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Three ways to try and heal a diseased muscle: macrophages, exon skipping and cell transplantation

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Muscular dystrophies have been the main interest of our group for many years. Here we will present three lines of research. The first one has to do with the role of macrophages in muscle regeneration. To this aim, we use the murine macrophage cell line J774 to obtain a serum-free, conditioned medium (mMCM) that we previously found to enhance the proliferation rate and the differentiation of rat and human myoblast (both normal and dystrophic) [1,2]. We are now characterizing its mechanism(s) of action in the murine model. We confirmed the pro-proliferative effect of mMCM on murine satellite cells, plus a pro-differentiation activity, and at the same time had a distinct anti-proliferative effect on primary fibroblasts from dystrophic muscle (i.e., from mdx mice). We also investigated the effects of mMCM on macrophages polarization, using human monocytes from blood that were differentiated and then stimulated to acquire either pro- or anti-inflammatory phenotype. Results so far indicate that mMCM seemed to have mixed effects, indicating that its composition might affect both populations. Similar findings were obtained in vivo, when the administration of mMCM to wt regenerating muscle seemed to decrease the number of both pro- and anti-inflammatory macrophages. Importantly, when applied in experiment of cell transplantation in dystrophic muscle, macrophagic factors led to much better grafting of donor wt satellite cells [3]. At the same time, we are also using the mdx dystrophic model to study the endogenous mechanisms that lead to the formation of dystrophin-positive fibers in the context of a dystrophic background, the so-called "revertant" fibers. Such phenomenon has been reported in man as well in animal models and is thought to occur thanks to alteration of the splicing mechanisms. We have analyzed a wide cohort of mdx mice of different ages and looked at different muscle types, showing that, as opposed to what had been suggested previously, the formation of new revertant fibers is a phenomenon that occurs throughout the animals' life. Besides, we found evidences that the number of revertant fibers increases with age not only in skeletal muscle but also in the heart, something that had never been reported before. We are now trying to clone satellite cells in which the 'reversion' phenomenon has occurred, in order to be able to study the biological mechanisms behind it.

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Congenital pseudo-myotonia in Chianina and Romagnola cattle and congenital myotonia in a New Forest pony: genetic homologs of human muscular diseases

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An inherited muscle disorder defined as "congenital pseudomyotonia" has been described in two important Italian cattle breeds Chianina and Romagnola and, as a single case, in a cross-breed calf in the Netherlands. Clinically the disorder is characterized by an exercise-induced muscle contraction. Cattle pseudomyotonia has been well characterized at both genetic and biochemical levels. By DNA sequencing of affected calves, we have provided evidence of mutations in ATP2A1 gene coding for sarco(endo)plasmic reticulum Ca²⁺-ATPase, isoform1 (SERCA1). Moreover we have demonstrated that cattle pathological muscles are characterized by a selective reduction in the level of SERCA1 expression. A New Forest foal has been evaluated for a muscular disorder: clinical findings include episodes of recumbency and stiffness with myotonic discharges on electromyography. The New Forest pony myotonia has been associated to a missense mutation in a well conserved domain of the equine chloride channel 1 (CLCN1) gene. The affected foal has been found to be homozygous and the mutation has revealed a recessive mode of inheritance within the reported pony family. On the basis of symptoms and of genetic confirmations, cattle pseudomyotonia has been defined as the true counterpart of human Brody disease, while the New Forest phenotype appeared related to human and goat congenital myotonia. Brody disease is a rare inherited disorder of skeletal muscle due to a SERCA1 deficiency, resulting from a defect of ATP2A1 gene. Human congenital myotonia results from recessive or dominant mutations of CLCN1 gene causing Becker's or Thomsen's disease, respectively. Our studies reflect the enormous potential of domestic animals to gain further insights into human medicine.

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Mitochondrial calcium signaling in the control of skeletal muscle homeostasis

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Skeletal muscle atrophy is due to a number of causes, such as disuse, denervation, aging, fasting, cancer cachexia. In recent years, lots of efforts have been made to understand the molecular pathways, and thus the potential therapeutic targets, of muscle atrophy. Mitochondria play a central role in skeletal muscle homeostasis, being the major source of ATP in oxidative myofibers. They have the ability to accumulate Ca^{2+} , behaving as buffers of the cytosolic $[Ca^{2+}]$ increase occurring during contraction. In addition, mitochondrial Ca^{2+} stimulates aerobic metabolism, and thus ATP production, essential for muscle activity. Finally, excessive Ca^{2+} accumulation in mitochondria can trigger cell death. The recent molecular identification of the Mitochondrial Calcium Uniporter (MCU), the highly selective channel responsible for Ca^{2+} entry into the mitochondria, has paved the way to novel experimental approaches, in which mitochondrial Ca^{2+} accumulation can be tightly regulated. Moreover, direct measurement and modulation of MCU expression and activity in different physiopathological conditions can now be performed [1-4]. Our preliminary data suggest that mitochondrial Ca^{2+} uptake plays a main role in muscle trophism. Thus, we wish to determine whether MCU represents a possible target of clinical intervention for the maintenance of muscle force. We also plan to explore whether the effects of mitochondrial Ca^{2+} uptake on muscle homeostasis (trophism and metabolism) vary according to the muscle type (slow-oxidative versus fast-glycolytic). Once the physiopathological effects of mitochondrial Ca^{2+} modulation have been clarified, we will focus our efforts in the dissection of the underlying molecular mechanisms. First we will try to understand how MCU activity is regulated in various conditions affecting muscle mass (e.g. denervation, exercise, overload, starvation). Next, we will explore the involvement of known or novel pathways in MCU-regulated muscle trophism.

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Skeletal muscle injury in Amyotrophic Lateral Sclerosis: first cause or effect?

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Amyotrophic lateral sclerosis (ALS) is a human progressive neurodegenerative disorder characterized by degeneration of motor neurons, muscle wasting and paralysis. There are familial (FALS 5-10%) and sporadic (SALS 90-95%). 20% of FALS is due to dominant mutations of superoxide dismutase enzyme (mSOD1) [1]. Studies on the pathogenic mechanism of ALS, have been focused (but not exclusively) on the mechanisms of neuronal damage that directly induce death (neuronal degeneration). In fact, much is known about the progression of the disease in cases of SOD1 mutations [2]. Neuronal injury in ALS belongs to four categories: excitatory glutamate toxicity, degeneration of mitochondria, oxidative stress protein toxicity. Skeletal muscle is sensitive to almost all of these categories which often end up with muscle atrophy. SOD1 expression (like expression of other genes and mutated proteins causative of the pathology) is not restricted to neurons, in fact other cells are involved in mechanisms that lead to disease progression or even to its establishment. Among tissues directly involved there is consensus on the importance of skeletal muscle [3]. The key evidence is that transgenic models with neuron restricted expression of mSOD show disease onset and progression only in few cases [4], while mice with muscle restricted expression of mSOD show signs of ALS similar to human. One suggestive working hypothesis is that motoneuronal degeneration might occur as a consequence of muscle wasting which causes neuromuscular junction loss [5]. Our goal will be to dissect pathogenic mechanisms of FALS in muscle cells and ex vivo muscle models by heterologous expression of mutant proteins to investigate muscle contribution to ALS.

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Presynaptic neurotoxins and the degeneration and regeneration of motor axon terminals

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Snake presynaptic PLA2 neurotoxins (SPANs) and the spider toxin α -Latrotoxin (α -Ltx) block the neuromuscular junction (NMJ) by inducing a flaccid paralysis. Despite their different biochemical activities these neurotoxins act similarly by inducing a massive calcium influx in the nerve terminal, altering the exo-endocytic balance, thus leading to paralysis. SPANs and α -Ltx -induced nerve terminal blockade is a reversible process: few days after toxins injection in the mouse hind limb the neurotransmission recovers completely. We wondered whether some molecules released by the paralyzed nerve terminal could help NMJ recovery, a process known to be mediated by both Schwann cells and muscle. We found that mitochondrial DNA (which has bacterial origin) and cytochrome c are indeed released in the supernatant of intoxicated primary neurons; these molecules could act as "alarmins" and activate immunocompetent cells as Schwann cells are, in order to remove cellular debris and initiate the regenerative program. These results could have relevant therapeutical implications for the comprehension of the complex regeneration process that follows a motoneuron insult.

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Water-environment as exercise setting to improve physical function in elderly

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In literature we found strong evidence supporting the use of water-based exercise for the improvement of aerobic capacity and strength [1,2]. Differently, moderate evidence highlighted the benefits on flexibility, and inconclusive evidence was found supporting the modification of body composition. These indications opened new research questions, beginning from the potential difference of the training in terrestrial setting versus aquatic environment. On the basis of these evidences, we developed a first study to assess the effectiveness of a 24-week exercise protocol aiming to detect potential differences between water and land settings on physical function in a group of healthy elderly by a twice-a-week exercise intervention. In addition dual-energy X-ray absorptiometry and peripheral quantitative computed tomography have been performed. Results indicated that both water- and land-based were beneficial in maintaining strength, while aquatic exercise was more effective to improve dynamic balance. We suggested that thermal swimming pools should be considered potential useful tools to enhance physical performance and maintain muscle mass in healthy elderly. The consideration that a warmer water temperature should be more beneficial for elderly subject, motivated a further study protocol evaluating the physiological responses during exercise in hot (HW) vs cold water (CW). We studied these two conditions in older subjects observing that heart rate was significantly higher when exercising in HW compared to colder condition (+15.9%, $p < .01$); additionally, systolic and diastolic blood pressures were found to be lower in the HW condition (-7.1% $p < .05$; -9.4%, $p < .05$). We suggest that a warmer environment may represent an additional stress to the body that should be considered when estimating the intensity of aquatic exercise in a training strategy.

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Muscle tissue micro engineering

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Tissue structure and function are known to be highly inter-related and the correct stimuli given at micrometric level are

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essential for the emergent properties of a multicellular tissue. The concept of “microscale tissue engineering” originates from the need of providing cells with the appropriate cues and with a controlled microenvironment in order to generate in vitro models of muscle tissue that accurately replicates the structure and function of the natural ones. In this scenario, we engineer the culture microenvironment to guide cell-substrate and cell-cell interactions to rationally guide human myoblasts behavior. Our interests are focused on healthy tissues and disease, in order to provide new tools for studying the physiology and the pathology of skeletal muscles. In particular, we adjust the mechanical and topological properties of the culture substrate, acting on the signaling involved on the mechanotransduction and on the cell-cell interactions. We designed a 2D substrate, with mechanical and topological properties (a 15 ± 2 kPa poly-acrylamide hydrogel micropatterned with 500 μm wide parallel lanes) able to guide the differentiation of human healthy and DMD myoblasts into myotubes exhibiting marked functional differentiation, highly defined sarcomeric organization and dystrophin expression in vitro within 12 days. Recently, we translated these results to a 3D culture system, in order to study human myogenesis in an in vivo-like physiological microenvironment. Micro-channels (80-160 μm in diameter, 10-15 mm long) within a hydrogel of poly-acrylamide and hyaluronic acid (12 ± 4 kPa) were developed. Human myoblasts were cultured for up to 15 days and tightly packed myotubes bundles have been obtained, expressing myosin heavy chain, α -actinin and dystrophin. It is worth to underline that such myotubes bundles can be extracted from the hydrogel scaffold and manipulated for surgical implantation. The 2D model of skeletal muscle has been already exploited for the in vitro test of stem cell therapy and for studying the effects of cyclic in vitro stretch. We studied the capability of human mesoangioblasts to restore dystrophin expression in our model of DMD skeletal muscle and we observed an increased dystrophin expression when DMD human myoblasts are co-cultured with human wild type mesoangioblasts. Moreover, an ad hoc cell-stretching device has been developed in order to accurately control human myotubes deformation along biaxial directions. A maximum cell deformation of 20% can be obtained by tuning the pressurization of microfluidic channels, with frequency from 0 to 1 Hz. With this device, we demonstrated that DMD myotubes have a higher stretch-induced membrane permeability compared to healthy myotubes, both subjected to 90 min cyclic deformation of 20%. These results show that in vitro models of human skeletal muscles, developed exploiting micro tissue engineering techniques, represent with high fidelity the natural tissue and are exploitable in a wide range of applications.

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ER processing of skeletal muscle proteins

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About one third of all cellular proteins pass through or are resident in the endoplasmic reticulum (ER) where they undergo proper folding and targeting. However, these processes may fail producing misfolded polypeptides, especially when single amino acid substitutions or in-frame deletions are present. The ER is therefore endowed by a sophisticated quality control system that drives nascent polypeptides towards native conformation and recognizes and funnels terminally misfolded proteins to degradation by the so called Endoplasmic Reticulum Associated Degradation (ERAD). The rigid rule of ERAD, however, can also eliminate misfolded but partially functional mutants leading to a de facto loss of function that can evolve in a disease [1]. This, as we recently demonstrated, is the unfortunate case of several missense mutants of α -sarcoglycan, causing type 2D Limb Girdle Muscular Dystrophy [2], as well as that of a single point mutant of the SERCA1 calcium pump, responsible for cattle pseudomyotonia. At present, no effective therapies are available for these severe myopathies and our efforts are devoted to the identification of new therapeutical approaches aimed to avoid the misfolded protein destruction. A “protein rescue strategy” will take advantage of the identification, that we have almost completed, of the ERAD components controlling the processing of α -sarcoglycan mutants. These components could become potential drug targets so as to control the effective degradation of mutants. Alternatively, a “protein repair approach” can be envisaged by using small compounds able to promote mutant folding and targeting. Promising results have been obtained with both approaches.

Funded by AFM and Telthon.

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Microgenomics of skeletal muscle fibers

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Skeletal muscle is composed by different myofiber types with a wide range of biochemical, structural, and functional properties. In the past years, biophysical analyses on isolated muscle fibers were extensively used to study the different contractile features of each myofiber type. To better clarify the complex mechanisms determining and regulating this heterogeneity, we applied genomic technologies at the level of single isolated myofibers (microgenomics). Fibers were

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prepared from soleus and EDL mouse muscles in order to obtain a comprehensive collection of fiber types classified according to myosin heavy chain (MyHC) isoforms. Transcriptomic analysis with microarrays produced expression profiles of myofibers free from the background of non-muscle cell transcription and identified a complete catalogue of genes differentially expressed among fiber subtypes, mainly coding for isoforms of the contractile proteins, metabolic enzymes, or proteins involved in Ca^{2+} homeostasis. Interestingly, myofibers grouped in 3 different clusters corresponding to slow, intermediate, and fast phenotypes that only partially fit with MyHC classification. In addition, we identified a limited set of transcriptional markers that can be used to unequivocally classify myofibers in one of the three clusters [1]. From classified myofibers, we investigated the different expression levels of the myomiRs. The correlation between mRNA and miRNA expression data will allow the definition of the transcriptional circuits involved in myofiber type specification. This microgenomic approach at single fiber level would allow a better understanding of the adaptive transcriptomic transitions occurring in myofibers under physiological and pathological conditions.

