the fast fiber types, i.e. 2A, 2X and 2B and the role of the different NFAT isoforms in activity-dependent fiber type specification is not known in detail. We have performed NFAT isoform-specific silencing by RNA interference and analysed the distribution of different NFATs in skeletal muscle in vivo. Our results indicate that graded recruitment of NFAT family members controls activity-dependent muscle fiber specification.

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Counteracting muscle wasting in a mouse model of ALS: the role of mIGF-1
A crucial system severely affected in several neuromuscular diseases is the loss of effective connection between muscle and nerve, leading to a pathological non-communication between the two tissues. One of the best examples of impaired interplay between the two tissues is the disease Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disease characterized by a selective degeneration of motor neurons, atrophy, and paralysis of skeletal muscle. However, skeletal muscle is an untested component of the pathogenesis of ALS. In this presentation I will discuss the contribution of skeletal muscle to motor neuron survival and the specific role of muscle-produced mIGF-1 to the maintenance of muscle integrity and of muscle-nerve interaction.

Pende Mario
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The mTOR pathway in muscle pathophysiology: from cell size to muscle integrity
The mammalian Target of Rapamycin (mTOR) protein kinase integrates signals from growth factors, nutrients and cellular energy status to regulate cell proliferation, survival, growth and metabolism. mTOR depletion impairs muscle fiber growth and integrity. The deletion of the mTOR substrate S6K1 is sufficient to suppress growth adaptations to nutrient availability. S6K1 deficient skeletal muscle cells have increased AMP and inorganic phosphate levels relative

to ATP and phosphocreatine leading to AMP-activated protein kinase up-regulation. Strikingly AMPK inhibition in S6K deficient cells restores cell growth and sensitivity to nutrient signals. Thus defects in mTOR signalling may underlie a broad range of myopathies.

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Ernest Gutmann - an example and friend
My memories of Ernest Gutmann and our friendship go back to 1964 when I visited him for the first time in Prague. His monography "The Denervated Muscle", published two years before, had made him known as a pioneer in muscle biology and had identified his laboratory as a European center for interdisciplinary research on neuromuscular interactions. Since then I stayed in close scientific contact with Ernest and we became close friends. As I will outline in my talk, our exchange of ideas has not ended with his death but found its continuation in collaborations with several of his students, above all with the work performed for many years together with Gerta Vrbová.

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Muscle MRI: state of the art in myopathies
The role of muscle MR in neuromuscular disorders is becoming increasingly relevant, as this techinique can not only reliably differentiate between neurogenic forms and primitive myopathies, but also identify specific patterns of selective muscle involvement in genetically distinct forms of muscle disorders, study the quantitative distribution of the total body adipose tissue and potentially monitor the efficacy of therapies. I will summarize the state of knowledge and discuss the results obtained by our experience in a specific metabolic disorder, that is glycogenosis II.

Protasi Feliciano
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Structural recovery of skeletal muscle fibers in absence of innervation in humans
It is generally accepted that severe muscle atrophy, as a result of a complete lesion of the conus cauda, is irreversible and ends in degeneration of the muscle tissue (denervated degenerated muscle, DDM). Contrary to this belief, here we report on the ultrastructural recovery of human muscle fibers in long-term DDM patients, which occurs in complete absence of nerve endings and simply under the influence of electrical stimulation (FES). Thus, despite the almost complete loss of muscle-specific structure, the long-term denervated fibers maintain the capability of a full differentiation program and can be rescued if appropriately stimulated. These studies may be of great importance for the rehabilitation and the general health of SCI patients.

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Skeletal muscle adaptations to hypobaric hypoxia: sensors, signals and changes in gene expression
Hypoxia has a deep influence of muscle fibre phenotype. Chronic hypobaric hypoxia determines reduction of fibre size and shift in myosin expression from slow to fast isoforms. In rats exposed to PIO2 □ 100 mmHg for 5 weeks, we found that endurance training can partially counteract the effect of hypoxia. The interaction between activity and hypoxia on the transcriptional activity of HIF-1 was studied in rat exposed to PIO2 □ 70 mmHg for up to 24 h. HIF-1 activity was increased by both contractile activity and hypoxia, suggesting that another pathway is responsible for regulation of myosin isoform in hypoxia.

