

Satellite Meeting of the 3rd AiM CONGRESS

7th Terme Euganee Meeting on Rehabilitation

First Japan/Italy Workshop on Molecular Treatments of Myopathies

Padova & Abano Terme, (Italy) June 12 - 15, 2003

Palazzo Bo, Padova & Centro Congressi delle Venezie, Hotel Alexander Palace, Abano Terme (Padova) - Italy

PROGRAM

Thursday June 12, 2003 – Padova, Palazzo Bo at The University of Padua

- 15.⁰⁰ Openings of the 3rd AiM Congress
15.¹⁵ Scarlato Lecture: "New antibodies in Myasthenia Gravis" Angela Vincent (Oxford, Uk)
16.⁰⁰ Coffee break and visit to *The Anatomical Theatre of the University of Padua*
16.³⁰ Long Term Denervation and Neuromuscular Rehabilitation
Sponsored by The 7th Terme Euganee Meeting on Rehabilitation
Chair: Reggie Edgerton (Los Angeles, USA) and Stefano Schiaffino (Padova, Italy)
16.³⁰ David J. Glass, New York, USA
"Intracellular signalling of muscle hypertrophy and atrophy"
17.⁰⁰ Bruce Carlson, Ann Arbor, USA
"The cellular environment of long-term denervated muscle"
17.³⁰ Reggie Edgerton, Los Angeles, USA
"Facilitating locomotor recovery following spinal cord injury"
18.⁰⁰ Humberto Cerrel-Bazo, Vicenza, Italy
"A task oriented approach by means of FES on SCI subjects"
18.²⁰ Helmut Kern, Vienna, Austria
"RISE: FES of long-term denervated muscles"
18.⁵⁰ Marzena Podhorska-Okolow, Wroclaw, Poland
"Muscle regeneration in human long-term denervation"
19.⁰⁰ Winfried Mayr, Vienna, Austria
"FES of Denervated Muscles: Technology and EU project RISE"
20.⁰⁰ Cocktail party and Visit to the Giotto Frescos in the Scrovegni Chappel
22.³⁰ Bus Transfer to Abano Terme

Friday, June 13, 2003 – Hotel Alexander Palace - Abano Terme

- 9.⁰⁰ 3rd AiM Congress

Saturday, June 14, 2003 – Hotel Alexander Palace - Abano Terme

- 9.⁰⁰ 3rd AiM Congress

Saturday, June 14, 2003 – Hotel Alexander Palace - Abano Terme

- 14.³⁰ Tissue and cell approaches to muscle engineering
Co-sponsored by the "International Cardiac Bio-Assist Association"
Chair: Stanley Salmons (Liverpool, UK) and Ugo Carraro (Padova, Italy)
14.³⁰ T. Partridge, London, UK
"Implantation of myogenic stem cells in adult tissues: past and future"
15.⁰⁰ M. Grounds, Perth, Australia
"Stem cells and muscle regeneration"
15.³⁰ J. Chachques, Paris, France
"Cellular cardiomyoplasty and angiogenesis: research and development"
16.⁰⁰ Coffee break

Saturday, June 14, 2003 – Hotel Alexander Palace - Abano Terme

- 16.³⁰ Tissue and cell approaches to muscle engineering, - Part two
Chair: Terrence Partridge (Lodon, UK) and Juan C. Chachques (Paris, France)
- 16.³⁰ S. Salmons, Liverpool, UK
“Avoiding Ischemic Damage in Muscles Redeployed as Functional Grafts”
- 17.⁰⁰ Gl. Rigatelli, Legnago (Verona), Italy
“Demand Dynamic Cardiac-Bio-Girdling”
- 17.³⁰ R.L. Kao, Johnson City, USA
“Fatigue Resistant Muscle by Cell Transplantation and Electrical Conditioning”
- 18.⁰⁰ F. Mazzoleni, Padova, Italy
“Skeletal muscle reconstruction: Clinical needs of a Plastic Surgeon”
- 18.³⁰ “The Terme Euganee Award on Skeletal Muscle Regeneration, Reconstruction and Engineering”
M Sandri: Regulation of muscle growth by different signaling pathways. Role of IGF1 and atrogen1
- 20.³⁰ The BAM Friends Dinner - Euganei Hill “Trattoria Monte Rua”

Sunday, June 15, 2003– Hotel Alexander Palace - Abano Terme

First Japan/Italy Workshop: Molecular Therapy of Inherited Myopathies

Chair: Salvatore Di Mauro (New York, USA) and Masafumi Matsuo (Kobe, Japan)

- 9.⁰⁰ R. Matsuda, Tokyo, Japan
“Negamicine therapy of Dystrophinopathies”
- 9.³⁰ M. Matsuo, Kobe, Japan
“Oligonucleotides against an exonic splicing enhancer sequence of dystrophin: therapeutic potential”
- 10.⁰⁰ T. Partridge, London, UK
“Deliberate exon skipping strategies in DMD therapy”
- 10.³⁰ K. Rossini and U. Carraro, Padova, Italy
“In vivo testing of anti-sense DMD therapy by exercise-induced muscle damage susceptibility”
- 11.⁰⁰ Coffee break

Molecular Therapies of Acquired Myopathies

- 11.³⁰ S. Di Mauro, New York, USA
“Mitochondrial myopathies”
- 12.⁰⁰ G. Peluso, Napoli, Italy
“Skeletal muscle metabolism in physiology and in cancer disease”
- 12.³⁰ G. Vescovo, Vicenza, Italy
“Carnitine reverses skeletal muscle myopathy in cardiac failure”
- 13.⁰⁰ R. Sabbadini, San Diego, Italy
“The Role of Sphingomyelinase and it's Adaptor Protein, FAN, in Ischemia/reperfusion Injury”
- 13.³⁰ Lunch

Physical Approaches in Management of Myopathies

- 15.⁰⁰ H. Kern, Vienna, Austria
“Physical therapies in post-traumatic myopathies: Mechanisms of action of underwater training”
- 15.²⁰ M. Ortolani, University of Padova, Italy
“Physical therapies in degenerative syndromes of the musculoskeletal system”
- 15.⁴⁰ U. Carraro, University of Padova, Italy
“Thermal therapy: Evidence of benefits in experimental myopathies”
- 17.⁰⁰ Visit and dinner in Montagnana

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Abstracts

[Temor1] SURFACE STIMULATION OF LOWER EXTREMITIES IN SPASTIC PARAPLEGIC SUBJECTS: STANDING UP AND STEPPING WITH A PC SUPPORTED EIGHT CHANNEL STIMULATOR

Manfred Bijak¹, Monika Rakos², Christian Hofer³, Dietmar Rafolt¹, Winfried Mayr¹, Friedrich Russold¹, Ewald Unger¹, Maria Strohhofer³, Doris Raschka³, Helmut Kern³

¹Department of Biomedical Engineering and Physics, University of Vienna; ²Otto Bock, Vienna, Austria; ³Department of Physical Medicine and Rehabilitation, Wilhelminenspital, Vienna, Austria

Surface stimulation of lower extremities in paraplegic patients with intact lower motor neuron is an established FES application to provide walker or crutch supported standing up from the wheelchair and walking (stepping) for short distances. Usually m. quadriceps and m. gluteus are stimulated for hip and knee extension while electrodes placed over the peroneal nerve elicit the flexion reflex for a flexion in hip, knee and ankle joint.

Constant current or constant voltage outputs of stimulators are switched on and off in a special sequence to get the required movement (standing up, stepping). The stimulation intensity is used to optimize the muscle contraction strength.

Due to the low number of (practicable) stimulated muscles the observed movement is unnatural and largely independent from the chosen stimulator.

A higher number of channels would significantly lower the usability of the system.

As another approach to improve the gait pattern we concentrate on stimulation parameter optimization. An eight channel surface stimulation equipment with PC interface was developed and included in a clinical trial.

The system offers a huge number of parameter variability for gait pattern optimization. The clinical trial will show which parameters have major influence on the movement.

First results demonstrate the importance of an amplitude ramp during stimulation onset. A smoother and more "natural" movement can be observed. For an adequate step length an overlapping stimulation of quadriceps and peroneal nerve during heel strike are crucial.

Additionally the fourth channel was used to activate adductor muscles. A better knee trajectory during standing up and better leg movement during the swing phase were achieved.