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Double face of autophagy in skeletal muscles during physical exercise

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Optimal autophagic flux is required for the maintenance of the integrity of myofibers, which are the basic contractile units of skeletal muscles. Both excess and reduced levels of autophagy are detrimental for muscle health; the former results in the loss of muscle mass, whereas the latter causes skeletal fiber degradation and weakness [6]. Mutations in any of the three genes that encode collagen VI, a skeletal muscular extracellular matrix protein, result in Bethlem myopathy and Ullrich Congenital Muscular Dystrophy in humans [4]. Collagen VI deficient (*Col6a1*^{-/-}) mice, the animal model of these diseases, are characterized by decreased muscle strength, skeletal muscle degeneration and a high rate of apoptosis. *Col6a1*^{-/-} mice revealed impaired activation of the autophagic machinery resulting in an accumulation of defective organelles such as dilated sarcoplasmic reticulum and swollen mitochondria that result in apoptosis and muscle wasting [1,3]. *Col6a1*^{-/-} skeletal muscles displayed an impairment of autophagy flux and lack of autophagosomes formation. The lack of autophagosomes is due to the lower induction of Beclin1 and to the constitutive activation of Akt/mTOR/ULK1 pathway. Forced

activation of autophagy cleared myofibers from dysfunctional organelles and ameliorated the dystrophic phenotype of the mice. These findings indicate that defective activation of the autophagic machinery plays a pathogenic role in congenital muscular dystrophies [1]. We also demonstrated that a proper regulation of the autophagy flux is fundamental for the homeostasis of skeletal muscles in response to physical exercise, which promotes mitochondrial biogenesis and function. We hypothesize that exercise might improve mitochondrial function by triggering autophagy in *Col6a1*^{-/-} mice. The notion that the reactivation of autophagy via physical exercise may ameliorate muscle myopathy is particularly relevant in the treatment and management of skeletal muscle-related diseases. In contrast to our original hypothesis, we found that while physical activity stimulates autophagy in normal muscles, neither long-term nor shorter spurts of intense physical activity stimulate autophagy in *Col6a1*^{-/-} mice. Physical training exacerbated the dystrophic phenotype of *Col6a1*^{-/-} mice, where autophagy flux is compromised. After training, skeletal muscles accumulate a large amount of swollen mitochondria, which determine an extremely high level of apoptosis in myonuclei. Conversely to wild-type control, autophagy was not induced in *Col6a1*^{-/-} muscles after either acute or prolonged exercise, and this led to the marked increase of muscle wasting and apoptosis. In a situation of altered autophagy in muscles, physical exercise seems to have more detrimental effects than beneficial ones [2]. Nonetheless, the study clearly shows that the activation of autophagy during physical activity in muscle cells is necessary for maintaining tissue homeostasis by preventing the accumulation of damaged mitochondria and myofibril degeneration [2,5].

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Interreg IVa “Mobility in elderly”

Rehabilitation studies in denervation, aging and oncology

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During the last decade we contributed to rehabilitation in aging studying 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 [9-12] 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. 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. 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) [13] 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 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. Based on our recent observation of the presence of a subclinical myopathy in patients affected with newly diagnosed colorectal cancer [14,15], 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

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Transcriptional changes in muscle with exercise and disuse

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We have used two models to change the activity pattern of skeletal muscle. Implantable stimulators have been used to add impulses to the voluntary activation of lower limb muscles, and tetrodotoxin (TTX) nerve block has been used temporarily to eliminate all activity to lower limb muscles and in some cases to allow a return of voluntary activity. These models demonstrate the amazing capacity of individual skeletal muscle fibres to dismantle and to rebuild their intracellular structures, and to modify the cellular components that determine their function as motors that power locomotion and transport. For example total silencing of the common peroneal muscle in rat over 14 days produces a 50% reduction in wet weight of muscle fibres, or almost 4% per day. Rebuilding is at a very similar rate once activity is restored. We have performed microarray analysis of rat muscles during disuse atrophy and recovery after restoration of activity using the Genechip 230 2.0 array in which 28,000 genes are represented. While muscle cells contain pathways

that can maintain homeostasis without changes in protein synthesis, transcriptional control is demonstrably important in adaptation to changes in activity. We found significant differential expression of between 1500 and 2000 genes with TTX block, and a similar number during recovery from block, whereas a comparison between sham operated and 7-day recovery of voluntary activity showed less than 100 differentially expressed genes. Among the genes that showed the greatest change in expression were myogenin and the HDAC family. We have investigated the ability of manipulation of the HDAC pathway to influence atrophy in myoblast culture. There are a number of recent studies in which the cellular pathways involved in mitochondrial biogenesis and fibre type modification have been investigated with various patterns of exercise in training humans and other mammals [1,2]. Even with the best planned studies including biopsy sampling, it is difficult in a human study to investigate the response space describing the relationship between pattern of activity, time, and changes in transcription. We have used PCR to follow key transcriptional pathways in separate groups of rats over the hours and days following a change in activity pattern. The data illustrate the variability in response between individuals but also that mean transcript levels show complex patterns of response over time that highlight the difficulties involved in interpreting and modelling the response space based on the restricted sampling points in human trials.

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The contribution of stem cell therapy to skeletal muscle remodelling in heart failure

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Aim of our study was to investigate whether stem cell (SC) therapy with human amniotic fluid stem cells (hAFS, fetal stem cells) and rat adipose tissue stromal vascular fraction cells-GFP positive cells (rSVC-GFP) was able to produce favourable effects on skeletal muscle (SM) remodelling in a well-established rat model of right heart failure (RHF). RHF was induced by monocrotaline (MCT) in Sprague-Dowley rats. Three weeks later, four millions hAFS or rSVC-GFP cells were injected via tail vein. SC differentiation was

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studied by double immunofluorescence. SM remodelling was assessed by Soleus Muscle fibre cross sectional area (CSA), myocyte apoptosis, myosin heavy chain (MHC) composition and satellite cells pattern [1-6]. Cytokine profile was evaluated. The hAFS and rSVC-GFP injection produced significant SC homing in Soleus (0.68 ± 1.0 and $0.67 \pm 0.75\%$ respectively), with a 50% differentiation toward smooth muscle and endothelial cells. Pro-inflammatory cytokines were down regulated to levels similar to those of controls. SC-treated (SCT) rats showed increased CSA ($p < 0.004$ vs MCT) similarly to controls and showed a shift towards the slow isoform with a leap of MHCI. Apoptosis was significantly decreased (11.12 ± 8.8 cells/mm³ hAFS and 13.1 ± 7.6 rSVC-GFP) ($p < 0.001$ vs MCT) and was similar to controls (5.38 ± 3.0 cells/mm³ p=ns). RHF rats showed a dramatic reduction of satellite cells (MCT: $0.2 \pm 0.06\%$ Pax7 native vs C $2.60 \pm 2.46\%$, $p < 0.001$), while SCT induced a repopulation of both native and SC derived satellite cells (rSVC-GFP $1.35 \pm 0.40\%$ Pax7 native and $1.25 \pm 0.33\%$ Pax7-SC injected; hAFS 0.60 ± 0.32 Pax7 native, 0.60 ± 0.34 injected, $p < 0.005$). In conclusion, SC treatment led to SM remodelling with satellite cells repopulation, decreased atrophy and apoptosis. Modulation of the pro- and anti-inflammatory cytokine milieu might play a crucial pathophysiological role. This observation opens a possible scenario for autologous transplantation of SC adipose-tissue stromal vascular cells in patients with cardiac cachexia.

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Patient results and mathematical methodology for EMG-based control of Deep Brain Stimulation in Parkinson and Essential Tremor

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We discuss an EMG-based design for closed-loop predictive on-demand control of deep-brain stimulation (DBS) in Parkinson (PD) and Essential Tremor (ET) patients. While presently DBS is applied in open-loop, it is generally recognized that closed-loop dynamic control of DBS in a manner that self-adapts to patient needs at any given moment is the final goal of DBS. Such control obviously is based on utilizing a reliable sensor for closing the loop, without which no control is possible. The natural sensor is one that reliably and continuously senses electrical activity such as neuronal spike signals or local field potential (LFP) at the site of stimulation, usually, the STN (sub-thalamic nucleus) or the VIM (ventral intermediate nucleus of the thalamus) in deep brain, to be subsequently processed in the IPG (implanted pulse generator) of the DBS system. This type of sensor located in deep brain involves many difficulties, such as reliability of secure placement such that contact is never lost, filtering of effects of the much stronger stimulation pulse (relative to electrical level of neural activity, let alone the mathematical aspects involved in any closed-loop control). We therefore proposed [1], [2], [3] using the raw surface-EMG (sEMG) from tremor affected limbs as sensors, noting its easy accessibility and the richness of that signal in terms of its integrating information from the firing of many motor-neurons. We also noted that tremor in PD patients is usually the first symptom to appear [4], while it is effectively the only symptom in ET. Although, pathological tremor is generated in the affected brain regions, but the related neuronal activity, wherever it originated, is sent to peripheral motor-neurons to cause tremor. The transmission delay from brain to the limb where tremor appears is of the order of 0.1 second, considering neuronal transportation speed (3-10 m/sec). The control approach we use is predictive control. Consequently, we predict the appearance of tremor at least 3-10 seconds before it actually occurs and once predicted to occur, we switch on DBS. This allows us to apply our method to any already-approved (by FDA or other regulatory authority), even in previously-implanted patients. Our resultant system communicates wirelessly with the already implanted IPG and hence avoids the need for additional surgery to incorporate the predictive sEMG controller in already implanted DBS system. The senior authors already demonstrated in 1975 a mathematical procedure capable of retrieving control of an artificial arm in 3 degrees of freedom from a single sEMG signal site, [5], thus illustrating the power of combining sEMG with powerful mathematical signal processing. Similar applications were published and utilized relating to using sEMG control of electrical stimulation in upper-motor-neuron paraplegics, for estimating desirable limb function from above-lesion sEMG. We also used sEMG sensors (in combination with others) to predict onset of sleep-apnea events [6]. The mathematical algorithm used [7] to predict onset of tremor ahead of its actual occurrence is based on

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incorporating several sub-algorithm in a decision tree. These algorithms each retrieve certain parameters from the sEMG signal such that the combination of these parameters determines tremor is to occur within a predetermined time-window, as follows:

(a.) Spectral (using Fourier and wavelet transforms) Measures:

- a.1. Mean frequency,
- a.2. Power at peak frequency
- a.3. Mean power in particular wavelet band

(b.) Entropy Measures:

- b.1. Wavelet entropy – Shannon Entropy measure
- b.2. Sample entropy

(c.) Recurrence quantification measure: Recurrence rate

For discrimination between tremor and voluntary movement and between ET patient states we also use an acceleration signal. Results. We carried out 190 test runs of 8 patients, 4 PD patients and 4 ET patients – all previously implanted.

The average outcome of these results was as follows:
PD (average over 4 patients): Accuracy = 80.2, Sensitivity = 100, p-value = 0.0097

ET (average over 4 patients): Accuracy = 85.7, Sensitivity = 100, p-value = 0.0078

The study was made under University of Illinois at Chicago (UIC) IRB approval.

Acknowledgements. This study would not have been possible without the help and advice of Dr Konstantin Slavin, Dept. of Neurosurgery, UIC. The support of Dr Daniel Corcos (Dept. Kinesiology, UIC) and of Dr. Leonard Verhagen-Metman (Dept. Neurology, Rush University Med. School, Chicago, IL) was also essential to this study.

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Bone and muscle assessment in patients undergoing total hip arthroplasty using HU based analysis

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Total hip arthroplasty (THA) is performed with or without the use of bone cement. The lack of reliable clinical guidelines for deciding which one to implement has encouraged this approach of joint clinical and engineering with the following objectives: 1. Validate quadriceps muscles and femur bone atrophy by extracting the mineral density from Computer Tomographic (CT) images. 2. Validate computational processes based on 3-D modeling and Finite Element Methods (FEM) [1,2]. A clinical trial was started, where 36 volunteer patients underwent THA surgery for the first time: 18 receiving cemented implant and 18 receiving uncemented implant. The patients were CT scanned prior-, immediately after and 12 months post-surgery. The CT data are further processed to segment muscles and bones and to create 3D-models for the simulation and for calculating bone mineral density (BMD). Furthermore quadriceps muscle density Hounsfield (HU) based value is calculated from the segmented file on healthy and operated leg [3]. These preliminary results indicate computational tools and methods that are able to quantitatively analyse patient's condition pre and post-surgery [4]. The BMD and muscle density measurement in correlation with the fracture risk analysis display a potential method for eligibility to receive non-cemented implant; the preliminary results show that also elderly that according with current clinical evaluation receives a cemented implant are suitable for the non-cemented type. The risk for structural failure during THA surgery is estimated by calculating femoral bone fracture risk index (FRI) as a ratio between compressive stress during surgery and estimated failure stress on bone. The correlations with the BMD observations during the clinical trial will assess and validate this potential predictor tool.

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Neural injury and regeneration

Accelerating axon growth after nerve injury

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Axons in the peripheral nervous system (PNS) conduct action potentials from one node of Ranvier to another, the myelin sheath formed by Schwann cells insulating the axons between the nodes. Accelerated conduction velocity due to the nodal conduction is also a feature of myelinated axons in the central nervous system (CNS) where the oligodendrocytic glial cells enwrap several axons to form myelin sheaths. After nerve injuries that disrupt axon continuity, the axons that are disconnected from their cell bodies undergo Wallerian degeneration with dissolution of the axons and their myelin sheath. The glial cells multiply but it is only the Schwann cells in the PNS that support the outgrowth and elongation of regenerating axons, the myelin products of the oligodendrocytes inhibiting axon growth in the CNS. The expression of growth associated genes in the cell bodies of injured peripheral nerves and the Schwann cells in the growth pathway facilitate axon outgrowth and regeneration within the distal nerve pathway after surgical apposition of the proximal and distal nerve stumps. Axons regenerate at the rate of slow axon transport, the rate being 1-3mm per day. However, there are substantial delays and asynchronous outgrowth of axons at the injury site. The Schwann cells and extracellular matrix are initially disorganized at this site before they gradually form Bands of Bungner of longitudinally orientated Schwann cells that extend throughout the endoneurial tubes that previously surrounded each axon in the distal nerve pathway. The Schwann cells are critical for axon regeneration, their presence and guidance being essential for axon regeneration to occur. At the time of nerve repair, a brief period of one hour low frequency electrical stimulation is sufficient to dramatically accelerate the outgrowth of axons across the injury site of surgically repaired peripheral nerves. As a result, the more synchronous outgrowth of axons results in accelerated regeneration through distal nerve stumps and their earlier reinnervation of denervated targets. Although the electrical stimulation does not accelerate the rate of axon regeneration once the axons reach the distal nerve stump, the accelerated axon outgrowth is functionally significant in the light of the transient expression of growth associated genes in the injured neurons that have not yet regenerated their axons to make functional target connections with associated progressive decay in their regenerative capacity. The growth supportive Schwann cell phenotype is also time-limited such that their expression of their growth supportive genes declines with time such that

they cannot support the regeneration of axons through the long distal nerve pathways at the relatively slow rate of axon regeneration. The accelerated axon outgrowth is associated with increased levels of neuronal cAMP and expression of the growth associated genes in the injured neurons. The transient expression has recently been shown to be prolonged by administration of anabolic steroids such that both when combined with brief periods of electrical stimulation, axon outgrowth and rate of regeneration are both accelerated. These findings in animal studies are very promising and proof of principle studies in human nerve injuries demonstrate dramatic increase and accelerated functional recovery.

