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Abstracts

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Electrical stimulation of denervated muscle in SCI: When and how

Kern H

Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and Dept. of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria
E-mail: helmut.kern@wienkav.at

During the last decade we contributed to rehabilitation in aging studying effects of physical exercise induced by Functional Electrical Stimulation (FES) in the special case of Spinal Cord Injury patients affected by complete injury of the Conus Cauda, a syndrome in which the denervated leg muscles are fully disconnected from the nervous system. Denervated human muscles become unexcitable with commercial electrical stimulators and undergo ultra structural disorganization within a few months from SCI, while severe atrophy with nuclear clumping and fibro-fatty degeneration appear within 3 and 6 years, respectively [1-4]. To counteract these progressive changes a novel therapy concept for paraplegic patients with complete lower motor neuron denervation of the lower extremity was developed in Vienna: home-based functional electrical stimulation of long-term denervated muscles (h-b FES). New electrodes and a safe stimulator for h-b FES have been designed to reverse severe atrophy by delivering high-intensity (up to 2,4 J) and long-duration impulses (up to 150 ms) able to elicit contractions of denervated skeletal muscle fibers in absence of nerves [5,6]. Specific clinical assessments and trainings were developed at the Wilhelminenspital Wien, Austria [7], based on sound evidence from animal experiments [8]. Main results of the clinical study on patients which completed the 2-year h-b FES training were: 1. significant increase of muscle mass and of myofiber size, with striking improvements of the ultra-structural organization; 2. recovery of tetanic contractility with significant increase in muscle force output during electrical stimulation; 3. capacity to perform FES-assisted stand-up and stepping-in-place exercise [9-13]. The study demonstrated that h-b FES of permanent denervated muscle is an effective home therapy that results in rescue of muscle mass, function and perfusion. Additional benefits are improved leg cosmetic appearance and enhanced cushioning effect for seating.

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Ultra-structural rescue of long-term denervated human muscle by Functional Electrical Stimulation (FES)

Boncompagni S (1), Carraro U (2), Mayr W (3), Kern H (4,5), Protasi F (1)

(1) CeSI - Center for Research on Ageing & DNI - Dept. of Neuroscience and Imaging, University G. d'Annunzio of Chieti, Italy; (2) Translational Myology Lab., Dept. of Biomedical Sciences, University of Padova, Italy; (3) Dept.

of Biomedical Engineering and Physics, University of Vienna, Austria; (4) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (5) Depat. of Physical Medicine and Rehabilitation, Wilhelminenspital, Vienna, Austria.
E-mail: s.boncompagni@phobos.unich.it

Muscle fibers and motor neurones are functionally and structurally dependent on each other (1). The interdependence of the two systems becomes obvious when the cross-talk between muscle and nerve is interrupted, i.e. in neuropathological conditions, or as a result of traumatic events such as spinal cord injuries (SCIs) (2). Denervation causes severe loss of muscle mass (atrophy), and significant functional and structural alterations of the fibers. Following Spinal Cord Injury (SCI), motor neurons do not reconnect easily to muscle fibers, probably because of dramatic changes which causes their almost complete degeneration. This argument raises some important questions that need to be addressed. Should the degeneration of muscle fibers be avoided to facilitate possible re-innervation events? Can extreme muscle wasting, due to denervation, be reversed in the absence of innervation?
Functional electrical stimulation (FES) is currently widely used in the treatment of many patients affected by peripheral nerve damage, incomplete SCI, and when muscle of the extremities are still connected to motor neurons, i.e. in "spastic" patients. However, the clinical applications of standard FES is not effective in promoting contraction in those patients in which muscle fibers are not connected to motor neurons and have undergone severe atrophy. For this reason, paraplegic patients affected by complete lesion of the conus cauda, usually do not receive any specific treatment and suffers severe secondary complications (osteoporosis, pressure sores, decubital ulcers, etc.) due to poor blood supply to the denervated areas.
Recently though, a new generation of stimulators for FES has been specifically designed with the aim of reversing muscle atrophy in paraplegic patients affected by complete lesion of the conus cauda (3). Clinical results indicates that muscle mass and function are significantly restored by this treatment. Human muscle biopsies from patients trained for prolonged periods of time with the newly developed FES stimulators, gave us the unique opportunity of studying the restoration of structure in long-term denervated fibers in absence of innervation.
FES stimulation induced surprising recovery of muscle fiber ultrastructure even in patients that had been denervated for prolonged periods before the beginning of FES training (up to 2 years) and whose muscles had almost completely lost muscle specific internal organization (4, 5). 90% (or more) of the fibers analyzed by EM showed a striking recovery of the ultrastructural organization of both myofibrils and Ca^{2+} handling membranes, i.e. transverse tubules and sarcoplasmic reticulum, deputed to activate the contraction of contraction. Interestingly, structural restoration of contractile elements and sarcotubular system follows a pattern that mimics some aspects normal muscle differentiation.
These studies proves that FES is effective in reversing muscle atrophy, and in restoring skeletal fibers ultrastructure even after extended denervation and inactivity. These results are of interest both from a basic biological perspective, since this recovery occurs in the absence of innervation, and from a clinical point of view, since the difficulty with poor recovery of long-term denervated muscle has been a longstanding problem in the field of SCI rehabilitation.

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Stimulators and electrodes for large and small denervated muscles with reference to nerve stimulation equipment. Design principles for surface and implantable devices

Mayr W (1), Hofer C (2), Krenn M (1), Kneisz L (1), Unger E (1), Kern H (2,3), Carraro U (4)

(1) Center of Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (3) Dept. of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria; (4) Translational Myology Lab., Dept. of Biomedical Sciences, University of Padua, Italy
E-mail: winfried.mayr@meduniwien.ac.at

The history of contemporary neuroprosthesis is based on grown comprehensive physiological knowledge developed under difficult experimental conditions over centuries and a technical breakthrough mid of 20th century with the Nobel awarded invention of the transistor. This milestone was the starting point for most of today known applications of Functional Electrical Stimulation (FES) from the very beginning relying on both implantable and non-invasive equipment.
Electronics developed with incredible speed towards miniaturization, operation speed, reduction of power uptake and increase in battery life, which supported growing complexity of stimulators and their control, whilst reducing

necessary size of devices. What remained constantly delicate and limiting was and is interfacing between technical solutions and living tissue. Special care is necessary in general as soon as materials and combinations of materials get in contact with an organism, as in enclosing implanted electronics, and problems multiply as soon as electrical current is delivered via a contact interface of an electrode. Finally biomaterials and their combination and processing are the decisive key features that decide upon success or failure of neuroprosthesis to much higher extend than level of complex electronic functionality.

The ultimate problem is safe delivery of electrical charge through appropriate electrode geometries. Electrodes are electron conductors, tissues are ion conductors. As soon as both get in contact, tissue acts as an electrolyte and electrochemical processes are initiated. Any electrical current across this interface enforces electrochemical processes, direct current is always associated with persistent electrochemical processes accompanied by oxidation and reduction, changes in pH, cell damage and electrode corrosion in dependence of its magnitude and duration. Charge balanced impulse currents, as needed for induction of action potentials in nerve or in muscle fibres, can be delivered safely, if their impulse charge is kept below the electrode material and size dependent "charge injection limit" (CIL). Below CIL charge can be shifted across the electrode tissue interphase and back without remaining electrolytic changes, higher amounts of impulse charge even compensated by equal charge backflow induce permanent changes in tissue milieu and electrode corrosion, in further consequence tissue damage.

To get some idea on the dimension of the problem, we can calculate CILs for various applications in nerve and muscle stimulation. For stainless steel 316L as a representative example CIL is given with 50µC/cm^2, which is the basis for the following exemplary calculations.

Delivering impulses for nerve stimulation via implantable electrodes requires at least an amplitude range to 2 mA at an impulse width of 1mA, which means 2µC per impulse and the need of a minimum electrode size of 2mm x 2mm (4mm^2). For surface electrode based nerve stimulation, again with an impulse width of 1ms, we need up to 100mA resulting in an impulse charge of 100µC and a minimal electrode size of 2cm^2. The latter condition should not be too difficult to comply with in practical applications, the restriction to minimally 4mm^2 contact size for implantable electrodes has severe consequences for applications where high selectivity or high resolution are needed, e.g. in retinal electrode arrays.

Direct muscle stimulation needs substantially wider impulses and higher intensities for both implantable electrodes and non-invasive surface electrodes. For implantable solutions we can consider up to 5mA at 50ms, which means to deliver 250µC and a minimum contact surface of 5cm^2 as a rough estimate. For surface electrodes we can calculate up to 200mA at 50ms or 10000µC via minimally 200cm^2.

Overall miniaturisation of electrodes is limited by CIL dependent size minimum versus necessary current (charge) delivery. The problem is limiting selectivity and resolution of electrode arrays for nerve stimulation in particular in implantable systems. The problem grows dramatically for direct muscle stimulation due to 100 times and more increase of necessary charge per impulse. For muscle stimulation the minimum size of implantable electrodes raises issues of too large foreign body parts integration in soft tissue, whereas non-invasive skin attached electrodes can induce skin damage in case of locally excessive current density e. g. by inhomogeneous contact pressure distribution. A secondary limitation in miniaturisation of implantable devices remains in necessary battery size despite all substantial technological advances, again more relevant in solutions for muscle than for nerve stimulation due to the substantial differences in delivery of electrical charge.

Examples for recent successful developments

RISE Stimulators and surface electrodes: Prototypes of stimulators and surface electrodes were designed and implemented in the Center of Medical Physics and Biomedical Engineering, Medical University of Vienna (Mayr) in close interdisciplinary collaboration with Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation & Dept. of Physical Medicine, Wilhelminenspital Wien, Austria (Hofer, Kern) [1-3]. Finally, a small series of devices was produced and allocated to enrolled subjects of the EU RISE Project. The stimulator delivers all the evolving stimulation protocols dictated by the EU RISE Project Strategies (based on early twitch- and, finally, tetanic-contractions), to recover long-standing muscle atrophy in Spinal Cord Injury (SCI) complicated with complete lower motor neuron denervation of the limbs, or at least of the quadriceps muscles [4-9]. EC approved products (Stimulette den2x and purpose constructed large safety electrodes) are now commercially available (Schuhfried, Vienna, Austria) [1-3]. Finally, a small series of devices were produced and for free allocated to enrolled subjects of the EU RISE Project. The stimulator delivers all the evolving stimulation protocols dictated by the EU RISE Project Strategies (based on early twitch- and, finally, tetanic-contractions), to recover long-standing muscle atrophy in Spinal Cord Injury (SCI) complicated with complete lower motor neuron denervation of the limbs, or at least of the quadriceps muscles [4-9]. EC approved products (Stimulette 2x and purpose constructed large electrodes) are now commercially available (Schuhfried, Vienna, Austria).

Denervated muscles of large and medium size may by stimulated with old bench electrical stimulators (e.g., the Phillips etc) or the MED-EL STI-WELL, but they may induce only twitch-contractions, since the lack the key features of the RISE-based electro-stimulator, very high outputs and very short inter-pulse pause in the train stimulation.

Small denervated muscle (up to the size of the human tibialis anterior) are stimulated by several commercial stimulator for denervated muscle (e.g., the MEDICAL TECHNOLOGY SPE1, Torino, Italy), since they are limited in to 25 mAmp output, for safety reasons and production aspects.

Implantable devices. The miniaturization of the electronics is opening tremendous chances, in particular for neuromuscular stimulation, but even for direct stimulation of denervated muscles. For the first case, please, see in the present Abstracts Volume: Jarvis J., Bijak M., Lanmueller H., Haller M., Unger E., Lindenthaler W. New Vienna/Liverpool implantable stimulator. European Journal of Translational Myology/Basic Applied Myology 2013; 23:this issue [10-11]. From it we excerpted the key statement: *An important*

step forward was made in 2012 with the introduction of a 4x4 mm device that has sufficient processing power to allow a fully remotely programmable device to be made small enough for implantation in the mouse.
This will open to researchers, analyzing mechanisms and potentials for applications of FES, the infinite world of molecular approaches in knock-out and over-espressing mice strains.
For the second case, several abstracts are presented in this EJTM/BAM Issue. In particular, the need, in vagus stimulation, for implantation of selective electrodes and implementation in electro-stimulators of selective stimulation protocols is extensively discussed by Rozman R, Pečlin P [12]. It is our hope that the new developments may provide hopes and results to many more patients than it is actually general belived.

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FES for large denervated muscles: Comments of patients and practical demonstrations

Fiorucci R (1), Piscioneri A (2)

(1) Città di Castello; (2) Milano, Italy
E-mail: info@avvrobertofiorucci.it;
E-mail: antonio_piscioneri@hotmail.com

Commenti di pazienti RISE

Hotel Augustus, Montegrotto Terme, 14 Novembre 2013.

Ringrazio il Prof. Carraro, il Prof. Kern, Il Prof. Cerrel-Bazo e la Dott.ssa Zampieri per questa opportunità. Il Prof. Cerrel-Bazo nel maggio 2003 mi comunicò che il Prof. Kern di Vienna cercava soggetti con muscoli completamente denervati per una sperimentazione europea, il Progetto RISE (Alzati!). Entrai così nel gruppo dei pazienti RISE, seguirono le visite a Vienna e la consegna di un prototipo di elettrostimolatore per applicazioni domiciliari quotidiane, domenica esclusa, controlli semestrali e check up completo ogni 3-5 anni a Vienna, con prelievi bioptici muscolari e loro analisi. Sono ormai quasi 10 anni che mi sottopongo ogni mattina, salvo rarissime eccezioni, all'elettrostimolazione dei muscoli interessati dalla lesione midollare, ma completamente denervati. La terapia dura circa 3 ore di elettrostimolazione, senza considerare le pause, e cioè per circa 4 o 5 ore totali. Durante l'elettrostimolazione ai glutei ed ai polpacci effettuo anche esercizi fisici per la parte non interessata dalla lesione; a giorni alterni nel pomeriggio aggiungo altra attività fisica per la muscolatura del tronco e delle braccia e la posizione eretta statica per almeno un'ora. Solo per posizionare gli elettrodi sui glutei ho bisogno di aiuto, mentre per il resto faccio tutto da solo. Prima dell'eletrostimolazione la mia sensibilità globale si fermava all'ombelico, oggi "sento" tutto il corpo, anche se a livello della pella nulla è cambiato. Sento il caldo ed il freddo e la pressione da contatto, nel senso che so individuare il punto esatto dove vengo toccato, sensazione che mi viene dal profondo, perché non ho sensibilità a livello della pelle. Inattivi per circa 3 anni dopo la lesione, i muscoli erano praticamente scomparsi. Il quadricipite della gamba destra ha iniziato a contrarsi solo dopo circa 7 mesi di elettrostimolazione, mentre per quello sinistro ci sono voluti più di 3 anni. Attualmente sono entrambi quasi allo stesso livello di risposta contrattile alla stimolazione elettrica. Con

Abstracts

l'elettrostimolazione muscolare ho raggiunto un notevole miglioramento anche nel funzionamento degli organi interni come l'intestino e mi sto adoperando per migliorare tutte le altre funzioni. L'elettrostimolazione migliora la circolazione del sangue, brucia i grassi e gli zuccheri al pari di tutte le attività fisiche, e forse anche di più, elimina le tossine permettendo al cervello di lavorare al meglio. Svolgendo un'attività prettamente intellettuale, mi trovo nelle migliori condizioni e, rispetto a molti miei coetanei, sono più giovane fisicamente e mentalmente. Prima del trattamento vedevo che la mia pelle era brutta e "vecchia", ora che sono realmente più vecchio, dopo l'elettrostimolazione la pelle diventa rosea, fresca, chiara e giovane, come se fosse quella di un'altra persona molto più giovane di me. Nel 2010 sono andato da Città di Castello a Vienna in auto guidando per oltre 900 km (4 ore di guida, 1 ora e ½ di sosta, 4 ore di guida) in andata e poi in ritorno senza alcun problema, come facevo spesso quando viaggiavo prima dell'incidente. Nel maggio di quest'anno, per cattiva alimentazione (reazione al glutine), ho avuto un blocco intestinale che mi ha costretto a stare seduto senza interruzioni sul vaso per 9 ore . Vi ricordo che dopo 4 ore di solito la pelle si piaga. Dopo queste 9 ore, ho elettrostimolato per 90 minuti i punti di carico. Nei giorni successivi, si era formato a livello superficiale un velo scuro di cellule morte, rimosse le quali, la pelle sottostante era rosea ed integra, senza alcuna piaga. Ho avvertito solo un pò di bruciore che è continuato per pochi giorni. Assumo acido alendronico soltanto la domenica ed avverto una qualche sensazione riparatrice della lesione alla colonna vertebrale, nervi compresi. Con l'elettrostimolazione ho riacquistato la cognizione completa del mio corpo anche al di sotto dell'ombelico, ed ho recuperato il controllo di alcuni muscoli del busto sotto il livello di lesione.

Credo che i risultati che io ed altri colleghi italiano abbiamo raggiunto con la elettrostimolazione domiciliare RISE siano così importanti da giustificare la richiesta al Sistema Sanitario Nazionale di diffonderne la conoscenza e di sostenerne i costi finanziari e di personale specializzato nell'assistenza mediante terapia "RISE" per quanti ne possono trarre beneficio, almeno nei casi idiscutibili che noi ben rappresentiamo. La nostra condizione incontra le stesse difficoltà che incontrano i malati di "malattie rare", ma non per questo i soggetti interessati devono avere meno diritto alle cure di altri malati. Lo stato di benessere che vi ho testimoniato è infatti condiviso da tutti gli altri amici paraplegici italiani in cura dal Prof. Kern, ma altri ne avrebbero bisogno e diritto. Alcuni di noi, Casotti, Lancelotti e Lecinni, oggi non hanno potuto partecipare per precedenti impegni. Oltre ad Antonio e a me, anche loro mandano saluti ed auguri di buon lavoro a voi tutti.

