Eur J Trans Myol/Basic Appl Myol 2014; 1: 65-90
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Abstracts

In vivo studies on muscle protein turnover in ageing

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Muscular Sarcopenia is a major clinical issue in ageing, and decreased muscle mass and strength are associated with physical frailty and risk of fall. Since the elderly population is expected to increase dramatically in the near future, knowledge of the underlying, causative processes leading to sarcopenia in ageing are of great importance also to understand how to counteract it. In this presentation, the most recent kinetic studies of muscle protein turnover in human ageing, using combinations of isotope infusions and muscle biopsy, are reviewed. By these techniques, the two key processes determining net protein muscle accretion, i.e. synthesis and degradation, can be dissected and selectively measured in vivo, both under basal conditions and following anabolic simulations such as nutrition and/or exercise. Although initial studies provided evidence of a decreased basal skeletal muscle protein synthesis in aged individuals,[1,2] with changes being detectable even in middle age,[3] subsequent reports were not able to confirm them.[4,5] Also basal muscle protein degradation, which is not easily measurable, did not show major changes in aged people.[6] In contrast, a defective, relentless and/or delayed response of skeletal muscle protein synthesis following nutrition, has been reported.[5] Skeletal muscle of aged individuals may require a greater proportion of essential amino acids (mostly leucine) to exhibit the same increase of muscle protein synthesis as that of younger people[7] Such an impaired response can be at least in part attributed to an impaired increase of muscle blood flow following amino acid administration. Mainteinance of blood flow with nitric oxide donors together with amino acid administration restored a normal muscle protein synthesis in ageing[8] An increased splanchnic sequestration of ingested proteins has also been reported, suggesting a decreased peripheral delivery of dietary-derived amino acids as a possible cause of the reduced anabolic response in muscle.[9] Ingestion of rapidly-absorbed proteins may favour protein accretion in the elderly[9] Following physical exercise, a defective response of muscle protein synthesis has been reported in aged individuals, being both delayed and less responsive to increasing workloads.[10] As concerns the possible interventions to counteract and/or to prevent sarcopenia, a regular physical activity supported by adequate and balanced nutrition appear as the most effective treatments. In contrast, there is no clear-cut suggestion for an increased protein requirements to sustain muscle protein anabolism in the elderly.[11,12]

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Multidisciplinary approach to myotonic dystrophy: clues to treatment

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Fatigue is a clinically and socially relevant problem, interfering with physical and social functioning, mental and general health perception. At present no effective treatment is available for DM1. It is well known, instead, that physical activity might optimise muscle and cardiorespiratory function, prevent additional disuse atrophy and deconditioning in people with muscle disease. Moreover, there is scientific evidence that physical training is not harmful for patients with neuromuscular disorders. Unfortunately, due to the limited number of clinical studies, it is not currently known whether the rehabilitation treatment is really effective and which is the most appropriate exercise program for each muscle disease. Five adult DM1 males patients aged between 39 and 67 years have been admitted to

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the IRCCS San Camillo Foundation for a 8-wk training program. Clinical diagnosis was confirmed by molecular analysis. All subjects performed aerobic training on a cycle ergometer five times a week for 30-60 minutes a day at the intensity range of 60-70% of the maximum heart rate. The intensity of the exercise for patients on β-blockers was prescribed using the Borg Scale (intensity of 11 to 13 of perceived exertion corresponding to mild to moderate work). A cardiological evaluation, standard 12 lead ECG, echocardiography, overnight oximetry study and spirometry were always completed before training. In the upper extremities, all subjects carried out moderate strength exercises with elastic bands three times a week for one hour a day. In the lower limbs, two patients performed Functional Electrical Stimulation while the others moderate conventional strength training. Patient 2, who had severe right tibialis anterior muscle weakness (MRC 3), used FES for foot drop three hours a day for only 4 sessions. NESS L300 consists of three small components that all communicate each other wirelessly. During deambulation, when the patient lift his heel, a gait sensor, positioned on the footwear, sends signals to a functional stimulation cuff located just below the knee. The stimulation unit on the leg cuff transmits electric stimuli to the common peroneal nerve with consequent contraction of the tibialis anterior muscle and foot dorsiflexion during walking. Rectangular pulses with pulse width of 300 μs and stimulation frequency of 45 Hz were adopted. Patient 3, with severe proximal and distal muscle weakness, performed a FES induced cycling training 5 times a week for a total of 15 sessions lasting 30 minutes. Patient was seated on a chair in front of a motorized cycle ergometer (RehaMove FES byke) . A current controlled 8 channel stimulator (Reha Stim 2) was used and surface electrode were placed on quadriceps, harmstrings, tibialis anterior and gastrocnemius of both legs. Before and after training, all patients performed the six minutes walking test (6MWT) that significantly increased. The outcome measures considered have been the variation of muscle strength assessed by MRC scale and the change from the baseline of the walking distance covered in six minutes at the end of the training program. The role of FES has never been investigated in DM1. Our preliminary report, the first in this area, might highlight the important role of FES in improving the strength in those skeletal muscles with MRC≤3 for which no effective restorative treatment is otherwise possible. This is of primary importance for patients because they could improve their ability to perform activities of daily living and reduce the risk of adverse events such as falls.

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Effects of two weeks of bed rest and subsequent rehabilitation on size and function of single muscle fibres

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Skeletal Muscle biopsies were obtained from the vastus lateralis before and after 15 days of bed-rest from seven young (age 20-30) and sixteen elderly (age 60-65) male subjects. A third biopsy was taken after 15 days of rehabilitation based on three sessions/week of exercise. All subjects were healthy normally active sedentary people. Single fibres were dissected, cross sectional area (CSA) and isometric force (Fo) during maximal activation were measured and myosin isoform determined with SDS_PAGE to classify fibres as slow, fast 2A, 2X and hybrid. Before bed rest, significant differences were present between young and elderly subjects as the proportion of slow fibres and slow myosin isoforms was greater in the elderly, while the average CSA and isometric force of single muscle fibres were greater in the young subjects. Average fibre CSA showed a decrease in both groups, which was followed, during rehabilitation by a recovery to initial values in the young but not in the elderly group. Average isometric force underwent to a decrease during the bed rest period without any increase during the rehabilitation period. Taken together the results point to the high sensitivity to disuse of muscle fibre function and size in the elderly and to the slow and incomplete recovery during the rehabilitation period.

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Molecular signalling response to short duration high intensity/low volume resistance training in human skeletal muscle

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Resistance training (RT) is one of the most important stimuli for muscle hypertrophy, but it may play also an important role on weight loss and fat acid (FA) oxidation increase. It

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has been largely demonstrated that RT affects anabolic signalling molecule phosphorylation but, considering the numerous variables of RT, the differences between training modalities has been till now poorly investigated. The aim of this study was to assess Akt, 4EBP1, S6 and AMPK, ACC signalling after a single bout of high-intensity resistance training (HIRT) and of traditional resistance training (TRT). 12 healthy subjects performed in two different moments and with different legs HIRT and TRT protocol. HIRT consisted in 2 sets of 6/2/2 reps with incomplete rest between (20") sets while TRT consisted of 4 sets x15 reps with 1'15" of rest between sets. Biopsies from the vastus lateralis were taken one week before training sessions (pre), immediately after (T0), 6 hours after (T6) and 24 hours after (T24) training. We utilized immunoblotting methods to analysis Akt, 4EBP1, S6, AMPK and ACC activity. Gene expression was measured by RT-qPCR. No significant differences were found at any of time points after exercise in AKT and 4EBP1 phosphorylation. There was a significant increase in S6 phosphorylation at T6 both in HIRT and TRT. S6-P remained at higher level even at T24. Exercise intensity does not seem to influence the response of AMPK after a single bout of training: pAMPK decreased after both kind of RT, with a greater decrease at T6 during TRT. On the other hand, pACC activity increased immediately after HIRT and decrease after TRT, no significant differences were measured at T6 and T24. mRNA analysis showed that HIRT seems to be more related to mechanical deformation (MGF), while the TRT seems to act on IGF-1 pathway. Our findings suggest that a less time commitment resistance training technique is, at least, equally effective to induce an increase of S6-P. The increase of the phosphorylated form of S6 without a concomitant increase of AKT-P could be explained by an AKT-independent S6 phosphorylation. The increase of the phosphorylated form of ACC in HIRT, but not in TRT, may suggests a greater FA oxidation with high intensity/low volume resistance training compared to traditional technique. This data seems confirm that the manipulation of different variables of RT induces different molecular and metabolic responses related to the activation of specific muscle signaling pathways..

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BMP-SMAD1/5/8-MUSA1 axis is crucial for cancer cachexia

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Cell size is determined by the balance between protein synthesis and degradation. This equilibrium is affected by hormones, nutrients, energy levels, mechanical stress and cytokines. Mutations that inactivate Myostatin lead to important muscle growth in animals and humans. However, the signals and pathways responsible for this hypertrophy remain largely unknown. Here we find that BMP signaling, acting through Smad1/5/8, is the fundamental hypertrophic signal and counteracts cancer cahchexia. BMP-Smad1/5/8 negatively regulates MUSA1, a novel ubiquitin-ligase, that is required for cancer-mediated muscle loss. These findings highlight the importance of BMP pathway in the development of novel therapeutic strategies for the control of protein breakdown and for muscle wasting disorders
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Use It or Lose It. A life-long-style of recreational sport activities increases reinnervation of aging muscle

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Age-related changes in skeletal muscle innervation, independent of patent peripheral neuropathies, are known to contribute to the decline in quality of life often reported in older population.[1-4] These changes and the mechanism(s) by

which they occur are not well understood.[5-7] We had the opportunity to examine the effects of lifelong high-level physical activity comparing cohorts of young adults and septuagenarians either sedentary or recreational sportsmen, collecting what is, in our opinion, strong evidence that aging atrophy is, at least in part, a result of progressive denervation that can be counteracted by lifelong high-level exercise. We used immunolabeling methods to analyze the fiber type composition of muscle biopsies harvested from two groups of of ~70 years seniors, either recreational seniors, who exercised regularly at high levels and had done so for the previous 30 years or healthy, sedentary seniors – age-matched subjects who limited their exercise to "everyday" activities; and active young sportsmen.

Main results are: 1. biopsies from young men seldom contain denervated, reinnervated or transformed muscle fibers; 2. biopsies from sedentary seniors contain both denervated, coexpressing myofibers and a few reinnervated clustered myofibers of the fast type; and 3. senior sportsmen present with a larger percentage of healthy, slow myofibers that appear mainly clustered in slow fiber-type groupings. Further analyses of the data reveal that there was no difference between the athletic and sedentary senior groups in terms of their (both very low) percentages of muscle fibers co-expressing fast and slow MHCs, suggesting that lifelong exercise does not simply induce motor unit transformation.[8-10] The recreational sportsmen had both considerably higher percentages of slow-type myofibers and greater numbers of slow fiber-type groupings, than the sedentary group. These data provide sound evidence that lifelong cycles of denervation/reinnervation occurred. It appears, therefore, that lifelong exercise allows the body to adapt to the consequences of age-related denervation and to preserve muscle function by saving otherwise lost muscle fibers through reinnervation by different, mainly slow, motor axons. We have to agree with the motto "Use It or Lose It...", as it is titled the presentation of our paper in the Journal of Neuropathology and Experimental Neurology 2014, in press[9-10].

