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ROLES OF NEBULIN AND ITS INTERACTING PARTNER MYOPALLADIN IN SKELETAL MUSCLE

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The giant sarcomeric protein nebulin spans the entire length of thin filaments in skeletal muscles and its critical role in skeletal muscle is demonstrated by mutations in human nebulin, which cause nemaline myopathy. We recently generated nebulin deficient (nebulin-/-) mice, which died within 10 days after birth due to reduced milk intake and muscle weakness. Our analysis of these mice revealed that nebulin is dispensable for myofibrillogenesis but plays a critical role in thin filament length regulation and in maintaining sarcomeric integrity during muscle contraction. To further study nebulin's role in skeletal muscle, we performed single fiber mechanical studies on 1-day-old mice, which revealed an important role of nebulin in modulating the actin-myosin interaction. After birth, nebulin-/- mice develop progressive Z-line abnormalities and the presence of structures resembling nemaline rod bodies observed in nemaline myopathy patients, suggesting an important role of nebulin in the Z-line. A SH3 domain within nebulin's C-terminal Z-line region has been suggested to be involved in signaling and binds to the striated muscle specific protein myopalladin, which in turn binds to α-actinin. In addition, myopalladin is also present in the nucleus and the I-band. To study the functional role of myopalladin in vivo, we have generated myopalladin knockout (myopalladin-/-) mice. The mice are viable but our preliminary studies reveal reduced stress generation and a significant decrease in skeletal muscle fiber size in myopalladin-/- mice compared to wildtype. In addition, myopalladin-/- mice develop progressively wider Z-lines starting from 8 months of age. These data suggest an important role of myopalladin in skeletal muscle.

EFFECT OF ORAL CREATINE SUPPLEMENTATION ON NEUROMUSCULAR FUNCTION

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Despite oral creatine supplementation has been proven to be effective in enhancing exercise performance, its effect on neuromuscular function is still uncertain. The present study aimed at verifying whether short-term creatine (Cr) supplementation would improve muscle contractile properties (as assessed by evoked and voluntary contractions), the force-velocity relationship and muscle fatigue during repeated bouts of exercise. 16 moderately active men (25±3 years) were assigned to a creatine (CRE) or placebo (P) group using a double-blind random design. Subjects assumed either 5g Cr + 15g maltodextrin (CRE) or 20g maltodextrin (P) 4 times a day for 5 days. Before and after supplementation, isometric maximal voluntary contraction (MVC), maximal twitch, force-velocity relationship and repeated dynamic fatiguing contractions were assessed in the elbow flexor were evaluated and mechanical and electromyographic (EMG) parameters recorded and analysed. Mean fibres conduction velocity (CV) was estimated from adjacent EMG signals and used as a parameter of interest. Peak torque of maximal twitch was 33.46% higher and time to reach the peak torque was 61.29% lower in CRE than P ($P<0.05$). Torque-angular velocity curve was improved after Cr supplementation. Mean fibres CV was on average 8.9% higher in CRE at all angular velocities after supplementation ($P<0.05$). Creatine supplementation did not affect EMG and mechanical parameters during the repeated exercise fatiguing protocol. Oral creatine supplementation enhances intrinsic and voluntary contractile capacity of skeletal muscle of young men. This could be related to an increased Ca2+ sensitivity and maximum Ca2+-activated force, which are associated to an increase in cellular water content needed to maintain osmolality.

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A MECHANICAL STUDY ON SINGLE INTACT MOUSE MUSCLE FIBRES

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The present knowledge of skeletal muscle contraction were derived mostly from mechanical and ultrastructural studies on single intact frog fibres. Similar studies on intact mammalian muscle have been hampered by the difficulties in isolating single fibres and thus, there is still a lack of information on this experimental model. The present work has been performed on single intact mouse (Swiss strain) fibres using techniques previously employed in studies on frog muscle. Single fibres, 25-40 μm wide and 0.5-0.8 mm long, were isolated from flexor digitorum brevis muscle in Tyrode

solution. Interest has been focussed on the force responses to fast ramp stretches applied during twitch and tetanic contractions. As in our previous data on frog fibres (Bagni et al., J Physiol, 2005), the results showed that during fast stretching tension rises steeply to a peak (critical tension, Pc) and then falls, before the end of the stretch, to a much lower level because of forced crossbridges detachment. During the tetanus rise Pc was directly proportional to tension developed, providing a valid method to estimate the number of attached crossbridges. At the end of the force transient produced by fast stretches, the force settled to a steady level exceeding the isometric level preceding the stretch. This excess of tension, referred to as "static tension" (Bagni et al., Biophys J, 2002), is due to the elongation of some unknown elastic sarcomere structure, outside the crossbridges. We speculated that titin could be one of these structure. Planned experiments on transgenic mice will offer the possibility to test this speculation.

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SKELETAL MUSCLE DAMAGE, REGENERATION AND INFLAMMATORY RESPONSES IN CACHEXIA

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Cachexia is a syndrome, associated to most chronic diseases, characterized by skeletal muscle wasting due to elevated pro-inflammatory cytokines such as IL-1ß, IL-6, TNF-α and increased proteasome-mediated proteolysis.

We hypothesized that skeletal muscle is particularly susceptible to damage in cachexia. As a model of cancer associated cachexia, we analyzed colon carcinoma-C26 bearing mice in the absence or presence of free exercise in wheel-equipped cages. Cachectic mice run significantly less than control mice. In EDL we observed that the combined effect of tumor and exercise results in a force reduction during a single maximal contraction. Both exercise and cachexia induced a significant increase of serum CK level. The combination of tumor load and free exercise increased the number of damaged fibers (i.e. EBD positive) in Tibialis anterior to about four times over the control, resting mice. Muscle repair, estimated by histological analysis of the number of fibers with central nuclei, exhibited no significant variations among treatments. This phenomenon occurs in spite of an observed increase in hematopoietic stem cells in the musculature of cachectic mice. Accordingly, cytokines reduced regeneration extent following experimental induction of a focal damage. In spite of the pro-inflammatory environment due to muscle damage and elevated cytokine levels, cachectic muscle were not enriched in neutrophils, macrophages nor lymphocytes. Overall this suggests that inflammatory cells do not contribute to muscle damage and that cachexia is a state of immunodepression. In conclusion, increased muscle damage associated to impaired muscle repair could contribute to muscle wasting in cachexia. Muscle damage might not be mediated by inflammatory cells, while an impaired contribution of precursor cells to muscle regeneration could account for the muscle repair deficit observed in cachexia.

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THE ENDLESS DIVING PROJECT: A CASE REPORT FROM WATER ENVIRONMENT TO DEEP SPACE

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The Endless Diving project was generated from the union between Sports and Science, between athletic gesture and scientific research, between sea and space; a multidiscipline approach to the study of the human factors during exposure to extraordinary underwater environment and the research of proper materials; various methods were applied both to the physiological modification that man could face during extreme conditions, as well as studies concerning psychophysical endurance tests. An athlete performed a continuous underwater scuba diving for 32 hours with a simple semi-dry one piece wetsuit, and absolute standard fittings as per booties, gloves, fins. Thanks to his extraordinary control of his own body and, above all, thanks to his innate mental discipline, he succeeded in managing every single minute of his immersion: a total amount of 34,000 liters of air consumption. Time and procedures have been thoroughly monitored to allow that everything worked at peak performance and that the athlete never had to face an emergency situation. In spite of the extended time of continuous use, the diving apparatus never pointed out any kind of problem concerning the CO and CO2, while an outstanding score in ergonomics was attained on physiological variations in propioceptors, EKG parameters, the HR and body temperature thanks to two permanent probes that took both inguinal and retroauricular temperature every 20' during the entiredive. Neurocognitive test and blood samples were performed pre and post dive. The "mission" is to evict all those pieces of information that can increase the safety during the subaquatic general activities and facilitate a long underwater permanence for professional divers as well. A professional research that allows to study even preventive techniques for Decompression Sickness (DCS) or circadian rhythms alterations with other further clinical implications, as their usefulness in explaining the aetiogenesis of some physiopathological moments of one's daily life. Here we present preliminary data.

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PROTEOME ANALYSIS OF ALTERED PROTEIN EXPRESSION IN SOLEUS AND GASTROCNEMIUS MUSCLE OF HINDLIMB SUSPENDED MICE

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In order to understand the adaptations to disuse induced atrophy we performed protein pathway analysis in control mice and mice subjected to 14 days of Hindlimb Unloading (HU). We analyzed soleus muscle as an example of a muscle undergoing a high degree of HU-induced atrophy. A marked slow to fast shift of MHCs isoforms was found following HU. The proteome map of soleus muscles of control and HU mice was defined and a differential proteome analysis was performed. More than 800 protein spots on each gel were detected by fluorescent staining. Proteome analysis of soleus muscle evidenced that only a little percentage of detected spots were differentially expressed in HU mice in comparison with control animals. Most of the differentially expressed spots were implicated in oxidative stress (Hsp, SOD1, PRDX6, CAH III). Data showed an up-regulation of SOD1 and a down-regulation of other defence systems: Hsp, PRDX6, CAH III, suggesting the presence of oxidative stress in hindlimb suspended mice and a damage of defence system against oxidative stress. We also performed the proteomic analysis of gastrocnemius muscle. The latter muscle showed a lower degree of HU induced atrophy and few changes in protein pattern. SOD1, PRDX6, CAH III were up-regulated, whereas no changes were found in HSPs. These data target the oxidative stress as one of the main mechanisms underlining disuse induced atrophy.