Mr. Roberto Fiorucci
E-mail: info@avvrobertofiorucci.it;

Comments of RISE patients

Hotel Augustus, Montegrotto Terme, November 14, 2013.
I would like to thank Prof. Carraro, Prof. Kern, Prof. Cerrel-Bazo and Dr. Zampieri for this opportunity to share my experience with you.
Prof. Cerrel-Bazo in May 2003 told me that Prof. Kern in Vienna was recluting subjects with completely denervated muscles for an European trial of home-based electrical stimulation. I accepted this opportunity, and after a visit in Vienna, I was enrolled in the "RISE" group of patients followed by Prof. Kern, which provided me with a prototype stimulator designed for everyday stimulation, except on Sunday. The protocol of treatment included frequent interim controls, complete check-up in Vienna approximately every 2 years, including muscle biopsies from stimulated muscles and further analyses of the permanently denervated muscles [1].
It is almost 10 years that I perform the home-based electrical stimulation every morning, except in rare occasions, stimulating the denervated muscles affected by the Spinal Cord lesion. Daily therapy lasts approximately 3 hours of stimulation, pauses excluded; and 4 to 5 hours in total, including pauses. During electrical stimulation of the glutei and calves, I also exercise the body parts not affected by the neural injury. In the afternoon of alternate days, I do in addition some physical activity for a minimum of one hour or more, to train the musculature of the trunk and of the arms in standing position. I need help only to place the electrodes on the glutei, while for the rest I do everything by myself. Before electrical stimulation, my body feeling was up to the navel, now I can "feel" the whole body, even though at the epidermal level nothing changed since lesion. I can feel the heat and the cold and the contact pressure, because I can indicate the exact point where I am touched. This feeling comes from the deep tissues, since the skin sensitivity is lost.
Being inactive for about 3 years since injury, the denervated muscles almost disappeared. The quadriceps of the right leg begins to respond to home-based electrical stimulation after about 7 months of treatment, while the left started to contract after more than 3 years of therapy. They are currently nearly at the same level of contractility. By electrical stimulation, I had also a considerable improvement of the functions of the internal organs, such as the bowel, and I am still working to further improve all of these functions. The muscle electrical stimulation improves blood circulation, burns fat at the same level of voluntary physical activities and perhaps even more. It induces the elimination of toxins, allowing the brain to work better. Since I do a purely intellectual job, I am now in the better conditions. I am physically and mentally younger compared to many of my peers. Before the electrical stimulation treatment my skin was bad and old looking mostly around the ankles, but after months/years of stimulation the skin is clear, young and pink.
In 2010 I went from Città di Castello to Vienna by car, a round trip driving for more than 900 km (4 hours driving, 1 hour and ½ of rest, and 4 hours driving again) without any problem, as it was before the injury. In May of this year, due to a bad diet (gluten), I had an intestinal block that forced me to sit on the pot for uninterrupted 9 hours. Usually, after 4 hours of impaction the skin forms ulcers. After the 9 hours of sitting, I did 1 hour and ½ of electrical stimulation on the contact points. During the following days, a dark layer of dead cells appeared on the surface of the skin, but removing this layer, the skin beneath it was pink, without any ulcer. I had just a little burning sensation for a few days.I take only alendronic acid on Sunday and I feel some amelioration on the lesion of the spinal cord. Thanks to electrical stimulation, I have not only the complete feeling of my body, but I can control some muscles of the trunk, under the lesion level. I think that the results obtained by the RISE home-based

electrical stimulation training are so important to justify the request to the Social Security System to spread the knowledge of this treatment. It is also reasonable to ask that it covers the costs of devices and of the personnel specialized in the "RISE" therapy for those patients that can benefit from it in the unequivocal cases that we here represent. Our condition is similar to that of "rare diseases" sufferers. Why the affected patients should have less rights for treatments than other patients? This condition of well-being is also shared by Leccini, Casotti and Lancelotti (today they could not come because of previous commitments) other paraplegic friends treated up by Prof. Kern. All of them, together with Antonio and I, thank you and wish you good work.

Mr. Roberto Fiorucci, Città di Castello, Italy
E-mail: info@avvrobertofiorucci.it

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Commenti di pazienti RISE

Gentile Dr. Carraro,
utilizzando in modo costante l'elettrostimolazione [1] non ho più avuto accenni di piaghe da decubito, ho una circolazione migliorata, la riduzione dell'osteoporosi, migliore funzione intestinale e tessuto muscolare aumentato e di conseguenza meno preoccupazioni ogni volta che collido contro oggetti o subisco lesioni cutanee. Non è semplice applicarsi tutti i giorni ma i risultati ottenuti e quelli che spero di raggiungere prossimamente mi spronano a continuare, inoltre vorrei che questa opportunità fosse parte delle procedure mediche standard da applicare a tutti i pazienti con lesione midollare come la mia e non solo ricerca di base e clinica. Purtroppo di norma nel nostro caso i muscoli denervati non vengono considerati degni di interesse e ci troviamo dopo pochi anni senza tessuto muscolare a far da cuscino ed impedire lesioni cutanee che spesso portano gravissime conseguenze, con l'unica prevenzione prospettata di un migliore cuscino antidecubito o operazioni chirurgiche per sanare piaghe oramai croniche. Ringrazio i teams del Dr. Kern e del Prof. Carraro, che mi hanno permesso di partecipare allo studio, persone che considero come amici oltre che scienziati della medicina.

Mr. Antonio Piscioneri, Milan, Italy
E-mail: antonio_piscioneri@hotmail.com

Comments of RISE Patients

Dear Dr. Carraro ,
using consistently electrical stimulation [1] I have no more hints of bedsores, but improved circulation , reduction of osteoporosis , improved bowel function and increased muscle tissue and, consequently, fewer worries whenever collide against objects or undergo skin lesions. It is not simple to apply every day, but the results obtained and those I hope to reach soon inspire me to continue. I take this opportunity to ask that electrical stimulation of denervated muscles became a standard medical procedure to be applied to all patients with spinal injury like mine and not only to those participating to basic and clinical research. Unfortunately, the norm in case denervated muscle injury is not to consider them worthy of managements. Indeed, after a few years we are remain without our muscle tissue, any more able to cushion our body and prevent skin lesions that often lead to very serious consequences with the only preventions proposed improved anti-decubitus cushion or surgery to heal chronic bedsores. I thank teams of Prof. Dr. Kern and Carraro , who have allowed me to participate in the study , people who I consider to be friends as well as scientists of medicine.

Mr. Antonio Piscineri, Milan, Italy
E-mail: antonio_piscioneri@hotmail.com

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FES for small denervated muscles. Educational reports

Case report I. Left leg polyomielitis syndrome worsened by three-year right leg partial denervation due to radiculopathy: extent of deficits and perspectives by FES

Marcante A (1), Zanato R (2), Stramare R (2), Kern H (3,4,), Zampieri S (3,5), Carraro U (3,5), Ricatti G, (5), Astolfi S (5), Masiero S (1)

(1) Physical Medicine and Rehabilitation, Dept. Neurorosciences, and (2) Radiology Unit, Dept. of Medicine, University of Padua, Italy; (3) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (4) Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria; (5) Translational Myology Lab., Dept. Biomedical Sciences, University of Padua, Italy.
E-mail: stefmasiero@unipd.it

We report on a case of a 74 years old man with a clinically evident atrophy of the right quadriceps femori caused by a radiculopathy L4-L5, spondylolisthesis L4-L5 and lumbar spinal stenosis. In 2010 an EMG examination showed a complete denervation of rectus femoralis muscle and a neurogenic sufferance of tibialis anterior and gastrocnemius medialis, so in the same year the patient underwent a surgery of vertebral stabilization L4-L5. After the surgery there were no improvements on pain and the patient also started to complain about a worsening of deambulation. Also before the radiculopathy the patient had an impaired gait as a chronic consequence of poliomyelitis in childhood. This disease provoked a flaccid paralysis of the left leg but the patient was able to walk using an knee-ankle-foot orthosis with ischiatic support and lived a normal life. The recent problem on the right leg after the surgery, was particularly relevant precisely because of the flaccid paralysis on the left. Based on these assumptions it is easy to understand why the patient underwent a second surgery, that was performed in 2013 and consisted in an L2-S1 stabilization. When the patient came to our attention, after several months of physiotherapy, he was almost unable to move using a walker. By electrostimulation test of right leg muscles, we found that while the posterior leg muscles and the distal anterior muscles were innervated and responded to both voluntary

and electrical stimulation, the quadriceps muscle did not responded to either neuromuscular stimulation (1 msec, 25 mAmp, 50Hz) delivered to small size 6x4 cm electrodes) or to electrical impulses for twitch-contraction of denervated muscle (300 msec, 1Hz), but limited to 20-25 mAmp due to pain induction. Even using very large electrodes 10x15 cm no contraction were observed with this pattern of stimulation even at the echomyographic observation. Despite this negative results, standing on experiences of the RISE project [1-9], the patient is stimulating his right quadriceps twice a day with a denervated-muscle pattern, using intermediate size electrodes to increase current intensity delivered to the muscle, and reduce discomforting sensations. Incoming changes in quadriceps excitability-contractility will be reported, in particular, dissociation of the two events.

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FES for denervated muscles. Educational case report II. Amyotrophic Neuralgic Syndrome: extent of contraction deficit and recovery by combining FES and other rehabilitation managements

Marcante A (1), Marziali A (1), Venturin A (1), Rossato E (1), Carraro U (2), Masiero S (1)

(1) Physical Medicine and Rehabilitation Unit, Dept. of Neurosciences; (2) Translational Myology Lab., Pept. Biomedical Sciences, University of Padova, Italy
E-mail: andrea.marcante@studentiòunipd.it

We report on a case of a 49 years man that was admitted in December 2009 with an evident muscle weakness and progressive atrophy of the left arm, preceded by pain on the left shoulder. Similar episodes occurred both to the patient at his twenties and to his father and son. EMG confirmed C5-C6 and C7-C8 total denervation of shoulder muscles. A similar episode at the right shoulder had a prompt remission by combined cortisone and electrotherapy. From January 2010 he was treated with an electrotherapy progressive protocol starting with exponential current 100-150 msec pulse duration, intensity 15 mAmp (delivered by the Neuroton stimulator, Phillips) to over-and infraspinatus, anterior, middle and posterior deltoid bundles, biceps and triceps brachii, extensor carpi and fingers. The patient recovered voluntary contractility of proximal muscles, that are no more electrostimulated. From six months after the clinical onset the patient was admitted to massotherapy and now is able to perform voluntary isometric and isotonic muscle potentiation with a left arm complete functional recovery.

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FES for small denervated muscles. Educational case report III: Steps toward a walking aid for unilateral denervated tibialis anterior

Zanato R (1), Marcante A (2), Masiero S (2), Kern H (3,4), Carraro U (3,5), Stramare R (1)

(1) Radiology Unit, Dept. of Medicine; (2) Physical Medicine and Rehabilitation Unit, Dept. of Neurosciences; (3) Dept. Biomedical Science, University of Padova, Italy; (4) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, (5) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria
E-mail: riccardo_zanato@yahoo.it

Electrostimulation of denervated muscle is still a controversial issue with lack of clinical studies based on patients with peripheral nerve lesions. We report on a case of a 26 years old man with a complete sciatic nerve injury related to a subtrochanteric fracture of the right femur caused by car accident in 2010. Femur fracture was correctly fixed with a long gamma nail but clinically, patient has still presented a complete anesthesia under his right knee and some pain on the gluteal region. The strength of the

hamstrings was almost spared, but flexion and extension of the ankle were impossible, with a severe impaired deambulation. In 2011 the patient underwent a surgery of neurolysis with removal of a voluminous neuroma and positioning of a 8 cm controlateral sural nerve graft. One year after the surgery the patient refers disappearance of gluteal pain, strengthening of the hamstrings but no improvement in the muscles of the shank, so we decided to perform an electrostimulation test to verify the response of tibialis anterior and tricep surae muscles. Using a Neuroton (Philips) stimulator, we applied a current of 10 to 30 mA with triangular monophasic waves, impulse length from 5 to 150 msec with a pause of 1 or 2 sec. The best muscular response with no pain for the patient was evident with a 20-25 mA current, impulse length of 150 msec and pause for 2 seconds. At home, the patient applied similar electrostimulation parameters using the electrostimulator SM1 (Demitalia, Medical Technology S.r.L., Torino, Italy) with the schedule: 2 sessions (lasting 30 minutes each) per day for the first month, then 5 sessions per week. After two months, the electrostimulation test revealed muscle contraction also with a 50 msec impulse length using a current of 25 mA. The denervated TA (at more than 1 year from sciatic nerve lesion and attempted surgical reconstruction) responded with twitches to adequate surface stimulation. Two months of "adequate stimulation" (150 msec Impulse Duration (ID), 25 mA) recovered excitability up to the point that a "tetanising" protocol may be attained, despite the fact that the twitch-training did not hampered the process of atrophy or improved kinetics of contraction/relaxation of the twitch induced by home-based surface Functional Electrical Stimulation (h-b FES). The next step in the process of functional recovery will be to recover mass and force of TA with a "tetanising" training (series of impulse trains of 2-3 second duration at intervals of 3 sec) against increasing resistance to dorsiflexion of the foot (by acting a "spring device" opposing foot dorsiflection). In conclusion the patient improved in two months of twitch-stimulation so much the excitability of the persistently denervated tibialis anterior that a tetanising protocol, with its therapeutic potential to be used in a “walking aid for denervated muscle”, would be the next “step” in the rehabilitation program.

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Performance of electrodes within the nerve cuff tested in vagus nerve stimulation and in vitro

Rozman R, Pečlin P

ITIS d. o. o. Ljubljana, Center for Implantable Technology and Sensors, Republic of Slovenia
E-mail: "Janez Rozman" <jnzrzmn6@gmail.com>

In recent decades, considerable efforts have been devoted to the development of neuroprostheses that interface the human autonomic nervous system with electronic implantable devices. Particular attention has been paid to vagus nerve stimulation (VNS) techniques that are to be used to treat, among others, a number of nervous system disorders, neuropsychiatric disorders, eating disorders, sleep disorders, cardiac disorders, endocrine disorders and pain.
Vagus fibers in the cervical distribution are made up of three types: 1. large-diameter myelinated A-fibers; 2. intermediate-diameter myelinated B-fibers; and 3. small-diameter unmyelinated C-fibers. The vast majority of vagus fibers are C; A and B-fibers are primarily cardiovascular and respiratory neurons. In the area of FES, multi-electrode cuffs have been used as stimulation electrodes as well as electrodes for the recording of an electroneurogram for more than 35 years. The trend in neural prostheses using selective nerve stimulation for electrical stimulation therapies however, is toward single-part systems having a large number of electrodes, each of which selectively stimulate neural tissue or record neural response.
In human case study, the cuff including thirty-nine platinum electrodes arranged in thirteen spiral sets, each having three electrodes (triplet), was temporarily installed on the mid-cervical left vagus nerve in the subject scheduled for carotid endarterectomy surgery. After the relevant position of the particular nerve compartment was identified, a profound slowing of the heart and the absence of cardiac contraction were elicited only using triplet1. The largest mean change in components of both ECG and mechanical events was elicited with quasitrapezoidal cathodic phase intensity ic = 2.5 mA, applied continuously before and shortly after the absence of cardiac contraction. It was shown that heart function was efficiently modulated by inducing varying degrees of slowing and blocking (sinus arrest) of the SA node and by concomitantly inducing varying degrees of slowing in AV conduction via selective stimulation of both particular compartments of the mid-cervical left vagus nerve and mainly innervating efferent B-fibers.
In experiments on an isolated porcine cervical left vagus nerve, a cuff containing a matrix of ninety-nine electrodes, was used. A quasitrapezoidal stimulating pulse was applied

to the nerve via an appointed triplet. The triplet and stimulus were configured to predominantly stimulate the B-fibres, minimizing stimulation of the A-fibres and by-passing the stimulation of the C-fibres. To assess which fibres made the most probable contribution to the neural response during selective VNS, the distribution of conduction velocity within the nerve was considered. It was shown that certain parameters and waveforms of the stimulating pulse, for which the contribution of the A-fibres to the neural response was slightly reduced and that of the B-fibres was slightly enlarged. However, in the present stage of development, fibre-type VNS remained rather limited.
In the study, the structural properties of a cold-rolled pure platinum 0.03-mm-thick foil used to manufacture the electrodes were also investigated. It was shown that the combined results of the resistivity and differential scanning calorimetry measurements provide good criteria for selecting appropriate thermal and mechanical working processes.
In in vitro study, cyclic voltammetry was used to investigate the electrochemical reactions at the electrode-electrolyte interface, to define a potential window within which the electrode could be safely used in selective VNS. Voltage transients retrieved during excitation with quasitrapezoidal biphasic current pulses however, were used to determine the maximum polarization across the electrode-electrolyte interface and to calculate cathodic charge injection capacity of the electrode. The results show that both, the tested stimulation pulse and electrode are suitable for efficient and safe VNS. The electrochemical properties of the electrode were also studied using the electrochemical impedance spectroscopy (EIS) technique. The equivalent circuit model of the interface between the electrode and neural tissue was extracted from the EIS data and simulated in the time domain using a pre-set current stimulus. The EIS results revealed capacitive charge transfer predominance, which is a highly desirable property.
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Vagus nerve stimulation and focal cerebral ischemia: a rodent model using an implantable electrical stimulator

D'Avella D (1), Denaro L (1), Carraro U (2), Jarvis JC (3)

(1) Neurosurgery, Dept. Neurosciences, and (2) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (3) School of Sport and Exercise Sciences, Byrom St Campus, Liverpool John Moores University, Liverpool, UK
E-mail: ugo.carraro@unipd.it

Considerable efforts have been devoted during recent years to develop devices to stimulate the human autonomic nervous system. In particular, techniques of vagus nerve stimulation (VNS) to treat a number of neurodegenerative and vascular central nervous system (CNS) dysfunctions, and neuropsychiatric, pain, sleep, eating, cardiac, and endocrine disorders. Our main goal is to extend published results of the effects of short term stimulation of the vagus nerve on the size of brain infarcts due to permanent or transitory arterial occlusion by an intra-arterial thin wire [1-3]. The Liverpool mini stimulator designed and developed by one of us will be used to stimulate the vagus nerve in the neck by implanting two loop electrodes sutured to the sternocleidomastoidus muscle. More selective cuff electrodes will be also tested. The encapsulated electronics, battery and optical switch will be implanted in the peritoneal cavity and the electrodes tunnelled to the neck. We are implementing the experimental plan by acute trials to evaluate the different components of the project in adult rats, that is: 1. the brain ischemia model; 2. the implantation in the peritoneal cavity of the mini electro-stimulator, tunneling of the wires to the neck, selection of the proper electrodes; 4. settings for acute and mid-term experiments. Preliminary results of acute experiments will be discussed.