[TEMOR2] EXERCISE-INDUCED MUSCLE DAMAGE: TIME-COURSE OF RECOVERY IN α -SARCOGLYCAN- AND DYSTROPHIN-NUL MICE

Donatella Biral¹, Anna Jakubiec-Puka², Ugo Carraro³, Romeo Betto¹

¹CNR Institute of Neuroscience, Laboratory of Muscle Biology & Physiopathology, ²Department of Cell Biochemistry, Nencki Institute of Experimental Biology, Warszawa, Poland, ³Department of Biomedical Sciences, University of Padova, Padova, Italy

Exercise-induced muscle damage of normal or dystrophic muscles of rodents is a reproducible experimental model to study the recovering capacity of muscle under physiological and pathological conditions. It allows to test *in vivo* the effects of a wide range of physical and chemical agents to be used to prevent or treat muscle injuries. Three-month old control (C57Balb/6J), α -sarcoglycan-null (α SGko) and dystrophin-null (*mdx*) mice were housed in cages provided of a rotating wheel, and allowed to spontaneously run the full night (about 16 hours). Activity of mice was quantified as covered distance (Km) and percent time of wheel rotation (%WR). At 0, 1, 7, 12 day post-running, mice were sacrificed and muscles from both legs removed. Muscle cryostat sections were stained with H-E and anti-embryonic MHC antibodies to establish the extent of myofiber death/regeneration. Surprisingly, no significant differences in muscle performance were observed in the three groups of mice, though SD were larger in both α SGko and *mdx* dystrophic mice in comparison to the control mice. Before running, fast and slow muscles of *mdx* mice presented, indistinctively, the majority of fibers with central nuclei, together with rare swollen, dying, fibers, foci of macrophage infiltration and areas with fibers expressing the embryonic MHC, evidence of muscle regeneration. While almost all fibers from fast and slow α SGko mice presented central nuclei, fibers with embryonic MHC were evident in slow muscles, only. An apparently higher number of swollen fibers were evident in α SGko than in *mdx* muscles. One day after running, muscles of running control mice showed modest edema and some infiltration of phagocytes. Fibers positive to embryonic MHC were as rare as in pre-running controls. In *mdx* muscles, one-day after running, new foci of macrophagic infiltration appeared, followed 3 days after by the presence of small embryonic MHC-positive fibers. After seven days, larger regenerating fibers were present, while, 12-day after running, the muscle was indistinguishable from that before running. In the α SGko-running mice, after one day, both fast and slow muscles showed diffuse infiltration and swollen fibers. Three days after running, diffuse areas of myotubes and small fibers were observed, with the evidence of embryonic MHC-positive cells also in fast muscles. After 7 days, several larger regenerating fibers were evident. In spite of the apparently earlier onset of exercise-induced muscle damage, after ten days the α SGko running mice showed muscles that appear less damaged than pre-running muscles. This result suggests that muscles of the α SGko mice may acquire higher recovery capacity after exercise-induced muscle damage as compared to *mdx* muscles.

The financial support of TELETHON ITALY (grant n. 1286) is gratefully acknowledged.

[TEMOR3] THE CELLULAR ENVIRONMENT OF LONG-TERM DENERVATED MUSCLE

Bruce M. Carlson

Institute of Gerontology, University of Michigan, Ann Arbor, Michigan USA

Although grossly, a denervated muscle appears to undergo a steady course of atrophy, the cellular environment within that muscle is, in reality, quite complex.

Within a week after denervation the satellite cell population doubles and continues to rise for the first two months before steadily falling thereafter. At the same time there is a differential atrophy of fast vs. slow muscle fibers. Nuclear death and even overall death of muscle fibers occurs in the months following denervation, but at the same time satellite cell activation and new muscle fiber formation are taking place. Muscle spindles and intrafusal fibers atrophy at a slower rate than do extrafusal fibers. Meanwhile, the microvasculature becomes greatly reduced and masses of collagen build up in both the interstitial spaces and within the intramuscular channels of the motor nerve branches.

Supported by grant PO1 AG-10821 from the NIH.

[TEMOR4] THERMAL THERAPY: EVIDENCE OF BENEFITS IN EXPERIMENTAL MYOPATHIES

Ugo Carraro, Katia Rossini

C.N.R. Institute of Neuroscience, Laboratory of Applied Myology of the Department of Biomedical Science, University of Padova, Italy

Exercise-induced muscle damage of normal or dystrophic muscles of rodents is a reproducible experimental model of muscle myopathy. It allows testing in vivo effects of a wide range of physical and chemical agents to be used to prevent or treat muscle injuries. The results of a series of 30 min swimming in warm water during the week preceding spontaneous running will be discussed. Evans blue dye (EBD) is injected two days before mice are housed in cages provided of a rotating wheel, and allowed to spontaneously run the full night (about 16 hours). Activity of mice is quantified as covered distance (Km) and percent time of wheel rotation (%WR). At stated times the mice are sacrificed and muscles are removed from both legs.

Muscle cryosections of one-day post-running are used to quantify permeabilized myofibers (dying muscle fibers). Anti-MHCemb monoclonals is used on cryosections of four-day post-running muscles to establish extent of myofiber regeneration.

Results confirm that pre-conditioning by physical activity in warm water significantly decrease muscle damage induced by abrupt increase of physical activity.

[TEMOR5] CELLULAR CARDIOMYOPLASTY AND ANGIOGENESIS RESEARCH AND DEVELOPMENT

Juan C. Chachques

University of Paris, France.

The goal of cellular cardiomyoplasty (i.e., intramyocardial cell grafting) is to limit the consequences of decreased contractile function and compliance of damaged ventricles following myocardial infarction. A cell-based therapeutic approach for post-ischemic scars is particularly attractive due to the potential for myocardial regeneration with a variety of myogenic and angiogenic cell types: skeletal myoblasts, bone marrow-derived mesenchymal stem cells, circulating blood-derived progenitor cells, smooth muscle cells, vascular endothelial cells, and embryonic stem cells. Cellular cardiomyoplasty using autologous skeletal myoblasts was performed by our group in 18 patients. Over 100 patients have been treated worldwide. Inclusion criteria consists of adult patients with a low ejection fraction, akinetic, non-viable post-infarction scar. Cell-based myogenic therapy seems to reduce the size and fibrosis of infarct scars, limit post-ischemic remodelling, and restore regional myocardial contractility in patients following extensive myocardial infarction. Techniques for skeletal myoblasts culture and ex vivo expansion using autologous patient serum (obtained from plasmapheresis) have been developed. These techniques yield over 300 million cells in 3 weeks, of which more than 70% are myoblasts. Cell viability at the moment of injection is greater than 90%. The main benefits of human-autologous-serum cell culture is that it can be performed without risk of prion, viral or zoonoses contamination. Traditional cell cultures techniques involve the use of fetal bovine serum for cell growth. Contact of human cells with fetal bovine serum results after 3-week in fixation of animal proteins on the cell surface, representing an antigenic substrate for immunological adverse events. After cell implantation an inflammatory reaction occurs in these cases with subsequent fibrosis. Malignant ventricular arrhythmias and sudden deaths have been reported following serum bovine-cultivated cell therapy, in many cases this complication required the implantation of cardioverter-defibrillators. The mechanism by which implanted cells improve heart function remains controversial. Several factors directly or indirectly contribute to the structural and functional benefits afforded by muscle cell transplantation. Muscle cell implantation increases regional elasticity and alters extracellular matrix preventing ventricular remodeling. Furthermore, cells grafted in the infarcted area contribute to decrease scar thinning and chamber dilatation (scaffolding effect). The mechanism explaining the transmission and propagation of electrical impulses from the native myocardium to the engrafted cells has not been elucidated. Response to a mechanical stimulus exerted by surrounding cardiomyocytes could be responsible for inducing this contraction. Thus, functional improvement is obtained from a combination of factors. While various donor cells have been studied to induce myogenesis after myocardial infarction, recent interest has arisen in promoting cardiac angiogenesis by the transplantation of vascular endothelial cells or endothelial progenitor cells (directly removed or mobilized from bone marrow). This approach retains an important potential as

an adjunct to myogenic cellular transplantation in inducing angiogenesis in injured myocardium, because muscular cells mortality after implantation in high fibrotic infarcted myocardium seems to be high since the oxygen and nutrients supply are limited within the scar. Thus, cell-based angiogenic therapy is in strong development for myocardial and limb ischemia due to the instability and adverse response to transfection vectors of angiogenic gene therapy, and the limitations of growth factor protein-based therapy, which present risks of systemic effects (inducing problematic angiogenesis in the retina, intimal arterial hyperplasia with development of arteromatous plaque, and potentiation of growth and metastasis of occult tumors). Cell transplantation is being recognized as a viable strategy to improve myocardial viability and limit infarct growth. Combined cellular transplantation with multisite cardiac pacing is actually under investigation by our groups. Following skeletal myoblast implantation in a experimental myocardial infarction model, atrial synchronized biventricular pacing was performed using epicardial electrodes. These studies showed improved cell distribution, development of myotubes and increased expression of slow myosin heavy chain isoforms (better adapted at performing cardiac work). In addition, this combined approach permits atrio-biventricular resynchronisation resulting in the improvement of heart failure symptoms. Electrostimulated cellular cardiomyoplasty should play an important role in transforming a passive cell-based procedure to a «dynamic cellular support».