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Mechanisms underlying exercise-mediated rescue of cachexia

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Cachexia is a multifactorial syndrome defined by an ongoing loss of skeletal muscle mass, with or without loss of fat mass that cannot be fully reversed by conventional nutritional support [1]. Cachexia is characterized by severe muscle atrophy and weakness, accounting for poor prognosis and worsening patients quality of life. Indeed, cancer cachexia accounts for about 20% of cancer patient's deaths. Recent

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studies showed that physical activity after cancer diagnosis ameliorates the prognosis, although the underlying mechanisms are still poorly understood. With the aim to delineate the pathways involved in exercise-mediated rescue of cachexia, we investigated the effects of spontaneous physical activity (wheel running) in colon carcinoma (C26)-bearing mice. All major diagnostic criteria for cachexia were reversed by exercise, including rescue of body weight, muscle atrophy and fatigue, ultimately leading to increased survival. These data suggested a potential use of exercise mimetics (that is, pharmacological treatments activating pathways physiologically triggered by exercise) to counteract cachexia. To test this approach, we treated C26-bearing mice with AICAR, an analog of AMP that is capable of activating AMPK-mediated pathways, thus mimicking endurance exercise adaptations in skeletal muscle [2]. Strikingly, AICAR treated mice looked healthier, lost significantly less body and muscle weight and show reduced expression of atrogenes in respect to vehicle treated mice. The molecular mechanisms by which AICAR can rescue muscle cachexia are under investigation. We first hypothesized that AICAR could increase PGC1 α expression and mitochondriogenesis, therefore promoting a shift towards a more oxidative status that may render skeletal muscle more resistant to wasting. This was not the case. We are now evaluating if AICAR rescue cancer cachexia by increasing autophagy in skeletal muscle. We propose that physical activity counteracts cachexia and reveal a potential use of exercise mimetics to ameliorate patient muscle wasting.

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Exercise-based rehabilitation in complicated patients with anemia

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After completion of cancer treatment, a relatively high percentage of patients (17-56%) present fatigue. It is associated with considerable impairments in cardio-respiratory fitness and is likely due to complex inter-relationships between direct effects of cancer on the affected organs, direct toxic effects of anticancer therapy on multiple systems (especially on heart, blood components, muscles, neuroendocrine system), as well as indirect consequences of therapy (physical deconditioning, depression). Anemia is one

of the most common manifestations, reaching a prevalence as high as 68-77% of patients in advanced stages of cancer. Likewise in heart failure patients, fatigue, impaired cardiovascular fitness and anemia are associated with an increased risk of cardiovascular morbidity and mortality. The effects of exercise training on these parameters and the adaptation mechanisms that could prevent or mitigate dysfunction in oncologic patients are still poorly known; so, some data are derived from the larger experience of exercise-based rehabilitation in cardiac patients. Exercise training modifies muscle fibers composition and muscle blood distribution, contrasts treatment-induced sarcopenia, and increases muscle mass and functional performance (independently of heart function); physical exercise reduces the risk of morbidity and mortality from other chronic diseases (such as diabetes and coronary artery disease), that could be induced or worsened by cancer therapy and whose effects could outweigh the initial cancer diagnosis. Observational studies and meta-analyses reports confirm that exercise-based interventions during or after cancer treatment are effective in reducing cancer related fatigue and anxiety, and - similarly to general population and chronic heart failure patients - improve quality of life and possibly increase survivorship. Likewise in coronary artery diseases, in oncology patients blood transfusions seem to be linked to increased or accelerated mortality, and erythrocyte stimulating agents - though effective in improving quality of life - could have an unfavourable effect on life expectancy. Individually tailored exercise training is well tolerated in complicated patients with anemia and allows similar physical improvements as in non-anemic populations; it can increase total Hb and red cell mass and thus enhance oxygen-carrying capacity to the working muscles and all other organs. It is reasonable to presume that a wider application of comprehensive exercise-based rehabilitation in oncologic patients could be worthwhile.

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A subclinical myopathy in early colorectal cancer

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Skeletal muscle is the major reservoir of body proteins and it can be particularly affected in conditions associated to altered protein turnover and metabolism such as cancer. Although severe wasting is seen primarily in patients with advanced malignancy or cachexia, some of them present degree of wasting at clinical presentation. It would be important to identify prognostic markers of cancer associated myopathy in order to prevent the severe muscle wasting that finally leads to cachexia. We collected skeletal muscle biopsies (rectus abdominis) from patients (n=50) affected with colorectal cancer at diagnosis, during open surgery for the excision of the tumor, and morphometric analyses showed that in the absence of muscle atrophy and local tissue inflammation, a high percentage of myofibers (median 13.1%) were abnormally nucleated presenting myonuclei located inside the myofiber rather than at the periphery. Histochemical analyses testing for the activity of myofibrillar ATPase, showed that these myofibers were predominantly of fast type, suggesting that this phenomenon was not randomly present, rather than fiber type specific. In addition, immunohistochemical stainings identified several small myofibers expressing two classical markers of muscle regeneration: the embryonic isoform of myosin heavy chain (MHC-emb) or the neural cell adhesion molecule (NCAM). Moreover, in cancer patients, we observed an inverse correlation between the number of internally nucleated fibers and the presence of node metastasis (N+) (ρ)=-0.30 (p =0.03). These features are typical sign of myopathy, that were not observed with such a frequency in a group of rectus abdominis muscle biopsies from non oncologic patients (n=25), undergoing surgery for benign non-inflammatory diseases (median=3%, p <0.0001 vs oncologic patients).

Analyses on serum samples collected prior to surgery testing markers of inflammation (C Reactive Protein, CRP), muscle enzymes, (Creatin Kinase, CK), soluble isoform of NCAM), and marker of protein turnover (prealbumin) showed that in the absence of systemic inflammation (CRP and CK values were in the normal range), in the prevalence of cancer patients the levels of prealbumin were below the normal range (200-400 mg/L), and the mean value was significantly lower compared to that detected in non oncologic patients (174.38±57.86 mg/L vs 264.00±69.73, p <0.001). Interestingly, the low levels of prealbumin were not

significantly associated with the body mass index in oncologic patients, and these patients had no body weight loss > 5% since 6 months prior to cancer diagnosis, that is one of the criteria to define the cachetic status of the subject.

Prealbumin, also known as transtiretin, is a typical marker of caloric and protein malnutrition, which has been already described in conditions characterized by proteins' deficit due to reduced food intake, malabsorption and coeliac disease, or increased catabolism (cancer, cachexia, AIDS, hyperthyroidism, hypercortisolism). Recently, prealbumin has been also described as a prognostic marker of cancer relapse in patients affected with colorectal cancer, but its association with the survival rate in these patients has not been clarified yet. Up to now, our data indicate that the subclinical myopathy we observed in patients affected with colorectal cancer appears to be associated with an early stage of cancer and it could be a side-effect of anti-cancer defense mechanisms (possibly pro-apoptotic, thus, non inflammatory) on the skeletal muscle, during the preinvasive stage of cancer. Cancer-related skeletal muscle wasting has profound effects on functional outcomes and quality of life. Evidence from recent publications indicates that repeated exercise may enhance the quality of life of cancer patients. When started early during the progression of disease, i.e. before the onset of severe myopathy or cachexia, at the stage in which the subclinical myopathy can be observed, regular physical exercise may prevent or control the progression of cancer-associated myopathy, possibly counteracting some of the mechanisms underlying muscle wasting.

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Rehabilitation treatment for lymphedema in postintervention breast cancer

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Lymphedema represents a clear and palpable increase in volume of a limb or a body segment, due to an interstitial lymphatic stasis. It's caused by a mechanical deficiency characterized by a low lymphatic flow as a result of lymph nodes and lymphatic vessels disorders, secondary to iatrogenic events like trauma, surgery, radiotherapy, physical insult and disuse. Lymphedema presents a large amount of proteins in the interstitial spaces and, when lasting for more than four weeks, leads to skin alterations characterized by proliferation of connective tissue and fibrosis. Lymphedema can be primary, due to congenital lymphatic deficiencies, or secondary to pathological and/or iatrogenic causes. The clinical signs are the following: skin colour appears nearly normal; in general it is monolateral; it affects both dorsum and fingers of hand and foot; it is painless; Stemmer sign is positive in the fibrotic phases; it does not improve in the antigravitational position. Lymphedema can have an early onset, soon after surgery and/or radiotherapy, or a late onset, months or up to twenty years after. Preservation of the limb, homolateral to the side of surgery, should be the priority in patient's management and requires the following cures: avoid excessive local heat, any compressive events, positions or activities that restrict venous and lymphatic circulations, risks of inflammation and infection. Moreover, it's essential to care the limb and prevent the complications using active mobilization. Treatment of lymphedema can be rehabilitative or pharmacological and its aim is to stimulate a correct hemolymphatic drainage. Rehabilitative treatment consists in: draining postures of upper extremity, strengthening and endurance exercises, passive and active mobilizations of upper extremity, manual lymphatic drainage with or without compression bandages and, if necessary, use of compression garment and pressure therapy. Furthermore, the rehabilitative management must include patient's education, daily living precautions and home exercise programme. Being lymphedema a chronic condition, rehabilitative treatment does not manage to resolve but only to limit the problem improving, however, patient's expectations and quality of life. In order to avoid or limit this complication an early approach of the rehabilitative team is fundamental. On the other hand, active collaboration of the patient in the self-management of lymphedema is necessary in maintaining treatment results and preventing aggravation. Because of its nature, lymphedema necessitates a multidisciplinary cross-sectional approach, involving several professionals like surgeons, oncologists, radiotherapists, radiologists, physiatrists, psychologists and physical therapists [1-3].

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Physical Medicine and Rehabilitation in Oncology. Therapeutic strategies in a key cancer center in Vienna, Austria

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Cancer is changing from a deadly to a chronic disease. New therapeutic modalities are helping to prolong the life span of the affected patients. Apart from new therapies regular exercise has proven to be safe and cost efficient [1-3]. In breast cancer patients, for example, the long term survival can be improved by about 50%! In a major cancer center we are trying to convince particular patients to use the tool of force and endurance training to make their life longer and self determined. After proper assessment and exclusion of contraindications we train these patients 30 times in 6 months on force in our hospital and guide them through a 3 times per week independent endurance training at home. With precise measurement and targets considering the metabolic situation and abilities of the patient we are able to follow the progress of the patients and motivate them to stay adherent to the training therapy. The program is complemented by psychological guidance and proper dietetic recommendations. In this article we try to give an insight in our considerations and our strategy to modify the behavior of these patients on a long term basis.

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Abstracts**Progressive un-coupling of mitochondria from calcium release units in ageing: implications for muscle performance**

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At the most basic level, skeletal muscle contraction requires Ca^{2+} and ATP and, thus, is under direct control of two important intracellular organelles: Ca^{2+} release unit (CRU) - specialized intracellular junctions, also named triads, which are involved in excitation-contraction (EC) - and mitochondria, the organelles deputed to produce the energy required for most cellular functions. It is now becoming clear that: a) CRUs and mitochondria interact functionally and structurally because entry of Ca^{2+} into the mitochondrial matrix is required to stimulate the respiratory chain, and increase ATP production; b) efficient Ca^{2+} uptake into mitochondria may strongly depend on their location with respect to sites of Ca^{2+} release. Recent studies from our laboratory have shown that many mitochondria in skeletal fibers are connected to CRUs by small structures, called tethers, electron dense linkers which keeps mitochondria in proximity of CRUs [2]. Miss-function of mitochondria and functional structural changes affecting the EC coupling apparatus have been both proposed to contribute to the age-related decline of skeletal muscle performance [1,3,5]. We have studied the morphology, frequency, and sarcomeric-localization of both CRUs and mitochondria in: A) Extensor Digitorum Longus (EDL) muscle from adult (4-12 months) and ageing WT mice (25 to 35 months of age); and b) in human biopsies from sedentary elderly subject and age matched sportman (65 to 75 years of age) using light, confocal, and electron microscopy (EM) to determine how EC coupling and mitochondrial apparatuses are affected by age and exercise. *Results*. A. Studies in mice revealed that: a) the number of CRUs/100 μm^2 (measured in longitudinal EM sections) in aging mice decreases significantly compared to adult mice: 93 ± 9 vs. 76 ± 8 , respectively ($p < 0.01$); b) the number of mitochondria-profiles/100 μm^2 also decreases with age: 54 ± 7 vs. 43 ± 6 , respectively ($p < 0.01$); c) in ageing fibers mitochondria are more frequently found at the A band of the sarcomere (2.0 ± 0.0 vs. 0.4 ± 0.0), away from CRUs. The miss-placement of mitochondria is likely the results of the decreased frequency of tethers: in ageing fibers their number decrease with age from 14/100 μm^2 in adult vs. 6/100 μm^2 in ageing mice. The above changes taken together cause a significant decrease in the number of functional CRUs-mitochondria couples: 39 ± 5 vs. 26 ± 5 (a decrease of about 33%). This considerable reduction of CRU/mitochondria couples may significantly contribute to the decrease of specific force and endurance of skeletal

muscle associated to ageing. A manuscript containing these data is ready to be submitted (D'Incecco et al., in preparation [4]). *Results B*. Studies in human Vastus Lateralis biopsies from sedentary elderly subjects confirmed general findings collected in mice (i.e. decrease in frequency of both CRUs and mitochondria and partial miss-placement of mitochondria). However, in human studies we also had the chance to compare samples from two groups of elderly individuals of 65-75 years of age (all males), sedentary and sportsmen (individuals who regularly exercised in the last several years of their life), in order to determine if structural changes in CRUs and mitochondria are just caused by ageing itself or if inactivity plays also a central role in the progressive decay of EC coupling and mitochondrial apparatuses. These studies revealed that both CRUs and mitochondria increase with exercise, mitochondria much more than CRUs. Number of CRUs/100 μm^2 : 16.0 ± 8.8 in sedentary vs. 21.6 ± 10.8 in sportsmen; number of mitochondria/100 μm^2 : 38.8 ± 20.3 in sedentary vs. 52.0 ± 21.3 in sportsmen. The combined increase of both CRUs and mitochondria results in largely increased frequency of CRU/mito pairs: 4.3 ± 4.5 in sedentary vs. 11.1 ± 8.3 in sportsmen. The results collected in our studies suggest that structure/organization of both EC coupling and mitochondrial apparatus: a) is affected by age; b) is better preserved in subjects which exercise regularly. In conclusion, the dramatic age-related decay affecting mitochondrial and EC coupling apparatuses in humans are, at least in part, caused by inactivity due to changes in life style.

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Abstracts

Decades of high level physical activity postpone age-related decline by increasing reinnervation of skeletal muscle

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Sarcopenia, the age-related impairment in skeletal muscle structure and function, is the major cause of frailty, fall-related injuries, increased morbidity and mortality in old age [3]. It is associated with a progressive loss of muscle mass, strength, and power. Histological changes seen in skeletal muscle from seniors indicate that denervation contributes significantly to muscle wasting. Denervation atrophy causes progressive accumulation and clustering of small, angular fibers with denervated features. A progressive loss of α -motoneurons and a decrease in the number of motor units are also observed [2,5]. These age related denervation events lead to reinnervation by the neighbor motor units (motor unit remodeling) that can increase in size, in association with fiber type grouping (one or more myofibers are completely surrounded by fibers of the same contractile phenotype). Several longitudinal studies have shown that regular physical exercise may extend life expectancy, reduce morbidity such as frailty, neurological disorders and reduce physical disability in aging [1,4]. Based on these findings, we intended to determine how a life-long exercise delays structural decline of muscle characteristics caused by aging, in particular investigating its effect on age-related denervation. Quadriceps muscle biopsies from a peculiar group of fifteen well-trained seniors that exercised regularly in their previous 30-40 years (senior sportsmen) and from fourteen sedentary elderlies with a normal lifestyle (sedentary seniors) were collected (70 ± 4.0 vs 71 ± 5.5 years, $p = n.s$). Quantitative histological analyses show that the average diameter of skeletal muscle fibers from Vastus Lateralis is significantly higher in senior sportsmen compared to sedentary seniors (61.22 ± 17.11 vs 51.41 ± 15.43 , $p < 0.0001$) and that the proportion of severely atrophic denervated myofibers with a mean myofiber diameter $< 30 \mu\text{m}$ is lower (2%), compared to the 6% observed in healthy sedentary elderlies ($p < 0.0001$). In all muscle biopsies from senior sportsmen, reinnervation events identified as fiber type groupings are observed, while they are detected in the 86% of healthy sedentary elderly. In particular, in seniors sportsmen slow fiber type groupings are detected almost exclusively (slow type $n=174$, fast type $n=3$), while in sedentary seniors the observed type groupings are of both slow ($n=19$) and fast ($n=20$) type. In senior sportsmen the higher prevalence of slow type fibers predominantly clustered in type groupings compared to fast type fibers (69% vs 31%, $p < 0.001$) 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 (177 vs 39, $p < 0.0001$) These observations suggest that reinnervated myofibers (in type groupings) are more common in senior sportsmen than in healthy sedentary elderlies. The low percentage of denervated myofibers and the higher mean myofiber diameter observed in seniors sportsmen suggest that decades of high level physical exercise have beneficial effects on age related decay also counteracting denervation atrophy by promoting reinnervation. All together, these findings suggest that a higher number of myofibers persists despite aging in senior sportsmen.