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Abstracts

Vagus nerve stimulation: a multi-purpose experimental model in rodents

Carraro U (1,11), D'Avella D (2), Vindigni V (3), Bassetto F (3), Masiero S (4), Danieli D (1), Mammucari C (1), Nori A (1), Sandri M (1), Volpe P (1), Zampieri S (1,11), Mosole S (1,11), Patruno M (5) Martinello T (5), Francini F (6), Musarò A (7), Protasi F (8), Gargiulo P (9,10), Kern H (11,12), Mayr W (13), Rozman J (14), Jarvis JC (15)

(1) Dept. Biomedical Sciences and CIR-Myo, (2) Neurosurgery, Dept. Neurosciences, (3) Plastic Surgery, Dept. Neurorosciences, University of Padua, Italy; (4) Physical Medicine and Rehabilitation, Dept. Neurosciences, (5) Dept Comparative Medicine and Nutrition, University of Padova; (6) Dept. Physiology, University of Florence; (7) Institute Pasteur Cenci-Bolognetti, DAHFMO-unit of Histology and Medical Embryology, IIM, Sapienza University of Rome; (8) CeSI - Center for Research on Ageing & DNI - Dept. Neuroscience and Imaging, University G. d'Annunzio of Chieti, Italy; (9) Dept. Biomedical Engineering, University of Reykjavik and (10) Dept. Science, Education and Innovation, Landspitali University Hospital, Reykjavik, Iceland; (11) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (12) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria; (13) Department of Biomedical Engineering and Physics, University of Vienna, Austria; (14) ITIS d. o. o. Ljubljana, Center for Implantable Technology and Sensors, Republic of Slovenia; (15) School of Sport and Exercise Sciences, Byrom St Campus, Liverpool John Moores University, Liverpool, UK.
E-mail: ugo.carraro@unipd.it

Considerable efforts have been devoted during last years to develop electronic implantable devices to stimulate human autonomic nervous system. In particular, devices and protocols for vagus nerve stimulation (VNS) to treat, a number of neurodegenerative and vascular central nervous system dysfunctions including epilepsy, pain, sleep, eating, cardiac, endocrine and neuropsychiatric disorders [1-4]. Based on our experience of the management by functional electrical stimulation of musculoskeletal deficit associated with Spinal Cord Injury [5-12], we are, thus, planning a multi-purpose experimental rodent model to investigate, first of all the side-effects of the VNS [13-16], and in knock-out and over-expressing mice models the effects and mechanisms of VNS in several thoracic and abdominal organs. The first goal of our ongoing rat and mouse study model is to establish the most efficient use of manpower and resources and the sequence of collection and fixation of organs from neck (larynx and high esophagus), thorax (lungs, heart, esophagus and diaphragm), and abdomen to perform structural, ultra-structural, metabolic and molecular analyses.

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ischemia: an experimental study in the rat. Brain Stimul 2012;5:1-10. doi: 10.1016/j.brs.2011.01.009. Epub 2011 Feb 22.

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Friday November 15, 2013

CBC SK-AT 2007-13 "Mobility in elderly" Mobility rehabilitation in denervation, aging and oncology: Introduction

Kern H, Mayr W, Hofer Ch, Löfler S, Fruhmann H, Burggraf S, Krenn M, Cvečka J, Sedliak M, Carraro U, Zampieri S

Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria
E-mail: helmut.kern@wienkav.at

During the last decade we contributed to rehabilitation in aging studying effects of physical exercise induced by Functional Electrical Stimulation (FES) in the special case of Spinal Cord Injury patients affected by complete injury of the Conus Cauda, a syndrome in which the denervated leg muscles are fully disconnected from the nervous system [1]. We are now extending our studies to application of h-b FES to the larger cohort of elderly. In order to assess the effects of exercise on aging rehabilitation, we are analyzing by morphometric light and electron microscopy and molecular biology quadriceps muscle biopsies from young (23 years) [2] and senior male subjects: sedentary elderly and senior sportsmen (a peculiar group of subjects that performed life-long sport activities) with a mean age of about 70 years. The group of sedentary seniors was also exercised for 10 weeks with two different types of training (leg press or electrical stimulation) and the analyses performed before and after the training period. Preliminary results confirm the effectiveness of h-b FES [Kern et al., revised manuscript submitted]. Based on our recent observation of the presence of a subclinical myopathy in patients affected with newly diagnosed colorectal cancer [3,4], we are now extending our approaches to oncologic rehabilitation. The factors associated with the subclinical myopathy at this stage of disease are unknown. A comprehensive study on the potential molecular mechanisms that are responsible for this cancer-associated myopathy could possibly provide new diagnostic and prognostic markers and new therapeutic and rehabilitation targets to prevent the severe loss of muscle tissue which characterizes late-onset cancer cachexia [5].

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Histology results in sedentary senior after 9-week of 3 times a week Electrical Stimulation

Zampieri S (1,2), Rossini K (1,2), Mosole S (1,2), Carraro U (1) , Löfler S (2), Hofer Ch (2), Kern H (2,3)

(1) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (3) Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria.
E-mail: sanzamp@unipd.it

The loss in muscle mass, coupled with a decrease in specific force, has been associated in aging with a reduction in muscle fiber size, shift in fiber composition. Alterations in metabolic and physiological parameters with corroborating modulations in gene expression were also described. Training and regular exercise attenuate the signs of sarcopenia, increasing muscle strength while decreasing fall risk. On the other hand, several pathophysiologic conditions limit the ability to perform voluntary physical exercise. We addressed whether electrical stimulation (ES) is an alternative intervention to improve muscle recovery. We analyzed at structural level the effects of ES training on quadriceps muscle biopsies from healthy seniors with normal life style (n=16), without routine sport activity, before and after 9-weeks of electrical stimulation [1]. ES was able to significantly improve muscle power (Torque pre 1.42±0.34 Nm/kg vs 1.51±0.38 Nm/kg, $p< 0.05$) and functional performances in terms of TUGT, chair rise test, Stair test, 10m walking test of seniors, and stimulates a significant increase in size of fast type muscle fibers (pre 46.53±14.04 vs post 47.54+/-15.79 μm, $p<0.0001$). Immunofluorescence analysis revealed an increased percentage of small N-CAM-positive expressing cells in post training biopsies, compared to pre-trained muscle biopsies, while we did not observe a significant increase of the number of myonuclei within

myofibers in ES-trained samples compared to pre training muscle biopsies. Thus, ES increases the density of activated myogenic satellite cells in sedentary seniors. Of note, no sign of fibrosis and/or inflammatory cell infiltration was detected in treated muscles, suggesting that ES maintains the integrity of senescent muscle. Our data provide evidence that ES is a safe method to counteract muscle decline associated with aging by electro-stimulating seniors only 3 times a week.

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Muscle activity controls the association between Ca2+ release units and mitochondria in skeletal fibers

Pietrangelo L (1), Ambrogini P (2), Sartini S (2), Kern H (3), Boncompagni S (1), Protasi F (1)

(1) 1CeSI - Center for Research on Ageing & DNI - Dept. Neuroscience and Imaging, Univ. G. d'Annunzio, Chieti, Italy; (2) Dept. of Earth, Life and Environment Sciences - Section of Physiology, Univ. of Urbino Carlo Bo, Urbino, Italy; (3) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation and Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria.
E-mail: fprotasi@unich.it

At the most basic level, skeletal muscle contraction requires Ca^{2+} and ATP and, thus, is under direct control of two major intracellular organelles: Ca^{2+} release unit (CRU), and mitochondria. CRUs are the sites of excitation-contraction coupling [1], the process responsible for triggering Ca^{2+} release from intracellular stores, i.e. sarcoplasmic reticulum (SR), in response to a propagating action potentials in the T-tubule membrane . Mitochondria are the powerhouse of the cell, being responsible for aerobic production of ATP. CRUs and mitochondria in skeletal fibers are functionally and structurally coupled: a) entry of Ca^{2+} into the mitochondrial matrix is able to stimulate the respiratory chain [2]; b) we have recently discovered that, in adult skeletal muscle fibers, mitochondria and CRUs are structurally linked to one another by small stands, or tethers [3]. Here we tested the following hypothesis: muscle activity improves/maintains the correct association between CRUs and mitochondria, which is challenged by ageing and inactivity. Using electron and confocal microscopy, we studied: a) ageing human/mouse muscle fibers and b) denervated rat muscle (by nerve crush). Our quantitative analysis shows that ageing (in humans and mice) and transient denervation (14 days, in rats) results in decreased association between CRU and mitochondria (2-to-3 folds decrease), whereas exercise and re-innervation either maintains or rescues the association between the two organelles (up to control levels). Functional implication of maintained/rescued correct-association between CRUs and mitochondria is potentially large: indeed, efficient Ca^{2+} uptake into mitochondria likely depend on their position in respect to sites of Ca^{2+} release.

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Surface electrical stimulation vs. proprioceptive strength training in enhancement of muscle strength and functional performance in sedentary seniors

Cvečka J (1), Sedliak M (1), Hamar D (1), Tirpáková V (1), Löfler S (2,3), Krenn M (4), Mayr W (4), Kern H (2,3)

(1) Faculty of Physical Education and Sport, Comenius University, Bratislava, Slovakia; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) Department of Physical Medicine and Rehabilitation, Wilhelminenspital, Vienna, Austria, (4) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria.
E-mail: cvecka@fsport.uniba.sk

Muscle strength is a crucial factor in maintaining functional independence in older adults. Decreased muscle strength is a risk factor for frailty and disability [1,2]. It seems that muscle strength is a key component in mobility in elderly and may impact the results in some clinical functional tests (e.g., chair rising test). Strength training has been shown to increase the muscle strength and to improve dynapenia [3]. On the other hand, several studies reported only weak influence of classical resistance training on more complex functional abilities (e.g., gait speed) [4]. Therefore, this study focused on the effect of alternative interventions on both muscle strength and functional abilities. Thirty subjects randomly assigned to either strength training (ST, age 71,66±2,97 years, height 167,4±11,52 cm, weight 72,33±15,02 kg) or electrical stimulation training group (EST, 70,21±3,25, 168,2±5,63 78,89±8,68) underwent an 8-week period of strength vs. electrical stimulation training. ST trained on a leg press device in isokinetic mode with proprioceptive stimulation. EST underwent stimulation of both quadriceps femoris muscles with an additional load placed on ankles (1 to 2.5kg). Both groups were tested one week before and one week after the training. Clinical functional test battery consisted of as follows: chair rising test, timed up-and-go test (TUG), 10-m maximum speed walking test, 10-m preferred speed walking test and balance test. Maximum isometric strength of knee and leg extensors was measured on a knee extension and leg press dynamometer, respectively. Muscle strength measured on the leg press dynamometer increased significantly in the ST group only by 28% ($p<0.01$). Knee

extension force measured on dynamometric chair improved significantly by 4,9% ($p<0.01$) and 8,2% ($p<0.01$) in ST and EST, respectively. The higher relative increase in leg press test compared to knee extension in ST can be ascribed to the training specificity, as the leg press device was also used during the training in this group. In clinical functional tests, chair rising improved significantly similar in both groups by 16,01% ($p<0.05$) and 16,54% ($p<0.05$) in ST and EST, respectively. TUG performance did not changed significantly in either group. Horizontal velocity of centre of gravity in balance test changed significantly in ST only by 13,64% ($p<0.05$). 10-m maximum and habitual speed walking performance did not change significantly either in ST or in EST. There was also a no correlation between functional test and strength level implicating other adaptation mechanism(s) besides muscle strength influencing functional performance. To conclude, similar effect was observed after both type of training for gaining the maximum strength. There was also similar effect of both ST and EST regimes on clinical functional tests. Strength training was superior for improving the static upright posture control compared to electrical stimulation.

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The role of strength for balance in elderly

Sarabon N (1,2), Loefler S (1), Fruhmann H (1), Burggraf S (1), Cvecka J (3), Sedliak M (3), Rosker J (2), Pisot R (2), Kern H (1)

(1) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (2) University of Primorska, Science and Research Centre, Institute for Kinesiology Research, Koper, Slovenia; (3) Faculty of Physical Education and Sport, Comenius University, Bratislava, Slovakia
E-mail: nejc.sarabon@zrs.upr.si

Elderly show impaired balance and muscle strength, which results from a combination of several underlying physiological processes, such as decline in muscle mass, changes in the central neural function, etc. Poor balance, impaired sensory-motor function and decline in strength are considered as reasons for falls in elderly [1-3]. Physical exercise has been shown to improve balance in an upright posture [3]. On the other hand, inactivity during bed rest studies results in decline in muscle mass and strength. Our aim was to make a better insight into the interplay between strength, balance and kinesthesia in elderly people. We conducted three separate studies: one epidemiological study and two intervention studies, a bed rest model inactivity study and a training study. Study 1: The purpose was to use a cross-sectional study design in order to test the relationship between maximal isometric strength and balance. Subjects (48 male, 53 female) over 65 years of age participated (66.8 ± 5.3 years; 168.7 ± 7.9 cm; 75.9 ± 13.8 kg). Body balance was evaluated via body sway in a semi-tandem quiet stance which was measured with a force plate using centre-of-pressure (CoP) derived parameters. Isometric dynamometers were used for testing maximal voluntary contraction strength of knee flexors, knee extensors, hip abductors and hand grip. Pearson correlations were calculated. The results showed surprisingly low correlation coefficients between strength and body sway velocity, amplitude and frequency (all $R2 < 0.22$). There was also no direction specific links between strength and body sway, such as thigh musculature and anterior-posterior sway or hip abductors and medial-lateral sway. Study 2: This study explored the effects of a 14-day horizontal bed rest (BR) without countermeasures on postural sway evaluated by rambling-trembling methodology, maximal voluntary isometric torque and precision of voluntary torque matching. Sixteen male subjects (59.6 ± 3.4 years; body height 173.3 ± 4.9 cm; body mass 77.3 ± 11.8 kg) were tested before, immediately after and two weeks after BR. The increase in frequency and amplitude after BR was comparable for both sway subcomponents (rambling and trembling) in medial-lateral direction. But in anterior-posterior direction, rambling increased more in frequency (-7% vs. +31%, $p < 0.05$) while trembling increased more in amplitude (+35% vs. +84%, $p < 0.05$). The drop in maximal voluntary torque after BR was present for plantar flexion ($p < 0.05$) but not for dorsal flexion. After the BR, the subjects were less precise in the dorsal flexion torque matching task ($p < 0.05$). All the observed parameters, except the dorsal flexion torque matching error, returned back to the pre-BR values after the two weeks of re-conditioning. Results of this study indicate that body sway subcomponents responded differently to BR. Based on these findings, it was not possible to draw clear assumptions on the effects of neural and structural changes on body sway. Study 3: With this study was to find out the effects of 2.5-month strength training of two different types on static balance in elderly subjects. Altogether, 74 subjects (74.3 ± 7.0 years, 169.6 ± 10.3 cm, 78.5 ± 16.1 kg) volunteered to take part in the study. They were randomly assigned to control group ($n = 19$), electrical stimulation group ($n = 27$) or leg press group ($n = 28$). Subjects in both the training groups were exposed to training 3x/week for a period of 9 weeks. In the electrical stimulation group the subjects received neuromuscular electrical stimulation of the anterior thigh muscles. In the leg press group the subjects performed strength training on a computer-controlled leg press machine using the mode of combined slow movements and superponated vibrations. Before and after the training period, static balance of the subject was tested by a quiet stance task (parallel stance, closed eyes, 3x30s. The three groups of subjects showed statistically significant differences ($p < 0.05$) regarding the pre-training vs. post-training changes in CoP velocity, amplitude and frequency. The differences were more pronounced for CoP velocity ($p = 0.000 – 0.001$) and amplitude ($p = 0.001 – 0.006$), while they were less evident

in case of mean frequency of the power spectrum (p = 0.03 – 0.21). The mean improvements were higher in the leg press than in the electrical stimulation group. To summarize, the results of this study indicate that training strength can improve balance in elderly subjects. The selected body sway parameters were namely improved after the training period in both experimental groups. The results of our studies indicate that no the relationship between strength and balance is far from straightforward. There is no simple relationship between them, however, activity/inactivity changes in strength seem to go hand in hand with the changes in improved/impaired balance.

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Improving stair-negotiating ability in older people

Maganaris C

Liverpool John Moores University, Liverpool, UK
E-mail: C.Maganaris@ljmu.ac.uk

The majority of falls in the elderly occur on stairs, and mainly during stair descent [1-5]. The physical injuries arising from such falls are of obvious concern, but equally important is the fear of falling and the loss of confidence and mobility that this brings. Therefore, it is imperative that effective measures are established to reduce the risk of stair falls and accidents in order to maintain independence and enjoy a good quality of life in old age. Exercise training programs designed to improve mainly muscle strength and balance can be effective for the reduction of falls in general, but they have two important limitations: 1) Generic and holistic physical activity programs are not focused on the specific muscle groups and functional deficits associated with stair negotiation and the prevention of falls. 2) There is no immediate feedback on functional abilities linked to stair negotiation ability and performance, and this reduces motivation and may affect participation and adherence. Furthermore, assessing and manifesting biomechanical changes beneficial for stair negotiation post-training requires specialized and not easily accessible equipment, so in effect the evaluation of the training program is possible only in terms of number falls encountered over a period of time following the intervention. This makes it impossible to make adjustments to the training program to optimise its effectiveness, before any accidents occur. In my talk I will suggest ideas and interventions that tackle these issues.

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The power of mitochondrial shaping machinery in controlling muscle mass

Favaro G (1), Romanello V (1), Scorrano L (1), Sandri M (1,2)

(1) Dulbecco Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy; (2) Department of Biomedical Sciences, University of Padova, Italy
E-mail: marco.sandri@unipd.it

Skeletal muscle is a tissue with high energy demand that requires an extremely organized and functioning mitochondrial network [1-3]. Therefore, mitochondria dynamics play a critical role in muscle homeostasis and function. However, only few genetic studies have explored the role of fusion and fission machineries in muscle physiology. Here we have investigated the role of DRP1 by generating a muscle specific knockout mouse. Ablation of DRP1 gene results in 100% lethality at newborn age. DRP-null muscles are smaller in size than controls because of inhibition of protein synthesis and activation of protein breakdown. DRP1 null muscles show also an alteration of myogenesis that contributes to the weakness. Knockout animals showed accumulation of lipid droplets and of abnormal mitochondria that are bigger in size with normal cristae. Therefore, mitochondrial shaping machinery is critical for muscle homeostasis and open a new set of potential therapeutic targets against muscle wasting.