The implantation of cultivated fetal atrial cardiomyocytes into the ventricular wall have been proposed as a biological cardiac pacemaker.

The aim of this approach is to transplant cardiomyocytes with a higher intrinsic rhythmic rate into the myocardium of the left ventricle. Therefore these cells could act as an ectopic pacemaker by functional coupling with host cardiomyocytes. Experimental studies showed survival of grafted cells, formation of gap junctions between donor and recipient cells, and spontaneous generation of electrical signals having the morphology of QRS-complexes of escape rhythm. Transplanting cardiomyocytes as cardiac pacemaker may open a new perspective for the treatment of cardiac arrhythmia, ranging from infants and premature babies with congenital atrio-ventricular block to patients with acquired blocks.

The major challenges for future research programs are the pre-conditioning for pre-differentiation of stem cells before transplantation, the improvement of host-cell interactions (mechanical and electrical coupling), and the optimization of the rate of surviving cells after myocardial implantation. The association of cell-based therapeutic angiogenesis prior to cellular myogenesis seems to be justified, in order to induce prevascularization of postinfarct scars. We hope that in the near future patient's autologous cells could be used as a reliable source for tissue regeneration. It is possible that periodically repeated catheter-based cell injection procedures could be necessary to progressively recolonise myocardial scars with living tissues. Myocardial regeneration by cellular cardiomyoplasty offers the promise of restoring ventricular function in patients with extensive myocardial infarction or idiopathic dilated cardiomyopathy.

[TEMOR6] MITOCHONDRIAL MYOPATHIES

Salvatore DiMauro, Eduardo Bonilla, Michelangelo Mancuso, Massimiliano Filosto, Sabrina Sacconi, Leonardo Salvati and Michio Hirano

Department of Neurology, Columbia University College of Physicians & Surgeons, New York, NY, USA

Mitochondrial diseases, defined restrictively as disorders due to defects of the mitochondrial respiratory chain, are notoriously heterogeneous, both clinically and genetically. Here, we review only disorders affecting exclusively or predominantly skeletal muscle (mitochondrial myopathies). As the respiratory chain is under dual genetic control, we consider first myopathies due to defects in mitochondrial DNA (mtDNA), distinguishing those due to defects of mitochondrial protein synthesis *in toto* from those due to mutations in protein-encoding genes. We then divide disorders due to nuclear gene defects into six groups and discuss mitochondrial myopathies due to genetic defects affecting: respiratory chain components; the protein importation machinery; the inner membrane lipid milieu; and intergenomic signaling. We conclude with some considerations on therapy.

[TEMOR7] FACILITATING LOCOMOTOR RECOVERY FOLLOWING SPINAL CORD INJURY

V. Reggie Edgerton

Departments of Physiological Science and Neurobiology, Brain Research Institute, UCLA, Los Angeles, California.

Three important properties of the spinal cord will be discussed that provide the possibility of using activity dependent rehabilitative strategies to regain the ability to stand and to step following spinal cord injury. First, the spinal cord can receive and interpret the sensory information from the lower limbs that is associated with weight-bearing standing and stepping. Therefore, when the body is placed in a weight-bearing posture, the spinal cord will respond by generating the appropriate activation signals necessary to execute successful standing and stepping. In essence, the spinal cord itself is capable of generating the appropriate activation patterns for standing and stepping.

The second important property to be discussed will be the ability to improve the functionality of these spinal circuits by practicing a specific motor task. Evidence will be presented which demonstrates the ability of mice, rats and cats to learn to stand and to step following a complete mid-thoracic spinal cord transection. It is clear that the glycinergic and GABAergic inhibitory systems within the spinal cord are modulated (both pharmacologically and by training) by spinal cord injury and by training. The ability of the complete spinal cord injured subject to regain standing and stepping capabilities is a function of the manner and degree to which these two systems are modulated.

The third important property is the responsiveness of the lumbosacral spinal cord to a combination of administration of a 5 HT agonist in combination with step training to facilitate learning to step. These studies in general demonstrate an ex-

tensive capability of the neuromotor circuitry within the spinal cord to be modulated in a controlled manner so that successful standing and stepping can be achieved with minimal or no input from the brain.

[TEMOR8] SKELETAL MUSCLE METABOLISM IN PHYSIOLOGY AND IN CANCER DISEASE

Anna Giordano, Menotti Calvani, Orsolina Petillo, Sabrina Margarucci, Gianfranco Peluso (1)

Scientific Department, Sigma Tau S.p.A., Pomezia, Rome and (1) Department of Experimental Oncology, National Cancer Institute, Naples, Italy

Skeletal muscle is a tissue of high demand and it accounts for most of daily energy consumption. The classical concept of energy metabolism in skeletal muscle has been profoundly modified on the basis of studies showing the influence of additional factors (i.e., uncoupling proteins and peroxisome proliferator activated receptors) controlling parameters, such as substrate availability, cellular enzymes, carrier proteins and proton leak, able to affect glycolysis, nutrient oxidation and protein degradation. This extremely balanced system is greatly altered by cancer disease that can induce muscle cachexia with significant deleterious consequences and results in muscle wasting and weakness, delaying or preventing ambulation and rehabilitation in catabolic patients.

[TEMOR9] THE ROLE OF STEM CELLS IN BUILDING NEW MUSCLE

Miranda D Grounds¹, Jason White¹, Nadia Rosenthal², Marie Bogoyevitch³, Cecilia Prele³

(1) School of Anatomy & Human Biology and (3) School of Biomedical and Chemical Sciences, the University of Western Australia, Perth, Western Australia 6009; (2) EMBL, Monterotondo, Rome, Italy

Skeletal muscle, even in aged individuals, normally has an excellent capacity for repair *in vivo* due to activation of reserve precursor cells called satellite cells. This regeneration is in marked contrast to the situation for adult cardiac muscle where damaged muscle tissue is usually replaced by scar tissue. The role of conventional precursors and of stem cells will be discussed in the context of clinical skeletal and cardiac muscle regeneration^{1,2} and also with respect to potential tissue engineering where construction *de novo* of large areas of muscle requires a tissue scaffold, blood supply³ and source of precursor cells to form the new tissue. Skeletal muscle precursors (myoblasts) are classically derived from reserve mononucleated satellite cells located on the surface of mature myofibres. It has also been demonstrated that myoblasts, and cardiomyocytes, can arise from 'non-muscle' sources including interstitial mesenchymal cells, cells associated with blood vessels and circulating bone marrow derived cells. All of these classes of cells may have stem cell properties¹. In addition, myoblasts

might be derived from de-differentiation of myonuclei in damaged mature myofibres (as is the case for amphibians): such reactivation of apparently postmitotic nuclei is also of considerable interest for adult cardiomyocytes. These various sources of muscle precursor cells and the important role of the local micro-environment (including IGF-1) for conversion of stem cells into the myogenic lineage will be discussed. In addition, the host immune response that results in the rapid and massive death of cultured donor myoblasts after intramuscular injection and the wider implications for transplantation of cultured cells into the *in vivo* environment will be considered^{4,5}.