Rigatelli Gian Luca
Rigatelli Gian Luca, Rossini Katia, Adami Nicoletta, Vindigni Vincenzo (1), Rossella Sferrazza (1), Mazzoleni Francesco (1), Kern Helmut (2), Carraro Ugo

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A new intravascular approach for minimally-invasive intramuscular massive muscle stimulation

Functional electrical stimulation of skeletal muscle is performed by cutaneous surface electrodes, transcutaneous needle electrodes or epineural implanted electrodes. From several months after injury, denervated muscle can only be electrostimulated by large transcutaneous surface electrodes or subcutaneous perimisial electrodes. We are exploring the hypothesis that customized endovascular electrodes via veins may be inserted in the denervated muscle up to the capillary tree. In spite of the success of the endocardial electrostimulation for cardiac pacing, risks of this approach are related to the: i) lower excitability of the denervated muscle in comparison to innervated tissue or myocardium; ii) thrombogenesis and embolism, iii) traumatic muscle damage related to muscle contraction. Some of these risks ask for new materials and technologies (possibly by nanotechnology approaches) and therefore of very new ideas and methods. On the other hand, we will show that, standing on sound biological basis, preliminary studies in rats and rabbits demonstrate the feasibility of the new concept. Plasticity of muscle tissue and of its vascular bed grants further testing of the concept.

Rodolico Carmelo

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Congenital myasthenic syndromes: clinical variability and molecular features in italian families

Congenital myasthenic syndromes (CMS) are inherited disorders in which the safety margin of neuromuscular transmission is compromised. They are caused by various genetic defects and are classified as presynaptic, synaptic basal lamin-associated and post-synaptic. We report our experience with eight unrelated Italian families with CMS. εT159P and εA411P homozigous mutations of the epsilon subunit gene of the acetylcholine receptor (CHRNE) were the most frequent molecular defects detected. A new heterozygous mutation (V506L and 1657_1659dupACC) of the cholineO-acetyltransferase gene (CHAT) were found in one family. Clinical differences between these groups of patients were evident. Four families had limb-girdle myasthenia with tubular aggregates.

Romeo Vincenzo

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PET – SPECT findings in Myotonic Dystrophy type 1 and type 2

We investigated: 1) 58 patients affected with DM1 through SPECT and a subgroup of 17 patients with PET; 2) 8 patients affected with DM2 through SPECT. The DM1/DM2 SPECT-patients were divided in 'normally' and 'abnormally' perfused. The 17 DM1 PET-patients were subgrouped in 'normal' and 'abnormal' performers. We used a three-degree semi-quantitative scale (mild, moderate, severe) to quantify hypoperfusion/hypometabolism. We localized focal abnormalities in frontal, parietal, temporal, occipital, sensory-motor, insular, basal ganglia, thalamic and/or cerebellar areas. 52/58 DM1 patients showed abnormal SPECT. 15/17 DM1 patients had abnormal PET. Mild hypoperfusion was mostly seen in left hemisphere. PET scans occasionally documented frontal lobe disturbances. The 8 SPECT scans in DM2 patients showed parietal involvement.Our study demonstrates a high prevalence of mild-moderate left frontal hypoperfusion/hypometabolism in DM1 patients. Parietal lobe involvement was documented in DM2.

Sferrazza Rossella

Sferrazza Rossella, Vindigni Vincenzo, Bassetto Franco, Mazzoleni Francesco, Adami Nicoletta (1), Biral Donatella (1), Caccavale Susy (1), Rossini Katia (1), Carraro Ugo (1). Unit of Plastic Surgery and (1) Laboratory of Applied Myology of the Department of Biomedical Science, Interdepartmental Research Center of Myology, University of Padova, Italy E-mail: *ross.sfe@libero.it*

Myogenic events in muscle massive crushing

The events of muscle degeneration and regeneration were studied in an experimental model of mechanical and myotoxic injury in rats. Rectangular patches, 24mm long and 8mm wide, of rat rectus abdominis (RRAMC) muscle were processed by mechanical crushing and injection of marcaine. In another group, three days after, the RRAMC were

autotransplanted (A-RRAMC). After 11 days RRAMC showed wide destruction of muscle tissue, infiltration by inflammatory cells and full presence of small regenerating myofibers, i.e., positive to an anti MHC-emb. 24-day RRAMC showed an almost complete reconstruction of the muscle tissue, with well packed large myofibers negative to anti MHC-emb, but distinct ATPases stainings (i.e., fast and slow reinnervated myofibers). Sections of 24-day A-RRAMC showed among fibrotic scar seldom regenerating myofibers, still positive to anti MHC-emb. In conclusion, after a massive mechanical-myotoxic damage, the vascular network continuity and the ordered connective tissue architecture maintain the potentials for muscle tissue regeneration and reinnervation, while vascular and nervous networks discontinuation by an muscle ischemic massive coagulative necrosis (satellite cells, included) confines the regenerative events to the rim, the patch being made of a fibrous scar tissue.