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FES4SEF-Italy or EU? Multidimensional model of prevention of falls in the elderly

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In elderly 70% of all falls occur during walking[1]. Indoor falls occur mainly during stair climbing or caused by obstacles, and outdoor falls while walking to the yard or on the road.[2] Many researches shows that the frequency of falls is related to the ability to anticipate and manage unexpected obstacles. These tasks however are tangled in elderly due to age-related deficits in postural control, balance, sensory input and the central organization of compensatory control processes. For mastering obstacles effectively, older adults need strength and power, especially in the lower extremities, a good balance performance and a fast reaction time.[3-5] The optimal training to reduce falling and to improve functional abilities linked to stair negotiation ability and performance was not still now well defined. Thus it is important to examine in details the validated function ability tests to better understand how to prevent falls and injuries and to better plan preventive physical activity. In addition it would be interesting to catch the early signals of falls tendency that might allow to design a personalized intervention based on multidimensional model. This model could enable to give more attention to one or more specific critical component: biomechanical, strength/power, postural or sensorial.

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RISE-3-Italy or EU? Further validation and new approaches

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The Rise-2-Italy started 7 years a go with the goal to test extension of the successful Vienna Strategy of home-based FES (h-b FES),[1-4] for complete and incomplete permanent denervated muscles of legs in cronic *Conus-Cauda* Syndrom to the apparently easier cases of partially denervated muscles due to trauma of the peripheral nerves. Five conus cauda were examined and enrolled. Peripheral nerves lesions in the observed cases were due mainly to monolateral injury of the sciatic nerve. One of the main results of the RISE-2-Italy has been the design and implementation of echomyographic approaches to repetibly follow evolution of the denervated muscles before and during h-b FES training. Further, in collaboration with Paolo Gargiulo of Reykjavik, Iceland 2D and 3 D color TAC post-analyses were successful designed and implemented making interpretation of the faint gry scales of TAC that document worsening by denervation and recovery by h-b FES, easy enough to be appreciated by family doctors and patients. Permanent denervated muscles were evaluated by ultrasound to monitor changes in morphology, thickness, contraction-relaxation kinetics and perfusion due to the electrical stimulation program of the Rise2-Italy project. In a case of monolateral lesion, 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 more in the middle third of the denervated muscle, reaching the same value as the contralateral innervated muscle. 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 the Vienna home-based Functional Electrical Stimulation strategy (h-b FES) the explored muscle shown electromyografic signs of re-innervation during the one-year of training. In conclusion, this pilot study confirms the usefulness of US Functional myography (Dynamic Echomyography) in the follow-up and the positive effects of h-b FES on denervated muscles. The procedure will have even higer impact if combibed with Stimulation needle electromyography (SNMEG) to test the electrophysiological properties of single muscle fibers. Despite the higher than normal energy of the delivered electrical stimuli of h-b FES the muscles shown electromyografic and dynamic echomyografics evidence of re-innervation during the years of training. Dynamic echomyografy confirm that fascicles of the TA muscle respond with twitch contractions to the electrical stimulation that elicit only contraction of innervated muscles (Impulse Duration 1 msec, Amplitude 25 mAmps). Interestingly, the hyperechogenic 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 dorsiflection 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.
Extending these experience to the new RISE-3 project by enrolling a new group of *Conus Cauda* and peripheral nerve lesions (either complete or incomplete) may open the possibility to design and implement a proposal to be submitted the a call of EU Horizon2020 that may have better chances to be funded.

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SAVe-ALS-Italy or EU? and FES4ALL (in need): Joining three weak options in a winning EU proposal

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Adult and aged population provide the vast majority of potential diseased people in Europe, the Americas and Japan. Their needs of mobility support and rehabilitation may be categorized as minimal (young light subjects during and after light traumatic events), medium (old subjects with impairments in the normal life activity and sarcopenia), severe (advanced sarcopenia in older old subjects, oncology, neuromuscular and skeletal disorders, subjects weaning from long hospitalization for heavy surgery and/or intensive care) and extreme (severe neuromuscular disorders, permanent flaccid paraplegia due to lower motoneuron injury in the spine, cachexia due to severe nutritional, metabolic, oncologic and septic conditions). The two previous speakers described either research proposals for a case of minimal need of interventions (FES4SEF) and a case of extreme need (RISE-3). I will here briefly describe a second case of extreme need (SAVe-ALS), and finally a comprehensive option to help people at large with purpose-designed strategies by FES based on implantable neuromodulators, like the heart or phrenic pace-makers, and embedded telecomunication networkings for long distance settings and monitoring: Functional Electrical Stimulation for all people in need (FES4ALL). I hope, indeed, to convince at least part of the audience that, after a short discussion of SAVe-ALS (Support Approaches for Ventilation in ALS), it is a more rational option to investigate the chances of success of a fourth option: joining session's three weak projects (and others) in a winning EU proposal: FES4ALL.

SAVe-ALS . Research on ethio-pathogenesis of SLA, on supports for survival of motoneurons, axonal regeneration and sproutings, and on transfer of animal results to patients' treatments are the main goals in ALS. Unfortunately, clinical relevant results are waiting for new concepts and discoveries to substantially influence the inexorable progression of the disease. Riluzole is the only drug that has been shown to (poorly) extend survival. Non-invasive ventilation prolongs survival and improves quality of life. The management of ALS is, thus, supportive, palliative and multidisciplinary.[1]

Awaiting the most wanted final solution, we will test in rodents and then in larger animal models of ALS a different approach, namely: Support of trophism of the denervated muscle fibers and evaluation of their potential contribution to power output by implanted neurostimulators and electrodes contacting the denervated muscle fibers, firstly in leg muscle and finally in the diaphragm to support ventilation of deteriorating animals. Based on the strong evidence of our EU Project RISE[,2] that proved recovery of ultrastructural organization, tetanic contractility and power output of degenerating myofibers in permanently denervated human muscles, we would like to transfer to ALS patients those results in collaboration with Antonio Musarò of Rome Sapienza University, Helmut Kern of the LBI, Wilhelminenspital, Wien, Austria, Winfried Mayr of the Medical University of Vienna, Austria, Jonathan Jarvis of the John Moor University of Liverpool, UK and Tessa Gordon of the Sick Children Hospital of Toronto, Canada. In the first year of a 3-year project we would like to validate the concept in ALS rodents3 comparing neuromodulation and direct stimulation of denervated muscle fibers of unilaterally sciatectomized posterior legs in wild and SOD-mutated mice. CIR-Myo of University of Padova will contribute with morphometry of immuno-fluorescence labelled experimental muscles,4-8 before and during the follow-up and Dr. Francesco Piccione, Dept. of Neurorehabilitation, I.R.R.C.S. San Camillo Hospital of Venezia-Lido,the clinical expertise and in the future the settings and the ALS sufferers for clinical experimentation. If the results of the rodent tests will be, as we expect, positive, in the next years we will test combined molecular and cellular approaches to support muscle fibers and the nerve-muscle units in larger animals and, then, in patients, hoping to substantially delay tracheotomy and need of positive pressure ventilation supports.

Our multidisciplinary group, supported by the European Project RISE: Use of electrical stimulation to restore standing in paraplegics with long-term denervated degenerated muscles (Contract no. QLG5-CT-2001-02191)] is the only in the world with the needed "evidence based" expertise [2,4-8] to design, implement and verify the hypothesis that permanent denervated skeletal muscle fibers may be recovered from severe atrophy, maintained in a trophic state that allow tetanic contractility and thus external work to support long term ventilation in ALS sufferers, before the critical phase of tracheostomy and positive pressure ventilation became mandatory.[1]

Combined with commercial devices for activation of the residual pool of phrenic axons, the long term goal of the actual project may substantially prolong both a decent quality of life and survival The relevance of the proposed preliminary animal studies described for ALS patients is self-evident.

FES4ALL. SAVe-ALS and RISE-3 are multidisciplinary complex strategies, that when the goal of a total implantable mini-neuromodulator will be reached, are addressed to alleviate the burdens of rare patients. Combining these two projects and others for severe needs, as extreme tests for FES, with FES4SEF (Functional Electrical Stimulation for Elderlies at Early Falls), that will extend to the global population of elderlies (soon or later we will be all at risk) may add to strong evidence of effectiveness of FES the bonus of attracting interest of industries and European Granting Agencies to provide funds to implement in 2015 the fascinating working hypotheses of FES4ALL.

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Development of tonic firing in rat muscles and control of body temperature

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Adult rats were kept in a climate chamber at stable temperatures near 26, 23, 20, or 17 °C. Continuous EMG recordings from electrodes implanted in soleus muscles displayed flat segments arising from single motor units firing stably between 20-30Hz when the rats were immobile at rest or sleep. The segments increased in duration and number with decreasing ambient temperatures ($P<0.001$). Most likely, plateau potentials in motor neurons, induced by activity in cold-sensitive serotonergic neurons in the brain stem, generated the tonic activity. The results point to an unrecognized mechanism for fine-tuning the body temperature around its set point. The mechanism involves heat generating tonic activity in slow fatigue resistant motor units. It operates independently of motor control mechanisms and continually at common ambient temperatures.

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Activity-dependent control of circadian gene expression in skeletal muscle

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Gene expression in all tissues displays circadian fluctuations under the control of an intrinsic clock and of extrinsic signals coordinated by the suprachiasmatic nucleus of the hypothalamus. However, the role of the local clock in muscle cell physiology and the relative contribution of intrinsic and extrinsic signals in orchestrating the circadian pattern of muscle gene expression are not known. To address these questions, we first disrupted the muscle clock by inactivating the essential clock gene, *Bmal1*, specifically in skeletal muscle. We found that insulin-dependent glucose uptake and glucose oxidation are impaired in mice with muscle-specific disruption of the muscle clock (Dyar et al, Mol Metab 2014). This result suggests that a major physiological role of the muscle clock is to prepare for the transition from the rest/fasting phase to the active/feeding phase, when glucose becomes the predominant fuel for skeletal muscle. Next, we examined the contribution of two major extrinsic factors, feeding and nerve activity, by time-restricted feeding and denervation, respectively. While the circadian oscillation of clock genes was drastically altered in *Bmal1*mutant muscles, a large fraction of circadian genes maintained their normal oscillation in knockout mice in response to feeding and/or nerve activity. Finally, we focused on activity-dependent cycling genes and found that the calcineurin-NFAT signaling pathway is a major mediator of nerve activity on circadian gene expression in skeletal muscle.