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Effects of electrical stimulation and strength training with proprioceptive stimulation on selected strength and functional parameters in elderly

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The term dynapenia has been recently introduced to refer to age-associated loss of muscle strength. This concept stresses that, with aging, loss of muscle function and strength is of higher importance and progresses at higher rate compared to loss of muscle mass [1]. 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 improve dynapenia [2]. However, there is less knowledge about alternative treatments for dynapenia e.g., surface electrical stimulation. Therefore, this study examined effects of an 8-week period of strength vs. electrical stimulation training on muscle strength and functional parameters in elderly. Twenty subjects were randomly assigned to either strength training (ST), age

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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±3.258, 168.2±5.630 78.89±8.681). Duration of both training regimes was 8 weeks. RT 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. Maximum isometric strength of knee and leg extensors was measured on a knee extension and leg press dynamometer, respectively. Muscle strength increased significantly in the ST group, only, with increase being 10% and 27% ($p < 0.01$) for knee and leg extension, respectively. A higher relative increase in leg press compared to knee extension may be a result of the training specificity, as the leg press device was also used during the trainings. A non-significant increase in muscle strength (5% in both tests) in EST could be explained by excluding voluntary neural activation in EST, which is a crucial part of the training adaptation during the initial phase of the strength training. In clinical functional tests, chair rising improved significantly by 17% ($p < 0.05$) and 14% ($p < 0.05$) in ST and EST, respectively. There was also a non-significant correlation between chair rising test and strength levels implicating other adaptation mechanism(s) besides muscle strength influencing chair rising performance. TUG performance did not change significantly in either group. 10-m maximum speed walking performance changed significantly in EST only ($p < 0.05$). To conclude, strength training was superior for gaining maximum strength in both training-specific and -non-specific tasks compared to electrical stimulation. There was similar effect of both ST and EST regimes on clinical functional tests. It seems that initial results and training adaptations measured by clinical functional tests may be more vulnerable to confounding factors e.g., body mass and health status, compared to laboratory strength tests.

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The effect of strength training on balance in elderly

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Falls and their consequences are a major health problem in elderly population [1, 2]. Poor balance has been recognized as one of many precursors of falls [2-4]. Moreover, sustaining balance in an upright posture involves reach sensory-motor processing which results in generating

appropriate timing and amount of muscle forces needed to counteract the constantly appearing destabilizing external forces. The aim of 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 (CG, $n = 19$), electrical stimulation group (ES, $n = 27$) or leg press group (LP, $n = 28$). Subjects in both the training groups (ES and LP) were exposed to training 3x/week for a period of 9 weeks. In the ES group the subjects received neuromuscular electrical stimulation of the anterior thigh muscles. In the LP group the subjects performed strength training on a computer-controlled leg press machine using the mode of combined slow movements and superonated 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 balance was quantified as the average velocity, amplitude and frequency of the center-of-pressure (CoP; total, medial-lateral and anterior-posterior) as detected with the force platform. Statistical analysis included descriptive statistics, tests of normality/homoscedasticity, and tests among the groups (ANOVA, ANCOVA and t-tests). The level of statistical significance was set at 0.05. 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 ($F = 4.9 - 9.6$, $p = 0.000 - 0.001$) and amplitude ($F = 5.6 - 8.5$, $p = 0.001 - 0.006$), while they were less evident in case of mean frequency of the power spectrum ($F = 1.6 - 3.6$, $p = 0.03 - 0.21$). The mean improvements were higher in the LP group than in the ES group. To summarize, our results provide supportive evidence to the existence of the strength:balance relationship. The selected body sway parameters were namely improved after the training period in both experimental groups. The training effect on static balance was bigger in case of training with voluntary contractions and less when neuromuscular electrical stimulation was used. This might indicate the role of recruiting central (supraspinal and spinal) processes and activation of functional kinetic chains for the better end effect.

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Abstracts

The power of mitochondrial shaping machinery in controlling muscle mass

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Skeletal muscle is a tissue with high energy demand that requires an extremely organized and functioning mitochondrial network. 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 OPA1 by generating a muscle specific knockout mouse. Ablation of OPA1 gene results in 100% lethality at newborn age. OPA1-null muscles are smaller in size than controls because of inhibition of protein synthesis and activation of autophagy-lysosome system. OPA1 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 smaller in size with dilated cristae and altered function. 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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The role of cytokine IL-6 in the physiopathology of skeletal muscle

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One of the crucial parameters of tissue regeneration is the microenvironment in which the stem cell populations should operate [1]. Stem cell microenvironment, or niche, provides essential cues that regulates stem cell proliferation and that directs cell fate decisions and survival. It is therefore plausible that loss of control over these cell fate decisions might lead to a pathological transdifferentiation or cellular transformation. Current advances in stem cell biology justify a cautious optimism, yet the presence of stem cells seems to be not sufficient to guarantee efficient tissue regeneration and repair [2]. Specific factors are required to trigger stem cells toward a specific lineage, to improve their survival, and to

render them effective in contributing to tissue repair. Studies on stem cell niche led to the identification of critical players and physiological conditions that improve tissue regeneration and repair. Among these, IGF-1 has been involved in the modulation of inflammatory response and in the regulation of muscle regeneration and homeostasis [1,3,4]. On the contrary, IL-6 is a multifaceted signalling molecule classified as a pro-inflammatory cytokine. It is commonly described as a pleiotropic cytokine, which is both produced by a variety of cell types, targets a variety of cell types, and has the capacity to induce several different intracellular signaling pathways. Circulating IL-6 levels are normally very low or undetectable and are dramatically increased in response to inflammatory conditions. In particular, chronically elevated levels of circulating IL-6 are associated with several pathological conditions such as cachexia, insulin resistance, heart failure, sepsis, etc [2]. We comparatively investigated the effects of high levels of this cytokine on muscle homeostasis, demonstrating that IL-6 affects muscle growth and plays a key role in the exacerbation of dystrophic muscle phenotype.

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The role of the Mitochondrial Calcium Uniporter (MCU) in skeletal muscle trophism

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Skeletal muscle atrophy is due to a number of causes, such as disuse, denervation, aging, fasting, cancer cachexia. In recent years, lots of efforts have been made to understand the molecular pathways, and thus the potential therapeutic targets, of muscle atrophy. Mitochondria play a central role in skeletal muscle homeostasis, being the major source of ATP in oxidative myofibers. They have the ability to accumulate Ca^{2+} , behaving as buffers of the cytosolic $[Ca^{2+}]$ increase occurring during contraction. In addition, mitochondrial Ca^{2+} stimulates aerobic metabolism, and thus ATP production, essential for muscle activity. Finally,

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excessive Ca^{2+} accumulation in mitochondria can trigger cell death. We wish to dissect, among these various possibilities, which is the prevalent effect of mitochondrial Ca^{2+} uptake elicited by contraction, and we aim at verifying whether novel therapeutic strategies against muscle atrophy may be forecasted by modulating mitochondrial Ca^{2+} uptake. The recent molecular identification of the Mitochondrial Calcium Uniporter (MCU), the highly selective channel responsible for Ca entry into the mitochondria, has paved the way to novel experimental approaches, in which mitochondrial Ca^{2+} accumulation can be tightly regulated. Moreover, direct measurement and modulation of MCU expression and activity in different physiopathological conditions can now be performed.

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Malignant Hyperthermia (MH) and Environmental Heat Stroke (EHS): understand the molecular mechanisms to develop therapeutic interventions

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Several skeletal muscle diseases have been associated to mutations in proteins involved in the excitation contraction (EC) coupling, the mechanism that links the transverse(T)-tubule depolarization to release of Ca^{2+} from the sarcoplasmic reticulum (SR) [8]. In particular, mutations in the gene encoding for ryanodine receptor type-1 (RYR1), the SR Ca^{2+} release channel, underlie several debilitating, life-threatening muscle disorders including malignant hyperthermia (MH) [10]. To date, MH is only seen as a clinical syndrome in which genetically predisposed individuals respond to volatile anesthetics in the operating room with potentially lethal episodes characterized by elevations in body temperature and rhabdomyolysis of skeletal muscle fibers [4]. However, virtually identical over-heating episodes have been reported in individuals also after exposure to environmental heat, physical exertion, or even during febrile illness [7]. Such episodes are described in literature as environmental/exertional heat strokes (EHS) [1]. The clinical presentation of these crises is indeed very similar to that of MH: hypermetabolic state, rhabdomyolysis, increased serum levels of CK, hyperkalemia, etc. [6]. Interestingly, some patients who experienced heat/exertion-induced MH episodes have family history of MH [3]. The life-threatening nature (and the frequency) of over-heating episodes, underscore the critical need for a deeper mechanistic understanding of these syndromes and for the development of new and effective treatments and drugs. This

requires, though, several important issues to be resolved: Specific Gap A) Mutations in RYR1 have been found in many, but not all, MH cases suggesting the potential involvement of additional genes in the pathogenesis of this syndrome. Specific Gap B) The relationship between classic MH and EHS over-heating episodes triggered by different stressors (heat, exertion, fever, etc.) is not yet widely recognized. Specific Gap C) The cascade of molecular mechanisms that from SR Ca^{2+} leak leads to rhabdomyolysis of muscle fibers are still unclear and needs to be fully elucidated. Thanks to the support of Telethon ONLUS (GGP 030289 and 08153), in the last years we have moved significant steps toward the solution of the specific gaps presented above. We have demonstrated in animal models that: a) MH episodes can result not only from mutations in RYR1, but also from mutations in proteins that modulate RYR1 function (like Calsequestrin-1, CASQ1) [9]; b) the mechanisms underlying hyperthermic episodes triggered by anesthetics and by heat are virtually identical, suggesting that these syndromes could be possibly treated/prevented using similar treatments [2]; c) during lethal MH/EHS crises Ca^{2+} leak from intracellular stores results in a feed-forward mechanism mediated by excessive production of oxidative species of oxygen and nitrogen (ROS and RNS) [5], which eventually will lead to depletion of the SR and to massive activation of Store Operated Ca^{2+} Entry (SOCE). Abnormal molecular mechanisms, once identified, are potential targets for prevention and treatment. In the next years we will attempt to develop/test drugs capable of: a) reducing SR Ca^{2+} leak; b) diminishing oxidative stress; and c) block SOCE in muscle fibers. Importantly, we have already been successful in curing CASQ1-knockout mice suffering of MH and EHS by administration of N-acetylcysteine (NAC), a potent anti-oxidant administered *ad-libitum* in drinking water.

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Epigenetic signature during differentiation of presomitic satellite cells

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The skeletal muscle adult stem cells (satellite cells SCs) are resident beneath basal lamina during entire life span and they are ready to activate themselves and regenerate new fibers. The SCs are responsible for muscle growth and preservation [3]. The skeletal muscles are composed of different type of fibres that can be distinguished by their expression of particular isoforms of myosin heavy chain (MyHC) proteins. Moreover, the skeletal muscle fibres have different embryological origin, the extraocular (EOMs) and jaw-closer muscles develop from prechordal mesoderm rather than somites (limb and trunk muscles) and MyHC proteins are used to identify them [1,2,4]. The different embryological origin of skeletal muscles could reflect the different regeneration/degeneration properties in healthy and/or chronic diseased muscles. We hypothesized that those differences may also endow the muscle associated satellite cells (SCs), responsible for muscle regeneration. The SCs were isolated from canine somitic (SDM) (Vastus Lateralis, Rectus Abdominus, Gluteus Superficialis, Biceps Femoris, Psoas) and presomitic (PSDM) (Lateral Rectus, Temporalis-M fiber, Retractor Bulbi and Masseter) muscles. We studied the proliferative and differentiative properties: population doubling level, fusion index, the expression of myogenic proteins, telomerase activity and skeletal muscle microRNA (myomiR) expression). We found interesting differences between somitic and presomitic satellite cells on differentiation ability.

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Cytoskeleton in injured skeletal muscles

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The extra-sarcomeric cytoskeleton (acto-miosin microfilaments and anchoring proteins) is involved in maintaining the sarco-membrane stiffness and integrity, and regulates the Ca^{2+} entry through the stretch activated channels (SAC). In contrast, intra-sarcomeric cytoskeleton (titin, nebulin and proteins of Z and M lines) is involved in maintaining the assembly of the sarcomeric acto-miosin proteins and the visco-elastic properties of the sarcomeres. Moreover, being intra-sarcomeric cytoskeleton bound by intermediate filaments (as desmin and ankirin) to costameric proteins and other proteins of the sarcoplasm, as receptors and ionic channels, it was expected that the passive and active electrophysiological and mechanical properties of skeletal muscle fibres may be affected by the modified architecture and functionality of the cytoskeleton. The aim of this research was to analyze the cytoskeleton alteration of the injured skeletal muscle due to mechanical overload (eccentric contraction) or atrophic state induced by denervation. The method used intracellular recording by conventional microelectrodes (50-70 M Ω) inserted in a single fibres of an isolated muscle bundle and records in voltage-clamp condition of the voltage-dependent L-type Ca^{2+} and SAC currents. The results demonstrated that in the two types of injured skeletal muscle conditions [1,4,6,7] there is a decrease in size of L-type Ca^{2+} current, that is an index of a reduced functionality of these channel proteins, and a positive shift of their voltage threshold and dependence. Moreover, muscle fibres showed an increase of the resting membrane permeability, of the SAC current as well as more depolarized resting membrane potential. This suggests a leaky sarcolemma and a reduced cytoskeleton expression/activity that in turn induces an increase of the intracellular Ca^{2+} concentration, which was confirmed by the negative e shift of the L-type Ca^{2+} current reversal potential. The shift towards more positive potentials of L-type Ca^{2+} current activation, directly involved in excitation-contraction coupling, was formerly observed in the slow skeletal muscle fibres of the frog by using ryanodine or ruthenium red. These

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treatments in fact, acting on RyRs, increased the number of L-type Ca^{2+} /DHPR receptors uncoupled to ryanodine receptors. Accordingly, the block of the interaction between DHPRs and RyRs by heptanol or octanol depressed in size L-type Ca^{2+} current [5]. In agreement to these findings, the shift towards more positive potentials of L-type Ca^{2+} current that we observed in injured skeletal muscle fibres could indicate an increased number of uncoupled DHPRs. In fact, it was demonstrated that a similar voltage shift was paralleled by an increased number of DHPRs uncoupled with RyRs in a subset of skeletal muscle fibres of old mice [2,3]. In conclusion, in injured muscle fibres the sarcoplasmic membrane, channel proteins and excitation-contraction coupling properties are paralleled by cytoskeleton alterations.