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[TEMOR10] CELL TRANSPLANTATION AND ELECTRICAL CONDITIONING FOR FATIGUE RESISTANT MUSCLE

Race L. Kao, Janet Davis, Elizabeth Lamb, William Browder
Department of Surgery, East Tennessee State University, Johnson City, TN 37614, USA

Congestive heart failure refractory to medical therapy can be managed with cardiac transplantation or artificial heart. Due to the scarcity of donor organs and unsatisfactory long-term outcomes of mechanical devices, skeletal muscle has been used to augment circulatory function. Dynamic cardiomyoplasty, skeletal muscle ventricle, aortomyoplasty, and muscle powered assist devices all require the fatigue resistant muscle to support the failing heart. However, muscle transformation by chronic electric stimulation commonly suffers fibrosis, atrophy, fatty degeneration, and diminished power. Masticatory muscles appear to be highly specialized in mammals and exhibit superior fatigue resistance. Satellite cells isolated from mastication muscles retain their phenotypic characteristics after transplanted into other muscles. Muscle biopsy (~1 g) from canine masseter muscle was used for satellite cell isolation and *in vitro* proliferation of the myogenic cells. Latissimus dorsi muscles from 12 dogs

were randomly assigned into three groups of treatments: 1) control, 2) electrical conditioning, 3) cell transplant + electrical conditioning. After two weeks of vascular delay and eight weeks of conditioning, at the end of fatigue test the power outputs were 0.21 ± 0.02 , 0.48 ± 0.07 , and 1.30 ± 0.09 watts with the muscle mass of 162 ± 14 , 119 ± 18 , and 164 ± 15 g for the control, electrical conditioning, and cell transplant + electrical conditioning groups, respectively. Transplantation of autologous satellite cells from jaw closing muscle and chronic electrical stimulation produced skeletal muscle of highly fatigue resistance with preserved mass and power output. This type of muscle could be used for circulatory support or as a permanent power source for assist devices.

[TEMOR11] "RISE": FES OF LONG-TERM
DENERVATED MUSCLES

Helmut Kern

*Ludwig Boltzmann Institute of Electrical Stimulation and
Physical Rehabilitation, Department of Physical Medicine,
Wilhelminenspital, Vienna, Austria*

Clinical work showed that electrical stimulation therapy with exponential current is able to slow down atrophy and maintain muscle trophism only in non-permanent flaccid paralysis. On the other hand, exponential currents are not sufficient for therapy of complete lesion of conus cauda patients with long-term denervated and degenerated muscle (DDM).

Therefore ten years ago we developed high intensity stimulation protocols for functional electrical stimulation (FES) of long-term denervated and degenerated muscles. At the beginning of the FES training, muscle twitches are elicited by biphasic pulses lasting 120-150ms, delivered directly by large surface electrodes. Due to its very low excitability, the denervated, degenerated muscle does not sustain tetanic contractions. Later on, taking advantage of the increased muscle excitability, repeated tetani of the muscles are elicited by stimulation with pulse duration of 30-50ms, stimulation frequency of 16-25Hz, pulse amplitudes of up to 250mA. This, finally, improves the structural and metabolic state reversing muscle atrophy/degeneration.

Denervated muscles showed an average increase of 66% in fiber diameter after 8-12 months of FES training and an increase in aerobic and anaerobic muscle enzymes up to the normal range of values. Even in the absence of axonal neurotrophic substances, FES was able to recover fiber trophism, and induce intracellular structural benefits in denervated muscles. The increment in muscle area as visible on CT-scans of quadriceps femoris was 10% in this group of patients. In the patients with conus cauda lesions, FES must be integrated into modern rehabilitation to prevent extreme muscle degeneration and decubital ulcers. Using FES we are able to improve muscle function and to induce positive trophic changes in lower extremities of our patients. Recovery of denervated and degenerated muscle by means of FES seems to be the result of two processes: re-growth of surviving myofibres and growth of regenerated new fibres.

In the European research project "RISE" we are now optimizing these rehabilitation strategies in humans. The under-

lying basic scientific knowledge of muscle regeneration from satellite cells/myoblast in long-term denervated, degenerated muscles is also studied by animal experiments.

[TEMOR12] EFFECTS OF UNDERWATER THERAPY
ON MUSCLE-SORE AND MUSCLE-TRAUMA IN
SPORT-INJURIES

Helmut Kern, Christian Hofer, Michaela Mödlin

*Ludwig Boltzmann Institute of Electrical Stimulation and
Physical Rehabilitation, Department of Physical Medicine,
Wilhelminenspital, Vienna, Austria*

The most frequent injuries in sports are lesions of muscles. Physical therapy consists of three different stages: 1. acute phase therapy; 2. enhancement of regeneration; 3. return to sport activity with increasing sport specific loading of the musculature.

Treatment during the acute phase of injury comprises compression, cooling, elevation, reduction of swelling and protection. Underwater therapy is essential in the enhancement of regeneration and return to sports. In underwater therapy we are using the physical and physiological effects of heating, unloading, water resistance and water pressure. Other benefits of this therapy are the reduction of edemas and the depression of inflammation.

Another important effect of this therapy after muscle injury is that EMG-activity decreases with increasing immersion into the water. This allows a very early beginning of active exercise therapy without the risk of re-injuring the muscle.

At the same time local or whole body Fangotherapy baths could/should be applied because of reduction of inflammation and heating effect. In athletes and healthy subjects this Fango baths do only slightly affect the blood pressure but there is a significant increase in heart rate observed. A Fangotherapy lasting for 20 minutes causes a steady increase of heart rate of about 60-70bpm up to 105-110bpm at the end of treatment. This demonstrates the increase of perfusion and thus the regeneration enhancing effect of additional Fangotherapy.

A faster return to sports is possible when through early unloaded movements in warm water the function of the injured muscles and the coordination of the whole body are completely restored.

[TEMOR13] DO PRO-INFLAMMATORY CYTOKINES
AND SPHINGOLIPIDS CONTRIBUTE TO THE MUSCLE
DAMAGE ASSOCIATED WITH ECCENTRIC EXERCISE?

Fred W. Kolkhorst¹, Robert E. Klepper², Michael J. Buono^{1,2},
and Roger A. Sabbadini²

¹*Department of Exercise and Nutritional Sciences and*

²*Department of Biology, San Diego State University, San Diego,
CA 92182, USA*

Following eccentric exercise, cytokines are produced that potentiate the inflammatory response. However, not all studies have observed increases in circulating cytokines following

strenuous exercise. Many cytokines stimulate the production of sphingolipid (SPL) mediators, some of which have been shown in skeletal muscle to modulate Ca^{2+} -release channels, produce apoptosis, and contribute to muscular fatigue. Moreover, SPLs may be novel inflammatory mediators. Accordingly, we conducted three pilot studies to develop an exercise model to investigate the role of circulating cytokines and sphingolipids in exercise-induced muscular damage. The first exercise model utilized active males running at -10% grade for 60 min at ~70% of HR_{max} . Only moderate muscle soreness and serum CK increases were observed; there were no significant changes in TNF- α , CRP, sphingosine (SPH), or sphingosine 1-phosphate (S1P). The second model used untrained males who performed 6 sets of 25 repetitions of eccentric arm curls at 80% of one maximal concentric curl. Subjects reported severe muscular soreness and a 32-fold increase in CK, which were strong evidence of skeletal muscle damage. However, we observed only non-significant trends in TNF- α , SPH and S1P. We hypothesized that the change in circulating cytokines occurred outside the times when blood was sampled. In a third test, we again used the eccentric arm curl model, but sampled blood immediately before and after exercise, and at 1- and 6-h postexercise, and at 1-, 2-, 3-, 5-, and 7-d postexercise. In this model, CRP appeared to peak at 5-d postexercise, although there were no changes in TNF- α or SPLs.

[TEMOR14] CAN NEGAMYCIN RESTORE DYSTROPHIN IN MDX MOUSE?

Ryoichi Matsuda, Masayuki Arakawa, and Masataka Shiozuka

Department of Life Sciences, The University of Tokyo at Komaba, Tokyo, Japan, E-mail: ryoichi@matsuda.c.u-tokyo.ac.jp

The ability of aminoglycoside antibiotics to promote readthrough of premature stop codon has attracted interests in these drugs as potential therapeutic agents in nonsense mutations. Barton-Davies et al. (J Clin Invest 104:375-381,1999) reported that gentamicin restored dystrophin function in skeletal muscle of mdx mouse, an animal model of Duchenne-type muscular dystrophy (DMD), which resulted from a nonsense mutation in the dystrophin gene. Here we report that negamycin treatment also restore dystrophin to skeletal muscle and cardiac muscles in mdx mouse. We established mdx muscle cell line by introduction of temperature-sensitive SV40-T antigen gene and treated these cells with negamycin. Dystrophin accumulation was confirmed by immunohistochemistry and immunoblot after partial enrichment of dystrophin-dystrophin associated glycoproteins.