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Activity-dependent synaptic competition at developing neuromuscular junctions

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I present experiments, performed by our research group in Verona, demonstrating that nerve impulse activity is important not only for the control of many properties of the entire muscle fibre but also for a much more localized regulation exerted at the neuromuscular junction. This relates to a striking process of competition and elimination of motor inputs, that denotes the physiology of development of the neuromuscular connections. In fact when the innervation is first established in the embryo, multiple inputs make synaptic contact with each muscle fibre, contributed by the collaterals of several motoneurons (poly-neuronal innervation). During early postnatal life a rapid suppressive process occurs that invariably eliminates all but one input, resulting in the mono-neuronal innervation that is distinctive of the adult muscle fibre. The same competitive process between redundant inputs is recapitulated during muscle reinnervation after nerve damage in adult life: in various experimental investigations we exploited this feature to ask whether the timing of nerve impulses in the competing inputs affects synapse elimination. Thus, we tested the effects of two types of firing in the competing inputs: synchronous or asynchronous, while keeping constant the total number of

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nerve impulses per day. This was achieved by chronic in vivo electrical stimulation in adult rats, using a strain (AO) in which the soleus muscle is innervated by two nerves, soleus and aberrant. The stimulation of the two nerves lasted several days, during reinnervation of the soleus muscle after their crush. In a final acute electrophysiological experiment the degree of polyneuronal innervation from the two nerves was determined in vitro. The results indicate that asynchronous activity of the two nerves promotes competition and input elimination; synchronous activity, in contrast, counteracts competition, so that polyneuronal innervation persists. This presynaptic spike-timing mechanism on muscle innervation must be distinguished from the effects of overall muscle activity, and their respective physiological role in development will be presented. In another experimental line performed in newborn rats, we have also shown that, perinatally, spontaneous motoneuronal firing is well synchronized in different motor units and that a few days after birth a rapid desynchronization occurs. Taking together these lines of evidence, we conclude that the timing of firing in different motor units has an essential instructive role in the development of neuromuscular innervation.

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Structure-function relationships in skeletal muscles. Lessons from ultrastructure

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Muscle fibers have a stereotyped organization of contractile myofibrils and membrane systems best defined by their ultrastructure. The sliding filament model (in 1945) established currently accepted principles of most cell motility. The four contributors, H.E. Huxley, J. Hanson; A.F. Huxley and R. Niedergerke are deceased and that era is closed. Basic mechanisms of motile proteins action and the signals that control maintenance of muscle integrity are major current questions. The membrane systems were introduced by the local stimulation experiments (A.F. Huxley and coworkers,1956- 1964), and by the electron microscopy of K.R. Porter (1967). Currently we deal with the key molecular components.

Functional domains of T tubules and SR, triads and peripheral couplings Transverse (T) tubules form a complete network between the myofibrils and are composed of two alternating segments. Junctional segments (jT) are in the shape of flat ribbons and are closely associated to SR elements to form triads. Non-junctional or free segments (fT) have a smaller, circular cross section. jT segments bear the voltage dependent calcium channels CaV1.1 or DHPRs that are essential in initiating e-c coupling and also probably in ECCE, a calcium entry that follows e-c coupling and is essential in overall calcium homeostasis. A component of Store Operated Calcium Entry (SOCE) has also been assigned to the jT segments, fT segments are the probable location of other channels and exchangers. The T network is of equal abundance in fast twitch and slow twitch fibers, but the proportion of jT segments is significantly lower in the latter (by ~40%). The SR is also separated into two sections: the free SR (fSR) and the junctional SR (jSR): fSR comprises the great majority of the SR surface area and it is dedicated to pumping calcium back into the SR during relaxation. To that effect jSR is very convoluted and more the 90% of the protein in the membrane is the calcium pump ATPase. In mammalian muscles fSR is composed of two segments opposite either A band or I-Z-I level. The two are not equivalent and they respond differently to aging and pathological stimuli. The jSR comprises a large protein complex involved in calcium release and its control.[1]

T-SR relationships and protein locations. RyR1, Cav1.1, Junctional feet and tetrads. An obligatory relationship that constitutes an essential link in skeletal type e-c coupling. RyR3: parajunctional position. Recent experiments prove that RyR3 enhances the fundamental Ca release event (S. Perni, S. Hollingworth, S. Baylor, unpublished). The bets subunit of DHPRs. Essential role in the stereospecific link between RyR and DHPR[2] In mammalian muscle: essential role in the expression of DHPR at dyad sites (Claudio Perez, unpublished).

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Sarcopenia: definition, diagnosis and functional/metabolic implications

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'Sarcopenia' is a term originally proposed by Rosenberg (1989)[1] to describe the 'loss of muscle mass associated with ageing. More recently, sarcopenia has been clinically described as 'the loss of muscle mass and strength' with advancing age.[2] Whereas the first definition truly reflects the meaning of this term, the second implies that muscle weakness is a surrogate of the loss of muscle mass associated with ageing. As a consequence different methods of clinical diagnosis of this condition have been introduced. Sarcopenia, in its traditional definition, is typically diagnosed from cut-off values of appendicular skeletal muscle mass (ASM) evaluated either by DEXA, MRI or bioelectrical impedance (BIA). With this approach, individuals with an ASM below 1-SD of a young reference population mean are classified as Type I sarcopenic and those below 2-SD are classified as type II sarcopenic. Accordingly, upon reaching the seventh decade, 47% of men and 59% of women have type I sarcopenia, while 6% of men and 9% of women have Type II sarcopenia. Instead, sarcopenia associated with muscle weakness is clinically diagnosed using an approach proposed by a self-proclaimed 'European Working Group on Sarcopenia in Older People' (EWGSOP)[3] According to this group sarcopenia should be diagnosed by screening participants for gait speed, and if this is $\leq$ 0.8m/s, then ASM is measured by DEXA. If gait speed is $\geq$ 0.8m/s, handgrip strength is measured and if above specific cut-off values, they are classified as non-sarcopenic, if below, ASM is measured by DEXA. If the DEXA ASM is below age-specific cut-off values, participants are classified as sarcopenic, if above, non-sarcopenic. These measurements are complemented by an assessment of functional performance through the Short Physical Performance Battery (SPPB). The fundamental flaws of the diagnostic method proposed by the EWGSOP are that, 1*) it does not actually diagnose sarcopenia but physical frailty instead*, and 2) *it fails to recognize that sarcopenia starts before the age of 65 yr*. Only frail people are in fact unable to walk at a speed <0.8 m/s, and in addition, a decline in muscle mass can be typically observed starting from the third decade of life.
Alternative, but highly accurate methods for diagnosing sarcopenia are those based on the *biochemical assessment of muscle mass.* The use of total body potassium (TBK) and 24-h urinary creatinine are classical approaches. More recently isotope tracer dilution (D3 creatine and 3-methylhistidine) measurement in plasma, urine or muscle have shown great promise as accurate measures of muscle mass.In addition to the classical *biochemical biomarkers* (inflammatory, hormonal, oxidative damage, AGEs) of sarcopenia, new exciting ones have been proposed, these include: procollagen type III N-terminal peptide (P3NP), Agrin c-terminal peptide (CAF), neuronal cell adhesion molecule (N-CAM), Homer proteins, Activivs/Bone morphogenic proteins (BMPs), follistatin, noggin and others.
Sarcopenia has important functional and metabolic implications. Functional consequences are the loss of muscle force, velocity and power, a slowing down of muscle contractile speed and a decrease in muscle force per unit area.[4] Metabolic consequences are related to the key-role of skeletal muscle as main disposal site of circulating blood sugar. The loss of skeletal muscle observed with sarcopenia is thus an important cause of insulin resistance and risk of onset of type II diabetes.[5]

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CBC SK-AT 2007-13 "Mobility in elderly" Muscle rehabilitation and training in older adults

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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 (SCI) patients affected by complete injury of the *Conus Cauda*, a syndrome in which the denervated leg muscles are fully disconnected from the nervous system [1].
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.[2-5]. 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.[6,7] Specific clinical assessments and trainings were developed at the Wilhelminenspital Wien, Austria,[8] based on sound evidence from animal experiments.[9] Main results[10- 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.[11]

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)[12] 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. End-point results confirm the effectiveness of h-b FES, [13-15] Main results demonstrate that: 1. biopsies from young men seldom contain denervated, reinnervated or transformed muscle fibers; 2. biopsies from sedentary seniors contain both denervated, coexpressing myofibers and a few reinnervated clustered myofibers of the fast type; and 3. senior sportsmen present with a larger percentage of healthy, slow myofibers that appear mainly clustered in slow fiber-type groupings. Further analyses of the data reveal that there was no difference between the athletic and sedentary senior groups in terms of their (both very low) percentages of muscle fibers co-expressing fast and slow MHCs, suggesting that lifelong exercise does not simply induce motor unit transitions. On the other hand, the recreational sportsmen had both considerably higher percentages of slow-type myofibers and greater numbers of slow fiber-type groupings, than the sedentary group. These data provide sound evidence that lifelong cycles of denervation/reinnervation occurred. It appears, therefore, that lifelong exercise allows the body to adapt to the consequences of age-related denervation and to preserve muscle function by saving otherwise lost muscle fibers through reinnervation by different, mainly slow, motor axons.[16-18] We have to agree with the motto "Use It or Lose It...", as it is titled the presentation of our paper in the Journal of Neuropathology and Experimental Neurology 2014, in press[19].

Based on our observations of the presence of a subclinical myopathy in patients affected with newly diagnosed colorectal cancer,[20,21] we are now extending our approaches to oncologic rehabilitation. The factors associated with the subclinical myopathy at this stage of disease are unknown. A comprehensive study on the potential molecular mechanisms that are responsible for this cancer-associated myopathy could possibly provide new diagnostic and prognostic markers and new therapeutic and rehabilitation targets to prevent the severe loss of muscle tissue which characterizes late-onset cancer cachexia.[22,23]

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Decline of skeletal muscle power with age: the world records of Master athletes point to a human lifetime of 110 years

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The power developed by skeletal muscles is declining with age. 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 then medical tests, e.g. from world records of Master athletes.[1-5] Master competitions are carried out in many sports: athletics, swimming, cycling, skating and others. In our study we have collected the absolute world records of most track and field events together with world records of all relevant master categories, as certified by the international athletic organizations at mid 2013. Masters are athletes competing within age classes of 5 years (35 to 39; 40 to 44; 45 to 49 and so on). Their performances can be normalized with respect to the absolute record: the normalized performances are thus represented by numbers ranging from one (world absolute records) to zero (null performance).
The baseline of the data (from 35 years up to 100 years) is very extended, especially in comparison with the age extension of most clinical tests. Furthermore, the data are associated with individuals with highly similar boundary conditions: athletes physically gifted for the specific performance at their best condition and top motivation. Such uniform boundary conditions are not available in any longitudinal or (even worse) cross-sectional study. The decline of the normalized performances with age have been analysed and compared.[6,7] Power decline of skeletal muscle power in all track and field events starts at the age of 30 with minor variance. The power developed by Master athletes declines initially very slowly in the short running events (about 0.5 % per year) and then the decline rate increases after the age of 70-80 years. In the long-lasting runs the decline rate is only slightly steeper. In the jumping events the decline rate is almost linear down to the age of 90 years and beyond (1.2-1.3% per year). In the throwing events the power developed by the Master athletes declines slightly quicker (about 1.5% per year). During the initial 40 years of decline (from 30 to 70) the power developed by the lower limbs appears to fall with age slower that the power developed by upper limbs. The situation is reversed in the second half of the decline phase, after the age of 70. Regardless of the decline trend, the power developed in all track and field events tend to zero at the age of 110 years. This is substantially in line with the actual survival of humans.