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Muscle reinnervation in a difficult-to-stimulate Conus-Cauda Syndrome

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We report a 41-year-old male patient (Rise2-Friuli-LS) who sustained a severe traumatic spinal cord injury in an accident at work (April 11, 2008). On clinical examination he presented with flaccid paraplegia (motor level D12). After discharge from Neurosurgery at 15 days from trauma he was admitted in our Spinal Unit. At the clinical examination was evaluated as A at the ASIA Impairment Scale (Motor level D12, anesthesia below D12). From an electrophysiological point of view somato-sensory evoked potentials and motor evoked potentials were silent. He had begun the rehabilitation program with the purpose to reach autonomy in wheelchair locomotion and activities of daily living (ADL). The physical rehabilitation was directed to the strengthening of upper limbs, trunk control and to preventing lower limb muscle atrophy with patterned Electrical Stimulation. Specific stimulation parameters (i.e., pulse width, train duration, between train intervals, method of application) were applied, but we could not obtain tetanic contraction of leg muscles (quadriceps and tibialis anterior). The training was completed by passive exercise at the cycloergometer and assisted body-weight support treadmill training. Gait over ground was not possible. At demission we verified autonomy in wheelchair locomotion and ADL, a good trunk control and an increment of aerobic performance (VO_{2max}). Meantime some reinnervation of leg muscles occurred demonstrated by minimal spontaneous activity of right foot muscles, but voluntary or electrical stimulation-induced muscle contractions did not appeared. A few months later FES-induced twitch dorsiflexion of the ankle were achieved by surface electrical stimulation of the tibialis anterior using the "Denervated Muscle Stimulation" program of the STIWELL Med4 device of MED-EL (Innsbruck, Austria), that delivers triangular or bidirectional impulses of 70 maps intensity and 150 msec duration. After this demonstration of maintained muscle contractility, the patient accepted the burden to travel to Vienna (January 21-23, 2009), to be analyzed and enrolled in the Rise2-Italy Program after signing the Consent and have performed a Computer Tomography analysis of his leg muscle that allowed to measure the thick subcutaneous fat layer and to evaluate the extent of muscle atrophy that underwent during the 9 months since Spinal Cord Injury. CT scan of left and right leg of Rise2-Friuli-LS demonstrates that the healthy macroscopic appearance was misleading, being due to overweight and to pseudo-hypertrophic lipodystrophy of denervated thigh muscles. The thick layer of subcutaneous

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adipose tissue, together with those separating denervated quadriceps and hamstring muscles is in part responsible of the difficulties encountered in leg muscle stimulation using commercial electrical stimulators for innervated muscles. Compared to the histological aspects of normal adult quadriceps muscle, the muscle biopsy of Rise2-Friuli-01LS after 9 months of permanent denervation shows that the muscle fibers are atrophic and the interstitial tissue is increased, but the fast and slow fiber types still distinct by ATPase histochemistry. Some groups of adipocytes of the infiltrating fat tissue were present. Morphometry of muscle fibers confirms that they are atrophic (50% decreased mean diameter), while Functional Echomyography of Tibialis Anterior shows that the muscles are poorly perfused at rest. From March 2009 Rise2-Friuli-01LS is performing home-based Functional Electrical Stimulation (h-b FES) for denervated leg muscles, using custom-designed electrodes, stimulators and protocols developed in Vienna, Austria for the EU Project RISE [1-7]. An early result of the training has been the impressive reduction of the leg edema. He achieved tetanic contractility and full knee-extension during the first six-months of training. After 3 years Rise2-Friuli-LS is still training his leg muscles. Meanwhile they became substantially reinnervated, providing strong evidence that h-b FES did not interfere to these most awaited events. This experience strongly suggests that more subjects affected with lower motor neuron denervation of skeletal muscles than those enrolled in the EU Project RISE will benefit of the Vienna Strategy for h-b FES, in particular those difficult-to-stimulate due to thick subcutaneous adipose layer that hamper excitation of denervated or poorly innervated muscles.

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Dynamic echomyography of skeletal muscle demonstrates that h-b FES in peripheral denervation does not hamper muscle reinnervation

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Permanent denervated muscles were evaluated by ultra sound (US) to monitor changes in morphology, thickness, contraction-relaxation kinetics and perfusion due to the electrical stimulation program of the Rise2-Italy project [1-4]. In a case of monolateral lesion of the sciatic nerve due to a surgical neurotomy during the removal of a hyperplastic lipenodes in the pelvis, morphology and ultrasonographic structure of the denervated muscles changed during the period of stimulation from a pattern typical of complete denervation-induced muscle atrophy to a pattern which might be considered "normal" when detected in an old patient. Thickness improved significantly. Contraction-relaxation kinetics, measured by recording the muscle movements during electrical stimulation, showed an abnormal behavior of the chronically denervated muscle during the relaxation phase, which resulted to be significantly longer than in normal muscle. The long-term denervated muscles analyzed with Echo Doppler showed at rest a low resistance arterial flow that became pulsed during and after electrical stimulation. As expected, the ultra sound measured electrical stimulation-induced hyperemia lasted longer than the stimulation period. Despite the higher than normal energy of the delivered electrical stimuli of Vienna home-based Functional Electrical Stimulation (h-b FES) the muscles

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shown electromyographic signs of re-innervation during the years of training. US confirm that fascicles of TA muscle respond with twitch contractions to the electrical stimulation that only elicit contraction of innervated muscles (Impulse Duration 1 msec, Amplitude 30 mAmps). Interestingly, the denervated component of the Tibialis Anterior activated by a protocol for denervated muscle responded at the same current amplitude to 150 msec long impulses every sec with twitch contractions. After a few months of twitch training, the impulse duration could be reduced to 50 msec, opening the possibility to induced sustained foot dorsiflexion by tetanic contraction. This may be achieved by shortening to 10 msec the inter-impulse pause. In conclusion, this pilot study demonstrates the usefulness of US myography in the follow-up and the positive effects of h-b FES of denervated/reinnervating muscles.

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Three-year RISE follow up of non- or poorly-compliant patients

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Three spinal cord injured (SCI) subjects with flaccid paralysis in the thigh muscles were treated with home based electrical stimulation for six years. The treatment was in the frame of the EU funded RISE project (QLG5-CT-2001-02191) and used its protocol [6]. That foresaw electrical stimulation of the quadriceps muscle group five days a week for one to two hours with different stimulation pattern. The stimulation treatment was targeting both fibres with small

diameter and bigger diameter with two different pulse rows. The goal of the treatment was to enhance the both size and force of the muscles with the final goal to enable the patients to stand up and stand in bars or equivalent balance aid. After an initial training period of some weeks the patients had the instruction to stimulate five times a week. They came for a follow up three times a year. Then they were accessed with some investigations one of which was a spiral CT [1-6]. On the basis of the CT data the volume, tissue density and shape of the muscles are monitored. Treatment compliance was varying. Patient one (P1) was not compliant. We could monitor a continuous decline of his muscles throughout the therapy years. The second patient (P2) was not compliant for the first two years, but started then and had considerable increase in volume, tissue density and force of rectus femoris muscle. The third patient (P3) has decubitus ulcers, broke his leg and other complications making it impossible to comply with the therapy. In this work the three patients are accessed three years after the end of the project. In that period they have had stimulators and been able to stimulate but rarely done so. The results show signs of a steady decline over the period. The three subjects are called to the hospital three years after project end for an assessment of first, their general health and second, for the monitoring of their leg muscles. This is done in a doctors interview with the patient (anamnesis), by looking on his skin and muscles, making a pendulum test with and without stimulating the quadriceps muscles with long pulsed and making a spiral CT of the legs. From the spiral CT data the rectus femoris muscle is segmented and its volume and density measured and the shape evaluated [1-5]. P1 is stimulating in the winter time when he needs to produce warmth in his body as he is outside in cold weather looking after his sheep. This is estimated to be less than ten times during the winter time. The lower leg is moved a little by the stimulated muscle, about 5 degrees from vertical. P2, the only one that had a considerable increase in volume (80%), has been stimulating in periods but not the last year. He has lost the volume of the rectus femoris muscle down to a stage considerably lower than at the start of the RISE project. Furthermore the average density is now 26 HU. P3 has not been stimulating. No movements are detected when the P2 and P3 quadriceps muscles are stimulated. In summary the monitoring shows in all three subjects a declining muscle volume, density and force. The muscles were declining in the period between observations. This is an expected result as the stimulation in the period was not taking place or only done occasionally. P1 did his stimulation for heat production. That effect should also diminish as the muscles decrease in volume. The monitoring work therefore is a documentation of the muscle degeneration. P2 had a considerable increase in muscle volume, density and force in the period of the RISE project. Now these values are considerably lower than that at the start of the RISE project demonstrating the progressive degeneration of the permanently denervated muscles and the reversibility of the eventual stimulation-induced muscle recovery if patients interrupt the treatment.

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Recovery of tetanic contractility: the first step toward a walking aid for unilateral peripheral denervation of tibialis anterior

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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 contralateral 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 triceps 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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Clinical effects of the exercise therapy in Amyotrophic Lateral Sclerosis patients

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Amyotrophic lateral sclerosis (ALS) is a fatal and progressive neurodegenerative disorder affecting motor neurons. If the reduced level of activity persists, cardiovascular deconditioning and disuse atrophy superimpose on weakness and muscle atrophy caused by the ALS itself [1]. The animal studies provided good basic scientific information about the effects of endurance exercise training in terms of survival and of reduction of the degeneration of the motor neurons in mice with SOD-1 induced ALS [2, 3]. In other studies, the neurons controlling fast fibres were noted to degenerate early in the course of ALS, whereas fatigue resistant neurons failed in the intermediate and slow twitch neurons in the later stages [3]. In the isometric muscular contraction, the force applied by the muscle is not sufficient to move the load, and as a result, the muscle does not shorten though its muscle tension or force of contraction increases [4]. Therefore, an isometric contraction should be proposed in order to avoid both the overuse weakness muscle and overstraining the twitch muscle fibres. In addition, moderate endurance training has been developed in order to minimize the fatigue, to facilitate the musculoskeletal endurance and to enhance the cardiopulmonary efficiency. Previous studies suggest an early subclinical involvement of the autonomic system in ALS [5]. The depressed sinus arrhythmia observed in no bulbar patients might be the result of a decreased state of physical condition resulting by a deconditioning. Bulbar patients, instead, show a more severe autonomic dysfunction than no bulbar patients. Several clinical trials have demonstrated the value of moderate exercise in improving the autonomic dysfunction and in inducing a cardiovascular reconditioning. Based on these available evidences from animal and human studies, strengthening and cardiovascular exercises may help maintain function and do not adversely affect disease progression in persons with ALS [6]. However, the current evidence is not sufficiently detailed to recommend a specific exercise prescription for ALS patients. In the present study, it has been designed an exercise program based on both moderate endurance training and isometric muscle contractions (in muscle strength in the mild

to moderate range with MRC score ≥ 3). The aim is to verify the clinical efficacy of this exercise protocol, through an objective assessment of the changes of muscular strength, the fatigue and the cardiovascular parameters. ALS pts admitted for rehabilitation treatment and enrolled with the inclusion criteria (clinically defined or probable ALS, the El Escorial criteria; mild-moderate disability; no heart and respiratory failures); 11 patients with ALS (7 males and 4 females aged 50 ± 13 years, range 24 ± 75 years) participated in the study; mean duration of the disease was 27 months (range 10 ± 66 months); at the time of the study, the patients had a total ALSFRS-R score from 21 to 41 (mean value, 31.7 ± 6.0). The exercise protocol consists in isometric muscle contractions, proposed in muscle strength in the mild to moderate range (with MRC score 3 and 4) and in a aerobic training by bicycle ergometry, arm-leg ergometry and/or treadmill with frequency of 4-5 sessions per week using an initial training intensity corresponding to 60–70% of maximum heart rate. Initial exercise duration of 15–20 min is recommended depending on the disability level of the ALS patient. We performed the following evaluations (before/after rehabilitation): ALSFRS-R (revised ALS functional rating scale), IB, Functional Independence Measure (FIM), Fatigue Severity Scale (FSS), strength measurements (MMT by dynamometer); analysis of HR variability and Oxygen Intake (VO_2 submax), six minute walking (6MWT) and ten meter walk test (10MWT). The project is a pivotal trial. 4 pts have presented a drop-out for rapid disease deterioration; the other 7 pts have concluded the study (see table).

Patients	age	gender	D. of disease	ALSFRS _r	FIM	Barthel	FSS
B. B.	71	M	38 mm	32	78	45	5.4
R S	54	M	10 mm	43	80	60	4.8
S B	65	F	26 mm	42	73	45	5.4
V G	72	M	32 mm	36	81	60	5.6
M D	53	M	16 mm	30	65	40	5.4
T M	71	M	48 mm	28	68	35	5.6
G D	65	F	19 mm	39	83	60	4.5

Decrease in fatigue as measured by a fatigue measurement tools at two months; the Fatigue Severity Scale and the change of the VO_2 submax have presented a reduction. Increase of muscle strength, as measured by the MMT dynamometry; the pts have presented an increase of MMT dynamometry scores; in 5 pts during the 6MWT the distance has presented an increase. In conclusion, the effects of therapeutic exercise in ALS patients are controversial and not well understood. The aim of this project has been to understand the clinical outcomes of moderate load endurance and isometric resistance exercise protocol. If confirmed, these findings may represent a translational step to establish exercise prescriptions and to improve the clinical practice in ALS.

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Molecular mechanism of action of botulinum neurotoxins at nerve terminal

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Seven different botulinum neurotoxins (BoNT/A to /G) enter the cytosol of peripheral nerve terminals, most notably of the neuromuscular junction (NMJ) and very specifically cleave protein components of the neuroexocytosis apparatus (SNARE proteins), blocking the acetylcholine release and causing a flaccid paralysis. For this reason, BoNTs are increasingly being used to treat a variety of conditions characterised by muscle hyperactivity. The toxins structure is structurally conserved among the serotypes and consist of two main chains linked together by a unique disulphide bond: the heavy chain (H;100 kDa) mediates the neurospecific binding, provided by the 50 kDa C-terminal part (HC) and chaperons through the 50 kDa N-terminal part (HN) the entry of the catalytic light chain (L;50 kDa). The mechanism of action consists of four steps: a) binding to nerve terminals, b) endocytosis, c) low pH-driven membrane translocation of L and d) L mediated cleavage of SNAREs. While the binding and the hydrolysis of SNAREs have been described in details, less is known about the vesicular compartment through which BoNTs are endocytosed. We recently demonstrated that BoNT/A is endocytosed at the active zones of presynaptic neurons through the recycling of small synaptic vesicles (SV) where was detected (one or two toxin molecules per SV) through immunoelectron microscopy [1]. Membrane translocation remains another poorly understood step. Biophysical studies with artificial membranes have indicated that, at low pH, the HN of BoNTs form protein conducting transmembrane channels, across which L chains can bypass the membrane. We recently developed a reliable method to study the translocation event across the plasma membrane of neurons [2], finding that they translocate very rapidly [3], and that the HN channel can be made of one, or at the most, two HN domains [1]. We also demonstrated that an intact SS bond is essential for translocation and therefore

it must be reduced on the cytosolic side. There are several disulphide bond-reducing systems in the cytosol of cells and by a pharmacological approach we found that specific inhibitors of thioredoxin reductase, inhibits the intracellular activity of BoNTs as well as Tetanus neurotoxin provide a strong indication that the thioredoxin system plays a major role in releasing the L chain of these neurotoxins after their translocation across the membrane of the endocytic vesicle assisted by their respective HN domains. We therefore propose such molecules as novel drugs effective for the treatment of tetanus and botulism patients [4].