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DETERMINATION OF CONTRACTILE PARAMETERS OF MUSCLE FIBRES FROM TWO SPECIES OF CARNIVORES: THE CAT AND THE DOG

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This study aimed to provide the first systematic comparison between the contractile properties of the fibres expressing common skeletal muscle myosin isoforms, slow and fast (2A, 2X) and the specialized fibres expressing M-MyHC (Myosin Heavy Chain) in two species of domestic carnivores, with great interest for biology as well as for veterinary medicine. The two species have a different modality of locomotion which reflects in different fibres metabolism; dog fibres are oxidative and likely fatigue resistant, whereas cat fibres are glycolytic and fatiguable; while the body mass of the adult cat is rather constant, a large diversity exists between dog breeds. In mammalian genome, the genes coding for various MyHC isoforms are grouped in clusters located in different chromosomes and form 3 subfamilies: a) subfamily of fast or type 2 isoforms (2A, 2X, 2B); b) subfamily of cardiac isoforms (beta/slow MyHC and alpha MyHC; c) the third subfamily is composed of only one isoform: the masticatory or M MyHC gene. Masticatory or M-MyHC is a isoform with expression restricted to muscles derived from the first branchial arch, in the first place, jaw closer muscles, with a clear inter-species variability. Only sparse information is available on the contractile properties of M fibres, i.e. muscle fibres expressing M-MyHC. In this study, in each muscle fibre isometric tension (Po) and unloaded shortening velocity (Vo) were determined during maximal calcium activation. MyHC isoforms expressed in the fibres used in mechanical experiments were classified on the basis of their migration rate in gel electrophoresis. The results obtained showed that in both species Vo increased regularly from slow to 2A to 2X fibres and that M fibres had Vo value similar to 2A fibres. M fibres showed Po values definitely greater than in any other fibre type both in cat and dog.

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ADP MODULATION OF ACTIN SLIDING VELOCITY ON SLOW AND FAST SKELETAL MYOSIN ISOFORMS AT DIFFERENT TEMPERATURES

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It is generally believed that the rate of ADP release from acto-myosin defines unloaded shortening velocity (Vo) of skeletal myosin. However, we have recently suggested that at least at low temperature (12°C) the rate of acto-myosin dissociation by ATP could play a significant role in defining Vo of fast, and not of slow skeletal myosins. Interestingly, the temperature dependence of ATP induced dissociation and of ADP affinity of acto-myosin for fast and slow isoforms suggested that, at temperatures above ~25°C, the ADP release can limit velocity of both isoforms. In this study we have used an in vitro motility assay (IVMA) approach and studied the effect of MgADP on the sliding velocity of actin (Vf) on slow and fast skeletal myosin isoforms from the rat in the absence and in the presence of 2mM MgADP. MgATP concentrations were varied in the range 0.01mM and 2mM, at 25 and 15°C and 50mM ionic strength. At 25°C the presence of MgADP decreased Vf of fast and slow myosin (Km of 73 and 32µmol/L respectively) and shifted the substrate concentration dependence toward higher MgATP concentrations (Ki of 215 µmol/L and 66 µmol/L respectively). At 15°C the effect of MgADP on fast isoforms yielded a Ki of 293µmol/L. The results suggests a more significantly role of ADP release in defining velocity in slow than fast myosin and, in fast

isoforms, at high than low temperature. The analysis on rat slow myosin at 15°C is ongoing.

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PLEGIC MUSCLES: PROGRESSION AND FES-REGRESSION OF MUSCLE ATROPHY/DEGENERATION

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Complete irreversible denervation of muscles results in a progressive loss of structural and functional properties of fiber, which finally ends in complete waste of muscle mass. In rodents repeated cycles of myofiber regeneration had been described during long-term muscle denervation and we recently shown that this also apply to human muscle in spinal cord injury (SCI). Enrolment in the EU Project RISE and follow-up of paraplegics with complete lower motor neuron lesion (CLMNL) were performed in Wilhelminenspital, Vienna, Austria, analyses of muscle biopsies in Padova, Italy. During the first two year post-SCI simple atrophy characterises the muscle biopsies. From three-year SCI the human denervated muscle presents severe atrophy, adipocytes and connective tissue (denervated degenerated muscle, DDM). Monoclonals against embryonic myosin show that regenerative events are present from 1- to 37-year post-spinal cord injury [1,2]. In 2-year FES-trained muscles larger than pre-FES round myofibers are present. They are accompanied by regenerative events, but at a lower rate than in DDM (myofiber per cryosection unit area: 0.8+/-1.3 in FES vs. 2.3+/-2.3 in DDM, mean+/- SD, p = 0,011). The average diameter went from 15.4 to 27.0, a 76% increase after two years of FES. All together, microscopic and ultrastructural analyses demonstrate that daily Vienna-FES of lower motor neuron denervated muscles in CLMNL paraplegics is safe and that the cosmetic and cushioning effects are granted in all persons, whatever the time from spinal Cord Injury (SCI) at the beginning of the treatment. Supported by EU Commission Shared Cost Project RISE (Contract n. QLG5-CT-2001-02191) and Italian Ministry of University (MIUR) PRIN Project Contract n. 2004061452-002.

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[2] Kern H, Hofer C, Modlin M, Mayr W, Vindigni V, Zampieri S, Boncompagni S, Protasi F, Carraro U. Stable muscle atrophy in long-term paraplegics with complete upper motor neuron lesion from 3- to 20-year SCI. Spinal Cord 2007 Oct 23.

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ACUTE EFFECTS OF PASSIVE STRETCHING ON A PREVIOUSLY FATIGUED SKELETAL MUSCLE

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Passive stretching (PS) has been shown to depress maximum muscle performance. However, few studies focused on the effects of PS on maximum force in a fatigued muscle. Thus, the aim of the present study was to evaluate the stretching-induced changes in the electrical and mechanical characteristics of a previously fatigued muscle during electrically evoked contractions. Eleven subjects (age: 21±2 yrs; body mass: 71±3 kg; stature: 176±3 cm; mean±s.e.m.) were tested twice. In both occasions, the calf muscles were fatigued with continuous electrical stimulation. During the first test, after fatigue the subjects underwent 3 tetanic electrical stimulations, before and after a bout of PS. On a different day, after fatigue the subjects were tested again before and after a resting period of the same duration as the PS protocol. During contractions, surface EMG, mechanomyogram (MMG) and force were recorded from the medial gastrocnemius muscle. From the 3 signals, the electro-mechanical delays were calculated (EMG-MMG, EMG-force and MMG-force, respectively). Fatigue induced a reduction in peak force in both testing sessions (-17.5±3.7 N and -18.0±3.4 N, respectively). Without PS, EMG, MMG and force parameters, together with electro-mechanical delays returned to their values before fatigue. With PS, however, EMG parameters returned to their values before fatigue, but MMG parameters remained depressed whilst peak force further decreased (-22±9%, $p<0.05$). Moreover, EMG-force and MMG-force delays were significantly lengthened by +10±3% and +12±4%, respectively. In conclusion, a bout of PS further decreases muscle maximum force generating capacity, affecting in particular the viscoelastic properties of the muscle–tendon unit. Thus, PS does not seem to be advisable as a method to recover maximum force after fatigue, especially when a following task is required.

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FOXO3 ACTIVITY IS MODULATED BY POST-TRANSLATIONAL MODIFICATIONS

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FoxO proteins are transcription factors that control cell cycle progression, DNA repair, muscle atrophy, stress resistance and apoptosis. These divergent functions are carefully regulated by post-translational modifications including phosphorylation, ubiquitination and acetylation. FoxO acetylation is mediated by acetyl-transferase CREB-binding protein (CBP)/p300 which are modulating FoxO transcription by interfering FoxO

binding to target DNA sequence. To verify the consequence of acetylation on FoxO3 transcriptional activity in adult skeletal muscle, we generated FoxO3 mutants in which the lysines residues of the DNA binding domain and of the nuclear localization sequence are replaced by arginine (KR) to block acetylation. In a second round of experiments we mutated the lysines into glutamine (KQ) to mimic a constitutive acetylation. We performed the luciferase assay using as readout of FoxO activity a FoxO-sensor constituted by six repeated FoxO binding elements driving a luciferase gene. Results confirm that acetylation inhibits FoxO transcription activity. Next we tested whether these mutants can affect a more complex condition like the atrophy program. Indeed acetylation reduced FoxO-dependent muscle atrophy by causing re-localization of FoxO proteins to the cytoplasm.