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Mitochondria association to Ca^{2+} release units is controlled by muscle activity

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At the most basic level, skeletal muscle contraction requires $Ca2^+$ and ATP and, thus, is under direct control of two major intracellular organelles: Ca^{2+} release unit (CRU), and mitochondria. CRUs are the sites of excitation-contraction (EC) coupling, the process responsible for triggering $Ca2^+$ release from the sarcoplasmic reticulum (SR) in response to a propagating action potentials in the T-tubule membrane. Mitochondria are the powerhouse of the cell, being responsible for aerobic production of ATP. CRUs and mitochondria in skeletal fibers are functionally and structurally coupled: a) entry of $Ca2^+$ into the mitochondrial matrix is able to stimulate the respiratory chain; b) in adult skeletal muscle fibers, mitochondria and CRUs are structurally linked to one another by small stands, or tethers (1). Here we tested the following hypothesis: muscle activity improves/maintains the correct association of mitochondria to CRUs, which is challenged by ageing and inactivity. Using electron and confocal microscopy, we studied: a) ageing human/mouse muscle fibers and b) denervated rat muscle (by nerve crush). Our quantitative analysis shows that ageing (in humans and mice) and transient denervation (14 days, in rats) results in decreased association between CRU and mitochondria (2-to-3 folds decrease), whereas exercise and re-innervation either maintains or rescues the association between the two organelles (up to control levels). Functional implication of maintained/rescued association between mitochondria and CRUs is potentially large: indeed, Ca^{2+} uptake into mitochondria and efficient ATP production likely depend on the correct association between the two organelles.

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Delaying aging of skeletal muscle: use it or lose it

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Aging is a multifactorial process influenced by genetic factors, nutrition, and lifestyle. In the last decades, the extended human longevity resulted in increasing numbers of senior individuals in the general population and in a consequent dramatic increase of health care costs.[1,2] A greater understanding of the mechanisms leading to impaired muscle function is of central importance for prevention of disability and for optimization of independence of the elderlies. Aging of skeletal muscle is characterized by a reduction of mass, and force, also known as sarcopenia that occurs at different degree in all individuals.[3] Beside severe reduction in fiber size, sarcopenia is the result of loss of motor neurons and motor units. Indeed, 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. These age related denervation events are accompanied by reinnervation from the neighbor motor units (motor unit remodeling) that increase in size, in association with fiber type grouping.[3] 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.[4-6] Based on these findings, to determine the relative contribution of inactivity and aging *per se* to this decay, we compared muscle function and structure of a group of well-trained seniors (average of 70 years) who exercised regularly in their previous 30 years and to age-matched healthy sedentary seniors with and active young men (average of 27 years). The results collected show that relative to their sedentary cohorts, muscle from senior sportsmen have a greater maximal isometric force and function, better preserved fiber morphology and ultrastructure and preserved muscle fibers size resulting from fiber rescue by reinnervation. All together, our results indicate that skeletal muscle of senior sportsmen is actually more similar to that of adults than to that of age-matched sedentaries indicating that long-term physical activity delays age-associated skeletal muscle decline, also promoting reinnervation of muscle fibers undergoing age-related denervation. Signaling pathways controlling muscle mass and metabolism are differently modulated in senior sportsmen to guarantee maintenance of skeletal muscle structure, function, bioenergetic characteristics, and phenotype. Thus, regular physical activity[7-9] is a good strategy to attenuate the rate[6] of age-related general decay of muscle structure and function,

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The influence of exercise program and functional electrical stimulation on functional, histological and molecular parameters in prostate cancer patients: rationale and design

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Prostate cancer is one of the leading male cancer diseases in incidence (2nd), mortality (6th), treatment costs (6th) and survival (1st) worldwide rates. Among patients with prostate cancer treated with androgen-deprivation therapy (ADT), appropriately prescribed exercise may ameliorate a range of treatment-induced adverse effects.[1] Especially resistance training has been shown to be effective type of exercise in prostate cancer patients when administered 6 month and later after the initiation of ADT.[2] Also functional electrical stimulation has been shown to have comparable effect to resistance training on ageing muscle in healthy elderly.[3] It is therefore plausible that electrical stimulation will be effective also in prostate cancer patients. However, most of the adverse effects of ADT take place in the initial weeks and months of the therapy, and significant reductions in both muscle size and muscle quality is observed during the first 16 weeks on ADT. Consequently, it could be expected that the efficacy of the resistance training and electrical stimulation to ameliorate ADT adverse effects in muscle is highest when exercise intervention is started together with ADT. The scientific evidence is, however, lacking and no one has so far looked into the cellular changes in the muscle during this first phase of ADT. Therefore, the main goal of the project is to study effects of resistance training and electrical stimulation in newly diagnosed prostate cancer patients. Moreover, the project design would allow examining currently poorly understood mechanisms of the adverse effects of ADT at the cellular level and whether exercise and electrical stimulation-induced adaptations of skeletal muscle tissue under ADT. Two groups of age-matched males (60-75 years old) will undergo either a 16-week resistance training period or 16-week of electrical stimulation. Both training groups will consist of newly diagnosed prostate cancer patients, clinical stage T2 and T3, receiving ADT (n=8). Both groups will be tested before and after the intervention period for selected physical performance parameters with focus on muscle strength. Importantly, muscle biopsies from vastus lateralis muscle will be harvested before and shortly after an acute resistance exercise loading undertaken both at pre- and post-training state in both training groups. Histological and intracellular parameters related to muscle cell hypertrophy/atrophy, metabolism, and secretory activity accompanied with blood parameters will be analyzed to explain the possible differences in adaptation mechanisms. Newly diagnosed cancer patients receiving standard treatment (including ADT) but no exercise intervention will serve as a control group (n=8). This will allow us to investigate the cellular mechanisms behind the observed adverse effects of ADT on muscle size and muscle quality.

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Stability of the lumbo-pelvic region and its role for fall prevention and healthy back

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Spinal stability, consequent biomechanical integrity of the trunk as well as general balance of the human body largely depend on well-timed and coordinated muscle activation of the lumbo-pelvic region. It has been shown that these functions can either decay (aging or injury) or improve (specific training).[1,2,3] Moreover, balance deficits have been shown to increase the incidence of falls in elderly population.[4] On the other hand lower back pain is one of the most common medical problem that associates with changes in neuromuscular functions of the lower trunk. The aim of this paper is to present two methodological studies focussing on lumbo-pelvic stability and discuss the importance of this field for healthy and active aging. Study 1: The aim of this study was to assess the sensitivity of body sway while sitting on an unstable surface by comparing: (1) specifically trained subjects (kayakers) and controls and (2) three age groups of subjects from general population. First part of the study included 16 kayakers and 16 healthy controls, while 36 healthy subjects from general population (12 in each group) were included in the age related part of the study. The three age groups contained subjects of age 12, age 22-27 and age 56-76, respectively. The body sway was assessed while the subject was sitting on a wobble board with hands across the chest and eyes open. A force plate was used to measure the centre-of-pressure movement during the task. Independent samples t-test was used to assess differences between kayakers and controls, while analysis of variance test with post-hoc t-tests was used to tests differences among the three age groups. The level of statistical significance was at $p < 0.05$. Statistically significant differences ($p < 0.05$) were found between kayakers and controls for majority of the parameters (centre-of-pressure velocity, amplitude and frequency). Similarly, the differences among the three age groups were observed for majority of body sway parameters. Results of this study are supportive about the development and aging related changes in the control of functional stability of the lumbo-pelvic region. Moreover, the results confirm our hypothesis about the trainability of the lumbo-pelvic stability through specific training. Study 2: Bed rest has been shown to have detrimental effects on structural and functional characteristics of trunk muscles. In addition to inactivity, aging has been shown to significantly affect trunk musculature and possibly their function to stabilize the spine. To assess the combined effect of bed rest and aging, this study evaluated the effect of fourteen days bed rest on anticipatory postural adjustments and postural reflex responses of the abdominal wall and back muscles. Sixteen men (59.6 ± 3.4 years) participated in the study. Postural activation of trunk muscles was measured using voluntary quick arm movement (anticipatory postural adjustments) and sudden loading of the arms (reactive responses). Measurements were conducted prior to bed rest, immediately after and fourteen days after bed rest. Immediately after bed rest, latencies of anticipatory postural adjustments showed significant shortening, for the lumbar erector and multifidus muscles ($p < 0.05$). After fourteen day recuperation period, anticipatory postural adjustments reached a near to complete recovery. On the contrary, reactive response latencies increased from pre-bed rest to both post-bed rest measurement sessions ($p < 0.05$). Results indicate an important effect of bed rest on neuro-muscular functions of the trunk stabilizers in elderly adults. However the effect was not uniform for the abdominal wall and back muscles. Moreover, there proved to be a significant deterioration of postural reactive responses that outlasted the 14-day post bed rest rehabilitation.

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Impairment of authophagy in muscle induces neuro-muscular junction degeneration and precocious aging

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The cellular basis of age-related tissue deterioration remains largely obscure. The ability to activate compensatory mechanisms in response to environmental stress is an important factor for survival and maintenance of cellular functions. A system that is often activated both in short and prolonged stress conditions is autophagy.[1] Autophagy is required to clear the cell from dysfunctional organelles and altered proteins and is reported to decline during ageing. This reduction might contribute to age-related organ dysfunction and, in general, to ageing. Here we report that specific autophagy inhibition in muscle has a major impact on neuro-muscular synaptic function and, consequently, on muscle strength that ultimately affects life span of animals. Autophagy-deficient animals show precocious deterioration of neuromuscular junction that leads to myofiber denervation. Inhibition of autophagy also exacerbates the aging-related features of muscle such as mitochondrial dysfunction, oxidative stress and profound weakness. Mitochondrial dysfunction and oxidative stress directly affect acto-myosin interaction and force generation but show a limited effect on stability of neuromuscular synapses. Conversely, autophagy inhibition alters Acetylcholine Receptor (AChR) recycling, MuSK clustering and expression of FGFBP1, a neurotrophic factor, that is critical for neuromuscular junction maintenance.[2] These results demonstrate that age-related deterioration of synaptic

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Electrical stimulation counteracts muscle decline of septuagenarians: Molecular mechanisms