Sampaolesi Maurilio
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Cell therapy in a large animal model for Muscular Dystrophy
The animal model specifically reproducing the dystrophin gene alterations and the full spectrum of human pathology is the Golden Retriever Dog model (GRMD). We report that intra-arterial delivery of wild type canine mesoangioblasts significantly ameliorates many symptoms of canine muscular dystrophy whereas autologous, genetically corrected cells are much less effective. Cells derived from blood vessels of human skeletal muscle can also regenerate skeletal muscle, similarly to canine mesoangioblasts. Human adult mesoangioblasts do not express endothelial markers, but instead express markers of pericytes, such as NG2 proteoglycan and alkaline phosphatase (ALP), and can be prospectively isolated from freshly
dissociated ALP(+) cells.

Sandri Marco
Cristina Mammucari1,4, Giulia Milan1,2, Vanina Romanello1,2, Eva Masiero1, Paola Del Piccolo1,Claudia Sandri,1,2, Stefano Schiaffino1,3,4 and Marco Sandri1,2,4. 1Venetian Institute of Molecular Medicine, 2Dulbecco Telethon Institute, Padova, Italy, 3C.N.R. Institute of Neuroscience, 4Department of Biomedical Sciences, University of Padova, Italy. E-mail: marco.sandri@unipd.it

Coordination of autophagy-lysosome and ubiquitin-proteasome systems during muscle atrophy
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 in vivo. FoxO3 activates the proteasomal system by inducing the muscle-specific ubiquitin ligases atrogin-1/MAFbx and MuRF1, however FoxO3-dependent autophagy is not affected by loss of atrogin-1 and MuRF1 or by blockade of the proteasome. Thus FoxO3 controls independently the two major pathways of protein breakdown in skeletal muscle, the ubiquitin-proteasome and autophagy-lysosome pathway.

Schaub Marcus C
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Long-term genome reprogramming by acute cardiac protective mechanisms
Patients with cardiovascular disease history bear a high risk for ischaemic complications during surgery. Volatile ether-derived halogenated anesthetics (VA) afford potent cardioprotection. In isolated beating rat hearts the signaling path via PI3K – PDK1 – PKB(Akt) – GSK3beta was cardioprotective by preventing the mitochondrial permeability transition pore from opening. In randomized clinical patient studies the use of sevoflurane proved to be cardioprotective during the operative period as well as in the long-term by affecting the gene expression profile. Sevoflurane anaesthesia better preserved postoperative cardiac function than the intravenous propofol and reduced DNA-damage signalling, activated the G-CSF survival pathway, and shifted the substrate metabolism toward the energetically more efficient glycolytic pathway.

Sorarù Gianni

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Skeletal muscle involvement and cognitive profile in SBMA patients and female carriers
Spinal and bulbar muscular atrophy (SBMA) is an adult form of X-linked motor neuron disease. We investigated clinical features, including cognitive assessment, and skeletal muscle histopathology of 6 SBMA patients and 5 female carriers. Our data showed muscle myopathic and neurogenic abnormalities in patients as well as in asymptomatic carriers. Neuropsychological evaluation pointed to a subtle cognitive impairment both in patients and carriers.

Spinazzi Marco
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A new OPA1 gene mutation associated with polineuropathy
Hereditary mitocondrial fusion disorders like ADOA, due to OPA1 gene mutations, and CMT2, due to MFN2 gene mutations, are recognized causes of optic atrophy, due to optic nerve fibres degeneration. CMT2 additional distinguishing feature includes axonal polineuropathy. We studied the clinical, laboratory, electrophysiological features, muscle biospy and OPA1 gene in two sisters affected by ADOA. EMG showed a motor-sensory axonal neuropathy in the index patient, while a mild demyelinating polineuropathy in her sister. Muscle biopsy showed neurogenic atrophy and mitochondrial granular reactions by oxidative stains within fibres. DNA analysis showed a new 38 base pair deletion between exon and intron 14.