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ASSEMBLY AND DYNAMICS OF SARCOPLASMIC RETICULUM DOMAINS IN SKELETAL MUSCLE CELLS

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The sarcoplasmic reticulum (SR) is a network of tubules and cisternae surrounding the myofibrils that is organized in two functionally and morphologically distinct domains, the longitudinal/free SR and the junctional SR (jSR). The longitudinal/free SR, dedicated to Ca2+ uptake, contains SERCA pumps and additional proteins, detected mainly in correspondence of the M-band and Z-disk. The jSR contains the Ca2+ release complex, composed of the Ca2+ release channel RyR1 and associated proteins, connected with the T-tubule network to form the triadic structures at the level of the A-I junction. How these sub-domains assemble is largely unknown. To characterize this process, we generated GFP-fusion proteins of representative proteins of the longitudinal/ free (SERCA, ank1.5 and InsP3R) and of the jSR (RyR1, triadin, junctin and junctophilins) to analyze the assembly and dynamics of these proteins by means of FRAP technique in undifferentiated and differentiated myotubes as well as in NIH3T3 cells. The results revealed that all GFP-proteins were apparently freely moving in NIH3T3 cells and in undifferentiated myotubes. Localization of longitudinal/free SR proteins near the M-band and/or Z-disk was accompanied by limited or absent reduction of their dynamic properties. The jSR was observed to organize in two steps. In the first, jSR proteins were recruited into randomly disposed clusters. In the second, the clusters relocated to form double rows at the level of the A-I interface that resemble the mature localization of triadic structures. The mobility of most of the jSR proteins analyzed was drastically reduced already when they organized in clusters and did not change when the jSR complexes were organized at the A-I junctional level. These results indicate that the organization of longitudinal/free and jSR domains follows independent pathways, and provide evidence for mechanisms of protein-protein interaction in the organization of proteins at jSR domains.

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HEAT AND ANAESTHESIA CAUSE A LETHAL CRISIS (MALIGNANT HYPERTHERMIA?) IN CS1 NULL MICE

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Our recently published results in mice lacking skeletal calsequestrin (Paolini et al. 2007; J. Physiol. 583:767) indicates that, in fast twitch fibers, CS1 is important for SR structure, Ca2+ storage and possibly to facilitate Ca2+ release. Because mutations in CS2 result in the same phenotype as mutations of RyR2 in cardiac muscle (CPVT) it suggested that mutations in CS1 may result in a similar phenotype as mutations in RyR1 (MH). We tested in vivo the sensitivity of CS1-null mice to heat-stress and to exposure to halothane. Surprisingly, both treatments were lethal in the majority of CS1 null mice, whereas identical treatments were well-tolerated by WT mice. These crisis were remarkably similar to those described as fulminant malignant hyperthermia (MH) episodes (Chelu et al. 2006, Faseb J. 20:329; Yang et al. 2006, Anesthesiology 105:1164) in knock-in mice carrying RyR1-MH mutations. To determine if these crisis were associated with functional alteration of skeletal muscle similar to MH muscle, we performed in vitro studies of adult EDL muscles and FDB fibers over a temperature range of 25-45°C. Whereas in CS1-null specimens, there is a balance between Ca2+ release and uptake at low temperatures, at temperatures above 37° Ca2+ accumulates in the cytosol causing progressive contracture while in Wt the balance between uptake and release is maintained for a wider range. This suggests that mutations of CS1 could indeed cause a syndrome similar to MH. These results may advance our understanding of the molecular mechanisms leading to malignant hyperthermia (MH) in humans and possibly provide an alternative genetic locus for linkage studies.

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EXPRESSION AND ANALYSIS OF MICRORNAS IN SKELETAL MUSCLE TISSUE

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MicroRNAs are non coding small RNA acting as negative gene regulators. They are transcribed as long primary transcripts, processed in the nucleus and exported to the cytoplasm where they are further processed into mature 21-23

nucleotide transcripts. MicroRNA modulation of gene expression occurs through two main mechanisms: or by translation block of traslation or by cleavage and degradation of the target mRNA. Many microRNAs are expressed in a tissue-specific manner suggesting a potential role in the physiology of the skeletal muscle. To address this point we analyzed the expression of selected microRNAs in skeletal muscle and in C2C12 myoblasts and myotubes. To detect the expression of small microRNAs transcripts, total RNA samples were separated onto a small denaturing polyacrylamide gel, blotted onto nylon filters and probed with labeled oligonucleotide synthesized on the basis of the microRNAs sequences. Some of these microRNAs were differentially expressed in undifferentiated or in differentiated C2C12 suggesting a possible role in myogenesis. Furthermore it was found that, in vivo, some microRNAs have a different expression pattern in skeletal muscle obtained from mice of different ages. Since microRNAs inhibit the expression of target proteins, it is currently investigating the interaction between the selected microRNAs and the mRNAs putative targets. Computational programs are available to predict microRNA targets based on the examination of the mRNAs 3'UTR region to detect potential binding site for the different microRNAs isolated and characterized. For this reason the 3'UTR of the proteins that are potential targets of the microRNAs was subcloned into a luciferase reporter system and analysed by co-transfecting these constructs with the corresponding pre-microRNAs.

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EFFECTS OF AEROBIC EXERCISE ON NONALCOHOLIC FATTY LIVER DISEASE: A CASE REPORT

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Nonalcoholic steatosis (nonalcoholic fatty liver disease, NAFLD) is now considered a metabolic pathway to advanced liver disease, cirrhosis and hepatocellular carcinoma. No established treatment exists for this potentially serious disorder. Current management of NAFLD is largely conservative and includes diet regimen, aerobic exercise, and interventions towards the associated metabolic abnormalities. However, there is insufficient evidence to make prescriptive recommendations on how much exercise and on the types of dietary modifications that are optimal, and how much weight loss is necessary to reverse the disorder (1). The precise mechanisms by which physical exercise may reduce hepatic steatosis remains unknown and needs to be extensively explored, even if it was suggested that it would stimulate lipid oxidation and inhibit lipid synthesis in liver through the activation of the AMP-activated protein kinase pathway (2). The aim of this study was to investigate the longer term effect of weight loss and aerobic exercise on quality of life in patient with NAFLD. We present a case report of a 30-year-old man with abnormal results from liver function tests who has completed 12 weeks of diet and exercise intervention. The subject of this case report experienced a good outcome using a combination of aerobic exercise and diet such a therapy for NAFLD. In short, these findings demonstrate that maintenance of weight loss and exercise in patients with liver disease results in a sustained improvement in liver enzymes, and quality of life. Treatment of patients should form an important component of the management of those with chronic liver disease. There is a need for further work examining the potential of physical activity and/or weight control as a preventive and/or therapeutic option in the treatment of fatty liver diseases.

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LOCALIZED EXPRESSION OF A MUTANT SOD1^{G93A} GENE CAUSES MUSCLE ATROPHY AND INDUCES PRE-SYMPTOMATIC SIGNS OF ALS

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Amyotrophic lateral sclerosis (ALS) is a disorder involving the degeneration of motor neurons, muscle atrophy and paralysis. However the specific cellular origins of ALS have remained difficult to define. We recently generated a novel transgenic mouse model expressing a mutant form of the SOD1 gene (SOD1^{G93A}) under the transcriptional control of the Myosin Light Chain promoter (MLC-1/3); this new mouse model develops muscle atrophy, associated with a significant reduction in muscle strength, alterations in the contractile apparatus, and mitochondrial dysfunction. We investigated the molecular pathway underpinning the muscle atrophy and we observed the activation of FoxO and NFκB, the upregulation of several atrophy-related genes, accompanied by accumulation of ROS that serve as signalling molecules to initiate autophagy, one of the major intracellular degradation mechanisms that we demonstrate to be determinant for the induction of muscle atrophy associated with SOD1 mutation. Although muscle-specific expression of SOD1^{G93A} transgene does not affect motor neuron survival, we observed in the spinal cord of the transgenic mice elevated levels of several markers associated with pre-symptomatic signs of ALS, such as microglial cell activation, protein nitration, and pro-inflammatory cytokines. Our data demonstrate that skeletal muscle is a primary target

of $SOD1^{G93A}$ -mediated toxicity and disclose the role of oxidative stress in the induction of muscle atrophy, underscoring the contribution of skeletal muscle to the pathogenesis of ALS.