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The loss in muscle mass, coupled with a decrease in strength and force output, has been associated in aging with reduction in muscle fiber size, shift in fiber composition, alterations in physiologic and metabolic parameters and modulation in gene expression. It is well documented that training and regular exercise attenuate the signs of sarcopenia, increasing muscle strength while decreasing fall risk. Nevertheless, pathologic conditions limit the ability to perform physical exercise and therefore the benefits from it. We addressed whether electrical stimulation (ES) is an alternative intervention to improve muscle recovery and have defined the molecular mechanism associated with improvement in muscle structure and function. Here we analyze, at functional, structural, and molecular level, the effects of 12 weeks of ES training on healthy seniors with normal life style, i.e., without routine sport activity. All subjects were healthy and declared not to have any specific physical/disease problem. We demonstrate that ES was able to improves muscle power and functional performances of seniors and stimulates an increase in size of fast type muscle fibers. At molecular level, ES induces overexpression of IGF-1 isoforms and modulation of MuRF1, a muscle-specific atrophy-related gene. ES also induces an up-regulation of relevant biomarkers of differentiating satellite cells, such as miRNA 206 and miRNA- 1. Interestingly, we also observed extracellular matrix (ECM) remodeling, which might guarantee shape and mechanical forces of trained skeletal muscle as well as maintenance of satellite cell function. Our data provide evidence that ES is a safe method to counteract muscle decline associated with aging by neurostimulating septuagenarians seniors only 3 times a week.
Trial Registration: ClinicalTrials.gov: NCT01679977

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An anti-oxidant treatment prevents/reduces formation of cores in a mouse model of Central Core Disease

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The Central Core Disease (CCD) and Malignant Hyperthermia (MH) are related skeletal muscle disorders in most cases linked to mutations in the ryanodine receptor type-1 (RYR1) gene, encoding for the Ca^{2+} release channel of the sarcoplasmic reticulum. CCD is characterized by hypotonia, proximal muscle weakness and presence of amorphous regions of myofibrillar disorganization (cores) lacking mitochondrial activity. In humans, the Y522S mutation in RYR1 is associated with MH susceptibility with cores. Knock-in heterozygous mice expressing this mutation (RYR1Y522S/WT) are viable, suffers of MH, and develop structural cores.[1,2] Previous studies indicated that oxidative stress likely represent a key event causing structural damage of skeletal fibers.[3]
Here we tested the hypothesis that reducing oxidative stress prevents/reduces structural damage and consequent formation of cores. We treated RYR1Y522S/WT mice from 2-to-4 months of age with N-acetylcysteine (NAC, a potent anti-oxidant) provided ad libitum in their drinking water (1% w/v) and analyzed skeletal muscle preparations by histology, electron and confocal microscopy. Analysis of un-treated RYR1Y522S/WT fibers from extensor digitorum longus (EDL) muscle confirmed the presence of mitochondrial damaged and severe structural alterations - previously defined as un-structured and contracture cores[1] - in a high percentage of fibers: respectively, 28% and 17% presented un-structured and contracture cores (3 muscles; 298 fibers). However, NAC treatment resulted in significant reduction of a) average size of apparently normal mitochondria (24% reduction); b) number of severely disrupted mitochondria; and c) relative mitochondrial volume (20% reduction). More importantly, although the fiber structure did not appear completely normal, frequency of fibers presenting un-structured and contracture cores, was also dramatically reduced, dowen to only 2 and 5 %, respectively (3 muscles;

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670 fibers). These results suggest that oxidative stress may play an important role in the development of cores in RYR1Y522S/WT mice and open to possible development of therapeutic strategies aiming to protect diseased muscle from damage.

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Coordination of energy demand and ATP production in the mitochondria of working cardiomyocytes

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(i) The calcium ion (Ca^{2+}) signal coordinates the energy demand and energy production in the working myocardium. (ii) The adenine nucleotide translocase (ANT) in the inner mitochondrial membrane (IMM) controls the traffic of ADP into the mitochondrial matrix for re-phosphorylation to ATP and its exit from the matrix. The IMM is impenetrable for peptides, metabolites, and charged ions; it thus strictly separates the mitochondrial matrix from the rest of the cell. Specific transport and channel systems exist for the traffic between cytoplasm and matrix. Ca^{2+} functions as intracellular second messenger regulating contraction, metabolism, energy production by oxidative phosphorylation in the mitochondria, sarcolemmal ion pump activities as well as protein expression by affecting the gene activity profile [1]. In order to fulfill its multifarious signaling tasks the Ca^{2+} level in the cytosol must be kept very low (~10–7 M) at rest, i.e. 20'000 times lower than outside the cell (extracellular Ca^{2+} concentration ~2 mM). Any uncontrolled, excessive Ca^{2+}entry leads to cell death (cardiac infarction, brain stroke). The Ca^{2+} signal for a heart beat comprises a rapid transient increase of Ca^{2+} by about 100-fold up to 10–5 M in the cytoplasm. This transient Ca^{2+} increase results from a combination of Ca^{2+} entry through the sarcolemmal voltage-gated L-type Ca^{2+}-channel (also called dihydropyridine receptor as the dihydropyridine drugs specifically inhibit Ca^{2+} entry through this channel) and the subsequent Ca-induced-Ca-release (CICR) through the ryanodine receptor (RYR), the Ca^{2+}-release channel of the sarcoplasmic reticulum (SR), where Ca^{2+} can be stored up to millimolar concentrations. Ca^{2+} can only function as messenger (i) against its low background level in the cytoplasm at rest, and (ii) by binding to Ca^{2+}-sensor proteins that reversibly can bind Ca^{2+} with an affinity in the range of KD 10–5 to 10–6 M. This is just the cytoplasmic Ca^{2+} level that may be reached during excitation. TroponinC (~18 kDa molecular mass) in striated muscle (skeletal and heart muscle) was the first such Ca^{2+}-sensor protein detected, and the ubiquitously present calmodulin (~17 kDa) in the cytoplasm was the second one [2,3]. Up to now over 200 genes coding for Ca^{2+} signaling proteins have been found in the human genome. These proteins all contain one or more specific Ca^{2+} binding motifs (helix-loop-helix, called EF-hands) developed early in evolution [4]. The entire cardiac ATP pool just suffices for a few heartbeats. It would be exhausted within one minute at rest and in 10 seconds at high workload [1]. Thus ATP needs to be furnished continuously. About one third of the cardiac energy production is used for maintaining the sarcolemmal Ca^{2+} and Na+ gradients as well as the proton (H^+) gradient at the IMM. The H^+ gradient at the IMM (130-180 mV, electronegative on the matrix side) produced by the electron transport chain (ETC) allows ATP production by the ATP-synthase F1 in the complex-V (FoF1). The heart generates anywhere from 6 up to 30 kg ATP per day (20-100 times its own weight) depending on the energy demand. In addition to the regulatory functions in the cytoplasm, Ca^{2+} enters the matrix through the recently described mitochondrial calcium uniporter (MCU) [5]. In the mitochondrial matrix Ca^{2+} stimulates several dehydrogenases of the tricarboxylic acid cycle (TCAC) as well as the rate-limiting enzyme of the ETC, cytochrome-c oxidase (COX, complex-IV), and the ATP synthase. Ca^{2+} entry into the matrix is driven by the membrane potential and reflects the Ca^{2+} concentration in the cytoplasm; it thus regulates mechanical contraction and energy metabolism in unison. Only a slight mismatch between energy demand and production might lead to disaster. Heart failure, in fact, is due to a shortage of fuel [6]. The isolated contractile structures from failing hearts remain perfectly functional when tested in vitro. The adenine nucleotide transporter (ANT ~33 kDa) presents the most abundant protein (5-10% of total protein) in the IMM. ANT belongs to the mitochondrial SLC25 carrier family whose members are nuclear-encoded and incorporated in the IMM. Analysing the mitochondrial phosphoproteome of rat hearts revealed only the ANT protein to vary in its degree of phosphorylation in dependence of exposure to ischemia. In particular, tyrosine residues Tyr186–Tyr190–Tyr194 display reversible phosphorylation. These tyrosines are arranged in a row running along the alpha-helix-4 at the inner side of the channel cavity. Molecular dynamics simulation suggests that the adenine rings of ADP successively interact non-covalently with the tyrosine residues and that phosphorylation of this tyrosine ladder facilitates the passage of ADP from the cytoplasmic side through the channel to the matrix [7,8]. As a corollary, the exit of the newly formed ATP through the same channel might also be enhanced. De-phosphorylation of the tyrosine ladder might reduce the efficient re-phosphorylation of ADP to form ATP. Details of structure and function of ANT will be discussed with regard to the regulation of energy production in the mitochondria by oxidative phosphorylation.

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How to stimulate muscles regarding their inherent adaptive capacity: Case reports

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The The amount of adaptation induced by electrical stimulation depends on the duration of stimulation, the frequency and pattern of stimulation, and the relationship between the pattern of stimulation and the natural firing rates (which vary significantly between species) routinely delivered to the muscle by the motor cortex. It is likely that the next phase of research into the relationships between pattern of activity and phenotype will be performed in small rodent species. The available technology has almost reached the stage at which any pattern of activation can be delivered to any motor nerve from a universal externally programmable stimulator. As an example of the care needed in extrapolating between rat and human, we emphasise with preliminary data from two studies the remarkable ability of rat muscle to 'absorb' activity with rather little change in fibre type, although fibre size seems to be more sensitive to the same amount of activation. We have stimulated rat tibilalis anterior with the pattern 200ms of 100Hz every 30 s, 24 hours per day. This equates to an average frequency of 0.66Hz. Based on continuous stimulation studies at similar average frequencies, we would not expect much change of fibre type. In fact we saw only about 10% in the volume percentage of the type 2b and 2a types by Tunell and Hart staining. On the other hand, this pattern of stimulation, though we had thought it might produce some fibre hypertrophy, caused approximately 16% reduction of wet weight. Presumably the stimulus of high force was present, but the amount of activity, or possibly the lack of long rest periods, caused any activation of protein synthesis to be overcome by a greater increase in protein degradation. As another example, we compared the effects of short bursts of high intensity exercise with longer bouts of lower intensity exercise in a simulation of the sprint-interval versus endurance tests made in humans. We used a sprint interval pattern of 100 Hz bursts, repeated every second for one minute every five minutes over a 20 minute session. The sessions were repeated every 48 hours. The equivalent constant frequency was 0.03 Hz. The 'endurance' regime was 20Hz continuous for one hour every 24 hours (equivalent constant frequency was 0.83Hz, or 28 times more impulses than the sprint interval pattern. There was very little change at the histochemical fibre type level with either regime. We will measure transcriptional responses to assess how the muscle interpreted these two challenges, but it is clear that a pattern based on a feasible human exercise programme must be intensified in order to make comparisons between similar exercise strategies in experiments in the rat. One significant problem is that although the cycle times must be intensified in rat, their circadian rhythms are determined by the same daily clock as humans.