Tasca Giorgio
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A microarray study of muscle transcriptome in patients with neurogenic atrophy
Aim of our study is to focus on both degenerative and protective mechanisms activated in patients with neurogenic atrophy. We performed a microarray analysis on muscle biopsies from 29 patients with different types of neurogenic atrophy (ALS, SMA, MMN, Kennedy disease, spine injury) and 7 controls. We obtained a list of more than 600 differentially expressed genes that were clustered into functional categories. Genes encoding NMJ components (nAChR subunits, ERBB3), developmental isoforms of myofibrillar proteins, extracellular matrix components (collagen type I, III and VI, TIMP1, MMP2, TGFB1 and 3) and proteins involved in cellular atrophy (including FOXO1A and cathepsins) were induced. We also observed increased transcription of genes related to protein synthesis (IGF1, EIF4A1 and a large set of ribosomal proteins). 4EBP1 expression was reduced, accordingly. Transcriptional programs activated in skeletal muscle in response to different types of neurogenic lesion share common aspects, including the induction of anabolic pathways, potentially reflecting the presence of both atrophic and hypertrophic fibers. Different degrees of deregulation correlate with the severity of muscle involvement.

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Time-course of muscle atrophy and recovery when reinnervation is from embryonic neurons transplanted into adult rat peripheral nerve.
Complete denervation induced by disease or trauma results in muscle atrophy that is both severe and progressive. To restore muscle function in adult rats, we have placed embryonic neurons into the peripheral nerve close to denervated muscles instead of into the spinal cord. Reinnervation occurs 4-6 weeks after transplantation of embryonic neurons of certain ages. The reductions in muscle atrophy that result from reinnervation are maintained long-term.

Vescovo Giorgio
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Heart failure and skeletal muscle reprogramming
It is well known that skeletal muscle can influence exercise capacity and symptoms in heart failure. Changes in skeletal muscle composition occurring in heart failure comprise qualitative changes with a shift from the slow, aerobic, fatigue-resistant myosin heavy chain 1 to the fast glycolytic myosin heavy chain 2a and 2b, decreased mitochondrial synthesis and a blunted oxidative metabolism. Together with these changes muscle bulk, which plays a pivotal role in

determining strength, force and endurance, which are in turn linked to exercise capacity, is significantly reduced. Cardiac cachexia represents the extreme spectrum of the muscle-wastage process.

Vindigni Vincenzo
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Towards an in vivo vascularized muscle construct
Tissue Engineering of skeletal muscle tissue still remains a major challenge. Every neo-tissue construct of clinically relevant dimensions is highly dependent on vascularisation. We started from preliminary observations collected thanks to in vivo experiments of clinically relevant muscle damage, applying a step-by-step approach to identify factors favoring the survival of autologous satellite cells and, thus, muscle regeneration in a rat model of full-thickness rectus abdominis muscle ablation. Patches have the mechanical properties to support the abdominal organs and represents an ideal in vivo model to study muscle regeneration events. Auto-grafting of the middle third of the rectus abdominis muscle results in scar. When autologous myoblasts are seeded in a homologous acellular matrix, the only regenerated myofibers are confined to the border of the patch. We succeed in regenerating myofibers in the graft by injecting marcaine in both the auto-graft and the surrounding muscles. Muscle regeneration seems to be the result of co-ordinate migration of angioblasts and satellite cell–derived myoblasts from the muscles surrounding the patch. The preliminary results strongly suggest that vascularization of the scaffold followed by co-ordinate proliferation of the seeded cells are mandatory events to allow myoblasts to migrate into the patch.