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How to stimulate muscle respecting its inherent adaptive capacity

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The maximum power output of a single skeletal muscle fibre can vary over at least an order of magnitude depending on its pattern of use. Its ability to produce continuous work can also be greatly enhanced or reduced by cellular changes induced by exercise or disuse. This is because of a fundamental cellular arrangement of sarcomeres and mitochondria in which a compromise must be achieved between power output and endurance. There is a considerable amount of experience now in stimulation of skeletal muscle, both in experimental situations and increasingly in therapeutic applications. Stimulation experiments in small animals are able to interrogate the extremes of the adaptive capacity of muscle [1], but a fundamental scaling of stimulation parameters is necessary in order to translate work in small animals to therapeutic stimulation in human patients and in larger veterinary patients. This scaling of parameters can usefully be related to the differences in the fusion frequencies of analogous muscles in small and large mammals [2]. A further

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complication arises when muscle is compromised by trauma which may involve loss of the normal maintenance of the motor end plate by the innervating motor neurones, and the voluntary activation of the muscle involved in normal behaviour. Stimulation of denervated or partially innervated muscle must take account of the loss of the normal background activity as well as the potential for reinnervation. There are good examples of the risk of over-stimulation especially when skeletal muscle is asked to perform an unaccustomed endurance task like cardiac or respiratory assistance. In these cases the potential adaptive change towards a slow phenotype must be taken into account. Fortunately there are also good examples and promising research concerning experimental strategies to reduce unwanted adaptive changes such as slowing and loss of power in therapeutic settings.

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Neuromuscular pathology in equine recurrent laryngeal neuropathy

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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 neurons in 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 [3,4]. 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. We hypothesised that severity of laryngeal dysfunction determined by resting video-laryngoscopy, would be correlated with severity of laryngeal muscle histopathology. Laryngeal function was graded from video-laryngoscopy recordings in 29 horses by 2 independent

observers. Subsequently, intrinsic laryngeal muscles (mm cricoarytenoideus dorsalis and cricoarytenoideus lateralis) were harvested and analysed for fibre types (by fluorescence immunohistochemistry for myosin heavy chain expression) [5], fibre type grouping and minimum fibre diameter. Morphometric analyses of % collagen (by immunohistochemistry for collagen V expression) and % fat (by oil red O) were conducted. The left cricoarytenoideus dorsalis muscle had significantly more collagen and fat than the right in horses with complete laryngeal paralysis. Discriminant analysis revealed significant overlap in muscle pathology between horses with different degrees of laryngeal function, with the exception of horses with complete left sided abductor paralysis. Fibre type grouping was observed frequently, particularly on the left side. Low grade histopathological changes were evident in many horses with normal, or near-normal laryngeal function at rest. In conclusion, laryngeal function - as determined by resting laryngoscopy - does not correlate with severity of histopathology and horses with normal laryngeal function may have subclinical RLN. Resting laryngoscopy may lead to inaccurate phenotyping in genome wide association studies and quantitative trait analyses. This study provides valuable information that will allow muscle histopathology to be used as an outcome measure in the future investigation of novel treatments for RLN, including functional electrical stimulation.

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Hounsfield based analysis of posterior cricoarytenoid muscles undergoing Functional Electrical Stimulation

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Horses have a naturally-occurring neuropathy leading to laryngeal hemiplegia with unilateral vocal fold paralysis leading to complete denervation. Functional electrical stimulation (FES) of the posterior cricoarytenoid (PCA) muscle may offer a more physiological treatment of this condition in future. In this study specific Hounsfield value (HU) from computed tomography (CT) technology have been processed using segmentation techniques to monitor PCA status during stimulation treatment [1]. In this model we performed a completed transection of the left recurrent laryngeal nerve with ligation to prevent reinnervation. Following a 12 weeks denervation period, 8 weeks stimulation was used in principal horses whilst control horse remained unstimulated. CT exam and 3-D modeling was employed to assess the status of the PCA muscle during the study [2]. In this study we analyze the HU density in 5 small volumes within the PCA muscle in order to minimize the influence of the artefacts generated by the electrodes implanted on the left PCA. For each horse the HU analysis is repeated at beginning of the experiment (0 weeks), when the FES treatment starts (12 weeks) and at end of the experiment (20 weeks). The density variation after 12 weeks denervation without stimulation is around -25%, the density variation in the following 8 weeks of FES is + 3,5%. The analysis of the PCA HU based density provides a metric to quantify state in PCA muscles undergoing different physiological conditions: Normal, Denervation and Electrical Stimulation. In Normal PCA muscles the mean HU value is between 40 and 60, in the denervated muscles the mean HU values decrease progressively to 20-30 HU after 30 weeks denervation. The PCA that are electrical stimulated seem to stop the density decline.

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Expression of Myosin Heavy Chain isoforms in laryngeal muscles in comparison with skeletal and special muscles

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Larynx in mammals is characterized by five intrinsic laryngeal muscles with complex movements involved in respiration, airway protection and phonation. These muscles, differently from limb and trunk muscles that derived from somites, originate from the branchial arches. In all species the laryngeal muscles have the capacity to express the transitional embryonic and perinatal isoforms in adult but at low level ([1-4]). In same species of mammals (horse and cow) the laryngeal muscles express only the three skeletal MyHC isoforms (type 1, 2A and 2X) ([3], [5]); other species (dog, cat and tiger) express also a faster isoform, not detectable in skeletal muscles, the 2B isoform [4,6,7]. Furthermore, in species where the 2B isoform is present in skeletal muscles (rat and rabbit), another isoform presumably faster is present, the EO MyHC ([8], [9]). In rat and human laryngeal muscles a different isoform (IIL MyHC) was described [10,11], but it is unclear if this new isoform correspond to EO in rat or to 2B in human or to the two novel isoforms identified by Rossi et al. [12] in EO muscles (codified by MYH 14 and 15 genes). Combining RNA expression, electrophoresis and immunoblot we demonstrated that the IIL isoform in human does not correspond to type 2 isoforms (2A, 2X, 2B, EO, embryonic and perinatal cluster), to cardiac isoforms (beta and alfa), to M isoform and to isoforms codified by MYH 14 and 15 gene, and therefore is probably a new isoform.

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Wavelet analysis of laryngeal EMG for estimation of different fibre type activation in normal horses and horses with a distal axonopathy

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Electromyography (EMG) is a technique used to measure the myoelectric signals generated by motor unit action potentials in muscle. Wavelet analysis of the EMG signal resolves the signal into its intensities in time and frequency. Recent studies have shown wavelet analysis to be a powerful tool for investigating recruitment strategies in locomotor activities, and for distinction of different motor unit fibre type activation [1,2]. In this study three distinct frequency bands likely relating to the muscle fibre types 1, 2a and 2x were identified in equine laryngeal muscles, both in normal horses and in horses with muscle atrophy associated with recurrent laryngeal neuropathy (RLN). Sequential recruitment of type 1 to type 2 fibres during exercise was demonstrated by wavelet analysis using implanted electrodes. In different grades of RLN, different recruitment patterns were identified between left and right cricoarytenoideus dorsalis muscles by wavelet analysis. Fibrillation potentials present in the left CAD muscle in RLN were all from type 2a and 2x fibres.

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Speckle analysis of transoesophageal ultrasound in laryngeal muscles during resting breathing and nerve stimulation

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Scattering, interference and reflection of ultrasound waves results in speckle formation. These speckles represent natural acoustic markers. Post-processing algorithms track the path of these speckles from frame to frame on 2D ultrasound CINEloops, allowing calculation of local tissue displacement and subsequent estimation of velocity and strain rate. Speckle tracking echocardiography is commonly used both in research and clinical setting. However, its application in assessing skeletal muscle contraction is limited [1-5]. Transoesophageal ultrasound imaging of the equine cricoarytenoid dorsalis muscle using a 10MHz transoesophageal probe has been validated previously [6]. Speckle tracking analysis of contraction of this muscle during normal breathing, secondary to nasal occlusion and in response to nerve stimulation at various frequencies, pulse duration and amplitudes would generate quantitative data regarding the muscle activity. Further, analysis in both longitudinal and transverse planes can distinguish regional variation in muscle contraction.

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Functional Echomyography in Healthy Subjects and Patients with Facial Palsy

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Even though the first reports of visualisation facial muscles with ultrasound are from 1988, there is still no standardized method for examination. The purpose of this study was to identify those facial muscles accessible for reliable identification and to provide normal values of size, echogenicity, contraction and perfusion. We used a HD11 XE ultrasound machine from Philips, Netherlands with two linear probes (L12-3; L15-7io). In healthy subjects all facial muscles were screened for visibility, separation from adjacent muscles, and reliability of landmarks. Bilateral scans of reliable muscles were performed in 140 adult volunteers (70 woman) (age: 18 to 92 years). In total, 14 different facial muscles were at least in some volunteers visible. In anatomical studies, the correct identification of all sonographic structures was confirmed. Seven muscles innervated by the facial nerve were easy reproducible. These were; frontalis, orbicularis oculi, orbicularis oris, depressor anguli oris, depressor labii inferioris, zygomaticus and mentalis muscles Together with temporalis and masseter muscle as controls, innervated by the mandibular nerve (V3), all muscles were measured in rest and in contraction. Additional to geometric parameters, echogenicity was measured. Based on the same examinations-protocol, 86 patients with acute and chronic facial pareses were scanned. The Inter-Observer-Reliability for the geometrical values was high ($p > 0.001$ – $p = 0.004$). Crosssectional area and muscle thickness showed clear gender differences but no age-dependence. The occur of facial palsy changed contraction immediately after onset, while echogenicity rised and size shrinked slowly in the first month after denervation. Crosssectional values below twice the standard deviation were reached. It was not only possible to demonstrate degeneration, but also normalisation after reinnervation followed surgical rehabilitation or spontaneous healing. In conclusion, the provided measurement protocol allows the

reproducible, efficient non-invasive diagnostic of pathologic changes of the facial muscles. Normal values for the facial muscles are provided. The protocol can be applied in clinical settings to quantify the success of surgical and conservative therapy.

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Implantable pulse generators for experimental studies - stimulation pattern flexibility versus technological limits

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The major characteristics of implantable pulse generators, used for experimental studies are easy handling, physical volume and life time [1-3]. The devices should work reliable over weeks without any intervention by the staff. Such a stand alone operation requires the use of primary battery cells to avoid interventions with external components for RF-powering or recharging. Usage of primary battery cells influences strongly the final device volume and life time, making power saving strategies essential. Each implantable pulse generator consists of circuitry for data transmission, a controller and an output stage. The latter two mainly determine the energy consumption while the contribution of the data transmission unit can be neglected due to the short time of activation in comparison to the implant live time. The energy consumption of the controller is roughly proportional to its operating frequency. Short pulse widths and a high stimulation frequency require a high clock frequency resulting in increased energy consumption. Another important factor in terms of battery life time is the relation between the time when the devices delivers stimulation pulses and the rest time. Reduced clock frequency or implementation of sleep mode during rest contribute significantly to energy saving. The selection of stimulation pulse width and amplitude is a further important fact to reduce energy consumption and efficiency of the output stage. The electric charge delivered with each pulse depends on the specific application and determines the minimum electrode tip area as well as the volume of the dc-preventing decoupling capacitors in the output stage. Pulse width and amplitude can be varied to achieve the required pulse charge. Therefore the amplitude can be used to optimize the efficiency of the output stage. In case of a constant voltage output, the amplitude should be slightly below the battery voltage or the integer multiple of the battery voltage. Similar to that, in case of a constant current output stage the product of stimulation current and electrode impedance should fulfil the above criteria. These technical facts, considered during

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study designing, can help to reduce volume and prolong implant live time.

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The short-term effects of antenna insulation thickness on path losses in wireless telemetry implants at microwave frequencies

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Various physiological parameters (e.g. glucose level, electrocardiogram, electromyogram, intracranial pressure [1]) can be monitored non-invasively by the use of biotelemetry links. The development of sophisticated ultra low power consuming transceivers allows the transmission of large amounts of data from the inside of the body to an external receiver in real time at microwave frequencies. Antenna impedance matching is crucial for obtaining an acceptable propagation link budget in a wireless telemetry link. The dielectric properties of biological tissue induce detuning to transceiver antennas when implanted into the body. To counteract detuning problems, implant antennas are coated with biocompatible insulating material. The study investigates the propagation losses of a wireless communication link at different insulation thicknesses of medical grade silicone in the 2.45 GHz ISM (Industrial-Scientific-Medical) radio band. The wireless link consisted of an implantable unit which was placed between two pads of tissue substitute material and an external receiver which was connected to a PC via USB. Predefined data packets were transmitted from the implant, the received packets were analyzed, packet errors and packet losses were logged and the received signal strength indicator values (RSSI) were recorded. Our results showed that the mean RSSI values of insulated transmitter antennas - embedded in tissue equivalent material - provide more safety distance to critical receiver sensitivity level than uncoated antennas. The conducted experiments let us conclude that with increasing thickness of the insulation layer, the antenna becomes less sensitive to detuning by adjacent tissue substitute material and we may expect less detuning after implantation in living tissue.

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A Neuroprosthesis for finger movement rehabilitation. Some results

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A neuroprosthesis for finger movement rehabilitation is under construction. It consists of a lower arm sleeve with integrated electrode matrixes for finger muscle stimulation, microprocessor based electronics for stimulating current control and communication with a user interface. The system has been under test and used for training of the finger muscles of one patient with spinal cord injury at level C6-C7. Voluntarily he has little or no mobility in the finger muscles, very limited mobility in the wrist but he can move his shoulder and elbow joints. Goal of the training is to enhance muscle force and enable finger movements. The training is ongoing for five years. Only the right arm is trained but not the left arm. Several different versions of the sleeve have been constructed and used for the training. Main goal of the sleeve construction is to enable the user to use it independently of the help from another person. Results of muscle monitoring show a slight increase in muscle tissue density. In contrast to the classical method of using a self-adhesive electrode [1,7], that is repeatedly replaced to find the best position on the arm for a certain finger movement, up to sixteen different electrodes were used. This way an electrode repositioning can be done by choosing a different one electronically [7]. As long as the training was done by an assisting person the electrodes could be adhesive. To make a tetraplegic patient independent in the training a sleeve with electrodes, that he can put on himself and take off, where designed and constructed. The new electrodes are smaller than usual adhesive gel electrodes, are 10 mm in diameter and are thin laser cut stainless steel plate to increase its surface area. They are placed in a silicone rubber cover and a sponge soaked in isotonic 0,9% NaCl solution. Hardware has been constructed for choosing active electrodes at each moment. The therapy lasted for about three

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and a half year. In this time the patient was stimulated 3 to 5 times a week, the first two years a stimulation frequency of 20 Hz was used but then changed to combined therapy with 6 and 16 Hz. Only the right arm has been treated. Partially the patient has done the training independent from help of another person and partially with the assistance and monitoring of others. For monitoring purposes a spiral computer tomography (CT) image has been made. The CT data was processed to measure the density and volume of the finger muscles and the result from different time points compared. A carbon fibre sleeve with integrated electrodes is constructed. It enables a patient with SCI at C6-C7 level and no voluntary finger movements to train his finger muscles independent from the help of another person. Furthermore the controlling electronic has been constructed. The CT data show that density has increased in the finger muscles tissue of both arms, also in the left untreated arm [3-6]. The increase is similar in both arms []. In the interval from 62 to 85 Hu a density increase is detected but at the same time a decrease in the interval from 39 to 62 Hu suggesting that some of the voxels in the lower interval have moved to the higher one. A sleeve with electrode matrix and controlling electronics is constructed that enables a C6 - C7 SCI person to train his finger muscles independent from help of another person. The density of the innervated finger muscles tissue is increased suggesting an effect of the electrical stimulation training. As a consequence of reflex stimulation [1,5], this happens on both sides, both in trained and untrained arm, that has to be investigated further.