Negamycin was less toxic than gentamicin. In order to study the functional aspects of the readthrough activity of negamycin, we showed that negamycin binds to partial sequence of eukaryotic ribosomal RNA A-site by mass spectrometry. These results suggest that negamycin is a new promising candidate for chemotherapy of DMD and many other genetic diseases caused by nonsense mutation.

[TEMOR15] INDUCTION OF DYSTROPHIN EXPRESSION IN MYOCYTES OF DUCHENNE MUSCULAR DYSTROPHY BY CREATING IN-FRAME MRNA WITH ANTISENSE OLIGONUCLEOTIDES

Masafumi Matsuo¹, Mariko Yagi¹, Makoto Koizumi², Kazuto Ishibashi¹, Yasuhiro Takeshima¹

¹Department of Pediatrics, Kobe University Graduate School of Medicine, ²Sankyo Co., Ltd. Japan*

Duchenne muscular dystrophy (DMD) is a rapid progressive skeletal muscle disease characterized by the complete absence of dystrophin but its treatment has not yet been established. Here, molecular therapy is proposed as a novel treatment for DMD whereby the correction of the translational reading frame transforms severe DMD into the milder form. We report the first evidence that oligonucleotides against an exonic splicing enhancer sequence successfully induced exon skipping and led to production of truncated dystrophin in myocytes from DMD.

In this study, a 31-mer phosphorothioate oligodeoxynucleotide (S-oligo) against the splicing enhancer sequence of exon 19 of the dystrophin gene was transfected to myocytes from a DMD case who has an out-of-frame deletion of exon 20. In his lymphoblastoid cells, the transfection induced exon 19 skipping in a large proportion of dystrophin transcripts, thereby creating an in-frame transcript lacking both exons 19 (88 nt) and 20 (242 nt). In his myocytes a proportion of dystrophin transcripts showed exon 19 skipping upon transfection. Markedly, more than 15 % of myocytes was stained positive for dystrophin concomitant with the appearance of an in-frame transcript, while no dystrophin-positive myocytes were identified without transfection. Since ethylene bridged DNA (ENA) has been reported to be more stable and higher binding affinity than S-oligo, its ability to induce exon 19 skipping was examined. Surprisingly antisense oligonucleotide containing ENA had stronger activity to induce exon 19 skipping than S-oligo. This new oligonucleotide is concluded as a new powerful tool for induction of exon skipping.

[TEMOR16] FUNCTIONAL ELECTRICAL STIMULATION (FES) OF DENERVATED MUSCLES: GENERAL TECHNOLOGICAL REQUIREMENTS AND THE EUROPEAN R&D PROJECT RISE

Winfried Mayr, Christian Hofer⁽¹⁾, Manfred Bijak, Dietmar Rafolt, Ewald Unger, Martin Reichel, Stefan Sauermann, Hermann Lanmueller and Helmut Kern⁽¹⁾

Department of Biomedical Engineering and Physics, Vienna University Medical School and (1) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital, Vienna

Recent experimental and clinical work gives strong evidence that functional electrical stimulation (FES) is a powerful tool for regeneration, functional restoration and maintenance of denervated musculature, a fact that for various reasons was not recognized in the past.

One reason was the lack of associated technology that in comparison to existing FES equipment for nerve stimulation has to

meet various completely different demands. Most of the few existing stimulators for denervated muscles provided by industry or published in conjunction with scientific studies are not sufficient for muscle restoration and maintenance due to their limited range of electrical parameters obviously with respect to the presently too restrictive EU regulations for stimulation devices.

In order to be effective a stimulator for activation of denervated skeletal muscles via surface electrodes requires biphasic long-duration impulses with a pulse width between 10 and 300ms and amplitudes of up to ± 100 V respectively ± 250 mA. These demands rise safety issues in the design and application of both the stimulator and the electrodes. In Vienna a sufficiently working prototype equipment was developed and clinically tested in preliminary experiments.

To make both the method and the technical equipment available for the patients the European R&D project RISE was started with November 1, 2001. Within the 4 year lifetime of the project the following objectives are addressed:

1. Definition of the range of electrical parameters and amount and intensity of training for safe (non-damaging) and effective FES training of denervated degenerated muscles (DDM).
2. Guidelines and protocol for clinical use of this new rehabilitation method..
3. Associated technical equipment and necessary modification of EU regulations for therapy of DDM.

[TEMOR17] MUSCLE REGENERATION AFTER SURGICAL ABLATION OF RAT RECTUS ABDOMINIS MUSCLE

Francesco Mazzoleni¹, Vincenzo Vindigni¹, Valeria Tomat¹,
Maria Elena Zanin, Marta Fabbian², Ugo Carraro²

(1) *Clinic of Plastic and Reconstructive Surgery, Department of Surgical Science, University of Padova;* (2) *C.N.R. Institute of Neuroscience, Neuromuscular Section, Laboratory of Applied Myology, Department of Biomedical Science, University of Padova, Italy*

Plastic surgeons could minimize the donor site defect after the use of the Transverse Rectus Abdominis Muscle flap (TRAM), based on recent developments of tissue engineering. The rat has been used as a surgical model, which, reproducing the considerable defect of this muscle due to TRAMF, allows to study the possible methods of functional repair.

We have performed a rectangular (20x8 mm) full thickness defect of the left TRAM in 18 adult rats. To repair the defect we used as "myogenic scaffold" a homologous muscle acellular matrix. This bio-matrix is useful to regenerate lost muscle by permitting proliferation and differentiation of muscle stem cells. We obtained sterile acellular matrix by two different methods: a) 3 cycles of freezing-boiling, and b) absolute alcohol fixation. In 9 rats we injected the scaffold with a suspension of autologous satellite cell obtained from TRAM. Rats were sacrificed 21 days after muscle reconstruction. By light morphometry interstitial tissue area, and fiber size distribution were determined. Myosin ATPases and immunohistochemistry were used to quantify fiber types, including regenerated myofibers. Total protein content, myosin-vs total protein and myosin-vs actin ratios are determined by SDS-PAGE to reliably complement or

substitute morphometry in quantifying muscle trophism in skeletal muscle biopsies. Crushed muscle and muscle infiltrated with marcaine 0.5% were used as controls.

This experimental model demonstrated to be well reproducible. The acellular patch was always found to be well integrated in the abdominal wall, despite different degrees of widespreading or fibrosis, related to different methods of scaffold preparation. Herniations of the abdominal organs were never seen. Therefore, the patch sustains the weight of the abdominal organs, so that the long-lasting phases (days) of muscle regeneration and reinnervation occur without abdominal complications. Macroscopically, marked neoangiogenesis from the peritoneal surface was observed. Finally, histological and immunohistochemical studies highlighted the presence of areas of muscle regeneration, usually with myofibers of small diameter, surrounded by scar-like fibrous tissue.

In a foreseeable future, the overall approach could be applied in the clinical practice. These experiments are preliminary to a long-term project to develop artificial myogenic scaffolds based on well known biomaterials, such as polylactide (PL), polyglycolide (PG), or their copolymers (PLG). On the other hand, we need optimized procedures to increase survival of injected myogenic cells *in vivo*, in particular when reconstruction of clinically significant amounts of muscle are needed, a case in which revascularization and reinnervation are essential processes.

[TEMOR18] THE ROLE OF SPHINGOMYELINASE AND IT'S ADAPTOR PROTEIN, FAN, IN ISCHEMIA/REPERFUSION INJURY

Nicole O'Brien, Roger A. Sabbadini

Department of Biology San Diego State University, San Diego, California USA.

Many of the cellular mechanisms responsible for cardiac cell death during ischemia and subsequent reperfusion have been elucidated, while others remain largely unknown. The sphingolipid-signaling pathway is one such mechanism by which cell death during ischemia/reperfusion (IR) may play a role. Exposure of cells to certain stressful stimuli, such as TNF- α and IR, causes activation of neutral sphingomyelinase (nSMase). Downstream effectors of sphingolipid-signaling pathway have been shown to be involved in apoptosis in the cardiomyocyte. This work concentrated on the location, presence and role of sphingolipid-signaling proteins like factor associated with neutral sphingomyelinase activation (FAN) and nSMase in HR induced cell death in the cultured cardiomyocyte. The novel protein, rat FAN, was discovered and along with nSMase1 was shown to be expressed in the cardiomyocyte. Furthermore, FAN and nSMase1 were found to have substantial co-localization using immunofluorescence and confocal microscopy. Importantly, TUNEL analysis determined that FAN and nSMase1 play a critical role in the signaling pathway leading to cell death in the hypoxia/reoxygenated cardiomyocyte. These results suggest a model for HR mediated cell death by which FAN activates nSMase1 leading to ceramide and sphingosine production and subsequent cell death.