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ENERGETICS OF KATA AND KUMITE: COMPARATIVE ASPECTS

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During training and competition Karate athletes use almost all muscles, but exists a significant difference in terms of style and technical content between the two different disciplines. Kumite is a non-contact fighting, while Kata consists in a predetermined series of movements performed with explosive swiftness against an opponent. The aim of our study was to compare the energy cost, aerobic and anaerobic, of these karate disciplines. On 12 (6 Kata and 6 Kumite) top level athletes (M = 6; F = 6) internationally ranked we determined: (i) the peak oxygen consumption (VO2 Peak) during conventional graded cycle ergometer test; (ii) the maximal anaerobic power (Wmax) by Bosco test and (iii) the maximal lactacid power by Wingate test. Moreover, the oxygen consumption (VO2k) was also measured by a portable breath-by-breath telemetric system during Kata and Kumite simulated performances. VO2 Peak both in male and female athletes was significantly higher in Kumite than in Kata athletes during simulated competition. On the countary, Wmax (squat jump and counter movement jump) and the maximal production of LA (Wingate test) were not significantly different between the two groups. At the end of the simulated performances the lactate production was found in Kumite athletes significantly higher than in Kata, both in males and females. In conclusion, it appears that Kumite and Kata athletes utilised at least in part, different mechanism of energy production, with a prevalence in the Kumite's athletes of aerobic metabolism.

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GLOBULAR ADIPONECTIN IS INVOLVED IN SKELETAL MUSCLE REGENERATION

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Adiponectin is an adipokine with antidiabetic and antiatherogenic action. The secreted hormone (named "full-length") is composed of a globular part and a C-ter tail, cleaved in the " globular" form (gAd). The hormone circulates in the plasma in associated structures (trimers, hexamers and high molecular weight forms) whose biological activities are poorly understood. A decrease of plasma adiponectin content correlates with obesity, diabetes and insulin resistance, conditions that can be reversed in mice by the treatment with exogenous adiponectin. The hormone exerts its antidiabetic action activating glycogen synthesis and glucose oxidation in the liver and glucose up-take and fatty acids oxidation in skeletal muscle. We herein show that chronic treatment of murine myoblasts with gAd induces myotubes formation and the expression of muscle typical lineage markers, through the activation of Akt, p38 and AMP-kinase (AMPK) pathways. In agreement with the signal transduction of the hormone in liver cells, the stimulation of gAd in differentiated myotubes produces a transitory burst of reactive oxygen species (ROS), which play a key role for the metabolic effects of the hormone. The elimination of the ROS through antioxidant treatments leads to a decreased activation of Akt, p38 and AMPK and greatly affects glucose-uptake. Finally, we observed that gAd is a proliferating agent for muscle stem cells. In particular, we show that gAd induces cell proliferation and chemotaxis in different stem cells populations, including reserve cells (a stem cells population of C2C12 murine myoblasts) and endothelial precursors. Our data propose a new function for gAd in skeletal muscle. Beside the well-known metabolic effect of gAd, we demonstrated that gAd is able to induce myogenesis and the proliferation of quiescent muscle stem cells suggesting an important role of the hormone for muscle regeneration after trauma.

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IMPROVEMENT OF STANCE AND MUSCLE PERFORMANCE BY MUSCLE VIBRATION IN ELDERLY WOMEN

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The effects of Quadriceps muscle mechanical vibration on body balance, leg extensor muscle power and vertical jump were studied in elderly women (age 65.2 years old + 6.9). Vibration was applied over 3 consecutive days, three times a day and each application lasted ten minutes. The subjects were separated into three subgroups: 1) a group in which vibration was applied on an isometrically contracted Quadriceps (VC), 2) a group in which vibration was applied on a relaxed Quadriceps (VNC), 3) a control group in which non vibration was applied (NV). The body balance was studied measuring displacement of the center of pressure in terms of length (mm), mean speed (mm/sec) and ellipse area (mm2). The muscle power was analysed using standardised vertical jump

test. The measurements were performed before and 1, 30 and 90 days after the stimulation. The VC group showed a significant remarkable improvement of all the tested parameters, as soon as 1 day after treatment, and lasting at least up to 90 days without decreasing. The other groups (VNC and NV) did not show any significant change. We thus concluded that this simple treatment can induce long-lasting improvement of muscle performance and balance in elderly women.

Brunetti O, Filippi GM, Liti A, Panichi R, Roscini M, Pettorossi VE, et al. Improvement of posture stability by vibratory stimulation following anterior cruciate ligament (ACL) reconstruction. Knee Surg Sports Traumatol Arthrosc 2006; 14:1180-1187.

Fattorini L, Ferraresi A, Rodio A, Azzena GB, Filippi GM. Motor performance changes induced by muscle vibration. Eur J Appl Physiol 2006; 98:79-87.

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THE MOLECULAR MECHANISM OF THE RESPONSE OF SKELETAL MUSCLE TO STRETCH

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The skeletal muscle not only works as a machine that provides body movement using ATP as energy source, but can also acts as a brake generating a high resistive force at low energetic cost when the load is suddenly increased above the isometric force (Katz, J. Physiol. 96:45,1939). This condition is experienced by leg extensor muscle when it has to decelerate the body at the end of a jump. The force enhancement induced by steady lengthening of active muscle is accompanied by increase in the stiffness of the half-sarcomere and by changes in the intensity of the 14.5 nm X-ray reflection that suggest recruitment of additional motors (Linari et al. J. Physiol. 526:589, 2000). The mechanism of stretch dependent recruitment and its kinetics were investigated in these experiments by using fast half-sarcomere level mechanics in intact fibres from skeletal muscle of the frog (Rana esculenta) to measure the changes in the half-sarcomere stiffness after step stretches of different amplitude (2-6 nm • hs-1). The results were interpreted with a mechanical model of the half-sarcomere where the contribution of the filament compliance, defined by X-ray diffraction, is taken into account. We show that additional motors are recruited already at the end of a 100μs conditioning stretch. The recruitment continues to increase during the 2 ms after the stretch. However, when plotted versus the axial distortion produced by stretch (Δz), the recruitment of new motors is the same for the same Δz, independently of the time elapsed after the stretch. Thus the distortion of the attached motors is the only factor that drives the recruitment. This mechanism allows the muscle to resist a stretch redistributing the force excess amongst the motors, so that the force imposed on each motor is the same as that generated during the isometric contraction.

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C-FLIP OVER-EXPRESSION REDUCES CARDIAC HYPERTROPHY IN RESPONSE TO PRESSURE OVERLOAD

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Activation of Fas signaling has been associated with the development of cardiomyocyte hypertrophy. In the present study we investigated the effects of increased expression of c-Flip, a natural modulator of Fas receptor signaling, in a mouse model of cardiac growth response to pressure overload. A transgenic mouse over-expressing c-Flip in the heart was generated in FVB/N strain. Echocardiographic, hemodynamic, histological and molecular analyses were carried out under basal conditions and after transverse aortic constriction (TAC)-induced pressure overload. Over-expression of c-Flip in ventricular hearth tissue was functionally silent under basal conditions affecting neither cardiac morphology nor basal cardiac function. Transgenic mice were then subjected to pressure overload by TAC procedure. Under such conditions c-Flip transgenic mice showed normal Left Ventricle (LV) function with a significantly reduced LV hypertrophy compared to wild-type mice and reduced induction of the cardiac "fetal" gene program. Further, analysis of intra-cellular signaling pathways indicated that c-Flip over-expression reduced phosphorylation of both the glycogen synthase kinase 3β (GSK3β) and Akt as compared to controls. Finally the reduction of the TAC-induced hypertrophy was not accompanied by significant apoptosis increase. Altogether, these findings indicate c-Flip as a key regulator of the cardiac response to ventricular pressure overload.

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A MULTI-ELECTRODE ARRAY APPROACH TO MUSCLE FIBERS CONDUCTION VELOCITY ESTIMATION DURING DYNAMIC EXERCISES

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The possibility of investigating motor control strategy from myoelectric signal has been variously addressed using needle or surface EMG (sEMG) with controversial results. Nevertheless, sEMG time-domain or frequency domain parameters provided some reliable informations about the phisical phenomena underlying sEMG signal generation. In general, it has been accepted that: 1. Amplitude content of sEMG reflects the level of muscle activation; 2. Spectral content of the myolectric signal provides indirect information related to the quality of active MU. However, sEMG spectral parameters are strongly influenced by a number of factors thus their reliability is largely questioned. On the other hand, muscle fibre conduction velocity (CV) is an important physiological parameter because it allows the non invasive assessment of motor units recruitment-derecruitment [1] and thus the modification of the peripheral properties of the neuromuscular system as a consequence of exercise. Estimation of CV from sEMG recording is a complex task and asks for a complex experimental set-up which uses multi-electrode array configurations. In this paper, we present CV estimation methods used in our laboratory and a set of the different results we obtained.