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Current versus voltage controlled electrical stimulation of the anterior thigh

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Neuromuscular electrical stimulation is well established for rehabilitation and diagnostic. The stimulator design is in most cases based on voltage controlled (VC) or current controlled (CC) output stages. VC devices are considered to be safer for transcutaneous applications because an electrode error does not lead to dangerous high current density. Whereas, the output force prediction of CC stimulators are more reliable, to due the fact of independency of the electrode impedance. In five subjects we compared both techniques during stimulation of the anterior thigh using surface electrodes (8 x 13 cm, STIMEX, schwa-medico GmbH). The controlled pulse shapes were rectangular and biphasic with a pulse width from 2 x 50 μ s to 2 x 1 ms. We increased the stimulation amplitude up to +/-60 V (CV) and +/-100 mA (CC) and measured the knee extension torque and evoked myoelectric signal of rectus femoris muscle. The equipment was custom-built for CV stimulation and the CC stimulation was applied by Stimulette DEN2X, Schuffried Medizintechnik GmbH -Austria. Using the CC stimulator the activation threshold decreases monotone with longer pulse width, e.g. in one subject from 75 mA at 2 x 50 μ s to 10 mA at 2 x 1 ms. The torque-amplitude graph shows an increasing slope for longer stimulation pulse widths. In contrast, during CV stimulation the activation threshold stays constant for pulse width longer than 2 x 300 μ s. Hence, the torque-amplitude graphs are also similar with pulse width longer than 2 x 300 μ s. At equal knee torque levels the pulse charge of VC and CC stimulation are alike, e.g. at 20 Nm the charge is 43.7 nC and 96.2 nC for a pulse width of 2 x 200 μ s and 2 x 1 ms, respectively. We conclude that for VC stimulation a pulse width of 2 x 300 μ s provides the most sensitive control characteristic via impulse amplitude variation, whereas pulse width variation is in general problematic. For CC mode both amplitude variation and impulse width variation provide a well adjustable control characteristic. This observation was

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investigated for single twitches and is limited to the specific electrode setup.

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The myositis caused by *Trichinella* spp.

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Myositis, either infective or autoimmune, is usually characterised by skeletal muscle inflammation, with different degrees of both damage and regeneration in the tissue. Among the possible causes for infective myositis parasites such as protozoa or helminths such as in particular *Trichinella* spp. can be responsible. At present, eight different species have been described, *Trichinella spiralis*, *Trichinella nativa*, *Trichinella nelsoni*, *Trichinella britovi*, *Trichinella murrelli*, and the recently described *Trichinella patagoniensis* (all encapsulating species) and *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis* (which, conversely do not induce the capsule formation) plus different genotypes [4,5]. This parasite has a special relation with the skeletal muscle, because of its unique intracellular localization in this tissue. After invasion by the parasite, the fiber muscle cell changes completely in morphology and biochemistry to become the parasite protective niche, otherwise called, nurse cell (NC). The long-lasting muscle infection of *Trichinella* is awarded by the keen interplay with host immune response, mainly characterized by a Th2 phenotype as shown by *in vitro* studies where cells collected from cervical lymph nodes or spleen cells of infected mice produced cytokines derived from such T subset, such as interleukin (IL)-5, IL-10, IL-13 but also IFN- γ after stimulation with somatic larval antigens as well as by the presence of parasite-specific IgG1 and IgE during the chronic

infection. Very important informations have derived from mice knock out (KO) for the different cytokines or haemopoiesis regulating genes. For example, in KO mice for IL-10 a crucial role of this cytokine was shown for the regulation of inflammation intensity. Muscular host immune response to *Trichinella* is partially regulated by the intestinal phase of the parasite which emphasizes the intensity of the following muscle inflammation compared to animals infected by synchronized injections of larvae directly into the muscle. In eosinophil-ablated mice such as PHIL and GATA⁻ animals, increased nitric oxide synthase II expression in macrophages is responsible for parasite damage (reviewed in [1]). Besides, modulation of the intestinal stage of the infection, using recombinant IL-12, increases the muscular parasite burden delaying adult worm expulsion from the intestine. Another Th1 adjuvant of bacterial origin called *Helicobacter pylori* neutrophil activating protein (HP-NAP), administered during the intestinal phase of trichinellosis, alters the muscular parasite burden, too [3]. Differences in the level of myositis are observed, depending on the different *Trichinella* species responsible for infection, in fact in mice experimentally infected with *T. pseudospiralis*, belonging to the non encapsulating clade, the inflammatory response is significantly lower than in those infected by either *T. spiralis* or *T. britovi*, both encapsulating species. Such difference is present not only around the parasite-NC complex, but also in non infected muscle [2]. In conclusion, a mutual adaptation between parasite and host immune response achieve a strategic compromise between two evolutionary forces pointed towards the survival of both species.

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Myotilin, Desmin and MHC-I expression in muscular disorders with protein aggregates

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Protein aggregates have been identified in muscle fibres in different neuromuscular disorders such as: Myofibrillar Myopathies (MFM), Inclusion Body Myositis and Myopathy (IBM), Becker Muscular Dystrophy (BMD), Limb Girdle Muscular Dystrophies 1A (LGMD1A) and 1F (LGMD1F). Pathological protein aggregates generally emerge as subsarcolemmal and/or sarcoplasmic inclusions. In addition, rimmed and non-rimmed vacuoles may be present in each one of these conditions and also up-regulation of Major Histocompatibility Complex Class I (MHC-I) is observed in some of them. In particular, there are similarities between IBM and MFM: midlife or late-onset clinical symptoms, apparently of both sporadic and genetic background, autophagocytosis by vacuole formation, which is frequent in IBM though rare in MFM, and presence of tubulofilamentous aggregates, which is almost regular in IBM but scantily found in DRM as beta-amyloid accumulation both in IBM and MFM. Therefore, differential diagnosis could be very complex. Trying to identify a morphological border between these diseases we studied diagnostic muscle biopsies from MFM and sIBM patients with histological, histochemical, enzyme histochemical, ultrastructural and immunohistochemical techniques using antibodies towards desmin, myotilin and MHC-I. This study has identified similarities and dissimilarities between these two different groups of protein aggregate myopathies and has given more insights into the effects of protein aggregation within muscle fibres.

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Caveolinopathy – Case histories of four persons, in two families

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The membrane protein Caveolin-3 regulates sarcolemmal stability and modulates different signalling pathways. Caveolin-3 deficiency leads to four skeletal muscle phenotypes: limb girdle muscular dystrophy (LGMD) 1C, isolated hyperCKemia, rippling muscle disease and distal myopathy. The CAV3 gene is mapped on chromosome 3p25, and most of caveolinopathies are transmitted with an autosomal dominant inheritance. Case histories of four patients, in two unrelated families are presented. In both families, the fathers and their sons were affected. Case histories. In the first family, the clinical symptoms were characteristic for rippling muscle disease. A 32-years old man has got high arch feet since his childhood, which has been operated several times, in first and second decades of his life. After operations, he has been suffering from a mild gait disturbance, without other complain. With physical neurological investigation no pathological findings were found, except percussion-induced rapid muscle contraction (PIRC). Serum creatine kinase levels were 2-3 times increased. Muscle biopsy demonstrated myopathy, and total absence of caveoline3 protein, by immunohistochemistry. The patient's mother and grandfather also had had foot deformities, without other complain and symptom. Tiptoe walking of patient's 1,5- years old son has been observed. The molecular genetic investigations found mutation of CAV3 gene, in exon 1(c.80G >A, p.R27Q), the same mutation in the case of father and his son. In the other family, the symptoms were characteristic for limb girdle muscular dystrophy (LGMD) 1C. A 55-years old man has been suffering from moderate proximal muscle weakness of shoulder girdle, and muscle cramps after exercise, primarily in the proximal musculature. His complain started in the second decade of his life, without serious deterioration. With physical investigation, a moderate muscle weakness was found in the shoulder girdle and in the pelvifemoral muscles, with expressed calf hypertrophy. Serum creatine kinase level was three times increased. Muscle biopsy showed increased number of the internal nuclei, an increase of the endomysial connective tissue, and total absence of caveoline3 immunostaining. The ultrastructural investigations demonstrated loss of caveolae at the sarcolemma and large subsarcolemmal membrane vacuoles. The molecular genetic investigation demonstrated CAV3 gene mutation, in exon 2, (c.159C>A, p.S53R), a gene mutation, which has not been previously described. The patient's 28-years old sons complain started about a year ago. He has been suffering from muscle cramps after exercise. With physical investigation, a slight muscle atrophy of the shoulder girdle and weak deep reflexes of upper extremities was found. Muscle histology did not demonstrate myopathy, but electron microscopy demonstrated the same ultrastructural alterations, as in the fathers case. Serum creatine kinase level was normal. Molecular genetic investigations is being performed.

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Hereditary Spastic Paraplegia: clinical effects of neurorehabilitation

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Electrostimulation Hereditary Spastic Paraplegia, also known as Spastic Paraplegias (SPGs) constitutes a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by insidiously progressive weakness and spasticity of the lower limbs (pure SPG), which may be combined with additional neurological and non neurological manifestations (complex or complicated SPG). SPGs are due to mutations in genes encoding for proteins involved in the maintenance of the corticospinal tract axons. The strong genetic heterogeneity is reflected by the fact that 52 gene loci associated with SPG have been discovered so far, involving X-linked, autosomal recessive, autosomal dominant and maternal inheritance. To date, 31 genes have been identified as causative [1,2]. Treatment is exclusively symptomatic. The effects of neurorehabilitation on SPG clinical manifestations are currently unknown. To evaluate the role of rehabilitation in SPGs, we considered the following outcome measures: improvement of muscle strength examined by MRC scale. Improvement of the 6-minutes walk distance (6MWT). Improvement of gait speed, evaluated by a reduction of the time required to cover 10 meter. Seven patients with clinically and/or genetically confirmed SPG have been recruited. Two patients had mutations in the gene encoding Spastin (SPG4), while one in REEP1 gene (SPG31). Genetic studies revealed mutations in SACS and Mitofusin genes in one subject. The diagnosis was confirmed by clinical and instrumental investigations in the other subjects. Patients have been admitted to the IRCCS Foundation Hospital San Camillo, where they performed intensive rehabilitation treatment consisting of daily aerobic (60% VO₂max) and strengthening exercise in addition to standard motor treatment. Two patients performed electrical stimulation in muscles with severe weakness (MRC <3). At the beginning and at the end of the rehabilitation program all patients underwent muscle strength evaluation according to the MRC scale, 6MWT, 10 meter walk test, spirometry, overnight oximetry study. Echocardiography, EMG, motor and somatosensory evoked potentials, brain and spinal cord

MRI were performed. Blood samples were collected for DNA, RNA and serum. At the end of the rehabilitative program we observed a statistically significant ($p=0.001$) improvement of muscle strength: mean MRC values vary from 3.46 ± 0.68 at the beginning of the treatment to 4.1 ± 0.69 at the end. The 6-minute walk mean distance varied from 193.4 ± 140 m to 208.35 ± 157 m ($p=0.04$). Time needed to walk 10 meter ranged from 18.22 ± 9.1 sec to 15.71 ± 7.88 sec ($p=0.005$). Aerobic and strengthening exercise may be an effective treatment for SPGs. We observed in the patients enrolled an improvement of muscle strength, fatigue tolerance and gait speed. Electrical stimulation may be a promising tool to counteract weakness in those muscles with MRC <3.

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POSTERS

P01 EMG of upper airway muscles in horses: quantitative and spectral analysis of thyrohyoid muscle activity during exercise

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Dorsal displacement of the soft palate (DDSP) is the most common cause of naso-pharyngeal dynamic collapse in horses and shares some etiological similarities with obstructive sleep-apnea. Thyrohyoid muscles (TH) dysfunction plays a central role in the pathophysiology of exercise-induced DDSP [2] which is thought to be associated with muscle fatigue. This is a preliminary report on the quantification of electromyographic (EMG) activity of the TH muscle in exercising horses and its correlation with the development of DDSP. Three adult Thoroughbred horses were evaluated in the study, two with a definite diagnosis of DDSP, one as control. All procedures were approved by the Institutional Animal Care and Use committee. One permanent 5F bipolar electrode was implanted into one TH of each horse. The horses were subjected to an incremental exercise test, while recording EMG, upper airway videoendoscopy and upper airway pressures. EMG data were recorded through a surgical implanted telemetric transmitter. Data from twenty consecutive breaths at the end of each speed interval were considered for the analysis. Mean electrical activity (MEA) [5], Median (MF) and Mean Power Frequency (MPF) were calculated. Data were normalized as a percentage of the value measured at an exercise intensity of 50% maximum heart rate (HR_{max}50). MEA increased with

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exercise intensity in the absence of displacement. Both horses with DDSP demonstrated a marked decrease in MEA immediately prior to the onset of displacement. In one of these horses spectrum analysis showed an initial increase in MPF, followed by a constant MF shift to lower frequencies. In normal horses, MEA activity of upper airway muscles increases with exercise intensity [2,6,7]. EMG data obtained from both affected horses presented here suggest that a component of TH dysfunction leading to DDSP may be produced by a decrease in muscle recruitment and this may result from muscle fatigue. The reasons for fatigue to occur in the TH muscle are unknown but could rely in a different fiber-type proportion between affected horses [3,4]. This requires further investigation through histologic evaluation. These findings give a potential rationale for a new management approach of horses with DDSP, by increasing the muscle strength and resistance to fatigue through functional electrical stimulation (FES) of the TH muscles [8].

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P02. A combined Sihler nerve stain & Technovit-injection technique for 3D-representation of the neurovascular architecture of the equine larynx

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Detailed information on the neurovascular architecture, i. e. the angioarchitecture and neuroanatomy of the equine larynx is essential for development of surgical techniques to implant probes for electrostimulation in patients with laryngeal paralysis. To get detailed information about the course and branching of arteries within the larynx we recently prepared 22 corrosion casts of the equine larynx. We also performed a Sihler staining (Mu and Sanders, 2010) on 4 larynges to reveal the course and branching pattern of the laryngeal nerves. Based on these specimens we were able to prepare a complete description of the neurovascular architecture of the equine larynx. Aim of this study was to demonstrate the spatial relationship between nerves and arteries as well as their position in relation the surrounding structures and the cricoarythaenoideus dorsalis muscle in particular. We focused on the surgical relevant dorsal aspect of the larynx. A combination of Sihler staining with a latex injection of the laryngeal arteries did not provide satisfactory results. So we replaced latex by Technovit 7143 in a modified technique. In two equine larynges of adult warm blood horses the arteries were injected with Technovit 7143 via the common carotid artery. Subsequently a Sihler staining was performed which revealed the principal branching pattern and also provided information on fine details of the regional branching of the laryngeal nerves within the cricoarythaenoideus dorsalis muscle. The first branch of the caudal laryngeal nerve enters the dorsal cricoarythaenoid muscle at its caudolateral and dorsal aspect. An arterial branch of the ascending pharyngeal artery reaches the muscle in this area but runs separate from the nerve. A second branch of the caudal laryngeal nerve runs close to the caudal laryngeal branch of the ascending pharyngeal artery along the lateral margin of the cricoid plate. The combination of corrosion casting and Sihler stain used in this study provides valuable information on the three dimensional relationship of arteries and nerves and surrounding tissues required for precise and correct positioning of probes for electrostimulation.