[TEMOR19] IMPLANTATION OF MYOGENIC STEM CELLS INTO ADULT MUSCLE: PAST AND FUTURE

Terence Partridge

Muscle Cell Biology Group, MRC Clinical Sciences Centre, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

Transplantation of precursor cells or stem cells has been so successful in the haematopoietic system, that we have been encouraged to try the same strategy for other tissues. In skeletal muscle, this was initially attempted in the form of grafts of tissue-cultured committed myogenic cells obtained from skeletal muscle. More recently, there has been great interest in the possibility of using more versatile precursor cells that can adopt a myogenic phenotype. Such cells show two properties that make them more attractive than myoblasts. First, they have been shown to be able to enter the muscle from the vascular system, raising the possibility of a systemic delivery to the widespread muscles of the body. Second, they are held to be capable of many cell divisions, providing the possibility of generating large amounts of muscle from small numbers of grafted cells. However, in practice, stem cells have not proven, so far, to exhibit these properties to any significant extent in skeletal muscle.

Future research will consist of identifying individual subsets of cells that show the best myogenic potential, and of identifying the signals that attract these cells to sites of incipient muscle regeneration and/or convert them to a myogenic fate. Part of the answer to these questions may lie in the fundamental cell biology of skeletal muscle, where the innate control mechanisms may play some part in limiting the efficiency of takeover by the grafted cells.

[TEMOR20] FUNCTIONAL LEVELS OF DYSTROPHIN EXPRESSION IN DYSTROPHIC MDX MOUSE MUSCLES BY ANTISENSE OLIGORIBONUCLEOTIDE MEDIATED SKIPPING OF THE MUTATED EXON

TA Partridge¹, QL Lu¹, S Fletcher², CJ Mann², SD Wilton²

¹ *Muscle Cell Biology, Medical Research Council Clinical Science Centre, Hammersmith Hospital, London, UK.*; ² *Experimental Molecular Medicine Unit, Centre for Neuromuscular and Neurological Disorders, Perth, Western Australia*

We have investigated the phenomenon of 'revertant' dystrophin-positive muscle fibres in the mdx mouse, showing that they are the result of a large variety of exon-skipping events. Individual colonies faithfully maintain their own particular pattern of skipped exons, implying that each is the product of a clone in which the 'revertant' event first occurred. Since, 'reversion' does not have the signature of a mutational event, we presume that it is an epigenetic phenomenon, an understanding of which might enable its use for therapeutic purposes. Since most DMD mutations in the dystrophin gene occur in the spectrin-like repeat region, which is largely dispensable, specific removal of one or more exons flanking the mutation so as to restore the reading frame would produce a Becker-like mRNA that could be translated into a shorter but still partially functional protein.

To this end, we have been using antisense oligonucleotides (AO) to disturb splicing of the dystrophin exons during pre-mRNA processing, and have previously demonstrated that 2'-O-methyl phosphorothioate AOs in mdx mouse muscle can induce removal from the mature dystrophin mRNA of exon 23, containing the nonsense mutation. The resulting in-frame transcript produces near full-length dystrophin. Refinement of AO design and delivery has induced exon skipping *in vitro* at 200 fold lower AO concentrations than previously. Improved *in vivo* delivery using a potent transfection protocol results in production of persistent dystrophin in large numbers of muscle fibres, sarcolemmal reassembly of components of the dystrophin associated complex and functional improvement of the treated muscle. This establishes practical feasibility of an approach applicable to a majority of cases of severe dystrophinopathy.

[TEMOR21] REGENERATIVE MYOGENESIS IN FES-INDUCED FUNCTIONAL RECOVERY OF HUMAN LONG-TERM DENERVATED MUSCLE

Marzena Podhorska-Okolow, Katia Rossini, Ugo Carraro

Institute of Histology, University of Wroclaw, Poland, C.N.R. Institute of Neuroscience, Neuromuscular Section, Laboratory of Applied Myology, Department of Biomedical Science, University of Padova, Italy

Following denervation, skeletal muscle undergoes rapid loss in both mass and contractile force. Myofibers undergo atrophy, with an accompanying series of changes in structure, biochemistry and physiology. Several-months denervation finally results in loss of myofibers, and their substitution by adipocytes and collagen sheets. Morphologic and molecular features of the long-term denervated muscle suggest that the original fibers are lost and those seen are the results of repeated cycles of cell death and regeneration. Markers of myogenic events in adult muscles are activated satellite cells and the presence of embryonic myosin and myogenic transcription factors.

Functional electrical stimulation of permanent denervated muscle recovers from severe atrophy the myofibers, and prevents apoptosis/necrosis and secondary degeneration. Recovery of muscle mass is the result of both re-growth of surviving fibers (FES biopsies present the chess board appearance of normal adult human muscles) and of regeneration of lost myofibers, which are detected by their content of embryonic myosin. The importance of these regenerative events in the FES rehabilitation protocol is related to the superior excitability and activation-contraction coupling capacity of early regenerated myofibers in comparison to long-term denervated, severely atrophic myofibers, which are hardly excitable before FES training.

At the ultrastructural level myofibers displayed typical features of muscle atrophy (angulated or round, small-size myofibers, with myonuclear grouping, myofibrillar changes, etc.), but accompanied by regenerative events (numerous activated satellite cells and myoblasts). The sarcoplasmic reticulum derived tubular aggregates (as "honey-comb" structures) were rarely observed, mainly in almost normal-size fibers. At electron microscopy we never find evidence of reinnervation. Furthermore,

the mitochondrial content is low (or very low in the severely atrophic myofibers), as expected in a muscle, which at best works against low load 20 min per day five times a week.

Taken together the results of clinical, morphologic and molecular analyses solidly demonstrate that two-year-long FES substantially reverses the severe atrophy of four-year denervated human muscles, and that generative/regenerative myogenic events significantly contribute to the functional recovery of long-term paralyzed human muscles.

[TEMOR22] DEMAND DYNAMIC BIO-GIRDLING IN HEART FAILURE IMPROVED EFFICACY OF DYNAMIC CARDIOMYOPLASTY BY LD CONTRACTION DURING AORTIC OUT-FLOW

GI Rigatelli, G Rigatelli, U Carraro (1)

cardiomyoplasty project unit, The Legnago General Hospital, Legnago (Verona) Italy; (1) Laboratory of Applied Myology, Department of Biomedical Sciences, University of Padova, Italy

The value of dynamic cardiomyoplasty has been brought into question by disappointing results produced by slow contraction-relaxation cycle and possibly degeneration of the latissimus dorsi muscle (LD) secondary to temporary tenotomy and chronic daily electrical stimulation. Objective of our study is to determine whether daily periods of rest introduced by demand stimulation in the continuous contraction protocol produce systolic assistance and improve clinical results. Twelve Dynamic Cardiomyoplasty patients (mean age 58.2 ± 5.8 years, M/F=11/1, sinus rhythm/atrial fibrillation=11/1) with dilated cardiomyopathy were enrolled in a randomized trial of Demand Dynamic Heart Bio-Girdling in a public regional teaching hospital. Periods of LD inactivity, each lasting several hours, were daily introduced by a heart rate-based demand regime. To avoid full transformation of LD, fewer impulses per day have been delivered, daily providing the LD with long periods of rest (Demand light stimulation). The contractile properties have been measured by transcutaneous non-invasive LD tensiomyogram interrogation (LD tensiomyogram). Bio-Girdle activation was synchronized to heart's beat by combining tensiomyogram and echocardiography. Clinic, echocardiographic and hemodynamic records, as well as aortic flow measurements by Doppler aortic flow wire were performed during the follow-up. Mean duration of the demand stimulation follow-up 40.2 ± 13.8 months. At five years, "Demand stimulation" shows: 1. No operative death; 2. 83% actuarial survival; 3. Highly significant 47.4% decrease of the NYHA class (from 3.17 ± 0.38 to 1.67 ± 0.77 , $p=0.0001$); 4. 41.6% improvement of LVEF (from 22.6 ± 4.38 to 32.0 ± 7.0 , $p=0.001$); 5. $7.5 \pm 3.0\%$ increase in aortic flow velocity peak in assisted vs. unassisted beats, and 6. Preservation of LD from slowness (TFF value 33 ± 7.86 at follow-up versus 15.8 ± 11.1 Hz just before switching from continuous to demand stimulation, $p=0.0001$) and muscle degenerative atrophy. In Dynamic Cardiomyoplasty the demand light stimulation maintains LD contraction properties over time, produces effective systolic assistance, and improves clinical results. Demand Dynamic

Bio-Girdling is a safe and effective treatment of end-stage heart failure in selected patients.