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INTERACTION BETWEEN JUNCTOPHILIN 1 AND DIHYDROPYRIDINE RECEPTOR

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Junctophilins (JPs) are a class of highly conserved proteins localized in junctional membrane complexes, formed between endo/sarcoplasmic reticulum (ER/SR) membrane and plasma-membrane (PM). In mammals, four JPs subtypes have been identified with a tissue-specific expression: JP1 is specifically expressed in skeletal muscle, JP2 in skeletal muscle and heart, JP3 and JP4 in the brain. In skeletal muscle, JP1 anchors the SR to the PM contributing to the formation and stabilization of the junctional membrane complexes, named triads, formed by the association of two terminal cisternae and one T-tubule. Triads are the site of excitation-contraction (E-C) coupling. At triads, the voltage gated Ca2+ channel dihydropyridine receptor (DHPR) directly induces the opening of ryanodine receptor (RyR) by a conformational interaction, resulting in Ca2+ release and in muscle contraction. It is known that, at triads, RyR1 interacts with many proteins like DHPR, triadin, junctin and calsequestrin. A recent study has shown that JP1 interacts with RyR1 and partecipates in regulating Ca2+ release from SR, but no interaction between JP1 and DHPR has been shown. We have observed that JP1 co-immuno-precipitates with RyR and DHPR. A direct interaction between JP1 and DHPR was also confirmed by GST pull down assay in skeletal muscle and in HEK 293 cells.

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KNEE FLEXORS-EXTENSORS COACTIVATION DURING VERTICAL JUMPS IS REDUCED IN VOLLEYBALL PLAYERS

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The aim of the present work was to verify the hypothesis that in skilled volleyball players the neuromuscular control around the knee joint during vertical jumps (CMJ) is more efficient than in non athletic subjects. Neuromuscular control was assessed in terms of agonist-antagonist coactivation, resistance to fatigue, mechanical power. Five male volleyball players and five male young healthy subjects matched for age, weight, height, BMI, were recruited for this study. The following tests were performed in random order: 5 single CMJ, 5 single squat jumps (SJ). At the end of single jumps test, subjects performed 30 repetitive CMJ (CMJ30). Surface EMG signals were recorded from Vastus Lateralis (VL) and Biceps Femoris (BF) muscles on both sides. Ground reaction forces and moments were measured with a force plate (Bertec, USA). Right knee angle was measured with an electro-goniometer (Satem, Roma). CMJ data provided better results than SJ in both groups and, as it was expected, volleyball athletes performed better than sedentary subjects in all tests. The CMJ30 test showed that athletes are much more resistant to fatigue than sedentary. Contrary to controls, athletes' sEMG shows a reduced coactivation of antagonist muscles (knee flexors) irrespective of the jump style. Present results seem to stand for a neural adaptations of the motor control scheme to training. Previous studies have suggested that muscle coactivation could be reduced by specific long-lasting training. This seems to be the case also for vertical jump training that seems to induce specific neural adaptations.

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NON STEROIDAL ANTI-INFLAMMATORY THERAPY IN DUCHENNE MUSCULAR DYSTROPHY

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The only beneficial pharmacological treatment in Duchenne muscular dystrophy (DMD) is the use of glucocorticoids but their mechanism of action is still unknown. These drugs exert anti-inflammatory effects but very few data are available to assess whether their anti-inflammatory activity is the explanation of their efficacy. The aim of this research is compare the effects of glucocorticoids and non steroidal anti-inflammatory drugs (NSAIDs) in mdx mice muscle, to evaluate if the control of inflammation could have a benefical effect on the pathology. Mice were daily treated with: methylprednisolone, aspirin and parecoxib (COX-2 selective inhibitor). Inflammation, necrosis and centronucleated fibers were evaluated in tibialis anterior muscle samples obtained from treated and untreated mdx mice (age: 30 days and 11 weeks). Inflammatory cells were conspicuous in mdx mice and extensive areas of infiltration were present. The administration of methylprednisolone and both NSAIDs reduced the inflammation area. All the treatments were also effective to reduce the necrotic area. The percentage of regenerating myofibers was not significantly different in untreated and treated mdx. Western blot analysis of tibialis anterior and diaphragms obtained from mdx mice was performed to evaluate myosin and collagen expression. Diaphragm strips were dissected to evaluate muscle mechanics and electrophysiology, maximun isometric tension, contraction kinetics and force developed with the fatigue protocol are not statistically different in muscle of treated and untreated mdx. These data suggest that chronic treatment with NSAIDs has a potentially beneficial effect on skeletal muscle morphology of mdx mice comparable with the effects of treatment with the glucocorticoid and we are currently evaluating if the block of inflammation could modify dystrophy progression.

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THE MECHANISM OF FORCE ENHANCEMENT BY SLOW LENGTHENING IN ACTIVATED FROG MUSCLE FIBRES

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It is well known that slow stretching of activated skeletal muscles enhances the force well above the isometric value. It is not clear however if force enhancement is due to an increase of crossbridge strain or crossbridge number or both. The mechanism of force enhancement during lengthening was investigated on single frog (Rana esculenta) muscle fibres by using fast stretches to measure the rupture tension (critical tension, Pc) of the crossbridge ensemble. Fast stretches were applied at various tension levels during the tetanus rise. Sarcomere length was measured by a striation follower device. Experiments were performed at 5°C. If the rupture force of the individual crossbridge is constant, Pc is expected to be directly proportional to crossbridge number while sarcomere length at Pc (critical length, Lc) should vary with the individual crossbridge tension. Pc and Lc values were compared with those obtained when the stretch was applied during slow fibre lengthening. The data showed that Pc was proportional to the tension generated by the fibre both under isometric and slow lengthening conditions. However, for a given tension increase, Pc was 6.5 times greater during isometric than during lengthening conditions. Isometric critical length was 13.04 ± 0.17 nm per half-sarcomere (nm hs-1) independently of tension. During slow lengthening critical length fell as the force enhancement increased. For 90% enhancement, Lc reduced to 8.19 ± 0.039 nm hs-1. These data indicate that the increase of crossbridge number during lengthening accounts for only 15.4% of the total force enhancement. The remaining 84.6% is accounted for by the increased mean strain of the crossbridges.

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STRUCTURAL DYNAMICS OF SKELETAL MUSCLE FIBRES IN DIFFERENT PHYSIOLOGICAL STATES BY SECOND HARMONIC GENERATION

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The high degree of intrinsic structural order in skeletal muscle allows imaging of this tissue by Second Harmonic Generation (SHG). As previously shown by fractional extraction of proteins (Vanzi et al., J. Muscle Cell Res. Motil. 2006, 27: 469-79), myosin is the source of SHG signal. The characterization of the polarization-dependence of the SHG signal provides very selective information on the orientation of the emitting proteins and their dynamics during contraction. We developed a line scan polarization method in order to perform measurements of a full polarization curve in intact muscle fibres from frog skeletal muscle (Rana esculenta, 10°C). These measurements allow the characterization of the dependence of the SHG polarization on different physiological states (resting, rigor and isometric tetanic contraction) over a wide range of sarcomere lengths (between 2.0 μm and 4.0 μm). The polarization data have been interpreted by means of a model in terms of the average orientation of SHG emitters. The different physiological states are characterized by distinct patterns of SHG polarization and the variation of the orientation of emitting molecules in relation to the physiological state of the muscle demonstrates that one part of SHG signal arises from the globular head of the myosin motor that cross-links actin and myosin filaments. The dependence

of the SHG modulation on the degree of overlap between actin and myosin filaments during an isometric contraction provides the constraints to estimate the fraction of myosin motors generating the isometric force in the active muscle fibre.

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ABNORMAL MATURATION OF THE SARCOTUBULAR SYSTEM IN SKELETAL FIBERS LACKING CALSEQUESTRIN-1: THE FIRST STEP TOWARD A MYOPATHY?

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The excitation-contraction (EC) coupling apparatus, a specialized system of membranes that finely controls the release/uptake of Ca2+ from the sarcoplasmic reticulum (SR), has a central role in the correct execution of the differentiation and maturation programs of skeletal muscle fibers. The major SR Ca2+ binding protein calsequestrin (CS) is expressed in two isoforms in skeletal muscle. CS2, abundant in fetal and neonatal stages, is down-regulated in the first few weeks after birth in the same critical period in which CS1 is up-regulated, remaining the only isoforms expressed in fast-twitch fibers. Interestingly, in the same time frame profound structural and molecular modifications transform neonatal fibers into mature. In the present study we examined the post-natal maturation of the sarcotubular system in EDL fibers from mice lacking CS1. Western-blot analysis indicates that CS2 expression does decrease postnatally, just as in WT EDL, leaving the majority of fibers without any CS. Structural maturation of the EC coupling units in CS1-null mice is characterized by an incomplete reorganization of the of the T-tubule from longitudinal to transversally oriented, as it would normally occur in WT muscle. Also frequency of longitudinal and multi-layered junctions, typical of early developmental stages, is higher in CS1-null muscle. The junctional SR often bears multiple rows of orderly arranged RyRs. Interestingly, multiple rows of RyRs are found in pre-natal skeletal muscle and also in cardiac muscle, which contains only RyR2. These findings suggests that CS1 is an important determinant of both spatial orientation and ultrastructure of CRUs and may be important for the correct execution of the maturation program in fast-twitch fibers.