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P03. 3D distribution of electrical field of multipolar intramuscular FES stimulation electrodes in perfused horse larynx model by robotic computer controlled needle potential screening acquisition system

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Motivation of these experimental measurements was to evaluate the electrical field distribution of implanted electrodes in three dimensions inside the tissue. Measurements were performed in a perfused ex vivo equine larynx to achieve results of physiologically intact tissue. Objectives of the measurements are the practical evaluation of the measurement system. Especially the reproducibility of the measurements, evaluation of the perfusion setup influences on field distribution and further investigation of the tissue changing effects of the measuring needle and the scanning process. A system to measure the electric field

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distribution inside the tissue has been developed, which contains a 3D linear motor system for the positioning of a measuring needle inside the tissue. At each discrete point electrical potential is measured and according to an interpolation the 3D electrical field distribution is calculated and visualized. We measured variations of scanning grids at same electrode configuration and electrode position to evaluate measurement reproducibility. The experiments were performed on perfused CAD muscle (Musculus cricoarytaenoideus dorsalis) of two horses. This enables an intact physiological state of the muscle for up to 6-8 hours. The perfusion setup shows no significant influences on the measurement system. The measuring needle causes a morphologically change in tissue (tissue shift) and thus a displacement of the field distribution measurements. In consideration of the reproducibility the different measurements, with the same electrode configuration and position, similar field distribution results are obtained. The experimental results show, that ex vivo measurements of the 3D electrical field distribution can be performed with this measurement setup. In future field distribution displacement will be reduced by an additional 3D force measurement and control system to evaluate the tissue shifts while inserting the needle. Further planned investigations are the optimization of electrode configuration and placement, to achieve a more selective stimulation and better therapeutic effects.

P04. Assessment of FES-threshold and optimal stimulation parameters in horses with naturally occurring recurrent laryngeal neuropathy (RLN)

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

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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]. Throughout 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. Sub-clinical denervation/reinnervation events are contributing mechanisms of muscle atrophy in aging

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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 elderly. 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. Strategies to accelerate muscle reinnervation across traumatic nerve gaps

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Regeneration of peripheral nerves is remarkably restrained across transection injuries, limiting recovery of function. Strategies to reverse this common and unfortunate outcome are limited. Remarkably, however, new evidence suggests that a brief extracellular electrical stimulation (ES), delivered at the time of injury, improves the regrowth of motor and sensory axons. (Singh et al *J Neurosurg* 2012). In this work, the authors explored and tested this ES paradigm, which was applied proximal to transected sciatic nerves in mice, and identified several novel and compelling impacts of the approach. Using thy-1 yellow fluorescent protein mice with fluorescent axons that allow serial in vivo tracking of regeneration, the morphological, electrophysiological, and behavioral indices of nerve regrowth were measured. The authors show that ES is associated with a 30%-50% improvement in several indices of regeneration: regrowth of axons and their partnered Schwann cells across transection sites, maturation of regenerated fibers in gaps spanning transection zones, and entry of axons into their muscle and cutaneous target zones. In parallel studies, the authors analyzed adult sensory neurons and their response to extracellular ES while plated on a novel microelectrode array construct designed to deliver the identical ES paradigm used in vivo. The ES accelerated neurite outgrowth, supporting the concept of a neuron-autonomous mechanism of action. Taken together, these results support a robust role for brief ES following peripheral nerve injuries in promoting regeneration. Electrical stimulation has a wider repertoire of impact than previously recognized, and its impact in vitro supports the hypothesis that a neuron-specific reprogrammed

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injury response is recruited by the ES protocol. Indeed, poor functional recovery after peripheral nerve injury is generally attributed to irreversible target atrophy. Gordon et al, in rats, addressed the functional outcomes of prolonged neuronal separation from targets (chronic axotomy for up to 1 year) and atrophy of Schwann cells (SCs) in distal nerve stumps, and whether electrical stimulation (ES) accelerates axon regeneration. Moreover, they studied whether in carpal tunnel syndrome (CTS) patients with severe non-nerve interrupting axon degeneration and release surgery ES accelerates muscle reinnervation (Gordon et al, *Neurol Res*, 2008). Reinnervated motor unit (MUs) and regenerating neuron numbers were counted electrophysiologically and with dye-labeling after chronic axotomy, chronic SC denervation and after immediate nerve repair with and without trains of 20 Hz ES for 1 hour to 2 weeks in rats and in CTS patients. Chronic axotomy reduced regenerative capacity to 67% and was alleviated by exogenous growth factors. Reduced regeneration to approximately 10% by SC denervation atrophy was ameliorated by forskolin and transforming growth factor-beta SC reactivation. ES (1 h) accelerated axon outgrowth across the suture site in association with elevated neuronal neurotrophic factor and receptors and in patients, promoted the full reinnervation of thenar muscles in contrast to a non-significant increase in MU numbers in the control group. They thus concluded that brief ES accelerates axon outgrowth and target muscle reinnervation in animals and humans, opening the way to future clinical application to promote functional recovery. Our goal is to verify published results on the beneficial effects of ES in the in vivo animal and human models, and to extend to humans the model of electrical stimulation of neural recovery after traumatic nerve transactions. Overall, the animal and human studies will take advantage of three potential approaches that will be also combined: 1. Cytokine seeded biomaterials; 2. Schwann cell delivery; 3. Electrical stimulation. Experiments will be performed in collaboration with the group of Nicola Elvassore, to identify effective biomaterials for bridging nerve gaps, and with Libero Vitiello lab to test combined strategies in rodent models. The eventual need for expansion of Schwann cell cultures of animal and human origin for the aims of the study will call in the collaboration with the group of Barbara Zavan. We will also count on the expert advice of Helmut Kern and Sandra Zampieri, Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria for ES protocols and of Paolo Gargiulo, Dept. Science, Education and Innovation, Landspítali University Hospital, and Dept. Biomedical Engineering, University of Reykjavik, Iceland for 3D color reconstruction of experimental muscles.

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P08. FES training protocols for the functional recovery of permanently complete denervated human muscles

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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.

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P09. Reliability of novel postural sway task test

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The purpose of this study was to examine the reliability of parameters obtained from a novel postural sway task test based on body movements controlled by visual feedback. Fifty-nine volunteers were divided into two groups. The first group consisted of young ($n = 32$, 16 females and 16 males, age: 25.2 ± 3.4 years) and the second group of elderly individuals ($n = 27$, 17 females and 10 males, age: 75.7 ± 6.9 years). Participants stood in parallel on a computer based stabilographic platform with the feet approximately a shoulder width apart, the toes slightly pointing outwards, the hands placed on the hips. The computer screen was placed approximately 1.5 meter from the platform at a height of subjects' eyes. An instantaneous visual feedback of participant's centre of pressure (COP) was given in a form of a blue cross visible on the screen. Participants were instructed to keep the blue cross driven by movements of their hips as close as possible to a predefined curve flowing on the screen. Out of the 6 parameters studied, only the average distance of COP from the curve line and the sum of the COP crossings through the curve line showed high reliability. Correlation between these two highly reliable parameters was -0.89 . There was also a statistical difference ($p < 0.001$) between young and elderly in both the average distance of COP from the curve line and the sum of the COP crossings through the curve. To conclude, the novel postural sway task provides a simple tool with relatively low time burden needed for testing. The suggested output parameters measured are highly reliable and easy to interpret.

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P10. Acquired multifocal myoclonus – Two case histories

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Myoclonus should be differentiated from other forms of muscle hyperactivity with central nervous system origin, such as dystonia, tremor, chorea and seizures, and from muscle hyperexcitability with peripheral origin, such as fasciculation, myokymia, rippling muscle syndromes and pseudomyotonia. Here we report case histories of two patients with symptoms of acquired, progressive multifocal myoclonus and cramps. Patient 1. A 48 years-old male patients complains started 6 years ago with cramps and involuntary muscle contractions in head and extremities. The symptoms slowly became more frequent, and occurred in the musculature of the abdomen. There were possible to elicit or increase the symptoms by voluntary muscle contractions and painful skin stimuli. The symptoms were complicated by excessive sweating. Physical neurological investigation found continuous irregular myoclonus-like twitching in the head, in the abdominal muscles, and in the muscles of the extremities. There was no found increase of the antibodies against several investigated proteins, except a threshold increase of kv.1.2 VGKC antibody. Neurophysiological investigation (EEG, EMG, ENG, SEP) demonstrated normal results. Muscle biopsy found diameter variation, II type fibre hypertrophy, and central nuclei, split fibres. Electron microscopy showed numerous vacuoles, which could be ascribed to dilated T-tubules. Increased amount of mitochondria and glycogen were seen under sarcolemma. Furthermore, bizarre, amorphous, electro dense subsarcolemmal lipid structures were seen. Antiepileptic drug treatments were ineffective, while plasma exchange resulted in several months symptom free episodes. Patient 2. A 45 years-old male patient complains started 5 years ago with involuntary muscle contractions in the right arm and in the abdominal musculature, which lasted about 30 minutes. Similar symptoms occurred two months after the first attack, and they became more and more frequent. Physical neurological investigation found continuous irregular twitching in the head, in the abdominal muscles, and in the muscles of the upper extremities. Skin stimulation by touch or pinprick elicited the symptoms, or increased the amplitude of twitching. A threshold increase of 1.1 kv. VGKC antibody was found. Neurophysiological investigation (EEG, EMG, ENG, SEP) demonstrated normal results. Muscle biopsy showed identical finding to the patient 1. Antiepileptic drug treatments were ineffective, while plasma exchange resulted in substantial recovery. In conclusions, two patients with acquired multifocal myoclonus has been presented. The clinical symptoms pointed to a possible brain stem origin, while electrophysiological findings directly did not support the localization. An autoimmune mechanism was postulated on the base of a threshold increased of VGKC antibodies. The usual antiepileptic therapies did not result in improvement. Plasma exchange resulted in substantial recovery in both patients. The histories of these patients emphasize the possible autoimmune pathomechanism of late onset nerve hyperexcitability disorders, and benefit of immunomodulating therapies. The muscle biopsy findings most probably were secondary to continuous muscle fiber hyperactivity, and not primary pathological alterations.

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P11. Age-related decline of muscle power in track and field master athletes indicates a lifespan of 110 years

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The power developed by skeletal muscles is surely declining with age after the growth of the youth. The characterization or the rate of loss is a question analyzed in many clinical research studies. Data useful to study the decline of the skeletal muscles power are largely available from sources other than medical tests, e.g. from track and field competitions of master athletes. Absolute world records of various events have been collected together with world records of all master categories. Masters are athletes competing within age classes of 5 years (39 to 39; 40 to 44; 45 to 49 and so on). The performance of master athletes can be normalized with respect to the absolute record: the normalized performances are thus represented by a number from one (world absolute records) to zero (null performance). The decline of the performances with age is analysed and compared. Most trend-lines show a linear decline down to 70 years. The average annual rate of power decline of analyzed events (running, throwing and jumping) is 1.25% per year. The events involving mostly upper limbs (shot put, javelin throw) show a declining rate higher than the disciplines where lower limbs are most important (long jump, track events). This study analyses the human decline within a coherent approach based on very extended baseline of data. The decline of skeletal muscle power starts at the age of 30 with minor variance. This conclusion is in line only with some of previous studies. The various trend lines tend to zero at the age of 110 years. This is substantially in line with the actual survival of humans.

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P12. Clinical effects of the exercise therapy in Amyotrophic Lateral Sclerosis patients

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Amyotrophic lateral sclerosis (ALS) is a fatal and progressive neurodegenerative disorder affecting motor neurons. If the reduced level of activity persists, cardiovascular deconditioning and disuse atrophy superimpose on weakness and muscle atrophy caused by the ALS itself [1]. The animal studies provided good basic scientific information about the effects of endurance exercise training in terms of survival and of reduction of the degeneration of the motor neurons in mice with SOD-1 induced ALS [2, 3]. In other studies, the neurons controlling fast fibres were noted to degenerate early in the course of ALS, whereas fatigue resistant neurons failed in the intermediate and slow twitch neurons in the later stages [3]. In the isometric muscular contraction, the force applied by the muscle is not sufficient to move the load, and as a result, the muscle does not shorten though its muscle tension or force of contraction increases [4]. Therefore, an isometric contraction should be proposed in order to avoid both the overuse weakness muscle and overstretching the twitch muscle fibres. In addition, moderate endurance training has been developed in order to minimize the fatigue, to facilitate the musculoskeletal endurance and to enhance the cardiopulmonary efficiency. Previous studies suggest an early subclinical involvement of the autonomic system in ALS [5]. The depressed sinus arrhythmia observed in no bulbar patients might be the result of a decreased state of physical condition resulting by a deconditioning. Bulbar patients, instead, show a more severe autonomic dysfunction than no bulbar patients. Several clinical trials have demonstrated the value of moderate exercise in improving the autonomic dysfunction and in inducing a cardiovascular reconditioning. Based on these available evidences from animal and human studies, strengthening and cardiovascular exercises may help maintain function and do not adversely affect disease progression in persons with ALS (6). However, the current evidence is not sufficiently detailed to recommend a specific exercise prescription for ALS patients. In the present study, it has been designed an exercise

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program based on both moderate endurance training and isometric muscle contractions (in muscle strength in the mild to moderate range with MRC score ≥ 3). The aim is to verify the clinical efficacy of this exercise protocol, thorough an objective assessment of the changes of muscular strength, the fatigue and the cardiovascular parameters. ALS pts admitted for rehabilitation treatment and enrolled with the inclusion criteria (clinically defined or probable ALS, the El Escorial criteria; mild-moderate disability; no heart and respiratory failures); 11 patients with ALS (7 males and 4 females aged 50 ± 13 years, range 24 ± 75 years) participated in the study; mean duration of the disease was 27 months (range 10 ± 66 months); at the time of the study, the patients had a total ALSFRS-R score from 21 to 41 (mean value, 31.7 ± 6.0). The exercise protocol consists in isometric muscle contractions, proposed in muscle strength in the mild to moderate range (with MRC score 3 and 4) and in a aerobic training by bicycle ergometry, arm-leg ergometry and/or treadmill with frequency of 4-5 sessions per week using an initial training intensity corresponding to 60–70% of maximum heart rate. Initial exercise duration of 15–20 min is recommended depending on the disability level of the ALS patient. We performed the following evaluations (before/after rehabilitation): ALSFRS-R (revised ALS functional rating scale), IB, Functional Independence Measure (FIM), Fatigue Severity Scale (FSS), strength measurements (MMT by dynamometer); analysis of HR variability and Oxygen Intake (VO_2 submax), six minute walking (6MWT) and ten meter walk test (10MWT). The project is a pivotal trial. 4 pts have presented a drop-out for rapid disease deterioration; the other 7 pts have concluded the study (see table). Decrease in fatigue as measured by a fatigue measurement tools at two months; the Fatigue Severity Scale and the change of the VO_2 submax have presented a reduction. Increase of muscle strength, as measured by the MMT dynamometry; the pts

have presented an increase of MMT dynamometry scores; in 5 pts during the 6MWT the distance has presented an increase. In conclusion, the effects of therapeutic exercise in ALS patients are controversial and not well understood. The aim of this project has been to understand the clinical outcomes of moderate load endurance and isometric resistance exercise protocol. If confirmed, these findings may represent a translational step to establish exercise prescriptions and to improve the clinical practice in ALS.

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