[TEMOR23] EXERCISE-INDUCED MUSCLE DAMAGE SUSCEPTIBILITY: A TOOL TO TEST IN VIVO ANTI-SENSE DMD THERAPY

Katia Rossini, Ugo Carraro

C.N.R. Institute of Neuroscience, Laboratory of Applied Myology of the Department of Biomedical Science, University of Padova, Italy

Awaiting more successful strategies of gene therapy, longer viability of dystrophic muscle is a major goal in Duchenne Muscular Dystrophies. An alternative approach to insertion of mini-genes is targeted-exon-skipping therapy to obtain shrunk dystrophin by antisense oligonucleotides against "splicing enhancer sequences", which in vitro selectively promote Becker-like Dystrophin mRNA and protein accumulation. We would like to test in vivo long-term efficacy and toxicity of oligos against the most clinically-relevant exons of human dystrophin gene. On the light of potential risks of gene therapy, major drawback of the antisense approach (i.e., life-long frequent administrations) represents a major advantage in safety, since this allows to temporary discontinue treatments in case of side-effects.

Littermates and mothers will be treated, and studied from two weeks after birth, analyzing leg muscles, after exercise-induced muscle damage tests, and finally respiratory muscles (diaphragm). Beside effects of oligo treatments on dystrophin expression at protein level, our micromethods allow to quantify muscle damage/regeneration on thin needle biopsies small enough to be performed on muscles of mice. We are confident that this approach to treat DMD is selective and effective, and that it will substantially prolong viability of the dystrophic muscle.

[TEMOR24] AVOIDING ISCHEMIC DAMAGE IN MUSCLES REDEPLOYED AS FUNCTIONAL GRAFTS

Stanley Salmons

Department of Human Anatomy and Cell Biology, The Sherrington Buildings, Ashton Street, University of Liverpool, Liverpool L69 3GE, Merseyside, U.K.

Functional adaptation (1) is the basis of several clinical applications that involve long-term electrical stimulation of skeletal muscle. In some of these, muscles are redeployed as functional grafts. For example, the gracilis muscle may be reconfigured as a urinary or anal neosphincter to treat incontinence, and the latissimus dorsi muscle (LDM) may be transposed into the chest to provide cardiac assistance in patients with heart failure.

The success of such procedures depends on the graft remaining viable. Chronic stimulation alone is not especially damaging; extensive damage, with fibrofatty replacement of muscle tissue, is invariably due to ischaemia. Raised intramuscular pressure during contraction can compromise blood flow. When the muscle is stimulated too frequently the muscle works under partially anaerobic conditions, which are unus-

tainable. Stimulation delivered in less frequent bursts allows more effective perfusion.

Ischaemia also results from the unavoidable sacrifice of arteries during mobilization. Incompletely perfused regions of the graft are then damaged during subsequent stimulation. Electrical stimulation of the muscle before mobilization can be used to enhance natural anastomotic connections, promoting recovery of blood flow to normal levels within days. For example, prestimulation of the LDM resulted in a 10.3-fold increase in flow after mobilization and recovery, compared to 4.5-fold for the more invasive vascular delay technique favoured by plastic surgeons (2). We now use prestimulation routinely to prepare the LDM for reconfiguration as a skeletal muscle ventricle.

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[TEMOR25] ECTO-ATPDASE ACTIVITY OF α -SARCOGLYCAN. POSSIBLE ROLE IN THE PATHOGENESIS OF SARCOGLYCANOPATHIES

Dorianna Sandonà¹, Stefano Gastaldello¹, Tiziana Martinello¹, Romeo Betto²

¹ *Department of Biomedical Sciences, University of Padova, Viale G. Colombo, 3, 35121 Padova;* ² *CNR Institute of Neuroscience, Laboratory of Muscle Biology & Physiopathology, Viale G. Colombo, 3, 35121 Padova, Italy*

Extracellular nucleotides are important signaling molecules modulating various physiological responses. Nucleotides, liberated in the extracellular fluids as a result of cell lysis, exocytosis or efflux from transport proteins, exert their action through the binding to specific cell surface receptors: P1 and P2, activated by nucleosides and nucleotides, respectively. A central role in modulating nucleotides signaling is played by a family of ecto-enzymes, ectonucleotidases (NTPDases), that sequentially degrade ATP to eventually form adenosine. Thus, activity of ectonucleotidases both terminate the signaling, generate new signaling molecules and mediate the salvage of purines. Striking past and recent evidences suggest the occurrence of extracellular ATP signaling in skeletal muscle, even though its physiology remains elusive.

We previously demonstrated that α -sarcoglycan is an ATP binding protein that attributes to the purified dystrophin complex the ability to hydrolyze ATP (Betto et al., *J Biol Chem* 19, 7907-12, 1999). As the putative ATP binding site is located on the extracellular domain, our data suggested that α -sarcoglycan could be an ecto-nucleotidase. Genetic defects responsible for the primary or secondary deficit of α -sarcoglycan generate severe muscular dystrophies, suggesting that α -sarcoglycan is recipient of an essential function for muscle physiology and survival. Therefore, we decided to further investigate the putative ATP hydrolyzing activity of α -sarcoglycan. During differentiation of C2C12 cells, the expression level of α -sarcoglycan rises in parallel to the level of total NTPDase activity of cells. Importantly, about 20% of the activity is inhibited by an antibody

specific for the extracellular portion of α -sarcoglycan. This result demonstrates both that α -sarcoglycan contribute to the ATP-hydrolyzing activity of C2C12 myotubes and also that these cells express other ecto-enzymes. To better characterize the activity of α -sarcoglycan, we then transfected the protein in HEK 293 cells, normally low in NTPDase activity. HEK 293 cells transfected with α -sarcoglycan demonstrate a large rise of activity, which requires as substrate ATP or ADP (at a ratio 3:1) while is not effective with other triphospho-nucleosides. Moreover, at variance with other NTPDases, α -sarcoglycan activity is stimulated by the contemporary presence of both Ca^{2+} and Mg^{2+} . Our results conclusively demonstrate that α -sarcoglycan is an enzyme that hydrolyze extracellular ATP, thus participating to the signaling of extracellular nucleotides. These finding offers new clues on the possible effects of the lack of α -sarcoglycan activity in the pathogenesis of muscular dystrophies.

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[TEMOR26] SIGNALING PATHWAYS IN HYPERTROPHY AND ATROPHY

Trevor N. Stitt, Michael Gonzalez, Esther Latres, Venus K.-M. Lai, Lorna Nunez, Frank J. Panaro, Lawrence Milosic, Roy Bauerlein, Erqian Na, Yekatarina Timofeyeva, Elizabeth Zlotchenko, George D. Yancopoulos, David J. Glass

Regeneron Pharmaceuticals, 777 Old Saw Mill River Road, Tarrytown, NY, 10591-6707, USA

Skeletal muscle hypertrophy occurs during an increase in work or load on the muscle. Insulin-like growth factor 1 (IGF-1) is a small protein growth factor which can induce skeletal muscle hypertrophy; however the signals downstream of IGF-1 which are required for hypertrophy are only now being identified. Investigations of the phosphatidylinositol-3 kinase (PI3K)/Akt pathway, which is stimulated by IGF-1, will be discussed.

Skeletal muscle atrophy occurs during pathological conditions such as cancer and AIDS, and as a reaction to decreases in activity and load. Two genes which have been demonstrated to be involved in the atrophy process are the E3 ubiquitin ligases: Muscle RING Finger 1 (MuRF1) and Muscle Atrophy F-box (MAFbx, also known as Atrogin-1), the latter being a member of the "SCF" family of E3 ubiquitin ligases. Mice lacking either MuRF1 or MAFbx were produced; skeletal muscle was spared during denervation, as measured by maintenance of skeletal muscle mass. These mice have now been analyzed further: for changes in other models of atrophy, for maintenance of muscle function; and for differences in gene regulation. These analyses demonstrate key differences between MuRF1 and MAFbx pathways.