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SYSTEMIC ADMINISTRATION OF ANTISENSE OLIGONUCLEOTIDES VIA ENCAPSULATED LIPOPLEXES CAN LEAD TO DYSTROPHIN RESTORATION IN CARDIAC AND SKELETAL MUSCLE OF MDX MICE

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Antisense-mediated exon skipping holds great potential for the treatment of Duchenne muscular dystrophy patients. We have recently explored the potential of systemic (intra-arterial) delivery of therapeutic morpholino oligos in aged mdx mice, whose phenotype resembles more closely that of DMD. We now present the preliminary results obtained with a novel formulation of lipid-complexed oligos, stable nucleic acid lipid particles (SNALP), delivered intra-venously. This particular system was chosen because it had been designed to specifically deliver its genetic cargo to areas of tissue inflammation and therefore appeared well suited for reaching dystrophic muscles. Our data showed that delivering just 35 μg of encapsulated 2-Me-O phospho-thioate ribonucleotides per animal was sufficient to notice an increase in the amount of dystrophin-positive fibers (over the revertant background) in all analyzed muscles. Importantly, it appeared that SNALP could deliver the therapeutic oligos also in the heart, a target that so far has eluded all protocols based on naked oligos alone. The absolute number of dystrophin-positive fibers in the cardiac muscle was smaller than that found in its skeletal counterparts, but the virtual absence of revertant cardiomyocytes in mdx animals made their presence even more statistically significant. Compared to morpholino oligos, however, the ribo-oligos used here appeared to be much less stable in the cells, as the levels of skipped mRNA fell below detection threshold within few days from injection.

EFFECTS OF DIFFERENT PHYSICAL TRAINING AGAINST SARCOPENIA: CELLULAR AND MOLECULAR ANALYSIS

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The loss of skeletal muscle strength in the elderly is a physiological status named Sarcopenia. Sarcopenia has a multifactorial origin linked to: oxidative damage of fibers (Fulle et al, Exp. Geront. 40:189, 2005), mitochondrial damage (Brunk UT, Eur J Bioch, 269:1996, 2002) hormonal reduced levels and reduced myogenesis (Beccafico et al., ANYAS (270906) 345, 2007). We analyzed the effects of three different physical training (endurance, resistance and local vibrational energy) on 65-85 years old people in order to identify on skeletal muscle the cellular and molecular pathways regulated by these training. The inferior limbs

strength has been evaluated with isometric and isokinetic test before and after the training. A nedlee biopsy (50-70 mg) was taken on vastus lateralis muscle before and after the training. We analysed: (i) the regenerative capacity of satellite cells (ii) the transcriptional profile and (iii) the specific tension development of single fibers. Each protocol increased the bilateral isometric strength after 12 weeks of training. The endurance training reduced the number of satellite cells and increased the CSA. The endurance and vibrational training increased the aerobic metabolism while the resistance training stimulated the creatine metabolism. Each training activated specific metabolic pathways as sarcomeric and cytoskeletal protein expression or ribosomal assembly. In conclusion, our results suggest that the training protocols have been effective on elderly people because each of them are able to stimulate a specific metabolism and specific signaling resulting in an increase of muscle strength.

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CONTRACTILE RESPONSE IMPAIRMENT IN SKELETAL MUSCLES OF ANK1.5 DEFICIENT MICE

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Vertebrate genome contains three ankyrin genes (Ank1, Ank2, and Ank3) from which by differential splicing multiple transcripts originate resulting in a large number of expressed proteins that participate in the organization of specific membrane domains by linking specific membrane proteins with intracellular cytoskeleton. Recently, a muscle specific variant of Ank1, ank1.5 has been shown to link obscurin, a myofibrillar protein, with the sarcoplasmic reticulum in striated muscles. To clarify the role of Ank1.5 in skeletal muscles we generated mice carrying null mutations that selectively affect the expression of the ank1.5 mRNA. Homozygote Ank1.5 mice are vital and fertile. To further understand the role of ank1.5 protein, the structure of the SR and the contractile properties of skeletal muscles from ank1.5 KO mice were analysed. Although staining of SR proteins revealed no apparent alteration in the organization of SR proteins ank1.5 KO mice, the contractile performance of the diaphragm was however weaker than in wild type animals, their fatigue resistance in an endurance test on the treadmill reduced and gait analysis showed stried length and stride frequency differences. Moreover some evans blue positive fibers indicated small necrosis in KO diaphragm muscles. These findings suggest that ank1.5 play an important, but yet not completely defined role, in muscle contraction.

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KNEE EXTENSOR MUSCLE BEHAVIOUR AFTER FATIGUE IN KATA AND KUMITE ELITE ATHLETES

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Karate athletes are divided in two main disciplines: Kata and Kumite. The former includes a choreographed patterns of isometric and explosive movements whereas the latter is a 3-min combat against an opponent. The 2 arts imply different training programs in order to enhance athletes' performance. This could have induced differences in skeletal muscle resistance to fatigue. Thus, aim of the study was to evaluate the knee-extensor muscle response to fatigue in a group of elite karate athletes. Fifteen athletes (8 Kumite and 7 Kata), with similar anthropometric characteristics and muscle-plus-bone area of the knee-extensors, performed a maximal voluntary contraction (MVC) before and after a fatiguing protocol. During MVC, the electromyogram (EMG), the force signals and the blood lactate concentration ([La]) were acquired. From EMG signal, the root mean square (RMS) and fibres conduction velocity (CV) were calculated. MVC was similar in the 2 groups. The fatiguing protocol lasted significantly longer in Kata than in Kumite (44±8 SD cycles and 31±11 cycles respectively). After fatigue, the MVC and EMG RMS significantly decreased in both groups to similar values, but no changes were found in CV. [La] was significantly higher after fatigue in Kata than in Kumite (4.9±0.73 mM and 3.9±1.3 mM, respectively). However, after normalisation by the number of fatiguing cycles, the difference in [La] disappeared. In spite of a similar MVC, Kata showed a higher resistance to fatigue compared to Kumite. The continuous isometric contractions typical of Kata may have induced changes in muscles compatible with a more resistant behaviour. On the contrary, Kumite, involving rapid dynamic contractions, could have lead muscles to faster but less resistant characteristics.

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SPHINGOSINE 1-PHOSPHATE DIFFERENTIALLY REGULATES SERUM-INDUCED PROLIFERATIVE RESPONSE IN C2C12 RESERVE CELLS AND MYOBLASTS

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In this study the effect of sphingosine 1-phosphate (S1P) on the proliferative response to low serum has been examined in two closely related cell populations such as C2C12 reserve cells and myoblasts. S1P was found to reduce labelled thymidine incorporation promoted by serum in C2C12 myoblasts whereas it enhanced DNA synthesis in response to

serum in reserve cells. By employing selective S1P receptor agonists and antagonist the co-mitogenic action of S1P in reserve cells was shown to depend on S1P1 and S1P3. Selective S1P1 agonist SEW2871 did not influence the stimulation of DNA synthesis in myoblasts, whereas it dose-dependently enhanced the proliferative response elicited by low serum in reserve cells. Moreover, the challenge with FTY720-P, which exhibits a comparable affinity to S1P for all the S1P receptors except S1P2, did not affect the serum-stimulated proliferation of myoblasts, but it increased in a dose-dependent manner labelled thymidine incorporation in response to low serum in reserve cells. The maximal efficacious amount of FTY720-P stimulated DNA synthesis more potently than SEW2871; interestingly, its efficaciousness was comparable to that of S1P, indicating a role not only for S1P1 but also for S1P3 in the proliferative response to S1P in reserve cells. Real time PCR revealed a specific expression pattern of mRNA S1P receptors in C2C12 reserve cells and myoblasts. However, S1P1 resulted the receptor subtype expressed at the highest levels in both cell populations. Collectively, obtained results suggest that the differential responsiveness to S1P in the two cell populations may depend on a different functional coupling of S1P receptors. The present study for the first time discloses a unique pleiotropic effect of S1P which is able to stimulate proliferation of muscle resident stem cells, such as reserve cells, and arrest the growth of committed progenitors cells, such as myoblasts, required for their subsequent myogenic differentiation.

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MITOCHONDRIAL HOMEOSTASIS AND ENERGY BALANCE CONTROLS MUSCLE MASS VIA AMPK PATHWAY

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FoxO family of transcription factors plays a critical role in regulating muscle size and when activated, coordinate the ubiquitin-proteasome and autophagy-lysosome systems and cause severe muscle wasting. However the substrates during muscle wasting of the two major proteolytic systems are still to be determined. Here we show that mitochondria are the target of autophagy-lysosome pathway. Foxo3 in adult muscle fibers causes a reduction in mitochondrial content, due to their destruction in autophagic vacuoles. Indeed mitochondria are reduced in two different models of muscle wasting (and FoxO prevents mitochondrial fragmentation and muscle loss). Furthermore mitochondrial fragmentation induced by either hFis1-DRP1 or Bnip3 pathways is sufficient to induce time-dependent muscle loss. Conversely inhibition of the fission machinery suppressed mitochondrial fragmentation and Foxo3-mediated muscle atrophy. Alteration of mitochondrial network activates AMPK pathway and AMPK inhibition restores muscle size in myofibers with fragmented mitochondria. These findings indicate that homeostasis of mitochondrial network affects muscle energy state which is an important determinant for muscle atrophy program.