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Abstracts

Insights into the molecular and cellular mechanisms of sarcopenia and into potential therapeutic approaches

Musarò A

Institute Pasteur Cenci-Bolognetti, DAHFMO-unit of Histology and Medical Embryology, IIM, Istituto Italiano di Tecnologia, Sapienza University of Rome, Italy; (2) Division of Rheumatology, Unit of Neuromuscular and Neurodegenerative Disease, Bambino Gesù Children's Hospital, Rome, Italy
E-mail: antonio.musaro@uniroma1.it

It is generally accepted that the primary cause of functional impairment in different myopathy is a cumulative failure to repair damage, resulting from sustained muscular activity, related to an overall decrease in anabolic processes [3]. The skeletal musculature is particularly susceptible to the effects of aging and diseases, undergoing a steady reduction in function and losing up to a third of its mass and strength. This decline in mass and functional performance is due to an overall decrease in muscle integrity as fibrotic invasions replace functional contractile tissue, and marked changes occur in muscle fiber composition, with a characteristic loss in the fastest most powerful fibers. Despite numerous theories and intensive research, the principal molecular mechanisms underlying the process of muscle wasting are still unknown. Current data point out that the development of muscle wasting is a multifactorial process and believed to be the result of both intrinsic factors, involving changes in molecular and cellular levels, and extrinsic ones, such as nutrition and exercise [1]. Many factors, including motor-unit remodelling, decreased hormone levels with consequent negative effect on protein synthesis, stress oxidative damage, chronic inflammatory response, alteration in satellite cells activity may all contribute to decrease in muscle mass and functional performance. We have evidences that either selective accumulation of oxidative stress in muscle [2] or serum accumulation of pro-inflammatory cytokines contribute to induce several signs of sarcopenia, including muscle atrophy, reduction in muscle strength, alteration in mitochondria activity and alteration in neuromuscular junction stability. It is well documented that exercise training and regular exercise can attenuate the pathological signs of sarcopenia, increasing muscle strength while decreasing fall risk. Nevertheless, certain pathologic conditions (e.g. osteoarthritis) limit the ability to perform physical exercise and therefore the benefits from it. An alternative effective intervention to improve muscle recovery is electrical stimulation (ES). We accumulated evidences about the positive effects of Electrical Stimulation (ES) on seniors, demonstrating that that electrical stimulation promotes muscle power, size of muscle fibers and functional performances of seniors.

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ERG1a K^+ channel induces expression of MuRF1, but not MAFbx-Atrogin1 or E3α-II, E3 Ligases

Pond A (1,2), Pratt E (2), Soderling I (2), Wang W-H (2), Hockerman G (2)

(1) Anatomy Dept., Southern Illinois School of Medicine, Carbondale, IL, U.S., (2) Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, U.S.
E-mail: apond@siumed.edu

The ERG1 K^+ channel is partially responsible for late phase repolarization of the cardiac action potential [1]. We have shown that the ERG1a splice variant is involved in the onset of skeletal muscle atrophy in hind limb suspended mice and that it up-regulates ubiquitin proteasome pathway (UPP) activity [2]. The UPP is a proteolytic pathway responsible for the majority of protein degradation that contributes to muscle loss during atrophy. In general, it is composed of three enzymes which function together to activate ubiquitin and then bind it to proteins which are, thereby, targeted for proteasomal degradation [3]. The final enzymes in the UPP, the E3 ligases, are the most diverse group and numerous isoforms have been identified in atrophying skeletal muscle, including MuRF1, MAFbx-Atrogin1 and E3α-II (also UBR2) [3,4]. Here, to further demonstrate that ERG1a has an effect on the UPP, using real time PCR we compared mERG1a, MuRF1, MAFbx-Atrogin1 and E3α-II expression levels in the skeletal muscle of hind limb suspended mice to those of skeletal muscle from mice ectopically expressing (a result of electro-transfer) the mERG1a gene. Interestingly, time course data demonstrate that, although mERG1a and both MuRF1 and MAFbx-Atrogin1 are up-regulated within 3 days of hind limb suspension, only MuRF1 is up-regulated in the mice ectopically expressing mERG1a. Expression of the non-skeletal muscle specific E3 ligase, E3α-II, is increased significantly after seven days of suspension, but is not induced after 7 days of mERG1a ectopic expression. Focusing on the skeletal muscle specific ligases, we detected up-regulation of MuRF1 protein in muscle from hind limb suspended mice and in the samples ectopically expressing mERG1a; however, while we did detect up-regulation of MAFbx-Atrogin1 protein in muscles from hind limb suspended mice, we did not detect any up-regulation of MAFbx-Atrogin1 protein in muscle ectopically expressing mERG1a nor did we detect up-regulation of MAFbx expression using a MAFbx luciferase reporter in these muscles. To further confirm our results, we infected C2C12 myotubes with an adenovirus containing the HERG construct and detected increased levels of MuRF1, but not MAFbx-Atrogin1, protein relative to appropriate controls. Finally, the FOXO3a gene encodes a transcription factor known to

induce MAFbx expression by binding directly to its promoter. We co-expressed an inactive HR-FOXO3a mutant with mERG1a in mouse gastrocnemius muscle and discovered that both the decreased fiber size and increased MuRF1 mRNA levels induced by mERG1a expression are NOT significantly curtailed by co-expression of mERG1a and inactive HR-FOXO3a, demonstrating that mERG1a does not modulate MuRF1 expression through FOXO3a. We conclude that the mERG1a K+ channel modulates expression of MuRF1, but not MAFbx-Atrogin1 nor E3α-II, and that the expression of MuRF1 is not modulated by FOXO3a. The mechanism by which mERG1a modulates MuRF1 expression may include other FOXO variants or other pathways such as the IKB-α/NF-κB pathway. It certainly merits further investigation.

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The genetic association with the variable response to resistance training

Erskine RM

School of Sport & Exercise Sciences, Liverpool John Moores University, Liverpool, UK
E-mail: R.M.Erskine@ljmu.ac.uk

Most chronic exercise studies have focused on the mean response to training, while the variability is assumed to be experimental error associated with the measurements or variation in the commitment of the participants. More recently, however, there has been an increasing awareness that the variable training response is a real phenomenon [2,3] and understanding the reasons for it may give insights into the mechanisms controlling muscle growth, changes in muscle force transmission, or changes in the oxidative capacity of the muscle. In addition, there could be practical benefits in knowing who might or might not respond, since exercise training is an important aspect of sport and rehabilitation. Consequently, there is considerable interest in determining whether variants (or polymorphisms) of particular genes are associated with the way in which human muscle adapts to both resistance and aerobic exercise training [1]. Furthermore, there is an increasing body of evidence to suggest that skeletal muscle and tendon/ligament injury has a genetic component [4,5]. However, any genotype-muscle/tendon phenotype association is likely to be polygenic, i.e. multiple gene polymorphisms, each providing its own contribution (some greater than others). Thus, there may be specific polygenic profiles that are advantageous or disadvantageous regarding the muscle's ability to respond positively or negatively to a particular type of chronic exercise. Identifying these profiles
could improve our knowledge of how skeletal muscle and tendon adapt to overload, disuse and ageing. This knowledge could then be used to inform personalised training programmes, thus improving overall public health and athletic performance.

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Gender differences in the decline of muscle power up to the most advanced age

Gava P (1), Kern H (2), Carraro U (1)

(1) Translational Myology Lab., Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria.
Email: "Paolo Gava" <paologavastra@alice.it>

We here present evaluation of gender difference in the decline of power of upper and lower limb muscles with age. Muscle power decline is drawn from the decline of the record performance of master athletes in different track and field events [1]. The analysis is based world records of master athletes competing within age groups of 5 years (from 35 to 39; from 40 to 44 and so on till the age of 100 years). The performances are normalized with respect to the absolute world record providing sets of dimensionless parameters ranging from one (absolute record) to zero (null performance) [2]. This approach to the decline of the skeletal

muscle power with increasing age, by selecting human subjects genetically well gifted, trained at their best by professionals, and fully motivated, produces results not affected by the most common confounding factors which weaken many studies dealing with aging [3]. The trend-lines indicate that the decline of muscle power starts not later than the age of thirty for men as well as for women. All trend-lines tend to zero at about the age of 110 years, in line with the present human maximal survival expectation. The decline of the power in the events involving most the lower limbs (running events) starts quite gently and accelerates as age increases while in the events involving most the upper limbs (throwing events) the decline starts more sharply and then slows down approaching the bottom line. Results from world records of master athletes show a different decline for males and females. Specifically, the power in the female running events declines more than in the male running events from the age of 50 onward. The diagram of the normalized power versus age is unambiguous. In the throwing events the trend-lines have an opposite behaviour: female decline is more rapid than the male decline in the initial phase while in the final aging phase the female and the male decline join together again. Interpretation of these results is difficult partly because the physical decline of males and females may be affected by a number of factors of various origin. Track and field world records of master athletes are surely linked to the social and cultural environment, included the economic situation. In general the diagrams indicate that female power declines more than the male power, even if in different age ranges. Finally a spot light on the running performances (both for males and females): in the shortest distances the decline is very, very gentle till a remarkable old age. This capacity to run fast for a short distance/time may reflect a human genetic characteristic developed to be able to escape predators.

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Exon skipping as a potential therapy for specific Laminopathies

Scharner J, Ellis JA, Zammit PS

Randall Division of Cell & Molecular Biophysics, King's College London, London, UK
E-mail: peter.zammit@kcl.ac.uk

Laminopathies are a heterogeneous group of diseases associated with mutations in A-type lamins and more than 300 mutations have been described to date. Pathogenic mutations are mainly missense, leading to unfavourable structural alterations and lamin A/C gain-in-function. The majority of mutations affect striated muscle, resulting in Emery-Dreifuss muscular dystrophy (EDMD) or dilated cardiomyopathy, while others result in lipodystrophy, neuropathy or premature ageing syndromes. Together with B-type lamins, the A-type lamins form the nuclear lamina: a proteinaceous network underlying the inner nuclear membrane. A-type lamins are encoded by alternative splicing of the LMNA gene to generate the predominate A-type lamin isoforms lamin A and lamin C. Lamin A/C are type V intermediate filament proteins and characterised by a tripartite organisation formed by an alpha-helical central rod domain flanked by a short N-terminal head region and a C-terminal tail domain. The central rod domain is characterised by multiple heptad repeat sequences, typical for coiled-coil alpha-helices. We hypothesised that removing complete heptad repeats containing a mutation, whilst retaining the reading frame may be an appropriate therapeutic strategy for laminopathies. For example, deletion of exon 3 or exon 5 (by 'exon-skipping') would shorten the central rod domain of lamin A/C by removing six complete heptad repeats but, unlike missense mutations, would not otherwise alter the structure of the alpha-helix. Therefore, detrimental effects of dominant missense mutations located in either exon 3 or 5 of LMNA, could be ameliorated by removing the entire exon [1,2]. To test this theory, we created cDNA constructs encoding lamin A and lamin C with a deletion corresponding to the amino acids encoded by exons 3 or 5 (lamin A-delta3, lamin C-delta3, lamin A-delta5 and lamin C-delta5) and lamin A constructs with EDMD-causing mutations in either exon 3 (lamin A-N190dup or N195K) or exon 5 (lamin A-S295P or S303P). Retroviral-mediated delivery of lamin A variants with EDMD-causing mutations into primary mouse embryonic fibroblasts (pMEFs) resulted in abnormal nuclear morphology with lamin A and B mislocalisation. Expression of lamin A-delta3 or lamin C-delta3 was similarly deleterious. By contrast, overexpressing lamin A-delta5 or lamin C-delta5 did not have such undesirable effects on the nuclei of wild type pMEFs. Furthermore, nuclear defects including abnormal nuclear morphology and aberrant lamin B and emerin localisation found in lmna-null pMEFs, could be rescued by introducing either lamin A-delta5 or lamin C-delta5, comparable to the effects of ectopic wild type lamin expression. No such rescue occurred with lamin A/C-delta3 or lamin A variants with EDMD missense mutations. Thus lamin A/C-delta5 functions as effectively as wild type lamin A. Therefore exon-skipping LMNA exon 5 provides a proof-of-principle for a potential therapy for laminopathies caused by pathological missense mutations within this exon. This is the first time exon-skipping has been proposed as a suitable therapy for diseases arising from missense mutations rather than deletions (e.g. DMD) and does not incur the limitations of current gene editing technology traditionally used for treating missense mutations.

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Abstracts

The effects of ageing on mouse satellite cells

Morgan JE

Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London, UK
E-mail: jennifer.morgan@ucl.ac.uk

Satellite cells are the principal skeletal muscle stem cells, responsible for muscle growth, repair and regeneration. The ability of skeletal muscle to maintain itself is diminished in old age, leading to a loss of skeletal mass and function (sarcopenia). It is not clear whether the reduction in the regenerative capacity of skeletal muscle with increasing age is due to defects in either the local or systemic environment, or in satellite cells themselves, or both. Age-related changes in Notch, TGFbeta, and Wnt signalling and in hormones, cytokines and growth factors (e.g. FGF2, IGF-1) may be contributory factors in the decline in muscle regenerative capacity with age. Aged muscle regenerates well when either grafted into a young host, or exposed to a young systemic environment, indicating that the environment is critical for effective muscle stem cell function. The number of satellite cells per myofibre differs across the lifespan and between the sexes of mice. The number of satellite cells per fibre is significantly increased in growing compared to adult and in adult compared to aged mice. Adult male mice have more satellite cells/fibre than adult females [3-5]. This reduction in satellite cell number may be responsible for the diminishment of muscle regeneration with age. Nevertheless, provided that the host muscle environment is appropriately modulated and similar numbers of donor satellite cells are grafted, satellite cells from both young (actively growing), adult and aged donor male and female mice can regenerate and self-renew with similar efficiency in a young or older host mouse [1,2,4]. Our data suggest the existence of two distinct populations of satellite cells. The first is responsible for muscle growth and routine muscle maintenance; this population decreases with age and is reduced in female compared to male mice. The second population is activated only in response to muscle injury, survives transplantation and its numbers are not altered by sex or age.

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Regulation of myoblast differentiation and hypertrophy

Stewart C, Erskine R, Sharples A

Stem Cells, Ageing and Molecular Physiology Unit, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK
E-mail: C.E.Stewart@ljmu.ac.uk

Skeletal muscle comprises ~ 45% of the healthy human body mass. It is critical for development, growth, metabolism, posture, locomotion, thermoregulation and provision of energy. Ageing, muscular dystrophies and cachexia are associated with muscle wasting and weakness, however, the mechanisms underpinning these losses may differ. Muscles hypertrophy (increase in size through an increase in the cross sectional area of individual fibres in response to e.g. loading, which ultimately leads to an increase in maximal force generating capacity) when protein synthesis exceeds protein degradation. Conversely, muscles atrophy following disuse, unloading or disease (decline in fibre size and cross sectional area and decline in peak force generating capacity) when protein degradation dominates. The adaptability of skeletal muscle, given its terminal differentiation, is thought to be achieved via activation of resident muscle stem cells. The regulators of synthesis, degradation and muscle mass are therefore likely to involve complex cellular, biochemical and genetic controllers. Our research focuses on the interactions of skeletal muscle stem cells with anabolic (Insulin-like growth factors) and catabolic (Tumour necrosis factor-alpha) agents and their roles not only in muscle maintenance with age/obesity/disease, but also in repair following injury [1-8]. Understanding the regulators (e.g. IGFBPs, PI3 kinase, MAP kinase, Adra1d, caspases and sirtuins), which influence survival, differentiation, migration or death of these cells is critical, since severe loss of functional muscle mass contributes to patient mortality. This presentation will provide information on the cellular and the molecular regulators of muscle stem cell growth, differentiation, survival and migration, with implications for health and disease.

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Hypoxia increases mouse satellite cell clone proliferation maintaining both in vitro and in vivo heterogeneity and myogenic potential

Urbani L (1,2), Piccoli M (1,2), Franzin C (1,2), De Coppi P (2,3), Pozzobon M (1,2)

(1) Fondazione Città della Speranza, Monte di Malo, Vicenza, Italy, (2) Department of Woman and Child Health, University of Padova, Padova, Italy, (39 Surgery Unit, University College of London, Institute of Child Health, Great Ormond Street Hospital for Children, London, United Kingdom.
E-mail: michela.pozzobon@gmail.com

Satellite cells (SCs) are essential for postnatal muscle growth and regeneration, however, their expansion potential in vitro is limited. Recently, hypoxia has been used to enhance proliferative abilities in vitro of various primary cultures. Here, by isolating SCs from single mouse hindlimb skeletal myofibers, we were able to distinguish two subpopulations of clonally cultured SCs (Low Proliferative Clones - LPC - and High Proliferative Clones - HPC), which, as shown in rat skeletal muscle, were present at a fixed proportion. In addition, culturing LPC and HPC at a low level of oxygen we observed a two fold increased proliferation both for LPC and HPC. LPC showed higher myogenic regulatory factor (MRF) expression than HPC, particularly under the hypoxic condition [1,2]. Notably, a different myogenic potential between LPC and HPC was retained in vivo: green fluorescent protein (GFP)+LPC transplantation in cardiotoxin-injured Tibialis Anterior led to a higher number of new GFP+muscle fibers per transplanted cell than GFP+HPC. Interestingly, the in vivo myogenic potential of a single cell from an LPC is similar if cultured both in normoxia and hypoxia. Therefore, starting from a single satellite cell, hypoxia allows a larger expansion of LPC than normal O2 conditions, obtaining a consistent amount of cells for transplantation, but maintaining their myogenic regeneration potential.

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Nutrient-gene interactions: manipulations of carbohydrate availability to maximize training adaptations

Morton JP

Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK
E-mail: J.P.Morton@ljmu.ac.uk

Traditional nutritional strategies for endurance training typically focus on the requirement for appropriate carbohydrate (CHO) intake before, during and after exercise so as to maintain a high daily training volume and intensity. However, in recent years our laboratory and others have collectively demonstrated that commencing training sessions with reduced CHO availability from both endogenous and exogenous sources apparently provides an enhanced stimulus to induce oxidative adaptation of human skeletal muscle. These data have therefore led to the development of the innovative train-low: compete high model, surmising that athletic populations should deliberately complete a selected portion of their training with reduced CHO availability but yet competition always be performed with high CHO availability. Despite the emergence of the train-low: compete high paradigm, the precise molecular mechanisms underpinning this enhanced training adaptation remain unclear. Although numerous laboratories have consistently observed that contraction-induced AMPK and p38MAPK signaling are enhanced in conditions of reduced CHO availability, their subsequent effects on the downstream regulation of the proposed master regulator of mitochondrial biogenesis, PGC-1α, remain equivocal. As such, the aim of recent studies in our laboratory has been to focus on identifying novel molecular signaling cascades that may contribute to the enhanced oxidative adaptation associated with CHO restriction. In this regard, we initially provided the first report that exercise increases p53 (traditionally known for its role as a tumour suppressor protein) signaling in human skeletal muscle in a time-course that appears consistent with upstream signaling through AMPK and p38MAPK. We further observed that p53 signaling is enhanced in conditions of reduced CHO availability (i.e. exercising in a glycogen depleted and fasted state) whereas adhering to traditional textbook sports nutrition guidelines completely suppresses this signaling cascade. Given the emerging role of p53 as a regulator of mitochondrial biogenesis and in the pathology of cancer, ageing and insulin

resistance, the apparent nutritional modulation of p53 activation may therefore be of relevance for both athletic and clinical populations.