Vita Giuseppe
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Nuclear factor kappa-B blockade reduces muscle degeneration and promotes muscle regeneration
Nuclear factor-κB (NF-κB) is an ubiquitous transcription factor regulating the expression of a plethora of genes involved in inflammatory, immune and acute stress responses. Several lines of evidence suggest a role of NF-κB in muscle degeneration and regeneration in Duchenne muscular dystrophy (DMD) patients and mdx mice. We investigated the effects of NF-κB blocking by IRFI 042 and PDTC on functional, biochemical and morphological parameters in mdx mice. IRFI 042 is a synthetic vitamin E analogue with a strong inhibitory activity on both oxidative stress/lipid peroxidation and NF-κB activation. PDTC is a synthetic antioxidant able to prevent NF-κB inhibitor (IkB-a) degradation. NF-κB inhibitors treated mdx mice showed: 1) an amelioration in functional parameters with an increased forelimb strength and strength normalized to weight, and decreased fatigue with PDTC; 2) a reduction of muscle necrosis and an enhancement of regeneration. Moreover IRFI 042 blunted NF-κB DNA-binding activity and TNF-α expression, reduced serum CK levels, decremented muscle conjugated dienes content and augmented muscle reduced glutathione. Our data suggest that oxidative stress/lipid peroxidation represents one of the mechanisms activating NF-κB and the consequent pathogenetic cascade in mdx muscles and that the inhibition of this cascade have beneficial effects on muscle function.

Vitiello Libero
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Systemic administration of morpholino antisense oligos in aged mdx mice: effects in skeletal and cardiac muscle
Antisense-mediated exon skipping holds great potential for the treatment of Duchenne muscular dystrophy patients. We report the systemic delivery of therapeutic oligos in aged mdx mice, whose phenotype resembles more closely that of DMD. Our procedure was based on either naked or lipid-complexed oligos. Upon ex vivo testing, treated EDL muscles displayed a 50% improvement in contractile strength compared to untreated controls. On the other hand, we found that the level and duration of the skipping effect in cardiac muscle were greatly decreased compared to skeletal muscle.

Vrbova Gerta

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Celebration of Ernest Gutmann's life and work. What was the secret of Ernest Gutmann's charm?
In my presentation I will try to answer this question. In his work Ernest liked to play with ideas, and assessed experimental finding in their wider biological context. In his work on skeletal muscle he acknowledged the decisive importance of innervation and neuromuscular activity in determining skeletal muscle properties, he nevertheless considered other regulatory factors. Indeed, he was one of the first scientists who suggested that trophic factors may regulate important events in muscle and motoneurone development and maintenance. Having noticed the tremendous importance of hormones on muscle survival in invertebrates he turned his attention to the effects of hormones on mammalian muscles linked to sexual activity. He and his colleagues discovered that the survival of such muscles is hormone dependent. Hormones led to aging, and Ernest's work on changes of the motor unit with age is a lasting contribution in this field. However what his work and the written papers can not convey is Ernest's passion for chasing nature's secrets, and his love for the truth that inspired all of us who worked with him.

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Nonquantal acetylcholine as a trophic muscle promoter in mammals
In addition to the well-established quantal release from nerve terminals there exists the so-called non-quantal release (NQR) of ACh at the rodent neuromuscular junction. The nonquantal leakage of transmitter is an important factor during synaptogenesis; it affects polyneural innervation of developing muscles, supports higher excitability of the endplate subsynaptic membrane and might promote postsynaptic receptor desensitization. NQR does even develop in vivo after anti-AChE injection and it might be one of the important factors determining synaptic failure and morphological changes on both pre- and postsynaptic parts of the endplate seen during anti-AChE poisoning.

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Autoantibodies in idiopathic inflammatory myopathies
Skeletal muscle is one of the tissues involved in immunoflogistic processes in patients affected with idiopathic inflammatory myopathies (IIM) such as polymyositis (PM) and dermatomyositis (DM). In genetically predisposed individuals, infectious agents and/or environmental factors can induce aberrant immune response with consequent flogistic reaction within the skeletal muscle. The autoimmune origin of the pathologic process is suggested by the presence of circulating autoantibodies which are specific markers of disease, although they are very rare. Our aim is to better characterize the autoantibody profile of IIM patients, also by the optimization of new and highly specific techniques for the detection of these autoantibodies.

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Trophic action of sphingosine 1-phosphate in denervated rat soleus skeletal muscle
The bioactive lipid sphingosine 1-phosphate (S1P) mediates a number of cellular responses, including growth and proliferation. Skeletal muscle possesses the full enzymatic machinery to generate S1P and expresses the transcripts of S1P receptors. Present work localizes the S1P1 and S1P3 receptors in skeletal muscle and shows that denervation causes down-regulation of the two receptors. The exogenous application of S1P counters the reduction of muscle mass caused by denervation, while the neutralization of the extracellular lipid with a specific anti-S1P monoclonal antibody worsens the atrophy. Our results suggest that S1P acts as a trophic factor of skeletal muscle.