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SERCA1 ACTIVITY IN THE CONGENITAL PSEUDO-MYOTONIA OF CHIANINA CATTLE

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Recently a congenital pseudo-myotonia was described in Chianina cattle. Chianina is one of the most important Italian breed for meat quality (fiorentina) and leather (Frau brand). Symptoms affect the musculature of the limbs and are exercise-induced stiffness and impaired skeletal muscle relaxation. Since the clinical aspect remembers myotonia but electromyography investigations resulted negative, symptoms could be due to an abnormal cytosolic Ca2+ concentration. In skeletal muscle Ca2+ is stored into the sarcoplasmic reticulum (SR). The RyR releases Ca2+ in the citosol and the SR Ca2+-ATPase (SERCA) pumps Ca2+ back into the SR, resulting in relaxation. RyR plays a crucial role in malignant hyperthermia (MH) which was described in human and porcine. Our clinical data excluded MH in Chianina breed, so we investigated SERCA. From fast twitch semimembranosus muscle of diseased animals, we prepared a crude microsomes membrane fraction (TM), selectively enriched in the main markers of junctional (RyR) and non junctional SR (SERCA). TM fractions were used for determination of SERCA ATPase activity, assayed by a spectrophotometer method. By comparison with control bovine muscle of the same species, ATPase activity in pathological muscles resulted reduced of about 50% in two specimens and almost absent in other subjects. Immunoblotting data with antibodies against SERCA1 isoform in the same fractions, confirmed the absence or reduction of expression of Ca2+-pump in pathological animals. Since the relation between the bovine skeletal muscle disorder found in our study and the human Brody's disease (a rare inherited disease associated to a mutation in the ATP2A1 gene encoding SERCA1 isoform) bovine muscle might be used as a suitable animal model.

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NEUROMUSCULAR CONTROL DURING ISOKINETIC KNEE EXTENSION IN TOP LEVEL KARATEKA.

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The motor task required in karate practice includes a fine static and dynamic control of movement, and a great ability to

perform technical actions as fast as possible (ballistic contractions). The neuromuscular response of knee flexor and extensor muscles during isokinetic contractions in 12 top level male karateka (Age:30±2 yrs) was explored in this study. Surface Electromyographic signal (sEMG) was recorded using 4 electrode arrays from the right vastus lateralis (VL) and biceps femoris (BF) during maximal isometric knee flexion and extensions (MVC), and during isokinetic contractions at different angular velocities (30°, 90°, 180°, 270°, 340°, 400°/s). Torque/Velocity (TV) curves was computed for both muscles. The activation level of VL and BF working as antagonist was quantified through normalized sEMG Root Mean Square value (RMSAnt%). Muscle fibre conduction velocity was computed for VL (VLCV) and BF (BFCV). Normalized T/V curves showed higher torque values for BF muscle compared to VL at any angular velocity except for 30°/s. VLCV and BFCV peaked at 30°/s (6.5±0.7m/sec and 3.8±0.5m/sec, respectively). CV showed a progressive decline as angular velocity increases; a slight increase in VLCV at 400°/s was observed. VL-RMSAnt% and BF-RMSAnt% ranged from 2.5% to 24.1% and from1.5% to 12.5% (MVC and 400°/s, respectively). These results highlight the peculiar neuromuscular control strategy adopted in karateka, which selectively activate agonist muscles with a minimal intervention of the antagonists. The higher normalized BF torque values obtained may be related to the functional specialization developed in these athletes. The increased VLCV observed at 400°/s may result from a consistent recruitment of fast MUs as it is expected in ballistic exercises.

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PROPERTIES AND ROLE OF TRANSIENT LOW VOLTAGE-ACTIVATED CA2+CHANNELS DURING MYOGENESIS

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During the first phases of myogenesis, transiently-expressed T-type Ca2+ currents have been identified, which mediate the influx of Ca2+ required for the fusion process as well as myotube contraction. Senescent myoblasts were also found to fuse more slowly and less efficiently than in earlier passages; they were smaller and thinner in size than the younger cells, and myotubes did not contract. In the present study, murine myogenic cells were maintained in culture until the stage of replicative senescence. T-current activity was recorded in myoblast and myotubes using the whole cell patch-clamp technique. A lower occurrence of T-type Ca2+ current was observed both in myoblasts and myotubes reaching replicative senescence, in parallel with a lack of fusion, identified by counting the number of nuclei per myotube under fluorescence microscopy by DAPI staining. In addition, in young myoblasts treated with the T-channel blocker, Ni2+, myoblast fusion was suppressed. Current clamp recordings were also performed with perforated patches in myotubes and revealed a sustained spontaneous rhythmic activity, highly dominated by membrane potential, initiated by T-type Ca2+ currents. Combining electrophysiological recordings with consecutive image acquisition, mechanical contractions were found to occur in response to single spontaneous action potentials. Such electrical activity in myotubes is suggested to have a trophic function during the maturation of skeletal muscle cells. In conclusion, these findings suggest that the T-type Ca2+ current could be a good target for physiological and pathological up- and down-regulators during myogenesis.

ACTIVATION OF RAGE IN MYOBLASTS AND RHABDOMYOSARCOMA CELLS RESULTS IN DOWNREGULATION OF PAX7 EXPRESSION

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RAGE (receptor for advanced glycation end products), activated by its ligand, HMGB1, stimulates myogenesis (Mol Cell Biol 24:4880-4894, 2004), and inactivation of RAGE in myoblasts results in reduced differentiation, increased proliferation and tumor formation (J Biol Chem 281:8242-8253, 2006). Also, enforced expression of RAGE in TE671 rhabdomyosarcoma (RMS) cells (that do not express RAGE) results in activation of the myogenic program, increased apoptosis and reduced proliferation, invasiveness and tumor formation (Am J Pathol 171:947-961, 2007). We show here that satellite cells (SCs) in situ and quiescent primary myoblasts do not express RAGE; however, RAGE is rapidly expressed in activated myoblasts in differentiation medium, and its expression is inversely related to the expression of Pax7, a marker of quiescent and proliferating myoblasts/SCs. Embryonal RMSs (ERMSs) originate from SCs and express low levels of myogenin and high levels of Pax7, and upregulated Pax7 has been suggested to contribute to ERMS genesis. We observed a direct correlation between RAGE and myogenin expression and an inverse correlation between RAGE and Pax7 expression in a panel of human ERMSs. Also, either the blockade of RAGE activity or inactivation of p38 MAPK results in upregulation of Pax7 expression in differentiating myoblasts, suggesting that RAGE might repress Pax7 expression via p38 MAPK. Further, RAGE signaling in RMS cells and myoblasts causes myogenin-dependent repression of Pax7 expression as investigated by chromatin immunoprecipitation. Our data suggest that RAGE has a role in repression of Pax7 expression, a prerequisite for SC differentiation, and that repression of RAGE expression or function in SCs might contribute to genesis of Pax7-dependent rhabdomyosarcoma.

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ELECTROPHYSIOLOGICAL CHARACTERIZATION OF DEVELOPING HUMAN FETAL CARDIOMYOCITES

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Readily available cultures of cardiomyocytes in vitro would be a valuable tool for research in allograft cardiac rejection. In this study, we first aimed to establish reliable cultures of human fetal cardiomyocites (Hfcm), retaining in vitro the features of a physiologically functional system. By patch-clamp analysis we showed that different ionic currents were detectable at various differentiation stages in this model, from primary culture (p0) to passage 9. In p0 our cells already expressed the majority of ionic currents: the outward delayed rectifier TEA-sensitive K+ currents, IKr and IKs, the transient outward potassium current 4-AP-sensitive, Ito and the inward rectifier, IK1. Being Ito and IK1 typical cardiac current our cells are just committed towards cardiac phenotype. In addition, in the early phase of differentiation (p0-p3) there was an increase of the membrane surface as evaluated by Cm, and a progressive hyperpolarization of the resting membrane potential. Starting from p3 the cells could elicit action potentials (APs) with the early transient peak related to Na+ current, INa. Moreover, there was a progressive change of K+ currents, that became constant from p4-p6. This suggests that Hfcm had reached a mature cardiac ventricular phenotype and this was also confirmed by the typical shape of APs. Moreover, Hfcm just in primary cultures expressed both homotypic and heterotypic gap junctions In conclusion, the key passages in Hfcm for cell growth were p2-p3, whereas the accomplishment of a mature phenotype occurred at p4-p6 since the following passages did not determine significant modifications. So far, we proved that we handled cultures spontaneously exhibiting and maintaining the acquisition of specific ionic channels along with the functional competence of mature cardiomyocytes, and still retaining the capability of proliferation, albeit with a finite life-span *in vitro*.