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Exercise, vitamin D and human skeletal muscle: is sarcopenia inevitable?

Close GL

Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK
E-mail: G.L.Close@ljmu.ac.uk

Over the last century, life expectancy has risen dramatically and for the first time in history there are now more people alive above retirement age than under the age of sixteen. This increased life expectancy brings with it many challenges for biogereontologists, one of which is dealing with the dramatic and somewhat debilitating effects of muscle frailty. As we age, our muscles become smaller and weaker, so much so that by the age of 70 muscle force can be reduced by approximately 30-40% thus affecting our ability to perform everyday tasks. From a cellular perspective, skeletal muscles from older adults exhibit electron transport chain (ETC) abnormalities oxidative stress and mtDNA deletions and mutations. Moreover, studies in aged rodents have clearly shown that skeletal muscles fail to adapt to exercise compared with muscles from younger rodents [1]. Perhaps the only effective treatment to attenuate these age-related problems is a regular physical exercise. Studies in highly trained masters athletes from our group [2] have demonstrated that lifelong training can preserve mitochondrial content and the ability to adapt to an acute exercise challenge (data under review). Moreover, we have recently reported that some, but not all, of the age related increases in genotoxic stress were also attenuated in muscles from highly trained older individuals [3]. Taken together these data suggest that physical exercise should be performed throughout lifespan to alleviate a variety of age-related problems, however despite being effective, exercise alone will not completely prevent declines in muscle function. Over the past decade there has been a growing interest in Vitamin D since it is now generally accepted that vitamin D deficiency is widespread, especially in older individuals [4]. Vitamin D is implicated in the regulation of a multiplicity of cellular processes, including those mediating human skeletal muscle function. Indeed, several studies in the elderly have clearly demonstrated improved markers of muscle function following vitamin D supplementation [5]. Although there is a growing trend to supplement with vitamin D, the appropriate endpoint serum 25[OH]D concentration for 'optimal' muscle function and the molecular mechanisms mediating improved muscle function with vitamin D supplementation are yet to be established [6]. The presentation will examine the magnitude of vitamin D deficiencies in the general population and investigate the efficacy of supplementation on skeletal muscle function.

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The effect of innervation on the development of fast twitch and slow tonic muscle fibres

Gordon T (1), Vrbova G (2)

(1) Division of Plastic and Reconstructive Surgery, The Hospital for Sick Children, 555 University Ave, Toronto, ON, Canada M5G 1X8; (2) Department of Cell and Developmental Biology, Faculty of Life Sciences, University College London, UK
E-mail: g.vrbova@ucl.ac.uk,

Most mammalian muscles are composed of fibres that are innervated at a single endplate and respond to nerve stimulation by a propagated action potential that initiates the contraction of the whole muscle fibre. In invertebrates frogs and birds there are two distinct groups of muscle fibres: those that are similar to mammalian twitch fibres and are activated by an action potential (twitch fibres) and those that are

innervated at several sites, do not conduct an action potential but are made to contract by the decremental spread of the depolarisation along the muscle fibre. This allows a much finer control since it is possible to produce force by only a section of the muscle fibre. In the wing muscles of the chick the fast contracting twitch fibres are segregated in the posterior latissimus dorsi muscle (PLD) and the tonic slow contracting ones are segregated in the anterior latissimus dorsi (ALD). A comparison between the response of the slow tonic and fast twitch muscles to cross innervation revealed that, unlike mammalian muscles the innervation of the tonic and phasic muscles cannot be interchanged and the nerve to PLD cannot successfully re-innervate ALD muscle fibres and vice versa [1]. Nevertheless, in newly hatched chickens transposition of the nerves from the fast twitch PLD into the ALD muscle and vice versa allowed these alien nerves to innervate the immature muscle fibres, indicating that the differentiation of the muscle fibres into tonic and phasic ones occurs after birth and depends on innervation [2, 3]. This is indeed the case because slow-tonic and fast–twitch muscle fibre properties of ALD and PLD muscles, respectively, emerged during development after their initial similarities shortly after innervation was established [4-6]. Moreover, when ALD and PLD muscles were removed from an adult chicken and the fragmented muscles containing only satellite muscle cells were transplanted, the properties of the differentiated muscles were transformed by the innervation that was determined by the motor nerve, the slow nerve providing multiple innervation at distributed endplates on the regenerated and re-differentiated PLD muscle and the fast nerve innervating the re-differentiated muscle at a single endplate resulting in slow and fast contractions of the cross-reinnervated dedifferentiated muscles [7]. The nerves to slow and fast muscle fibers not only differ in their properties but also in their quantal release of transmitter, the terminals of nerves to the slow tonic muscle fibres releasing less acetylcholine than those of the fast twitch fibres of the chick [8,9]. This difference accounts for the different pattern of innervation because tubocurarine that reduces the endplate potential amplitudes in the fast-twitch PLD muscle, when administered before differentiation of properties, resulted in multiple innervation of the normally fast-twitch PLD muscle. This experiment was consistent with the explanation that the electrical and contractile properties of developing muscle fibres are determined by their innervation and depend on the quantal release of acetylcholine of the nerve terminals. The slow tonic muscle fibers with several endplates are normally activated by terminals that release small amounts of ACh not sufficient to elicit an action potential, while the terminals to the twitch fibres release sufficient Ach to elicit an action potential.

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Mitochondrial Functional Alterations in Aging Muscle: Cause or Effect(or)?

Picard M (1,2), Ritchie D (3), Thomas MM (3), Wright KJ (3), Hepple RT (1,2)

(1) McGill University Health Centre, Montreal, Canada; (2) Dept. of Kinesiology, McGill University, Montreal, Canada; (3) Faculty of Kinesiology, University of Calgary, Calgary, Canada
E-mail: russell.hepple@mcgill.ca

Mitochondria play a key role in myocellular homeostasis by regulating energy supply, reactive oxygen species (ROS) signaling, and intrinsic pathways of apoptosis [1]. For this reason, dysfunction of mitochondria is hypothesized to contribute to the atrophy of aging skeletal muscle, where this dysfunction is posited to occur in part by accumulating damage to the mitochondrial genome (mtDNA) [2]. Whilst recent evidence has demonstrated functional alterations of mitochondria in aging muscle, it remains unclear the degree to which these changes are actually causing the pathophysiology of aging muscle versus being a down-stream effector that is responding to an alteration in the aging cellular environment (no intrinsic mitochondrial defect per se). The significance of this distinction is that if upstream changes are in fact responsible for modulating mitochondrial function in aging muscle, targeting mitochondria as a therapeutic strategy may have limited benefits. In considering possible modulators of mitochondrial function in aging muscle, amongst the best-known changes in aging muscle are alterations in the motor unit, which manifest in part as destabilization of the neuromuscular junction and eventually a progressive failure of reinnervation and resulting accumulation of denervated muscle fibers in advanced age [3]. Indeed, experimental denervation is well-known to result in increased mitochondrial ROS production [4] and recruitment of mitochondrial-mediated pathways of muscle atrophy [5]. However, the extent to which denervation may be responsible for altered mitochondrial function with aging

is unknown. In this respect, we have recently shown that mitochondrial functional alterations in muscles of contrasting atrophy severity and fiber type composition in aging rats not only segregate in part by fiber type (with slow but not fast muscle showing a respiratory deficit), but also are also inconsistent in several respects with the effects of accumulated mtDNA damage [6]. For example, whereas progressive mtDNA damage causes a reduction in protein contents of mitochondrial electron transport system complexes [2], normal aging is associated with an unchanged or elevated expression of these proteins [6]. On the other hand, our analysis suggests that at an age where muscle atrophy is severe and associated with a large accumulation of denervated myofibers, mitochondrial phenotypes are more consistent with the known effects of denervation on mitochondria. For example, both experimental denervation and normal aging cause an increase in mitochondrial ROS production. On this basis, our working hypothesis is that when aging muscle atrophy becomes severe, and thus, clinically relevant, mitochondrial functional alterations are largely driven by denervation, making mitochondria an unlikely therapeutic target in treating aging muscle atrophy. Indeed, restoring mitochondrial function may simply prevent atrophy of denervated myofibers in severely atrophied aging muscle, which could in fact further exacerbate the problem by placing a greater load on the remaining innervated muscle fibers.

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The influence on muscle growth in aged animals and diet mediated starvation by myostatin

Patel K

School of Biological Sciences, University of Reading, UK
E-mail: ketan.patel@reading.ac.uk

Myostatin is a potent inhibitor of muscle growth. Manipulation of myostatin function allows us to address major issues related to muscle biology. Here we address two of them. Firstly we will present data related to sarcopenia by demonstrating that neutralisation of myostatin activity through a single application of virus expressing the propeptide molecule is able to not only stop the muscle wasting process, but promote muscle growth in aged mice. Secondly we examine the consequences of acute starvation diet in animals displaying hypertrophic muscle. We show that the mechanisms regulating the response to starvation are markedly different in hypertrophic mice compared to wild type animals. We suggest that results from these studies not only further our understanding of basic muscle biology but also offer mechanisms to treat condition suffered by humans.

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Bone morphogenetic protein controls muscle mass and dominates over myostatin signaling

Sartori R (1), Schirwis E (2), Blaauw B (1), Bortolanza S (1), Zhao J (3), Enzo E (1), Stantzou A (2), Mouisel E (2), Toniolo L (1), Ferry A (4), Stricker S (5), Goldberg AL (3), Dupont S (1), Piccolo S (1), Amthor H (2), Sandri M (1)

(1) Dulbecco Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy; (2) UFR des Sciences de la Santé, Université de Versailles Saint-Quentin-en-Yvelines, Montigny-le-Bretonneux, France; (3) Department of Cell Biology, Harvard Medical School, Boston, MA, USA; (4) Université Pierre et Marie Curie, Institut de Myologie, Unité mixte de recherche UPMC-AIM UM 76, INSERM U 974, CNRS UMR 7215, 75013 Paris, France; (5) Development and Disease Group, Max-Planck-Institute for Molecular Genetics, Berlin.
E-mail: helge.amthor@uvsq.fr

Cell size is determined by the balance between protein synthesis and degradation. This equilibrium is affected by hormones, nutrients, energy levels, mechanical stress and cytokines. Mutations that inactivate Myostatin lead to

increased muscle growth in animals and humans. However, the signals and pathways responsible for this hypertrophy remain largely unknown. Here we find that signaling by Bone morphogenetic proteins (BMP), acting through Smad1/5/8, is the fundamental hypertrophic signal and dominates Myostatin signaling. Inhibition of BMP signaling causes muscle atrophy, abolishes the hypertrophic phenotype of Myostatin knockout and strongly exacerbates the effects of denervation and fasting. BMP-Smad1/5/8 negatively regulates a novel gene (Fbxo30) that encodes an ubiquitin ligase, which we named Muscle Ubiquitin-ligase of SCF complex in Atrophy-1 (MUSA1) that is required for muscle loss. Collectively, these data identify a critical role for the BMP pathway in adult muscle maintenance, and uncover the regulation of BMP signaling as the rate-limiting step of muscle growth and atrophy.

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How and why slow muscle fibers prevail in aging human muscle

Mosole S (1,2), Zampieri S (1,2), Kern H (2,3), Pond A (4), Carraro U (1)

(1) Translation Myology Lab., Dept. of Biomedical Sciences, University of Padova, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (3) Dept. of Physical Medicine and Rehabilitation, Wilhelminenspital, Vienna, Austria; (4) Anatomy Dept., Southern Illinois University School of Medicine, Carbondale, IL, United States
E-mail: ugo.carraro@unipd.it

Age-related changes in skeletal muscle innervation, independent of patent peripheral neuropathies, are known to contribute to the decline in quality of life often reported in our older population [1-4]; however, these changes and the mechanism(s) by which they occur are not well understood. We had the opportunity to examine the effects of lifelong high-level physical activity on a cohort of recreational sportsmen and to collect what is, in our opinion, strong evidence that aging atrophy is, at least in part, a result of progressive denervation that can be counter-acted by lifelong high-level exercise. We used immunolabeling methods to analyze the fiber type composition of muscle biopsies harvested from three groups 1) senior sportsmen - seniors of ~70 years, who exercised regularly at high levels and had done so for the previous 30 years; 2) healthy, sedentary seniors – age-matched subjects who limited their exercise to "everyday" activities; and 3) active young sportsmen (age ~26 years). Our main results demonstrate that: 1. biopsies from young men seldom contain denervated (0.2±0.5 %), reinnervated or transformed muscle fibers (0.5±0.6 %); 2. biopsies from sedentary seniors contain both denervated (2.6±1.9 %), coexpressing myofibers (1.8±1.7 %) and a few reinnervated clustered myofibers of the fast type (3.0±4.7 %); and 3. senior sportsmen present with a larger percentage of healthy, slow myofibers (up to 68.5±14.1 %,) that appear mainly clustered in slow fiber-type groupings (7.9±7.4 %). Analyses of the data reveal that there was no difference between the athletic and sedentary senior groups in terms of their (both very low) percentages of muscle fibers co-expressing fast and slow MHCs (0.6±0.6 %), suggesting that lifelong exercise does not simply induce motor unit transformation [9-11]. However, the sportsmen had both considerably higher percentages of slow-type myofibers and greater numbers of slow fiber-type groupings [5-8] than the sedentary group. These data provide sound evidence that lifelong cycles of denervation/reinnervation occurred. It appears, therefore, that lifelong exercise allows the body to adapt to the consequences of age-related denervation and to preserve muscle function by saving otherwise lost muscle fibers through reinnervation by different, mainly slow, motor axons. These important observations demonstrate that regular physical activity is a good strategy to attenuate the age-related muscle functional decline associated with aging and thus advocate for a life-style of high-level physical exercise.

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Neuromuscular pathology in equine recurrent laryngeal neuropathy

Piercy RJ, Tulloch K, Goodwin D, Perkins JD

Comparative Neuromuscular Disease Laboratory, Royal Veterinary College, Royal College Street, London, UK
E-mail: "Piercy, Richard J" <rpiercy@rvc.ac.uk>

Recurrent laryngeal neuropathy (RLN) is a common equine distal axonopathy associated with neurogenic atrophy of the intrinsic laryngeal muscles (particularly on the left side) due to loss of motor neuronal innervation of axons within the recurrent laryngeal nerves [1,2]. The aetiology is unknown, but genetic and environmental factors are thought to be involved. Prevalence is very high, with severity varying from sub-clinical involvement to total paralysis of certain laryngeal muscles. Markedly-affected horses when exercised, develop laryngeal paresis, inspiratory stridor, low PaO2 and poor performance. The most widely accepted treatment is prosthetic laryngoplasty, but the procedure has varying success rates and complications are high: consequently, the disorder has a major impact on horse racing worldwide. Determining variation in degrees of neuromuscular pathology according to clinical severity could have a significant impact on our ability to identify horses suitable for novel forms of therapy designed to replace, support or mimic intrinsic laryngeal muscle function, such as functional electrical stimulation [3]. Furthermore, examination of neuromuscular pathology might be a sensitive technique for identification of unaffected animals and to grade disease severity for genetic studies and quantitative trait analyses. Pathological evaluation of the condition can be separated by the various components of the functional unit of contraction – i.e. the motor unit - and its associated structures. For RLN, this means the motor neuron cell bodies in the nucleus ambiguous in the caudal brain stem, the peripheral nerve itself, the neuromuscular junctions and the intrinsic muscles of the larynx. We, and previous groups have examined the caudal brain stem of affected horses but have not found convincing evidence for neuronal cell death on the left side in comparison with the right, though so far, examination has been conducted at a rather superficial histological level. In the left and to a lesser degree, the right recurrent laryngeal nerves, there is a progressive loss of myelinated axons as you extend more distally. In the most severely affected horses, (Grade 4 at rest; Grade C at exercise) there can be almost total loss of myelinated axons in nerve fascicles in the distal left recurrent laryngeal nerve, and marked loss of myelinated axons on the right side in the distal region closest to the larynx: typically nerve fascicles are left with extensive inter-axonal fibrosis and frequent Renault bodies. The neuromuscular junction morphology has not been evaluated in detail and at the microscopic level in horses with RLN – however one could expect that morphological abnormalities in neuromuscular junctions on the left side would be an early marker of disease and might be useful in pathological evaluation of sub-clinical cases. Evaluation of the intrinsic laryngeal muscles supplied by the recurrent laryngeal nerves confirms the marked disparity between severity on left and right sides in affected horses [4,5]. Classic signs of neurogenic muscle atrophy are identified, consisting of angular atrophy, group atrophy and fibre type grouping. In the most severe cases, there is extensive fat infiltration (or replacement) and substantial endomyseal and perimyseal fibrosis. Changes are most severe in the principal left adductor muscle, cricoarytenodeus lateralis, perhaps due to the greater number of large diameter (fast) axons to this muscle in comparison with the abductor muscle. Age of onset and degree of clinical progression in this disorder still remains to be clarified: since sensitivity of diagnosis depends substantially on the technique used. In particular, there is quite marked day-to day variability in laryngeal grade assigned to horses and our results, and those of others suggest that most, perhaps all, horses have sub-clinical evidence of RLN as detected by muscle histopathological evaluation. Nonetheless, previous work has documented signs compatible with RLN in foals of a few weeks of age. An additional question that remains unanswered is whether other nerves are affected in this disorder. Early work documented both nerve and muscle pathological features compatible with a peripheral neuropathy in long nerves in the limbs of affected horses, but this was not supported by more recent work. Unfortunately, probably too few horses have yet been examined to conclude that RLN is a disorder that uniquely affects left and right recurrent laryngeal nerves and further work is required in this area. In summary, RLN is a fascinating and enigmatic disease of horses that causes substantial losses to racing, and with it, major welfare issues to affected horses, give the current surgical treatments and the not insubstantial morbidity that can affect treated horses in terms of chronic low grade particulate aspiration caused by an inability to fully close the rima glottidis on swallowing.