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Scar wars – A new hope

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Vocal fold (VF) scarring is the most frequent cause of poor voice after vocal fold injury and results from a disruption of the layered vocal fold lamina propria. It leads to a significant impairment in vibration characteristics by altering the viscoelastic properties and results in a hoarse, often breathy and little sustainable voice. Reasons for scarring comprise vocal abuse, chronic inflammation, radiation therapy, external or internal trauma. The changes have a considerable impact on quality of life and may professional speakers (teachers, call centre agents, etc.) force to quit their jobs.[1] Modern societies depend to a smaller degree on manual labor but largely on communication skills. Today the success of a person is defined in terms of her/his ability to communicate effectively. Treatment of fibrotic and scarred VF is still an unresolved chapter in laryngology. Big hopes are placed in laryngeal tissue engineering, especially in the activation of

local stem cells.[2] This presentation is thought to give a comprehensive overview about different aspects of stem cell therapy for the larynx. Recent studies also by our group proved the presence of stem cells in form of side population cells (SP cells) in the VF. Under normal circumstances stem cells rest inactive in the peripheral maculae flavae and migrate after trauma to the region of the lesion with a peak after 5 – 7 days. Various kinds of cells including stem cells have been injected in VF injury with promising results, but so far only in animal trials and most of them with only little distance from time of injury to time of injection. Kanemaru reported enhanced wound healing by injection of autologous bone marrow derived mesenchymal stem cells (MSC) prior to injury in Beagle VF. Groups in Sweden used human embryonic stem cells in injured canine VF. All trials showed promising healing tendencies, as well as superior outcome parameters compared to control groups. Injection of stem cells lead to elevation of anti-fibrotic parameters such as HGF (hepatocyte growth factor) and HA (hyaluronic acid), while collagen levels decreased.

Laryngeal tissue engineering has emerged in the last decade and has enlarged our knowledge about the micro-physiology and micro-pathophysiology of the VF. Cell therapy is probably the most powerful tool in regenerative medicine. The underlying concept is to inject multipotent regenerative cells in a scarred or atrophied vocal fold, where they start production of extracellular matrix proteins.[3] The extremely complex structure of the vocal fold, especially the delicate layered structure of the lamina propria, makes this a challenging field which can only be handled by a close cooperation of physicists, molecular biologists and bio-engineers.

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Chronic electrical muscle stimulation following nerve injury and immediate repair enhances reinnervation

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The use of chronic electrical muscle stimulation for treating partially or completely denervated muscle has been met with controversy. Our previous work demonstrated that a moderate stimulation paradigm significantly increases the numbers of reinnervated motor units following long term muscle denervation and subsequent nerve repair.[1,2] More recently, this same paradigm was used to investigate early effects following 2 weeks of muscle stimulation after nerve injury and immediate repair. The results from this study showed that stimulation significantly increased the number of reinnervated motor units as well as accelerating axon outgrowth into the distal nerve stump.[3] Extending the duration of muscle stimulation to a period longer than 2 weeks has not been previously explored. Research question: Does a moderate electrical muscle stimulation paradigm delivered chronically improve reinnervation and functional outcome measures following nerve transection and immediate repair? Six groups of Thy1-GFP transgenic male rats were subjected to tibial nerve transection and immediate repair using two epineurial sutures. One group of rats underwent daily electrical stimulation of the denervated gastrocnemius muscle with a paradigm comprising of 600 equally separated contractions throughout one hour, delivered 5 days per week. Rat gastrocnemius muscles were electrically stimulated for either 1, 2, or 3 months and then underwent terminal assessments which included evaluating muscle force, contractile properties, motor unit numbers, and wet weight. Rats in the 3 month group were serially assessed for skilled locomotion with the tapered beam test. Behavior in the same rats was also assessed with a walking track analysis that measured gross changes in reinnervation using the tibial functional index. Muscles were then harvested for immunohistological examination of motor end plate reinnervation. Muscles that received daily electrical stimulation had a significantly greater number of motor units for all three time points (1, 2, and 3 months) as characterized using electromyographic methods. Mean motor unit sizes were significantly smaller in stimulated muscles suggesting that muscle stimulation may inhibit terminal sprouting as reported by others.[4] This may allow for a more natural course of reinnervation resulting in improved functional recovery.[5] Indeed, skilled location tests showed that stimulated muscles enhanced and maintained recovery at levels no different from those in normal functioning rats whereas non-stimulated controls became progressively worse and did not recover to baseline. The tibial function index showed no significant differences between groups. Chronic treatment of denervated muscle using electrical stimulation significantly enhances muscle reinnervation and functional recovery. As the muscle continues to become reinnervated, tailoring the stimulation paradigm to improve muscle force and fatigability may lead to shorter recovery times and reduce extensive physiotherapy and rehabilitation requirements.

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A new Liverpool/Vienna implantable neuromuscular stimulator: update

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The miniature programmable device presented in previous Padua Muscle Days has been tested successfully in prototype form and is nearing full testing in its intended radio-frequency programmable form.[1] It will be small enough for implantation in mice and rats, and will provide stimulation both programmable in terms of daily pattern and adjustable in amplitude non-invasively. We will give an update of progress to date.

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Muscle stimulation in a complex neuromuscular pathology: recurrent laryngeal neuropathy in the horse

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The laryngeal muscles are concerned with protection of the airway during swallowing, opening of the airway to allow unrestricted inspiration (and therefore capacity for exercise) and the production of the voice. Clinically important reduction of these functions is surprisingly common, and increases in the elderly. The causes of damage include elective surgery (during ventral cervical fusion to address spinal cord compression, and sometimes unavoidably in cancer surgery), thoracic or cranial tumours that may impinge on the laryngeal structures or their innervating neurones, and trauma. There is an additional related problem in horses, because of a relatively common recurrent laryngeal neuropathy.[1] This causes reduction in maximum exercise potential, most obviously a problem in race horses, but also affecting the usefulness and saleability of sport horses because of abnormal respiratory noise. There is therefore an opportunity for veterinary clinical research to address the problem in the equine population that may also indicate potential means of intervention in human clinical laryngopathy. Equine RLN is a progressive neuropathy interpreted at the histological level as a mix of denervation, reinnervation indicated by type grouping, and fibrosis. Cricoarytenoid dorsalis (CAD) muscles removed from severely affected horses show, in the advanced stages of the disease, near complete fibrosis and therefore inability to provide an opening force on the arytenoid cartilages. Since RLN is progressive, there is a mixture of fibres in affected laryngeal muscles, from at one extreme, normal muscle fibres with a normal activity pattern and therefore normal exercise capacity, through to affected but not fully denervated muscle fibres that may have reduced efficacy of activation, and therefore reduced exercise capacity, and at the other extreme, fully denervated fibres that perform no useful work to open the airway. Stimulation to improve function may address the active but weak fibres, but also the denervated fibres if we use intramuscular stimulation with sufficient current to activate those denervated fibres.[2] We have done experiments to investigate whether the function of the laryngeal muscles in equine RLN can be changed by electrical stimulation and whether stimulation of the laryngeal muscles during a period of denervation enhances or reduces the chance of re-innervation (native or after surgical reinnervation) and long-term function. In common with stimulation studies on denervated human and rabbit muscles, we have noted a normalisation of fibre size distribution in stimulated CAD. We have also shown that muscle function measured by videoendoscopy during treadmill exercise responds to periods of training and periods of de-training although we are aware that this is an indirect measure affected by the coupling of muscle force to arytenoid movement and muscle loading due to negative inspiratory pressures tending to close the airway. Volumetric and functional ultrasound measurements have also shown positive effects of muscle stimulation, but it is clear that, just as with the studies on human denervated limb muscles, the greatest benefits are on innervated muscles fibres and those that are not in an advanced state of degeneration at the onset of stimulation.[3-5]
The choice of stimulation pattern has been based on the desire to produce hypertrophy without excessive slowing in the muscles, together with choices of stimulation frequency based on function-frequency tests. In general, the amount of stimulation needed to produce a worthwhile change in muscle performance is higher than our initial estimates suggested.
The work on equine larynx has been in parallel with pilot clinical trials in human patients, in whom stimulation of the opening muscles has in several cases shown striking benefit in terms of quality of sleep and exercise capacity.[6]

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Correlations between muscle atrophy/degeneration in aging associated to life style and comorbidities on a preliminary set of data from the AGES-Reykjavik Study

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Amount and quality of skeletal muscle and heart failure are dependent to life style, i.e, physical activity, and eventual diseases.[1,2] Further, there are mutual interactions among the two apparatuses.[3,4] Muscle atrophy is well established to occur during malnutrition, bed rest, unloading, or partial unloading model, spinal cord injury, stroke or tendon reconstruction.[5-7] Muscle atrophy, obviously, leads to reduced levels of force and power development, with strong dysfunction in mobility, balance and related additional risks of falls. The literature offers a good knowledge base concerning the progression of muscle atrophy in response to upper motor neuron (UMN) lesions in human patients. Muscle mass decreases significantly during the first few months after UMN damage with ultrasound revealing a loss of up to 40% of the mass in the first month post injury, but after the first few months post injury, when a new life style is stabilized, the atrophy process slows down, resulting in a stable atrophy (50% of initial muscle mass) with the onset of spastic incomplete paralysis for up to twenty years after SCI.[6] The atrophy is much more severe when the lower motor neuron (LMN) is damaged. Two of the Authors contributed to design and develop of the elegant strategy of the Vienna home-based Functional Electrical Stimulation training for muscle recovery in this second case (LMN lesions), where muscle atrophy end in full degeneration of muscle tissue.[7-9] Our aim is to associate the muscle amount/quality as percentage of atrophy or degeneration, primarily to heart failure and secondly to other co-morbidities.
We propose to assess the muscle quality using the CT data, and to evaluate the percentage of atrophy/degeneration within the muscle volume based on Hounsfield values (HU). CT data from healthy muscles can be displayed within an interval of 40 and 90 HU, though within a normal muscle volume there are other tissue elements such as water, connective tissue and fat, which are coded with much lower or higher HU value. The different tissue elements express their specific HU value if such tissue completely occupies the voxel volume; otherwise the HU number will be an average of different values. This fact explains the wide range of values present inside a dataset and suggests the definition of various intervals to study muscle structural changes. Therefore, to estimate the tissue composition in the muscle volume, and assess the muscle atrophy within the volume we will divide the HU distribution in four HU intervals: [min; –10], [–9; 20], [21; 40] and [41; max] representing, respectively, fat, connective tissue/ water, low/atrophic and normal muscle tissue.[8,9]
On the other hand, less is known about the correlations between muscle amount/quality, heart failure and life style.
During our experience with denervated muscle we found indications of correlated effects between muscle recoveries (increase of muscle density and decrease of atrophy in quadriceps muscle) and increase of Bone Mineral Density in patella bone. In that project we could assess muscle quality in complete denervated and atrophied muscles and follow the recovery process through electrical stimulation treatment and associated bone structural changes.
Based on this work and potentials offered by the AGES-Reykjavik Study we propose to investigate the amount and quality of muscle tissue in the enrolled subjects.
An index of muscle tissue amount (muscle atrophy), whether normal or degenerating is derived using imaging techniques from the ratio between the bone volume of total body or of an appendix and the related muscle tissue. The hypothesis is that the degree of muscle amount (atrophy) or degeneration (as index of muscle quality) within the muscle volume may be related to physical conditions (life styles), heart failure and to co-morbidities . The parameters needed for the execution of the study are based on parameters extracted from the left and the right femur, muscle parameters crossing the hip joint extracted from the left and right side and finally information about the subject from the AGES-Reykjavik Study.