[TEMOR27] THERMODYNAMICS & KINETICS
OLD LAWS & NEW MEDICINE FOR HUMAN DISEASES

Richard Strohmman

University of California at Berkeley, Department of Molecular and Cell Biology, Berkeley, CA 94720-3206 E-mail: strohman@uclimk4.berkeley.edu

The search for new laws in biology is associated with the beginnings of molecular biology and with Max Delbruck who brought the idea from physics that living systems, while ultimately reducible to universal physical laws, displayed qualities not shared by non-living matter, and might harbor new laws unique to life itself. The rich history of 20th century molecular biology has included a failure to find such laws (1,2), and that failure is seen as the major factor driving biological research to find so called genetic laws from which would come understanding of life and of our many diseases, inherited or otherwise. And of course, this failure has prompted the question: "if not in the genome ... and organisms are clearly programmed in some sense of that word ... then where is the program and what is its nature? Fifty years after the start-up of molecular biology the philosopher Thomas Nagel, at the Ciba Conference on *The Limits of Reductionism in Biology* in 1997 (1), reflected on this stubborn absence of understanding in biology. He concluded: "...our finite mental and computational capacities mean that we either cannot grasp the ultimate physical explanation ... or we can't fruitfully link the old universal physical laws to higher order phenomena." Therefore, he said, repeating Delbruck, perhaps biologists needed to discover new laws for life.

The nature of the linkage between physical laws and phenotypes of living matter has now begun to take on new dimensions, although one such key juncture has been known for some time: the laws of thermodynamics and kinetics are "causally" linked to the phenotypes of organisms through the agency of dynamical systems. Sadly this essential point has been all but ignored in the rush to find agent-based genomic-proteomic explanations. Looking back, that substitution of agents (genes and proteins) for agency (self organizing open systems of gene-encoded proteins) must be recognized as an epistemological error of great moment. Nevertheless, *normal science* (3) has proved true to form and molecular biology has now, inadvertently, followed the genotype-phenotype trajectory to an end point identified as open, self-organizing, molecular systems within which controls-constraints are distributed among many interacting subsystems each with robust behavior. The quasi-universal metabolic system represented by the Chart of Metabolism known to all biochemists is a *prime* example of such a system.

I will summarize the work carried out recently by Richard Veech at the NIH (USA), who was unable to attend this meeting, and his collaborators. They have applied metabolic control analysis (MCA) directly to living systems, including *in vitro*, *in situ*, and *in vivo* models of human muscular dystrophy (heart and skeletal muscle), and other complex diseases. This work strongly suggests that Duchenne's Muscular Dystrophy (DMD) is caused by a gene (dystrophin)-related defect hitherto unexplored. That defect is located in a primary energy deficiency: a failure to metabolize glucose. While the precise

link between the gene defect and the metabolic cause of muscle energy loss and cell death is still unknown, ongoing research clearly shows the defect to be corrected in the models mentioned by a simple substitution of ketone bodies for glucose. This hypothesis for ketone reversal of gene-related cell death in Alzheimer's and Parkinson's disease has already been tested and is confirmed for model systems. Therefore, the possibility exists for a therapy for DMD based on routine use of physiological levels of ketones suitably chemically modified to allow direct feeding and efficient assimilation.

[TEMOR28] PHYSICAL THERAPIES IN DEGENERATIVE SYNDROMES OF THE MUSCULOSKELETAL SYSTEM

Andrea Venturin, Marco Ortolani

Dipartimento di Specialità Medico Chirurgiche, Medicina Fisica e Riabilitazione, Azienda Ospedaliera – Università di Padova

Physical therapies represent an helpful treatment in patients affected by degenerative syndromes of musculoskeletal system only if considered as a part of a rational therapeutic program and not an alternative to pharmacologic or not-pharmacologic approaches. Unfortunately, acritic over-use of standard physical therapies is common in every-day practice. Prescriptions need a correct knowledge of different approaches and techniques, proper applications, eventual side-effects and specific contraindications, and extent of achievable advantages. Therefore, in Italy, only doctors with a speciality degree in Physical and Rehabilitation Medicine have permission to prescribe them.

Physiokinesis treatments of degenerative syndromes of musculoskeletal system are meant to: 1. Reduce or resolve pain. 2. Prevent or decrease muscle contractures. 3. Maintain or increase joint function. 4. Stimulate muscle trophism 5. Recover psychologic temper. These goals could be achieved by rational use of exogen and endogen thermal therapies, cryotherapy, electrotherapy, massotherapy and specific kinesitherapy.

Massotherapy has an essential utility among physical therapies because of its effect on skeletal muscle, improving vasal tonus, secondary to mechanical stresses on muscle synapses and muscle fibers. It is helpful for muscle contractures, for hypotrophy and represent a pre-treatment for the following kinesitherapy that is really an essential component of any therapeutic program in subjects with degenerative syndromes of musculoskeletal system. Too often, injuries appear in these patients after wrong prescriptions or even more due to self-prescribed physical exercises.

[TEMOR29] PHARMACOLOGICAL APPROACH TO THE PREVENTION OF SKELETAL MUSCLE MYOPATHY IN HEART FAILURE

Giorgio Vescovo, Luciano Dalla Libera (1)

*Internal Medicine II, S. Bortolo Hospital, Vicenza, Italy;(1)
CNR Institute of Neuroscience, Unit for Neuromuscular Biology and Pathophysiology, Department of Biomedical Sciences, University of Padova, Italy*

Heart Failure is a syndrome leading to a skeletal myopathy with muscle atrophy and shift toward fast contracting fibres. These changes produce a limitation of exercise capacity due to early appearance of fatigue and dyspnoea. It has been shown that the major cause of atrophy is muscle waste due to skeletal muscle myonuclei apoptosis. Apoptosis is triggered by circulating cytokines and their second messengers, in particular sphingolipids. We used several pharmacological approaches to block apoptosis. Thalidomide, an inhibitor of TNF α biosynthesis, was used without success. More successful was the attempt with blocking the Angiotensin II receptors in that apoptosis and atrophy could be prevented. Another useful approach was the block of the apoptotic cascade involving sphingomyelinase and caspases. This can be successfully done both in

vivo and in vitro with a natural occurring substance: carnitine that has been shown to be able to block both sphingomyelinase and mitochondrial caspases. Apoptosis could be inhibited with subsequent prevention of the myopathy (atrophy and MHCs shift) and improvement of exercise capacity and symptoms. We have also recently shown that high doses of GH, able to increase the circulating levels of IGF-1, can block apoptosis, prevent atrophy and preserve from the shift toward the synthesis of fast myosins.

We therefore suggest that it is possible to block the apoptotic cascade and prevent muscle waste and atrophy, the next step is to push research into heart failure in humans and maybe to other myopathies in which it has been shown that apoptosis plays a determinant role in producing myocyte loss and see whether the improved muscle trophism can be reflected in an amelioration of exercise tolerance.

Recent studies have also demonstrated that the post-transcriptional regulation of fibre type and size are mediated through two different pathways: the calcineurin FK-506-FKB and the mTOR Rapamycin-FKB receptor respectively. Fibre type is also mediated by the transcriptional co-activator PGC-1 α . These observations open up the avenue for selective pharmacological interventions able to modulate separately fibre type and size.

“CARDIAC BIOASSIST ASSOCIATION”

History: Founded in year 2000 - <http://www.cb2a.org>

First Congress: Lübeck, Germany. May 24-26, 2001

Second International Congress of the Cardiac Bioassist Association

PROGRAM AND ORGANISING COMMITTEE

Directors: JC Chachques, MD, PhD and JN Fabiani, MD.

Location: European Hospital Georges Pompidou, 20 rue Leblanc, 75015 Paris, France.

Date : October 9-11, 2003

Call for abstracts: deadline June 15, 2003 (information: j.chachques@brs.ap-hop-paris.fr)

PROGRAM

Session 1: Experimental models of heart failure: What is new ?.

Session 2: Cellular myocardial support, angiogenesis, gene therapy.

Session 3: Tissue-engineered cardiac muscle and valves.

Session 4: Dynamic cardiomyoplasty, aortomyoplasty, atriomyoplasty.

Session 5: Ventricular containment, myosplint, radiofrequency heating.

Session 6: Ventricular restoration, endoventricular patch plasty, mitral annuloplasty, reduction ventriculoplasty.

Session 7: Atrio-biventricular resynchronization by multisite cardiac pacing.

Session 8: Heart transplantation. Ventricular assist devices.

Honored Lecture. Professor Alain Carpentier: Past, present and future of Cardiac Bioassist.

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