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Abstracts

Case Selection and effect of functional electrical stimulation on reinnervation in a preclinical laryngeal model

Cheetham J (1), Jarvis JC (2), Cercone M (1), Soderholm LV (1), Mitchell LM (1), Sledziona M (1), Ducharme NG (1)

(1) Dept. of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA, 2. School of Sport and Exercise Sciences, Tom Reilly Building, Byrom St Campus, Liverpool John Moores University, Liverpool,UK
E-mail: jcheetham@gmail.com

Denervation produces progressive loss of muscle mass and regrowth of peripheral axons may take many months. Recent work in functional electrical stimulation (FES) of denervated muscle shows atrophy can be prevented. Attitudes towards electrical stimulation to reduce atrophy differ between rehabilitation centers, partially due to the fear that FES may reduce the probablility of reinnervation. We present an equine preclinical model to test the effect of FES on reinnervation following cold injury to the recurrent laryngeal nerve (RLN) [1,2]. The posterior cricoarytenoid muscle was instrumented and stimulated daily for six months. Matched controls were instrumented similarly and remained unstimulated. Data on time to reinnervation (evoked potentials, EMG, rheobase); functional recovery (force-frequency relationship,loaded function during exercise); and the extent of return to the pre-denervated state of full innervation from the RLN (immunohistochemistry for fibre type and fibre integrity, retrograde labeling to assess the source of reinnervating axons) will be presented. Data on the effects of FES on the intrinsic laryngeal musculature of horses with naturally occurring disease, and a novel screening tool for selecting cases suitable for FES will also be presented.

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Early reinnervation is improved by daily electrical muscle stimulation following nerve injury and immediate repair

Willand MP, Zhang JJ, Chiang C, Gordon T, Borschel G

Division of Plastic and Reconstructive Surgery, Department of Surgery, The Hospital for Sick Children, Toronto, ON, Canada
E-mail: mike.willand@gmail.com

The use of chronic electrical muscle stimulation for treating partially or completely denervated muscle has been met with much controversy. Our previous work has shown that a moderate stimulation paradigm can significantly improve the numbers of motor units following long term muscle denervation and subsequent nerve repair [1]. More recently, this same paradigm was used to investigate long term recovery following nerve transection and immediate repair with no negative impact on reinnervation. However, early effects of this treatment were not evaluated [2] Research question: Does a moderate electrical muscle stimulation paradigm improve early outcome measures following nerve transection and immediate repair? Two groups of Thy1-GFP transgenic male rats were subjected to tibial nerve transection and immediate repair using two epineurial sutures. One group of rats underwent daily electrical muscle stimulation of the gastrocnemius with a paradigm comprising of 600 equally separated contractions throughout one hour, delivered 5 days per week. Rat gastrocnemius muscles were electrically stimulated for 2 weeks and then underwent terminal assessments which included evaluating muscle force, contractile properties, motor unit numbers, and wet weight. Muscles were then harvested for immunohistological examination of motor end plate reinnervation. Muscles that received daily electrical stimulation had a significantly greater (2-fold) number of motor units as characterized using electromyographic methods (8.7 ± 0.9 vs. 3.0 ± 0.4, $p<0.01$). This result was confirmed when muscle end plates were visualized showing a much greater number of intramuscular axons and innervated end plates. Muscle weights, forces, contractile, and fatiguing properties were no different between groups. These results provide preliminary evidence that the improved early reinnervation may be due to antidromic depolarization of axons proximal to the repair site. Short term stimulation of the proximal nerve stump following injury has been shown improve axon outgrowth across the injury site [3]. Early treatment of denervated muscle using electrical stimulation can significantly enhance early muscle reinnervation. As the muscle continues to become reinnervated, tailoring the stimulation paradigm to improve muscle force and fatigability may lead to shorter recovery times and reduce extensive physiotherapy and rehabilitation requirements.

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Abstracts

Interest of combining posterior cricoarytenoid muscle reinnervation and functionnal electrical stimulation in horses with naturally occuring disease

Mespoulhes-Rivière C (1,3), Lechartier A (1), Rossignol F (2), Verin E (3), Marie JP (3), Cercone M (4), Cheetham J (4), Ducharme NG (4)

(1) National Veterinary School of Maisons-Alfort, Maisons Alfort, France, (2) Equine Clinic of Grosbois, Boissy Saint Léger, France, (3) CHU de Rouen, GRHV, EA380, France, (4) Department of Clinical Sciencs, College of veterinary Medicine, Cornell University, Ithaca, New York, USA.
E-mail: cmespoulhes@vet-alfort.fr

Treatment of vocal fold paralysis through selective motor reinnervation has been described with variable techniques and results in different animal species and in human patients. Recent experimental work demonstrated the interest of electrical stimulation of a nerve distal stump after transection, with promotion of motoneuron regeneration and earlier functional recovery. Combination of both techniques is currently being investigated in naturally occuring laryngeal hemiplegia in the equine patient, and preliminary data will be presented. Functional electrical stimulation (FES) of the posterior cricoarytenoid (PCA) muscle has been shown to preserve muscle fiber excitability and contractile strength. The effects of FES in surgically reinnervated PCA muscle will also be discussed.

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New Vienna/Liverpool implantable stimulator

Jarvis J (1), Bijak M (2), Lanmueller H (2), Haller M (2), Unger E (2), Lindenthaler W (3)

(1) Research Institute for Sports and Exercise Sciences, Liverpool John Moores University, Liverpool L33AF, UK; (2) Center for Medical Physics and Biomedical Engineering, Medical University, Vienna, Austria; (3) Med-El, Innsbruck Austria.
E-mail: hermann.lanmueller@meduniwien.ac.at

Stimulation of individual muscles or small groups of muscles via their motor nerves allows us to investigate the relationship between activity and phenotype without the confounding factors associated with voluntary or imposed exercise. It is a simple matter to activate all the motor units in the dorsiflexor muscles of the lower hind limb, for example, by stimulating the common peroneal nerve with electrodes placed close to the nerve, supplied by an implantable device placed in the abdominal space [1]. The development of suitable stimulating devices has been influenced by the progressive miniaturisation of commercially available microcontrollers. An important step forward was made in 2012 with the introduction of a 4x4 mm device that has sufficient processing power to allow a fully remotely programmable device to be made small enough for implantation in the mouse. Furthermore, the device can also be programmed to produce patterns with multiple nested timers to simulate some of the patterns that are claimed to be particularly effective in human training protocols [2]: for example, burst stimulation at 100Hz, 1s on, 2s off for 30s, 3 times over 20 minutes, repeated once per day. The Vienna group is developing a suitable radio frequency interface based on printed receiving coils in the circuit board of the stimulator, and a desktop transmitting coil. We are also developing a tablet-based user interface so that the amplitude and pattern of stimulation can be downloaded within a few seconds while the subject is placed over the transmitting coil. This also makes possible the investigation of progressive training regimes in which the intensity is increased weekly, for example, and allows us to test rationally the effect of periods of inactivity within a training programme.The use of the new microcontroller brings some technical challenges associated with encapsulation for long-term implantation because the distances between conductors are so small (approximately 0.1 mm). We have begun to use parylene coating applied by vapour deposition, and this seems promising.

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An implantable, modular telemetry system for bio-signal recordings in large animals

Kneisz L, Unger E, Lanmüller H, Bijak M, Mayr W

Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria
E-mail: lukas.kneisz@meduniwien.ac.at

To facilitate more sophisticated investigations on bio-signals we have developed an implantable telemetry system which is capable of recording various bio-potentials thanks to its modular design. The analog front end which offers a specific bandwidth of interest to be amplified with a desired gain is combined with a telemetric unit which also performs an analog-to-digital conversion of the signal. It is possible to acquire signals from two different channels with a sample rate of 1020 samples/s per channel and a resolution of 12 bit. In addition a multitude of information is utilized by simultaneous recordings with an integrated 3-axis accelerometer. The bidirectional communication link that is capable of transferring data between the implant and an external host station (a laptop or tablet PC) over several meters is based on the Bluetooth standard. The antenna of the implantable device was realized as a printed, meandered inverted-F antenna as that type showed the best performance in terms of size and efficiency for our purposes. Power supply is provided by a battery (3.6V, 1600 mAh) which enables an operating lifetime of more than 40 weeks if measurements are performed for 10 minutes a day. Dedicated software offers visualization of the data and allows the user to set recording commands. The results of two laboratory experiments and one field experiment are presented.

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Abstracts

Neuromuscular Stimulation Training for Sedentary Elderly

Krenn M (1), Hendling M (1), Haller M (1), Fruhmann H (2), Löfler S (2), Kern H (2,3), Mayr W (1)

(1) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (3) Department of Physical Medicine and Rehabilitation, Wilhelminenspital, Vienna, Austria.
E-mail: winfried.mayr@meduniwien.ac.at

A stimulator for neuromuscular electrical stimulation was designed, especially suiting the requirements of elderly people with reduced cognitive abilities and diminished fine motor skills. The ageing of skeletal muscle is characterized by a progressive decline in muscle mass, force and condition. Muscle training with neuromuscular electrical stimulation reduces the degradation process. The discussed system is intended for evoked muscle training of the anterior thigh.
The portable stimulator consists of a control unit and two stimulation modules. Each stimulation channel is designed modular and contains a power output stage to generate biphasic-rectangular, charge balanced and voltage-controlled pulses and a measurement module. To help elderly people to handle the stimulator by themselves the user interface is kept very simple. The compliance monitoring was composed of set amplitude value, stimulation pulse voltage and current shape. Especially the current information, i.e. charge, is an important parameter for monitoring muscle activation while using voltage-controlled stimulators. Also basic information like date and number of successfully completed training series are recorded. The evaluation software is programmed in Visual Studio C# (Microsoft, Redmond, USA). It allows the analysis of a specific training series up to the whole training. The compliance data of five subjects were evaluated from a current study. Stimulation training was performed for 9 weeks, with 2 sessions per week in the first two weeks and then increased to 3 sessions per week. A training session consisted of 3 series of training separated by 5 minutes breaks. A series took 6 minutes in the first two weeks and then 10 minutes which consisted of 75 contractions of one thigh (stimulation time: 3.5 s; off time: 4.5 s). Of all subjects during the whole training the mean value and standard deviation for the stimulation amplitude was 18,81 V (2,58 V) with the corresponding stimulation current 57,33 mA (5,78 mA). Whereas, the variability over the whole training of a subject was +/-12% and +/- 15% of the stimulation voltage and current, respectively.
Monitoring of the training is important for evaluation of the study success, especially concerning home based training [1-4]. Therefore, electrode interface abrasion, wrong or insufficient usage of stimulator can be detected.
Trial Registration: ClinicalTrials.gov NCT01679977.

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Image processing and segmentation in preparation for the simulation of electrical field during Functional Electrical Stimulation of musculus cricoarythenoideus dorsalis

Gargiulo P (1,2), Pétursson V (2), Magnússon B (2), Otto S (3), Martinek J (4), Reichel M (4)

(1) Dept. of Science, Landspitali University Hospital, Reykjavik, and (2) Dept. of Biomedical Engineering, University of Reykjavik, Iceland; (3) Institute of Veterinary Anatomy, University of Leipzig, Germany; (4) Dept. of Biomedical Engineering, Univ. of Appl. Sciences Technikum Wien, Austria
E-mail: paologar@landspitali.is

Distribution of the electrical field is very important to activate muscle and nerve cells properly. However the simulation of the electrical field based on anatomical data, is a challenging process and requires several preliminary steps. In this work we describe the techniques employed to prepare the simulation of electrical field during Functional Electrical Stimulation of musculus cricoarythenoideus dorsalis. Spiral computed tomography images and special computational tools are used to isolate cricoid cartilage and cricoarythenoideus dorsalis muscles from surrounding laryngeal elements. The CT scan data are imported into a special image processing and editing computer program called MIMICS [1]. In this software environment, muscles and cartilages are isolated using the Hounsfield (HU) scale which allows discrimination amongst different tissues based on their linear attenuation coefficient. To select and visualise only the region of interest a threshold based on HU values is defined. A maximum and minimum value is established and individual pixels are selected if their value falls in between the threshold values. Different false colours are used to label the elements in the data sets such as: muscle, bone, cartilage, fat, air and metal [2,3]. The results from the image processing and segmentation work are exported in DICOM format, where each segmentation mask is superimposed to the original data. In this it can be easily post processed. Finally material properties can be easily assigned to each segmentation element and the finite element analysis can start.

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New model systems for activation of muscle in culture

Stewart, CE, Sharples, AP

School of Sport and Exercise Sciences, Tom Reilly Building, Byrom St Campus, Liverpool John Moores University, Liverpool,UK
E-mail: C.E.Stewart@ljmu.ac.uk

Degeneration of skeletal muscle (SkM) with age (sarcopenia) is a major contributor to functional decline, morbidity and mortality. Methodological implications often make it difficult to embark on interventions in already frail and diseased elderly individuals. Using in vitro aged (Sharples et al., 2011) and three-dimensional (3D) bioengineered skeletal muscle constructs (Sharples et al 2012; Martin et al 2013) may more accurately characterise the SkM niche. Furthermore, an in vitro model would provide greater experimental manipulation with regard to gene, pharmacological and exercise (mechanical stretch⁄electrical stimulation) therapies and thus strategies for combating muscle wasting with age. Initial studies investigated morphological and immuno-histological (desmin) analyses together with transcript changes in matrix metalloproteinases (MMP2, MMP9), myogenic regulatory factors (MyoD, Myogenin), insulin-like growth factor members (IGF-I, IGF-IEa, MGF, IGF-IR, IGFBP2, IGFBP5) and myostatin (qRT-PCR). 3D bioengineered constructs incorporating aged cells had reduced myotube size and diameter vs. control constructs. Morphology suggested aged muscle constructs also showed reduced ability to attach to the collagen matrix also with reduced transcript expression of remodelling matrix metalloproteinases MMP2 and MMP9. Aged constructs were characterised by reduced peak force development over 24 hrs after cell seeding, reduced differentiation/hypertrophic potential shown by reduced IGF-I, IGF-IR, IGF-IEa and MGF mRNA expression. Increased IGFBP2 and myostatin expression in aged constructs also suggested impaired differentiation/reduced regeneration potential. This study provides an important 3-D model for studying ageing muscle phenotypes in-vitro. It consolidates key findings in monolayer cultures of rodent and human muscle cell ageing and further provides important insight into the impact of multiple divisions (artificial ageing), independent of stem cell niche, on the cellular/molecular mechanisms underpinning degeneration. Current studies are applying these models to investigate the impact of mechanical stimulation and repeated insults (atrophic and hypertrophic) on skeletal muscle cell memory, epigenetics and adaptation.

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How to stimulate muscle respecting its inherent adaptive capacity

Jarvis J

School of Sport and Exercise Sciences, Tom Reilly Building, Byrom St Campus, Liverpool John Moores University, Liverpool,UK
E-mail: J.C.Jarvis@ljmu.ac.uk

There is an increasing interest in the use of electrical stimulation to activate muscles in many different clinical or sports related situations in which voluntary activation is either impossible because of neural damage, inconvenient because of lack of time, or painful because of joint damage. Electrical stimulation can provide a hypertrophic stimulus to restore muscle mass after denervation [1-3]. It can provide some degree of cardiovascular training in paralysed persons [7], improvement of thigh muscles in patients with refractory heart failure [5] and it is claimed to provide accelerated recovery from intense exercise, perhaps by improving blood flow, although this is an area that needs further work. There is interest in the application of electrical activation when muscle strength has fallen below the critical point that allows safe standing and walking, which may ensue quickly after bed-rest enforced by a fracture of one of the long bones, especially in the elderly. There is also, however, considerable confusion over the mechanism by which electrical stimulation has its effect. For example, the idea that fast muscles 'respond' to high frequencies, whereas slow muscles 'respond' to low frequencies is commonly stated but inaccurate.
The following simple framework can help us to select appropriate patterns: Which muscle or muscles need to be activated and is the nerve supply intact?
On the basis of these answers we can select an appropriate pulse shape, taking account of the electrode system and the capacity of the stimulating device.
What effect do we require in the muscle? Is the primary goal to improve endurance, or to improve size, or to improve strength? In which species is the muscle?
On the basis of these answers we can select an appropriate activation pattern, taking account of experimental results that indicate how much activation is necessary to promote mitochondrial biogenesis for example [4], and whether we

should use stimulation that produces tetanic contractions, or simple oscillation of the muscle. There has been considerable recent interest in genetic predisposition to response in terms of changes in oxygen uptake to training, and the concept that some individuals have a much smaller cellular response to the same pattern of training than others [6]. It remains to be seen whether there is a similar variability among individuals in the response of individual muscles to training, but we have shown consistent relationships between the amount of activation and the response of individual rat muscles with group sizes of about 6, so it is unlikely that we will need a full genetic analysis before we can decide what pattern of stimulation to use.

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The impact of intramuscular-inserted hooked wire electrodes and specific stimulation parameters

Arnold D (1), Guntinas-Lichius O (2)

(1) Institute of Systematic Zoology and Evolutionary Biology with Phyletic Museum, FSU Jena, Germany; (2) University Hospital, Friedrich-Schiller-University Jena, Germany
E-mail: d.arnold@uni-jena.de

The importance of FES (functional electrical stimulation) with hooked wire electrodes increases in the diagnostic and therapy methods in the last years. These new potential approaches make it necessary to impale the target muscle and stimulate them. In our pilot experiments we determine how intense the target muscle was injured by repeated impalement with hooked wire electrodes and subsequent electrical stimulation. The triceps brachii muscles of 10 female rats were investigated. Typical signs of mechanical muscle injury and subsequent regeneration can be observed. We can assume that the stimulation protocol had no undesired side effects on muscle structure and function.
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Measurement of the 3D electrical field distribution with different multiplexed stimulation electrode configurations in a constant pressure ex vivo model of the equine larynx

Otto S (1), Diekow Ch (2), Gänz P (2), Michler JK (1), Koch KP (2), Mülling ChKW (1)

(1) Institute of Veterinary Anatomy, University of Leipzig, Germany; (2) Technical Department, University of Applied Sciences Trier, Germany
E-mail: sven.otto@vetmed.uni-leipzig.de

The objective of the study presented here was to improve the experimental setup used by Martini et al. [3]. Specific improvements were: 1. the usage of a constant pressure perfused ex vivo model of the equine larynx with the aim to reduce the reported changes in tissue morphology; 2. the implementation of different multiplexed stimulation electrode configurations

Abstracts

Functional electric stimulation (FES) is an innovative therapeutic approach for the treatment of recurrent laryngeal neuropathy in horses [1], [2]. The measurement of the three dimensional (3D) distribution of the electric field around the implanted stimulation electrodes [3] is sufficient to improve the outcome of FES. Horses were euthanized in consent with the national council´s guidelines of animal care (protocol number: T66/13). Subsequent to euthanasia, the larynx was dissected and two quadripolar stimulation electrodes were implanted into the cricoarythenoideus dorsalis (CAD) on each side of the larynx. The organ was fixed in an acrylic glass tub filled with prewarmed Tyrode solution (33°C) and connected to a closed constant pressure perfusion system. This was used instead of a constant flow perfusion system Martini et al. [3] used for their experiments. The perfusion solution was gassed with Carbogen (95% O2, 5% CO2). The larynx was perfused with warmed perfusion solution (37°C) and a constant perfusion pressure of 9.81 kPa. Subsequently, myogenic autoregulation was verified.
We used the same method described by Martini et al. [3] for the measurement of the 3D electrical field distribution but with a modified measuring system. A multiplexer was added to the experimental setup in order to allow a parallel measurement of the 3D electrical field distribution in different configurations of the implanted stimulation electrodes. The electrical potential inside the tissue could be measured for 17 different stimulation electrode configurations on each discrete testing point. 10 different configurations were used with 1 stimulation electrode and 7 with both stimulation electrodes on the left respectively right side of the larynx. This approach reduced the duration of measuring 17 times. The larynx showed no visible signs of tissue swelling and maintained myogenic autoregulation. The experimental setup developed by Martini et al. [3] was improved successfully. This was achieved by enhancing the system with a multiplexer. In addition to that, the application of a constant pressure perfusion of the equine isolated larynx was able to reduce the described tissue swelling effects [3].
Acknowledgement. This work was supported by a grant from Med-El, Innsbruck, Austria.