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Estimation of signal strength in application of Acousto-Electric-Interaction Effect to monitor denervated muscle stimulation

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Electrical activity of muscles is commonly mapped with surface electrodes placed on the skin or with fine wire or needle electrodes placed inside the muscle. Surface electrodes are simple in use and can be used repeatedly without any harm to the patient. But they do not give detailed information on what muscles are active or what part is active if the muscle is partly denervated. EMG form deep lying muscles are difficult to reach or even out of range. Using intramuscular electrodes increases selectivity and even deeper lying muscles can be reached. Their disadvantage is that each electrode pair is only capable of local EMG registration. In order to map the EMG activity of a whole muscle many stiches have to be made. They are therefore not suitable for muscle monitoring on a daily basis. Additionally people with paralysis and reduced blood flow and metabolism, wound healing has slowed down making frequent stiches unacceptable. Methods for registration of muscle activity that are based on CT, MRI or PET scans or incorporate radioactive materials are more complicated, not suitable for bedside monitoring and costly. Ultrasound imaging gives information on the shape and movement of muscles but does not give information on what muscles are causing the movement. A novel technique to map electrical current in muscles is the Ultrasound current source density imaging (UCSDI).[1] It is based on the Acousto-Electric-Interaction effect (AEI). An acoustic wave traveling through material modulates its electric resistivity. Therefore when an ultrasound wave crosses electrical current in the material a high frequency voltage signal is generated. This signal is termed AEI signal. By focusing the ultrasound wave to a focus point inside the muscle or tissue will give the strongest AEI signal from that location. Moving the ultrasound focus in the tissue gives a way to map the electrical current distribution in the tissue. As little as two electrodes can be used to for the registration of the AEI signal. Groups working on the UCSDI have been targeting the heart and brain diseases.[1,2] We suggest the use of UCSDI to map the electrical activity of denervated muscles to monitor electrical stimulation therapy. [3] That way the portion of the muscle reached by the electrical stimulation can be detected. Also the monitoring of denerveated muscle in the state of re-innervation can be made given that the sensitivity of the method is sufficient. That way the effectiveness of a therapy can be monitored on a daily basis. The method is non-invasive since the ultrasound source and electrodes can be placed on the surface of the skin, again given that the AEI signal strength is sufficient. In this work we examined in a mathematical way the relation between the electrical current intensity and ultrasound wave amplitude on one hand and the AEI signal strength on the other hand. The results suggest that the AEI voltage signal is strong enough for application on the thigh muscles. Since the expected electrical current density used in stimulation therapy and the acoustic wave amplitude can be adjusted in a wide range the associated AEI signal strength is also varying in a wide range. In fact it is in order of two magnitudes higher than what is expected with no injected current, as reported by Gudjonsdottir et al.[3] The conclusion is that UCSDI is a promising technology to monitor both electrical stimulation therapy and electrical activity of muscle fibres.

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Vagus nerve stimulation to limit aura of focal cerebral ischemia: rodent model and implantable neuromodulator

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Considerable efforts have been devoted during recent years to develop devices to stimulate the human autonomic nervous system. In particular, techniques of vagus nerve stimulation (VNS) to treat a number of neurodegenerative and vascular central nervous system (CNS) dysfunctions, and neuropsychiatric, pain, sleep, eating, cardiac, and endocrine disorders. Our main goal is to extend published results of the

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effects of short term neuromodulation of the vagus nerve on the size of brain infarcts due to permanent or transitory arterial occlusion by an intra-arterial thin wire.[1-3] The Liverpool mini neruromodulator designed and developed by Jonathan C Jarvis will be used to stimulate the vagus nerve in the neck by implanting two loop electrodes sutured to the sternocleidomastoidus muscle. More selective cuff electrodes will be also tested. The encapsulated electronics, battery and optical switch will be implanted in the peritoneal cavity and the electrodes tunnelled to the neck. We are implementing the experimental plan by acute trials to evaluate the different components of the project in adult rats, that is: 1. the brain ischemia model; 2. the implantation in the peritoneal cavity of the mini neuromodulator, tunneling of the wires to the neck, selection of the proper electrodes; 4. Neuromodulation settings for acute and mid-term experiments. Prototype protocols of neuromodulation will be discussed and preliminary results of acute experiments will be reported.

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Neuromodulation in motor nerve trauma: Accelerating traumatic nerve repair and sustaining muscle trophism

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The process that follow an axonal injury is known as Wallerian degeneration. It is characterized histologically by axonal blebbing and fragmentation into ovoids.[1,2] Its clinical electrophysiology correlation is generally taken to be failure of electrical conduction by the nerve distal to the site of injury and the onset of muscle denervation potentials.[3-7] In turn, this information is used to prognosticate the time course and extent of recovery in response to therapeutic interventions. An example would be the case of a 47 year old man with a complete right median nerve lesion at the wrist, following a cutting injury of the right forearm. The complex trauma also caused a lesion of the radial artery, a total cross-section of the deep flexor tendon of the first toe and an injury to the flexor carpi radialis tendon. Three hours after the trauma, the patient underwent a surgical suture of artery, tendons and neurorrhaphy of the median nerve. After 10 days of healing, the patient was submitted to a neuromuscular stimulation protocol 5 times a week, each session lasting 30 minutes, using these parameters: pulse duration 0.3 msec, current intensity < 25 mAmp (according to pain sensation), stimulation frequency 50 Hz for 1 second and pause of 4 seconds. After 1 month of this training protocol, the patient reported an improvement of subjective sensitivity of the second and third fingers of the hand. At that time, we performed a dynamic echomyographic scan of the abductor pollicis brevis (ABP) that showed initial muscular atrophy (innervated left hand 15 mm vs. denervated right hand 16.5 mm) and an increased echogenicity of the muscle, both accepted evidence of denervation. Few days later, to avoid muscular wasting, we decided to combine the neuromuscular electrical stimulation protocol with direct stimulation of the denervated tenar muscles, using the following parameters: rectangular biphasic waves, pulse duration 150 msec and pause of 2 seconds, applied every day for 30 minutes. Two months after the trauma, the needle EMG exam of the ABP muscle showed low level of spontaneous activity (fibrillations) and no voluntary recruitment of motor units (fasciculation). The nerve conduction studies showed no sensitive response of the median nerve with orthodromic stimulation of the first and third fingers.
After additional 4 months, the EMG showed minor modifications of the spontaneous activity, further increase of subjective sensitivity of the second and third fingers of the hand, but absence of volitional activity of the tenar muscles. On the other hand, under dynamic echomyography, all the tenar muscles responded with clear contractions when directly stimulated at 10 mAmps, a threshold far from pain sensations. It is worth mentioning that electrical stimulation achieved the goal of maintaining the left denervated tenar muscles (14 mm) almost at the thickness of the contralateral innervated left hand (16 mm) and without major worsening during the period from 2 to 5 post-denervation months.
Taken together the results are evidence that combining fibrillation analyses to monitor the denervation phase and dynamic echomyography to look for fasciculation and follow rate of atrophy and effectiveness of the electrostimulation therapy, the last can be finely tuned to patients' needs.[1-3]

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Dynamic Echomyography combined with EMG analyses of fibrillation/fasciculation increase sensitivity and specificity in the follow-up of muscle denervation atrophy and neuromodulation of denervation/reinnervation

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From 2008, in the Rise2-Italy project we have proposed and implemented for patients suffering with permanent or transitory Lower Motor Neuron denervation a new protocol of quantitative ultrasonography (we named it Dynamic Echomyography to stress the functional components of the analyses) to evaluate changes in Tibialis Anterior, Deltoid, and Quadriceps muscles undergoing home-based electric stimulation, both neuromuscular stimulation or direct surface stimulation of denervated/reinnervating muscles. Before and during h-b FES training for periods lasting one year in the case of a subject with lesion of the right sciatic nerve, 6 months for a subject suffering left brachial palsy and 8 months for a Spinal Cord Injury ASIA A subject suffering incomplete Conus Cauda Syndrome, we analyzed the denervated/reinnervating muscles comparing, when possible, them to the contralateral normal muscles using ultrasound to record information on: 1. Gross morphology and sonographic structure; 2. Thickness; 3. Dynamic properties of voluntary and electrical stimulation-induced contraction-relaxation cycles. 4. Short-term and long-term modifications of the arterial perfusion in response to volitional and electrical stimulation-induced contractions. Morphology and ultrasonographic structure of the denervated muscles changed during the period of h-b FES training from a pattern typical of muscle atrophy to a pattern which might be considered "normal" when detected in an old patient. Thickness improved significantly more in the middle than in the proximal and distal thirds of the denervated muscles, reaching in one year measurements in the first subject approximately the same thickness of the contra lateral innervated muscle. In all the measurements, arterial perfusion of the denervated muscles showed a low resistance pattern with Doppler ultrasonography at rest, and a pulsed pattern after several months of home based electrical stimulation, more similar to the trifasic high-resistance pattern of the innervated muscles. Contraction-relaxation kinetic, measured by recording the movements during electrical stimulation, showed an abnormal behavior in the denervated muscles, in particular during relaxation, which resulted significantly longer than in the normal muscle, in agreements with many experimental animal models and clinical human observations. The very high current energy needed to activate the denervated muscles according to the Vienna h-b FES strategy are strong evidence that the explored muscles were still denervated. If they recovered volitional function, the energy of muscle activation went down to the values of motor point stimulation of innervated/reinnervated muscles.
All together, these observations confirms both usefulness of Dynamic Echomyography in the follow-up and the effectiveness of h-b FES of denervated and reinnervating muscles. In the latter case, Dynamic echomyography provide information on the muscles or muscle parts that respond to stimulation.We are confident that combining sound invasive "time zero" and "end-point" analyses (tissue biopsy and 2D and 3D Color CT macro-morphometry with noninvasive, bed-side repeatable EMG (including fibrillation/fasciculation recording) and ultrasound analyses,[1-4] some of the following open questions will find final answer: 1. Reliable evaluation of the extent of muscle denervation and reinnervation; 2. Reliable quantification of the progression of atrophy to degeneration in longstanding muscle denervation; 3. Influence (positive or negative) of electrical stimulation on muscle reinnervation (in well defined sub-groups of patients), contributing to the much-needed evidence-based approaches in Physical Medicine and Rehabilitation.

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Myofiber-motoneuron interactions in ALS

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One of the crucial systems severely affected in several neuromuscular diseases is the loss of effective connection between muscle and nerve, leading to a pathological non-communication between the two tissues. One of the best examples of impaired interplay between the two tissues is the disease Amyotrophic Lateral Sclerosis (ALS).
Whereas the steps leading to the pathological state are well defined, several fundamental issues are still controversial: are the motor neurons the first direct targets of ALS? What is the contribution of muscle, if any, to the pathogenesis of ALS?
These questions raised from the following considerations: i) ALS is a genetic disease in which the contribution of different cells and tissues - either neuronal or non-neuronal - cannot been excluded; ii) skeletal muscle is a relatively unexamined tissue that potentially contributes to ALS; and iii) the retrograde-talk muscle-to-nerve is extremely important to determine if and to what degree muscle plays a role in the progression of the pathology and to develop alternative therapeutic approaches. We accumulated evidences demonstrating that muscle selective expression of SOD1 mutation caused pathological alterations in skeletal muscle, including mitochondrial impairment and alteration in the functional interplay between muscle and nerve, inducing pre-symptomatic sign of ALS. The pathogenic mechanisms associated with ALS will be discussed.