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IN VITRO ENGINEERING OF RABBIT ARTICULAR CARTILAGE: ORGANOTYPIC CULTURE IN RELATIVE MICROGRAVITY

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Chondrocytes represent the unique cell type of cartilage and play a fundamental role in extracellular matrix synthesis. The characterization of their functions in vitro is often limited by the low availability of cells, that constrains to amplify them in monolayer; this condition is known to induce cell dedifferentiation, characterised by a switch from chondrocyte- to fibroblast-like phenotype. Several in vitro models have been developed for cartilage engineering, but they are still limited by a gradual loss of tissue-specific functions. Our aim was to develop in vitro models that could preserve such functions. Since it is well known that cell growth, morphology, and differentiation are greatly controlled by the composition of cellular environment, we explored the possibility to establish 3D cultures of cartilage fragments alone, or to introduce them into an amorphous bone-made scaffold. The 3D culture was performed by an innovative device, the Rotatory Cell Culture System (RCCSTM), which creates a microenvironment known as “relative microgravity”, where shear forces and turbulence, known to limit 3D cultures, are reduced to a minimum. We expected that this particular 3D microenvironment, by allowing the maintenance of tissue-like organisation, could support the preservation of the chondrocyte differentiated phenotype. Our results demonstrated that the RCCSTM bioreactor allows the maintenance of cartilage fragments for several days, keeping their organisation as in the native tissue and without any sign of suffering (necrosis). Moreover, the Alcian Blue staining showed that cells were able to express cartilage-specific proteoglycans even after several weeks of culture. These data indicate that the RCCS-based 3D organotypic culture of cartilage represents a potent tool for studying the properties of this tissue; it may also be a suitable alternative to the poorly effective existing models used to prepare bioengineered chondrocytes for therapeutic use in orthopedy.

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CONGENITAL PSEUDO-MYOTONIA IN CHIANINA CATTLE

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The authors present a muscle function disorder observed in 11 Chianina cattle. The clinical picture is mainly characterized by an exercise-induced muscle contracture which prevents animals from performing muscular activities more intense than a simple walk at a slow pace. In fact, when stimulated to move faster, the muscles immediately become stiff and "freeze up" temporarily, inducing a rigid and uncoordinated gait or bunny hopping. The stiffness disappears as soon as the exercise ceases. If the exercise is prolonged, the stiffness freezes the locomotion and the animals fall on the ground like a log. The animals do not pass out or lose consciousness as in fainting. Retraction of the bulbi and prominence of the third eyelid are other common findings during these startle-elicited crises. Biochemistry usually shows slightly increased levels of creatine kinase, lactate dehydrogenase, aspartate amino-transferase and L-Lactate. Standard needle electromyography showed normal spontaneous insertion activity and voluntary electrical activity. Routine histological preparations as well as more specific histochemical and histoenzymatic stains carried out on bioptic samples of the semimembranosus muscle failed to show any remarkable and helpful findings. Genealogical evaluation of the affected animals revealed common ancestors which might suggest a genetic aetiology. On the basis of the clinical findings and of the scanty results obtained by collateral exams, we have adopted the term "Congenital Pseudo-myotonia". As regards human medicine, the disease in some way resembles the Brody's disease, a muscular disorder characterized by painless muscle contracture and exercise-induced impairment of muscle relaxation due to a defect of calcium reuptake.

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MORPHOLOGICAL STUDIES OF COSTAMERE STRUCTURE IN DYSTROPHIC SKELETAL MUSCLE SINGLE FIBRES. A TIRFM ANALYSIS

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In contractile cells, as cardiomyocytes and skeletal muscle fibers, the interactions between the cytoskeleton and the plasma membrane require complex protein structures called costameres. These structures are oriented transversely, over the Z and M lines of myofibrils, and together with other longitudinally oriented structures form a rectilinear lattice. To determine whether the costameres are altered in muscle dystrophies, particularly those caused by mutations of proteins forming the connection between cellular cytoskeleton and extracellular matrix (as dystrophin, integrin, laminin and collagen VI), in skeletal muscle fibres of dystrophic mice the sarcolemmal surface was examined by "Total Internal Reflection Fluorescence Microscopy (TIRFM)". This is an imaging technique based on the total internal reflection of an incident light ray at glass-culture medium interface. This particular kind of sample illumination is achieved when the incident ray surpasses the critical angle, depending on the mismatch between refractive indexes of observation and culturing medium, according to Snell's law. At the interface, an electromagnetic evanescent field with an exponential intensity decay is generated, enables to specifically stimulate only fluorophores which lie in a very thin region, tipically ranging from 80 to 200 nm. Thus, TIRF provides an excellent signal-to-noise ratio, especially in thick samples, and a higher (at least 2-fold increase) axial resolution respect to confocal microscopy. We have analysed costamere structure in living and fixed single muscle fibres using FM1-43 and antibodies specific to membrane proteins. Work in progress is focussed on the comparison between muscle fibres of several mice strains as mdx (lacking dystrophin), Col6a-/- (lacking collagen VI) and control muscle fibres of C57 (wild type). The experimental is represented by intact adult fully differentiated muscle fibres dissociated from FDB muscles. These cells prove to be very suitable to study the sarcolemmal organization.

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MOLECULAR MECHANISMS REGULATING SKELETAL MUSCLE HOMEOSTASIS: EFFECTS OF V1A AVP RECEPTOR OVER-EXPRESSION

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The maintenance of a working skeletal musculature is conferred by its remarkable ability to regenerate after mechanical or pathological injury. However most muscle pathologies are characterized by the progressive loss of muscle tissue due to alterations of healthy skeletal muscle homeostasis. In particular cachexia is a severe syndrome consisting of marked skeletal muscle atrophy, characterized by a dramatic loss of muscle mass associated with a compromised regenerative ability. Arg-vasopressin (AVP) is a potent myogenesis promoting factor and activates both the calcineurin and CaMK pathways, whose combined activation leads to the formation of transcription factor complexes in vitro [1-3]. The local over-expression of V1a AVP receptor (V1aR) in injured muscle results in enhanced regeneration. V1aR over-expressing muscle exhibits: early activation of satellite cells and regeneration markers, accelerated differentiation, increased cell population expressing hematopoietic stem cell markers and its conversion to the myogenic lineage. Moreover we investigated the role of V1aR over-expression in animals undergoing cachexia as a result of

muscle over-expression of a specific cytokine (TNF-α) [1]. In these conditions, the local V1aR over-expression counteracts the negative effects of cachexia on muscle, as demonstrated by morphological and biochemical analysis. In particular, the presence of V1aR results in increased Pax-7, myogenin and myosin expression levels both in wild type and in cachectic muscles. Finally, we demonstrate that V1aR over-expressing muscle increases the calcineurin and IL-4 expression levels, and induces the phosphorylation of FOXO trascription factors, inhibiting the expression of atrophic genes. This study highlights a novel in vivo role for the AVP-dependent pathways which may represent a potential gene therapy approach for many diseases affecting muscle homeostasis.

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LOCALIZATION OF S1P RECEPTORS IN RAT SOLEUS MUSCLE. ROLE OF SPHINGOSINE 1-PHOSPHATE ON SKELETAL MUSCLE TROPHISM

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Sphingosine 1-phosphate (S1P) mediates a number of cellular responses, including growth and proliferation. The aim of this work was to localize S1P receptors in rat skeletal muscle and to investigate whether S1P exerts a trophic action on muscle fibers. RT-PCR and Western blot analyses demonstrated the expression of S1P1 and S1P3 receptors in adult soleus muscle. Immunofluorescence revealed that S1P1 and S1P3 receptors are localized at the cell membrane of muscle fibers and in the T-tubule membranes. The receptors also decorate the nuclear membrane. and were expressed in the satellite cells. The possible trophic action of S1P was investigated by utilizing the denervation atrophy and in vivo regeneration models. Denervation of rat soleus muscle, analyzed 7 and 14 days after motor nerve cut, produced the down regulation of S1P1 and S1P3 receptors. The continuous delivery of S1P, through a mini osmotic pumps, to the denervated muscle significantly attenuated the progress of denervation-induced muscle atrophy. During regeneration of rat soleus, induced by bupivacaine, expression of S1P1 receptor progressively increased between 3 and 7 days after degeneration, while that of S1P3 progressively decreased. The direct injection into the regenerating muscle of S1P (100 µl of 50 µM) determined an accelerated growth of regenerating fibers. In fact, in the presence of S1P, the mean cross sectional area of the 3-days regenerating fibers was significantly higher (+ 29.4 %) than in the contralateral regenerating muscle. In conclusion, the results indicate that S1P plays a significant role in muscle fiber trophism and development.

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