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Simulation of the electrical field during Functional Electrical Stimulation of musculus cricoarythenoideus dorsalis – a feasibility study

Martinek J (1), Otto S (2), Diekow C (3), Gargiulo P (4), Reichel M (1)

(1) Dept. of Biomedical Engineering, Univ. of Appl. Sciences Technikum Wien, Austria; (2) Institute of Veterinary Anatomy, University of Leipzig, Germany; (3) Technical Dept., University of Applied Sciences Trier, Germany; (4) Dept. of Science, Landspitali University Hospital, Reykjavik, Island.
E-mail: martin.reichel@technikum-wien.at

One therapeutic method to treat Recurrent Laryngeal Neuropathy (RLN) in horses can be performed by Functional Electric Stimulation (FES). Distribution of the electrical field is very important to activate muscle and nerve cells properly. Current method to optimize the stimulation effect is to use quadripolar electrodes either for anode or cathode and testing electrode configuration until best possible optimum is reached. For better understanding and finding of maximum possible activation of musculus cricoarythenoideus dorsalis a simulation model of the actual entire setting is currently in development. Therefore the geometric model is built from CT-data of a dissected and perfused larynx containing the implanted quadripolar electrodes. The geometric model is the basis for a finite difference method containing of voxels with corresponding electrical conductivity of the different types of tissue due to threshold segmentation of the CT-data. The acrylic glass of the electrolytic tray, a metallic reference plate and anodic as well as cathodic current sources represents system boundaries. Changes of electrode configuration can be realized by repositioning anodic or cathodic current sources, respectively. Model validation is done by measurement of the 3D electrical potential distribution by a differential amplifier controlled by a Labview-application with a micromanipulator-controlled placement of the needle electrode. The 3D measurement data of the electrical field and the CT-data are geometrically referenced by the placement of 3 fiducials in certain positions of the larynx. A customized multiplexer is switching defined configurations of the FES-electrodes automatically. Preliminary results show, that changes of electrode configuration leads to significant different voltage distributions and can be well presented by CT-slices with super positioned equipotential lines – a MatLab graphical user interface visualizes the results in freely selectable slices of the 3D geometry. Calculated potential distribution can be referenced to the measured potential distribution by the help of the fiducials positions, estimated in both data sets. Current work show, that the conditions for the measurement of the 3D potential distribution and the CT-data has to be improved due to avoiding geometry distortion. Further improvements could be done in increasing measurement speed and procedure optimization of potential distribution measurement to reach higher resolution within proper measurement time. For further calculation of nerve or muscle fiber activation and its optimization, fiber paths referenced to the potential- and the CT-data have to be estimated.
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Abstracts

EMG biofeedback muscle training with a wireless signal transmission: Design and methods

Helgason T (1,2), Thorgilsson B (2,3), Einarsson E (2,3), Eiriksson A (3)

(1) Landspitali – University Hospital, Reykjavik, (2) University of Reykjavik, (3) Kine ehf, Hafnarfjordur, Iceland
E-mail: thordur@landspitali.is

Training of movement coordination is mostly done with oral instructions immediately after the movement has been performed. The movement is done voluntary and the trainee interpreters his trainers instructions for the movement correction. The process is iterative, the movement and instructions are repeated until satisfactory results are achieved. This is a common practice in training of athletes as well as in rehabilitation after injury. Biofeedback has proven its efficiency in training. It gives the trainee an augmented sensing of the movement and, if designed properly, the error he makes or the distance from his training goal. In this work a small 26 g EMG unit was developed that is placed directly above the targeted muscle and transmits the EMG signal wireless directly from that location to a signal processing computer located within 50 m. The computer processes the signal in real time and produces a biofeedback signal in form of colour columns on a screen or in acoustic form. The columns are projected on a wall where the trainee can see it while doing his movement. The system works in several different modes, each with its own focus. In Strength mode the focus is on the muscle contraction strength reflected by the amplitude of the EMG signal. Looking at the columns the trainee gets immediate feedback on the firing of a muscle and its intensity. Levels of the signal strength can be programmed that change the colour of the column and gives an acoustic feedback if the strength goes above some minimal level and another one if the strength goes above some maximal level. In Ergo mode the focus is again on the EMG signal level. The muscle rest is the goal. Length of work pauses and their frequencies are monitored. A rule of rest is defined and the distance from this overload is communicated on the screen and with acoustic signal in real time. In Motor Control Mode the timing between muscle contractions is monitored, the muscle synergy. For example if two muscles are supposed to contract simultaneously a feedback is given if the time between them goes over some defined level and on how much. Speed mode focuses on muscle contraction or relaxation speed. Speed is an index of strength. The ability of relaxing a load slowly is an index of good muscle control. As a case the captain of the Icelandic women´s national team was diagnosed with an impingement syndrome of the shoulder. By having the signals from her shoulder muscles, anterior deltoid, upper trapezius, infraspinatus and serratus anterior displayed on the wall during training she could see the muscle activation in real time. She learned how to activate the different muscles and helped her to develop a movement pattern that was pain free.

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Electrical Stimulation in neural repair: First experience in Padova

Vindigni V (1), Dalla Venezia E (1), Marcante A (2), Masiero S (2), Mosole S (1,3,4), Zampieri S (3,4), Carraro U(4), Bassetto F (1)

(1) Plastic, Reconstructive, Aesthetic Clinic and (2) Rehabilitation Clinic of the Dept. of Neurosciences; (3) Translational Myology Lab., Dept. of Biomedical Sciences, CIR-Myo Interdepartmental Research Center of Myology, University of Padua, Italy; and (4) Ludwig Boltzmann Institute of Electrical Stimulation, Austria
E-mail: vincenzo.vindigni@unipd.it

Trauma of peripheral nerves can entail different disorders: strength and muscular balance deficit, sensibility impariments, sympathy function loss, pain. It results in motility deficits and rigidity development, algodystrophic alteration, protection sensation deficit and then further trauma risks. The type of trauma first of all determines management and prognosis. A sectioned nerve has no chance of spontaneous healing and discontinuity must be surgically repaired. Sprain nerve damage or significant distance between stumps have worse prognosis than clear local nerve cut. Mixed nerves have poor functional regeneration. Sensitive terminations degenerate more rapidly than motor ones. Outcome is also conditioned by general patient conditions. Young patients have better recovery. Diabetes, cardiovascular, immunity and other systemic disease can complicate the procedure. In the Operative Unit of Plastic and Hand Surgery of the University of Padova,(Italy), during the last three year 78 neurorrhaphy were performed. Just 2 patients were submitted to a secondary repair of a great segmental loss with neural graft. 76 procedures were executed in urgency room, until 3 hours after the trauma. 62 were performed on digital nerves and 14 on forearm nerves. The patients were followed up for 15 days to 2 years. Surgical repair consisted in the suture of the ends of the nerve. Early repair is the first choice, if permitted by a clean wound and no nerve segmental loss, because extensive nerve retraction has not occurred yet. Otherwise a delayed primary repair can be performed within 14 days. Secondary repair, two weeks after until 2 years after injury, is still indicated but achievement decrease with delay. Neurorrhaphy is executed by micro sutures carried out around epineurium, to accomplish group fascicular repair or every single fascicle through the perineurium and it is performed after aligning the nerve ends according to fascicular pattern and epineurial landmarks. Reconstruction after peripheral nerve injury may require management of segmental defects in the damaged nerve. Donor nerve for grafting is usually patient sural nerve. Regeneration of peripheral nerves is remarkably restrained across transection injuries, limiting recovery of function. Strategies to reverse this common and unfortunate outcome are limited. However, new evidence suggests that electrical stimulation (ES), delivered soon after injury and repair, improves the regrowth of motor and sensory axons [2]. Further reports describe positive effects of electrical stimulation on repair of peripheral nerve injuries either in experimental or clinical settings [1-6]. We will test electrical neurostimulation as described in [2-5] on patients undergoing

early neurorrhaphy. The first case (RISE-2 ESNR-Pd01 (BD) is a male who underwent a glass windov injury of the right forearm. October xxx, 2013the patient underwent medial nerve complete section and microsurgical suture in urgency room 3 hours after the trauma. After 10 days (???) of healing, the proximal stump of medial nerve was submitted to surface Electrical Stimulation (30 min, 0.3 msec pulse duration trains (1.5 sec ON, 4.5 sec OFF), delivered at intra train 50 Hz, and < 25 mAmp, settled according to the patient pain threshold but observing the contractile response of the forearm muscles, 5 days per week. The train had been delivered by the SPE1 stimulator, Medical Technology, Turin, Italy. Before and after the 30 min stimulation period, voluntary minimal movements of the right hand and fingers were observable. During the first weeks of electrical stimulation no adverse effects were observed. From day-zz post injury, the patient was admitted to standard hand physiotherapy.

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Stem cells and tissue engineering for myocardial regeneration and ventricular support

Chachques JC

Cardiac Research, Pompidou Hospital and Alain Carpentier Foundation, University of Paris, France
E-mail: j.chachques@egp.aphp.fr

Cardiomyopathy induces geometric remodeling of ventricular cavities, which change from a natural elliptical (conical) shape to a spherical shape. Ventricular chamber dilatation and spherical deformation are important causes of morbidity and mortality of patients with congestive heart failure. Results of cardiac cell therapy showed that cell bio-retention and engraftment within infarct is low and that extracellular matrix degradation contributes to adverse left ventricular remodelling. Stem cells have the ability to differentiate into various types of lineages which makes them attractive for the regeneration of myocardial tissue. Stem cell differentiation can be achieved by extrinsic physical stimuli (electrostimulation, magnetic fields), cyclic compressive strain, as well as by chemical (cytokines) and biological/genetic stimuli (cell co-cultures, genetic manipulations). Electrostimulation is a safe method to induce physical and biochemical changes in stem cells moving toward cardiac cells. Electrical stimulation is probably a major physiological stimulus in the heart for inducing cardiac differentiation. One of the most interesting applications of this concept is the Latissimus Dorsi Dynamic Cardiomyoplasty procedure, consisting in the electrical stimulation of muscular skeletal tissue grafted onto the heart for inducing myosin change to support heart failure patients. In this surgical approach the Latissimus Dorsi muscle flap transposed into the chest is wrapped around the ventricles to improve contraction and to limit heart dilatation in patients presenting ischemic and non-ischemic cardiomyopathies. Ventricular restraint therapy using polyester mesh wrap and nitinol devices have been used for heart failure patients who develop oversized, dilated hearts due to increased filling pressures. Adverse effects like diastolic function restriction and lack of improvement of systolic function, without evidence of myocardial healing open the door to associate a new approach based on biological regenerative therapy & myocardial tissue engineering. Tissue engineering and nanobiomaterials (containing nanoparticles smaller than 100 nanometres) emerge as new therapeutic tool becoming a promising way for the creation of "Bioartificial Myocardium". Biohybrid scaffolds have been implanted onto the heart for long-term cardiac support and myocardial regeneration, these implants should provide a suitable environment for cell homing, growth and differentiation, as well as mechanical support to the heart. Semidegradable nanofibers should allow the implanted cells to progressively interconnect, organize and contract, partial degradation of the elastomers forming the implants should reduce the risk of development of chronic fibrosis involved in the restriction of diastolic function. Perspectives and Clinical Implications. Bioactive molecules & nanobiotechnologies may contribute for the creation of a new « Bioprosthesis » for ventricular support and myocardial regeneration. Future research should include the creation of semi-degradable "ventricular support bioprostheses" designed with the concept of helical myocardial bands and models for left or right ventricle diseases with controlled degradation in response to the physiology of the left or right heart. Bioactive implants (bioprostheses) may reduce the risk of death or heart failure (HF) progression. Long-term effectiveness studies of bioactive implants evaluating the efficacy to prevent HF progression are needed to guide future clinical translation.

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Bioactive implant development to assist cardiac recovery

Semino CE, Castells C

Tissue Engineering Laboratory, Department of Bioengineering, IQS-School of Engineering, Ramon LLull University, Barcelona, Spain
E-mail: semino.c@gmail.com

Acute myocardial infarction (AMI) normally happens when blood supply to the heart is interrupted. Current treatments under development consist in cellular therapy where stem cells are implanted in scaffolds and grafted onto infarcted ventricles with the hope that cells will contribute to the generation of new myocardial tissue. We propose that this mechanism could be enhanced by the application of a "bioactive implant" onto the pathologic cardiac area. The development of the first prototype was possible by the coordinated action of all research groups of RECATABI consortium (EU grant CP-FP: 229239-2, http://www.recatabi.com), demonstrating the first proof of concept of the main hypothesis of the project. We therefore present here results in a small animal model (mouse) that indicate that the approach suggested is feasible. The main aim of this project was the development of a functional bioactive implant to assist or improve regeneration/restoration of the infarcted myocardium. The bioactive implant was successfully attached to the infarcted area in a small (mice) and big (sheep) animal model. Loaded stem cells started an active migration into the host tissue suggesting that it could contribute to induce regeneration, including possible angiogenesis and cardiac tissue remodeling. We present results suggesting that our platform promoted remodeling of necrotic areas of the injured tissue as well as recovery of functional cardiac parameters (i.e. Ventricular volume, Ejection fraction, etc.). We believe that one-time application of a bioactive implant will have a great impact in cardiac tissue recovery.

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Biomimetic and biofunctionalization approaches in cardiovascular tissue engineering for stem cells differentiation: getting closer to nature, getting closer to patient

Spadaccio C

University Hospital UZ Leuven and Ziekenhuis Oost Limburg (ZOL) Genk, Belgium
E-mail: cristianospadaccio@gmail.com

Despite the mediatic drive associated to stem cell therapy for cardiovascular disease, scientific literature is increasingly revealing its limitations and drawbacks. Non homogenous population recruited throughout clinical trials performed, no standardized technical guidelines on patient selection, contraindications, type of cells, cell dosage immunogenicity, timing and degree of expectable benefit on patient prognosis, are conspiring against its clinical translation. The most recent randomized control trials have failed to produce the same results as uncontrolled observational studies, and showed little improvement, particularly after longer follow-up times. Clearly, tissue regeneration cannot disregard the importance of extracellular matrix which is providing not only a structural support to tissue but also a crucial biological signaling able to preserve cells viability allowing their proliferation and differentiation. For this reason strategies of tissue engineering (TE) using resorbable materials have been developed. In the panorama of TE the idea of a biomimetic approach based on the simulation of the ECM along with the guidance of stem cells differentiation aided by a growth factor is emerging as a new perspective. The goal to concentrate and spatially organize a biological mediator within a three-dimensional environment mimicking the native tissue histoarchitecture has been recently pointed out. It allows exploiting the well-known benefits of the use of stem cells in regenerative medicine, enabling cell differentiation in a familiar and convenient 3D microenvironment and preserving cells from harmful factors present in the injured myocardium. Differentiation strategies in these 3D systems are discussed with particular orientation towards the clinical translation of this therapeutic resource.

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Cardiac progenitor cells: current and future directions for cell therapy

Chimenti I

Dept. of Medical and Surgical Sciences and Biotechnologies of "Sapienza University", Latina, Italy
E-mail: isotta.chimenti@uniroma1.it

Cardiac cell therapy is a promising regenerative treatment for the increasing number of heart failure patients in Western countries [Ptaszec LM, Lancet 2013]. Among multiple adult stem cells tested, resident cardiac progenitor cells (CPCs) seem to have the most effective therapeutic potential [Li TS, JACC 2012]. Despite encouraging results in the first clinical trials with intracoronary infusion of cell suspensions (CADUCEUS and SCIPIO trials), many hurdles (e.g. low cell engraftment and differentiation) still need to be overcome [Forte E, Stem Cell Rev 2011]. Recent studies suggest that 3D cell culture systems and optimal extracellular microenvironment can improve survival and differentiation compared to routine 2D cultures, paving the way to cardiac tissue engineering (TE) protocols. In fact, CPCs cultured as cardiospheres (CSs, spontaneous 3D niche-like structures) [Chimenti I, Methods Mol Biol 2012] have higher paracrine and regenerative potency compared to cell monolayers (CS-derived cells or other 2D-growing CPCs) [Lee ST, JACC 2011], which might be further enhanced when embedded in scaffolds for TE, also mechanically supporting the extracellular matrix [Chimenti I, Biomaterials 2011]. Moreover, the use of bioreactors, designed for mimicking the physiologic environment, represents an effective and scalable approach to further overcome 2D cultures limitations [Massai D, J Healthcare Eng 2013]. Research efforts might need to take a step backward “from bedside to bench” in order to investigate deeper the biology of CPCs and how to modulate their fate.

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In vivo application of adult stem cells and platelet rich plasma: recent experiences and future perspective in veterinary orthopaedics

Patruno M (1), Maccatrozzo L (1), Perazzi A (2), Iacopetti I (2), Martinello T (1)

(1) Dept. of Comparative Biomedicine and Food Science, and (2) Dept. of Animal Medicine, Production and Health, University of Padova, Italy
E-mail: marco.pat@unipd.it

The application of adult mesenchymal stem cells (MSC) and/or platelet rich plasma (PRP) into a damaged tendon aims to improve the poor reparative mechanisms that occur naturally in the regular dense connective tissue. In our study we examined the effects of autologous MSC derived from peripheral blood (PB-MSC), PRP and a combination of both for improving the regeneration of injured digital flexor tendons of sheep. The success of applications was evaluated at 30 and 120 days post application comparing clinical, histo- and immunohistochemical features. Concerning tendon morphology and extracellular matrix composition significant differences were found between all treated groups and their corresponding controls (placebo). Especially the use of MSC led to a better quality of the regenerated tissue. On the contrary, the combined use of PRP and MSC did not produce a synergistic regenerative response.