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SAVe-ALS - Ventilation by total implantable options recruiting denervated myofibers

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Research on etio-pathogenesis of SLA, on supports for survival of motoneurons, axonal regeneration and sproutings, and on transfer of animal results to patients' treatments are the main goals in ALS, but clinically relevant results are waiting for new concepts and discoveries to substantially influence the inexorable progression of the disease. Riluzole is the only drug that has been shown to extend survival. Non-invasive ventilation prolongs survival and improves quality of life. The management of ALS is thus, supportive, palliative, and multidisciplinary.[1]
Awaiting the most wanted final solution, we will test in rodent models of ALS a different approach, namely: support of trophism of the denervated muscle fibers and evaluation of their potential contribution to power output (and therefore mobility) by implanted neurostimulators with electrodes contacting the denervated muscle fibers, firstly in leg muscle and finally in the diaphragm to support ventilation of deteriorating animals. Based on the strong evidence of our EU Project RISE,[2,3] showing recovery of ultrastructural organization, tetanic contractility and power output of degenerating myofibers in permanently denervated human muscles, we would like to transfer to ALS patients those results to support by direct stimulation of the denervated muscle fibers ventilation in patients,[4] in collaboration with Antonio Musarò of Rome Sapienza University, Helmut Kern of the LBI, Wilhelminenspital, Wien, Austria, Winfried Mayr of the Medical University of Vienna, Austria and Jonathan C. Jarvis of Liverpool John Moores University, UK, Tessa Gordon of the Child Hospital of Toronto, Canada. In the first year of a 5-year planned research we would like to test these concepst in ALS rodents comparing neuromodulation and direct stimulation of denervated muscle fibers of unilaterally sciatectomized hind limbs in wild and SOD mice. I.R.C.S.S.

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of San Camillo Hospital of Venezia-Lido Italy will contribute with morphometry of immuno-fluorescence labelled experimental muscles before and during the follow-up. If the results will be, as we expect, positive, in the next few years we will test combined molecular and cellular approaches to support muscle fibers and the nerve-muscle units in larger animals and, then, in patients, hoping to substantially delay tracheostomy and the need for positive pressure ventilation.[1]
The Italian, Vienna and the Liverpool John Moores University groups have a longstanding collaboration on functional reactivation and repair of denervated muscles.[2]
Under expert supervision of Antonio Musarò,[5] mutated mice will be divided in two groups: the control-untreated mice and treated mice (i.e., with or without neuromodulation). Morphological, morphometric, molecular and functional analysis will be performed at different ages both at the onset of the disease and at paralysis stage both in Venice-Lido and Rome. We will evaluate whether the progressive training developed in Vienna and the devices developed in Vienna and Liverpool, adapted to ALS needs from existing prototypes will delay the progression of the disease. The denervated muscle will be chronically stimulated to sustain trophism and contractility of permanently denervated muscle fibers, both prexisting or regenerated,[6-9] in particular in the diaphragm to support ventilation.
Novelty and Relevance for patients: During the last decade our international group contributed to rehabilitation in neuromuscular disorders by studying exercise training for patients affected with traumatic complete injury of the *Conus Cauda*, a syndrom that fully disconnect leg muscles from the spinal cord.[2,3] A novel strategy was developed in Vienna. New electrodes and a safe stimulator for home based FES have been designed to reverse muscle atrophy by delivering high-intensity and long-duration impulses able to elicit contractions of denervated skeletal muscle fibres in the absence of nerves. Specific clinical assessments and training programmes were developed at the Wilhelminenspital Wien, Austria. Total implantable neurostimulator for rabbit and rat experiments were developed in collaboration with the Vienna Bioengineers in the Jonathan C. Jarvis Lab in Liverpool. These are now down-sized to mini-implants for mouse experiments. The EU RISE longitudinal human trial demonstrated that home-based FES of permanent denervated muscle is an effective therapy that results in rescue of muscle perfusion, mass and function.[2,3]
Our multidisciplinary group, supported by the European Project RISE: Use of electrical stimulation to restore standing in paraplegics with long-term denervated degenerated muscles (Contract no. QLG5-CT-2001-02191)] is the only consortium in the world with the required "proven" expertise[2,3] to design, implement and verify the hypothesis that permanent denervated skeletal muscle fibers may be recovered from severe atrophy and maintained in a trophic state that allows recovery of tetanic contractility and thus of external work to support long term ventilation in ALS sufferers, before the critical phase of tracheostomy and positive pressure ventilation becomes mandatory.[1,4]
Combined with commercial devices for activation of the residual pool of phrenic axons, the long term goal of the actual project may substantially prolong both a decent quality of life and survival. The Relevance of the proposed preliminary animal studies here described for ALS patients is self-evident.

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Excitation-Contraction Coupling is affected in Ankyrin 1.5 Null Mice

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Excitation-contraction coupling (ECC) is the process whereby depolarization of the T-tubular membrane (TT) triggers the Ca^{2+} release from the sarcoplasmic reticulum (SR) into the sarcomeric space that leads to muscle contraction. The first step in ECC is a voltage-sensing process that is the change of orientation of charged molecules within the dihydropyridine receptors/L-type Ca^{2+} channels (L-CaC) of the TT. This is detected as intramembrane charge movement (ICM) and in normally polarized skeletal muscle fibres of frog as well as mammal it shows three components (Qβ, Qγ and Qh). Qγ is the charge related to L-Ca^{2+} channel and RyR opening.[1] Therefore, DHPR is thought to have two actions: it is a voltage-operated L-CaC and a voltage sensor for RyR opening. Notably, the L-CaC current (ICa,L) activation and inactivation are multistep mechanisms with slow and fast kinetics.[2] Remarkably, the skeletal muscle

machinery involves a reciprocal interaction between sarcoplasmic and ECC proteins mediated by cytoskeleton.[3] Moreover, sarcomeric proteins are bound, by intermediate filaments (as desmin), to costameric proteins. Ankyrins are anchoring proteins localized in the Dystrophin Glycoprotein Complex at the costameric region. Thus, Ankyrins may play a role in ECC. The aim of this work was to evaluate the involvement of ankyrin 1.5 in the ECC in extensor digitorum longus, EDL, and diaphragm, DIA, muscle by using Ank 1.5 null mice. Membrane potential was recorded in current-clamp condition by a microelectrode inserted into a single muscle fibre, whereas the passive properties of the fibres and both ICM and ICa,L were evaluated in voltage-clamp condition in isolated fibre segments using the double Vaseline-gap method.[1] No significant changes of the above parameters were observed in EDL and DIA fibres of young Ank 1.5 null mice but these were reduced in DIA muscle from old mice. So, DIA from Ank 1.5 null fibres had affected ECC since showed a reduced Qγ charge paralleled by a decrease in size of ICa,L and a faster inactivation kinetics. In conclusion, Ankyrin 1.5 inhibitory action on ECC of DIA is muscle- and age-specific since it was observed only in DIA muscle from old mice. Interestingly, the results observed in DIA muscle from Ankyrin 1.5 null mice are observed in atrophic muscles with altered cytoskeleton as in old, dystrophic and denervated muscles.

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The role of myopalladin and associated myopathies in mammalian skeletal muscle

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The half-sarcomere, the functional unit of skeletal muscle, is able to produce power at high efficiency or resist a sudden increase in load with low metabolic cost, due to the integrated function of myosin II motors cross-bridging the thick and the thin filament and cytoskeleton proteins acting as a scaffold. While the relation between structure and function of the actomyosin motor has been extensively studied both in vitro and in situ,[1-3] much less is known about the other sarcomeric proteins. The importance of cytoskeleton proteins is illustrated by the identification of mutations in many of the corresponding human genes in patients with skeletal myopathies. Here we report a study on the role of myopalladin (MYPN), a protein located in the Z-line and the I-band. MYPN gene mutations have been identified in patients with limb-girdle dystrophy as well as dilated, hypertrophic and restrictive cardiomyopathy.[4,5] To provide insights into the physiological role of this protein and the mechanisms leading to myopathy, the mechanical performances of skeletal muscles and/or single fibres from wt and knockout (KO) mice were determined. In EDL muscle, in which the motor is mostly the 2B isoform, the absence of MYPN (i) decreases the isometric force (T0) by 48% and the cross-sectional area (CSA), calculated by the muscle wet weight, by 21%; (ii) decreases the shortening velocity and thus the power at any load < T0 and (iii) does not affect the curvature of the force-velocity relation. In skinned fibers from the same muscles T0 is reduced in proportion to CSA, indicating that the CSA of EDL muscle is overestimated in KO mice with respect to control.[4] Thus the reduced muscle performance in KO mice is due to the reduction in fibre dimension while the kinetics of actin-myosin interaction is unaffected. Injection of an adeno-associated virus (AAV) vector expressing the wt form of MYPN results in a substantial rescue of muscle performance.
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Ventricular torsion and cardiac suction effect. The electrophysiological analysis of the cardiac band muscle

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The Torre: nt Guasp concept postulates that the ventricles are formed by a continuous muscle band that begins at the level of the pulmonary valve and extends to the aortic root, limiting in this way the two ventricular chambers. This specific anatomical arrangement would support the interpretation of two fundamental aspects of left ventricular

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dynamics: 1) the torsion mechanism and 2) the physiology of rapid diastolic filling by the suction effect.

To investigate the electrophysiological basis of this mechanism, the left ventricular activation sequence was studied by 3D electroanatomical mapping (EAM) in five patients, during radiofrequency ablation of arrhythmias associated to probable abnormal epicardial pathways. As the descending band segment is endocardial and the ascending band segment is epicardial, two approaches were used to perform the mapping.

Conclusions: 1) 3D endo-epicardial mapping shows electrical activation of the apical loop concurrent with synchronic contraction of the ascending and descending band segments; 2) The simultaneous and opposing activation of the ascending band segment to the starting point of its radial activation from the descending band segment, at the point where both band segments cross, is consistent with the clockwise and anticlockwise ventricular torsion of apical and basal areas; 3) Late activation of the ascending band segment, compatible with persistent contraction of the ascending band segment during early isovolumic diastole (basis of the suction mechanism) is produced without need to postulate electrical activation beyond the QRS.

The novel activation sequence of the Torrent Guasp band found in this study would explain the previous process triggering the ventricular torsion and suction mechanism. Moreover, this work demonstrates that activation of the ascending band segment completes the QRS. This finding explains the persistent contraction of this muscle segment during early diastole, rejecting the traditional concept of passive relaxation.

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