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Abstracts

Friday November 15, 2013

POSTERS

01. The short-term denervated hemidiaphragm: a mixture of "hypertrophy" and "denervation" features

Carraro U (1), Mosole S (1,2), Zampieri S (1,2), Nori A (3), Gobbo V (4), Germinario E (5), Danieli D (5)

(1) Translational Myology Lab., Dept. Biomedical Sciences, University of Padova, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) Excitation-Contraction Unit, Dept. Biomedical Sciences, University of Padova, Italy; (4) Neuroscience Unit, Institute of Medicine, C.N.R., Padova, Italy; (5) Muscle Contractility Unit, Dept. Biomedical Sciences, University of Padova, Italy.

Unilateral sectioning of the phrenic nerve in the thorac causes a transitory hypertrophy of the denervated hemidiapragm, followed a month later, by atrophy [1]. This seemingly unusual manifestation of the loss of innervation is attributed to the chronic, periodic stretching of the denervated myofibers by contraction of the still functioning contralateral hemidiaphragm [2,3]. During the hypertrophy there is a considerable accumulation of proteins. A significant increase in the rate of protein synthesis was observed in vivo as soon as 1 day after nerve section [4,5]. The enhanced rates of protein synthesis arise at least in part from the extra availability of RNA, ribosomes, the activity of polyribosomes, the capacity of cell sap to support incorporation of aminoacids, though the rate of disaggregation of the polysomes after in vitro incubation of denervated muscle appered to be slower than the control preparations [6]. Since the role of initiation factors in the control of ribosome activity has been suggested to explain these results, we measured the first step in the initiation of protein synthesis that appear to be rate limiting for protein synthesis in several experimental models. This control indeed operates to a varying extent in different cells and may be induced or repressed causing increased or decreased peptide chain inintiation [7], in particular in muscle from starved rats [8]. We contributed to these studies demonstrating that: 1. at 2 days post-phrenicotomy there is a +18% increase of muscle weight and a +24% increase of total RNA; 2. the eIF-2 initiator Factor and deacylase activities of the postribosomal supernatant were more than doubled, in comparison to unoperated controls, in the denervated hemidiaphragm during the early stages of its transitory hypertrophic response to denervation [9].We are comparing the effects of frenicectomy of the left hemidiaprams with those of left sciatectomy of EDL and SOL muscles, to reduce to a minimum the inter-individual differences that are much lower in inbred animals, but not eliminated.The effects of very early denervation [10] on contractile properties or rat skeletal muscle occur over the period from 2 to 6 days, when the twitch: times to peak and half relaxation are prolonged [11,12]. Similar behaviors we are observing in the denervated hemidiaphragm from aged rat (30 months old), despite it is not undergoing the extent of atrophy observed in the denervated EDL. Immunoflurescence analyses will be performed to described at fiber type levels if the responses are muscle specific and/or myofiber specific.

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02. Assessment of muscles, connective and fat tissue, using µCT data: feasibility study for meat quality control

Gargiulo P (1,2), Örlygsson G (3), Rizzi C (4,5), Carraro U (6)

(1) Dept. of Science, Landspitali University Hospital, Reykjavik; (2) Dept. of Biomedical Engineering, University of Reykjavik, (3) Dept. of Materials, Biotechnology and Energy, Innovation Center Iceland, Reykjavik , Iceland; (4) Dept. of Biotechnology, University of Verona, Italy; (5) Istituto Italiano Assaggiatori Carne, Torri del Benaco, Verona, Italy; (6) Translational Myology Lab., Dept. Biomedical Sciences, University of Padua, Italy.
Email: paologar@landspitali.is

Meat eating traits, such as tenderness and juiciness, are known to be linked to total fat levels, and to the associations between intra-muscular connective and fat distribution. The visual appearance of the fat could additionally affect the consumers overall acceptability of the product and therefore the choice and the selection of meat products before buying [1,2]. Bioimaging, image processing and 3D modeling has showed a fundamental role in assessing denervated muscles during electrical stimulation [3,4]. Similar techniques have been recently employed to monitor Extracellular Matrix Mineralization in biological scaffolds using X-ray µCT technology [5]. A combination of these two approaches was used to analyse meat samples with the General Electric nanotom x-ray µCT system, this system has 200 nm detail detectability. The aim is to study the feasibility of developing an alternative methodology for meat quality assessment based on image processing that could be in the future correlated with specific sensory analysis. A salted-smoked-fermented meat sample (Tiroler-speck, a typical product of the north part of Italy) of 10×10×1 mm was scanned with step of 3,5 µm using µct technology. The µct data scan data are imported into a special image processing and editing computer program called MIMICS [6]. In this software environment we isolate: muscles, intra muscular connective tissue and fat tissues. the discrimination between tissues is possible because of the different linear attenuation coefficient and consequent gray value intensity. False colors are assigned to the muscles, connective and fat tissues within the sample and the amount of each tissue have been quantified [7]. Moreover, it was also possible to isolate few single muscle fibers of 40-60 µmm diameters. Correlation of the 3D color analysis of each sample product with expectations of buyers may open large application to the imaging approach.

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03. Gender differences in the decline of muscle power up to the most advanced age

Gava P (1), Kern H (2), Carraro U (1)

(1) Translational Myology Lab., Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria.
Email: "Paolo Gava" <paologavastra@alice.it>

We here present evaluation of gender difference in decline with age of power of upper and lower limbs muscle. Muscle power decline with increasing age is drawn from the decline of the record performance of master athletes in different track and field events [1]. The analysis is based on the declining word records of master athletes competing within age groups of 5 years (from 35 to 39; from 40 to 44 and so on till the age of 100 years). The performances are normalized with respect to the absolute world record providing sets of dimensionless parameters ranging from one (absolute record) to zero (null performance) [2]. This approach to the decline of the skeletal muscle power with increasing age, by selecting human subjects genetically well gifted, trained at their best by professionals, and fully motivated, produces results not affected by the most common confounding factors which weaken many studies dealing with aging [3]. The trend-lines of all normalized performance indicate that the decline of muscle power starts not later than the age of thirty for men as well as for women. All trend-lines tend to zero at about the age of 110 years, in line with the present human maximal survival expectation of both males and females. The decline of the power in the events involving most the lower limbs (running events) starts quite gently and accelerates as age increases while in the events involving most the upper limbs (throwing events) the decline starts more sharply and then slows down approaching the bottom line. Results from world records of master athletes, while confirming previous results [4,5], show a different decline for males and females. Specifically, the power in the female running events declines more than in the male running events from the age of 50 onward. The diagram of the normalized power versus age is unambiguous. In the throwing events the trend-lines have an opposite behaviour: female decline is more rapid than the male decline in the initial phase while in the final aging phase the female and the male decline join together again. Interpretation of these results is difficult partly because the physical decline of males and females may be affected by a number of factors of various origin. Track and field world records of master athletes are surely linked to the social and cultural environment, beside the economic situation. In general the diagrams indicate that female power declines more than the male power, even if in different age ranges. Finally a spot light on the running performances (both for males and females): in the shortest distances the decline is very, very gentle till a remarkable old age. This capacity to

run fast for a short distance/time may reflect a human genetic characteristic developed to be able to escape predators.
Acknowledgements. EU Project Interreg-IVa, Mobility in Elderly, HK; Institutional funds UC.

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04. Nuclear wandering and nuclear grouping in human and rodent denervated skeletal muscle

Rossini K (1), Biral D (1), Zampieri S (1,2), Mosole S (1), Löfler S (2), Kern H (2), Carraro U (1)

(1) Translational Myology Lab., Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria
E-mail: krossini@tin.it

We will describe in details the time course of denervation-induced morphological changes affecting the human denervated skeletal muscle, resulting from the analyses of our world-unique cirMYO-Bank of human denervated muscle biopsies. These morphological changes last longer (in years) than generally accepted; the mid- and late-phases of denervation-induced atrophy and degeneration presenting two very contrasting myofibers populations: beside those severely atrophic due to loss of sarcomeric structures and with clumps of internalized myonuclei [5,6], large fast-type muscle fibers continue to be present four-to-six years after Spinal Cord Injury [2]. Throghout these phases in the denervated muscle several events of muscle fiber regeneration occur that are the outcomes of satellite cell activation, proliferation and fusion to aneurally myotubes and myofibers [5,7]. These are stages of the myofiber development characterized by centrally localized nuclei, and in adult muscle fibers, central location of the nucleus is too often taken as marker of myofiber regeneration
On the other hands, experiments in rodents [4] and observation in humans suggest that the normal elicoidal distribution of subsarcolemmal myonuclei is a dynamic process that actively and life-long maintain the nuclei in their peripheral position [3,1]. The percentage of severely atrophic myofibers showing nuclear clumps between the third and the sixth year after LMN lesion in humans is up to the 27% [6], while the percentage of big fibers is approximately of the 2% [2]. The percentage of morulae abruptly decreases after the sixth year onward, when fibrosis takes over to neurogenic muscle atrophy. Example of nuclear internalization will be provided in the peculiar case of muscle biopsies harvested from rectus abdominis of patients bearing colorectal cancer at the clinical onset of the tumor [9]. How and why this peculiar and exclusive distribution is maintained in the skeletal muscle myofibers remains to be described and their evolutionary advantage understood.

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P06. Scalp Potentials Changes due to Fontanels in an Infant's Head Model

Gargiulo P (1,2), Belfiore P (3), Friðgeirsson EA (3,4) , Vanhatalo S (5), Ramon C (3,6)

(1) Dept. of Science, Landspitali University Hospital, Reykjavik, Iceland; (2) Dept. of Bioengineering, Reykjavik University, Reykjavik, Iceland; (3) Dept. Biomedical, Electronic and telecommunication Engineering, University Federico II of Naples, Italy; (4) Dept. Development CE and IT, Landspitali University Hospital; (5) Dept. Children's Clinical Neurophysiology, Helsinki University Central Hospital, Helsinki, Finland; (6) Dept. Electrical Engineering, University of Washington, Seattle, WA, U.S.A
E-mail: paologar@landspitali.is

Our objective was to study the effects of fontanel on neonatal scalp EEGs. We used a 3-D finite element method (FEM) model generated from 110 segmented axial MR slices of an infant. The hexahedral voxel resolution was

0.938×0.938×1mm. Majority of the tissues were identified that included: scalp, fat, muscle, dura layer, CSF, cerebellum, gray and white matter, and hard and soft skull bone. The electrical activity of the whole cortex was represented by 2,048 dipoles. The dipole intensity distribution was in the range of 0.0 to 0.4 mA meter with a uniform random distribution to represent the spontaneous brain activity. Each dipole vector was oriented normal to the local boundary between gray and white matter and they were pointing outward from white to the gray matter. Simulations were performed for two models. In one model the conductivity of fontanel was equal to the conductivity of CSF (fontanel present) while in the other model it was equal to the hard skull bone (fontanel absent). The electrical conductivities of various tissues were obtained from the literature. Using an adaptive FEM solver, the potential and flux distributions in the whole head model were computed and scalp potentials (EEG) were extracted. Spatial contour plots of potentials on the scalp surface were made. Relative difference measure (RDM) and magnification factor (MAG) were computed between the two models. If the scalp potentials from two models are the same, the RDM and MAG will be zero and unity, respectively. Ten trial runs were performed with different uniform random distribution of dipolar intensities. Averaged over ten trials, the RDM was 0.062±0.016 and the MAG was 1.1±0.31. These values suggest that the two models yield very similar results. Closer inspection of spatial potential topographies, however, indicated up to 15% higher values above the fontanel area in the model with fontanels. This suggests a notable but local effect of fontanels on the EEG potentials in newborns.

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P06. Reinnervation of muscle fibers in aging muscle: Indirect immuno-histochemical evidence

Mosole S (1,2), Zampieri S (1,2), Rossini K (1,2), Löfler S (2), Kern H (2,3), Carraro U (1)

(1) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, and (3) Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria
E-mail: ugo.carraro@unipd.it

Although denervation has long been implicated in aging muscle, the degree to which it causes the loss of myofibers seen in aging muscle is unknown [1-3]. To address these questions, we quantified in senescent patients (either sedentary or with a life-long history of amateur sport) the percentage and size of denervated and innervated muscle fibers in a leg muscle using both in situ co-expression of fast and slow myosin heavy chain and the ATPase assay. Quantitative histological analyses show that the average diameter of skeletal muscle fibers from Vastus Lateralis is significantly higher in senior sportsmen compared to sedentary and that the proportion of severely atrophic denervated myofibers with a mean myofiber diameter < 30 μm is lower, compared to those observed in sedentary elderlies. In all muscle biopsies from senior sportsmen, reinnervation events identified as fiber type groupings were observed, while they were detected in the 86% of sedentary elderly. In senior sportsmen the higher prevalence of slow type fibers predominantly clustered in type groupings compared to fast type fibers suggest that the amount of endurance exercise that these subjects performed lifelong, induces an increment and a strengthening of the oxidative muscle metabolism. The total number of fiber type groupings detected in seniors sportsmen was significantly higher compared to that observed in sedentary seniors. In summary, our study provides a quantitative assessment of the contribution of denervation/reinnervation events to muscle decline in aging. A renewed focus on these aspects, in seeking for their clinical relevance and to understand causes and mechanisms may identify new targets for therapy/rehabilitation of aging muscles.

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P07. FES training protocols for the functional recovery of permanently complete denervated human muscles

Löfler S (1), Mayr W (2), Moedlin M (3), Burggraf S (1), Kern H (1,3)

(1) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation Vienna, Austria; (2) Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria; (3) Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Vienna, Austria
E-mail: stefan.loefler@wienkav.at

In denervation it was generally believed that no effective treatment was available for muscle that has undergone severe atrophy resulting from a chronic denervation injury. Under the Gutmann's [1] view of the trophic influence of nerves on muscle, the effect of a mimicking approach, electrical stimulation, played an important role, but over the years the value of electrically stimulating the denervated muscle has been disputed because of the difficulties to obtain strong contraction by electrical stimulation and of its possible unfavorably effects on any remaining potential for reinnervation. In the last 15 years, we studied the possibility to effectively train permanently denervated human muscles by means of Functional Electrical Stimulation (FES). The

results of the EU Project RISE [2-4] show a new perspective in stimulating muscle fibers in the absence of nerves and after prolonged denervation, enabling: i) restoration of muscle fiber ultrastructure; ii) recovery of conduction velocity of the excitation-contraction apparatus up to a level that allows tetanic contractility; and thus iii) astonishingly recovery of fiber size, muscle mass and FES-induced force.
Our training strategy is based on two combined stimulation programs. Within continuous clinical assessments, the stimulation parameters and training protocols should be progressively modified according to the patient's time span of denervation, the current condition of muscle and function. At the beginning of the treatment, biphasic stimulation impulses of very long-duration (120-150 ms, 60-75 ms per phase) at high intensity should be applied to improve membrane excitability and muscle structure. The next period of the routine daily training consists of combined stimulation patterns one eliciting single twitches (impulse duration of 120 ms) and the other tetanic contractions (2 – 3 s bursts with an impulse duration of 36-50 ms and impulse pause of 10 ms). After tetanic contractility is achieved and the subject is able to provide full extension of the leg during stimulation of the quadriceps muscles, the ankle should be progressively loaded following the training theory for healthy people. Finally, few patients who have achieved a good muscle and functional condition can be able to stand and perform step-in-place and walking exercise with stimulation to train the cardiovascular system, upper body, sense of balance and thigh muscles.

[1] Gutmann E, editor. The denervated muscle. Prague: Publishing House of the Czechoslovak Academy of Sciences, 1962.

[2] Kern H, Hofer C, Mayr W, Carraro U. European Project RISE: Partners, protocols, demography. Basic Appl Myol / European Journal of Translational Myology 2009; 19: 211-216.

[3] Kern H, Carraro U, Adami N, Hofer C, Loefler S, Vogelauer M, Mayr W, Rupp R, Zampieri S. One year of home-based daily FES in complete lower motor neuron paraplegia: recovery of tetanic contractility drives the structural improvements of denervated muscle. Neurol Res 2010; 32: 5-12.

[4] Kern H, Carraro U, Adami N, Biral D, Hofer C, Forstner C, Mödlin M, Vogelauer M, Pond A, Boncompagni S, Paolini C, Mayr W, Protasi F, Zampieri S. Home-based functional electrical stimulation rescues permanently denervated muscles in paraplegic patients with complete lower motor neuron lesion. Neurorehabil Neural Repair. 2010; 24: 709-721.

P 8. Myosin isoforms in small and large mammals: SDS PAGE demonstrates the co-migration of slow, but not fast Myosin Heavy Chains (MHC) from different mammals

Carraro U, Rizzi C, Rossini K

Myosin isoforms are used as markers of heterogeneity and plasticity of skeletal muscle fibers and motor units. We here describe a sensitive method that separates in nanogram or microgram amounts the myosin heavy chains (MHC) of immature, and fast and slow adult muscles. Though the method is assembled from published procedures (SDS-PAGE, peptide mapping in the presence of SDS, silver stain) for the logical extensions introduced the end-product is a powerful tool to separate and characterize these high molecular weight biopolymers until now inseparable from complex mixtures. The method reveals the heterogeneous nature of the MHC from embryonic muscles and the surprisingly fact that slow type MHC from several small and large mammals comigrate in SDS PAGE , a behavior that suggests strong similarity, despite the interspecies differences of contraction speed are larger than the intraspecific differences among slow and fast muscles.

[1] Carraro U, Catani C. A sensitive SDS-page method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. Biochem Biophys Res Commun 1983:116,793–802.

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P 9. Ribosomal profiles of denervated muscle

Carraro U, Catani C, Margreth A

Experiments are reported showing that following 8 days of denervation the function of the protein-synthesizing machinery, operating in the rat muscle fibres, is altered, probably as a consequence of decreased amounts of ribosomes and actively translated mRNA. The data obtained show that the amount and availability of the soluble factors involved in the process of protein synthesis are markedly decreased, thus suggesting that the amounts of ribosomes, mRNA and soluble factors are regulated in a concerted fashion when muscular protein synthesis is decreased after denervation.of leg muscles or increased after denervation hypertrophy in the hemidiaphragm.

[1] Carraro U, Catani C. iIF-2 initiation factor activity in postribosomal supernatant of hypertrophying rat diaphragm. FEBS Lett 1980;110:173-176.

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