SESSIONE 1

SKELETAL MUSCLE MECHANICS AND BIOPHYSICS

EFFECTS OF HYDROGEN PEROXIDE (H2O2) ON ELECTRICAL PROPERTIES OF SKELETAL MUSCLE CELLS

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Reactive Oxygen Species (ROS) are known to be implicated in pathological events but also considered to play a role in physiological signalling.

To investigate the possible effects of ROS generation on electrical membrane properties, Hydrogen peroxide (H_2O_2) was exogenously applied at low doses (100 µM) to mouse skeletal myotubes in culture and electrophysiological recordings were performed in perforated and whole-cell patch-clamp techniques.

Under voltage-clamp conditions, in most myotubes, current-voltage relationships revealed various electrophysiological properties, but mainly inward membrane rectification beyond *ca*. -70 mV, independent on the pre-set resting potential. Such rectification is unaffected by tetrodotoxin and blocked by Ba^{2+} 100 µM.

Under current clamp conditions, an anode break Na^+ spike was observed on return to the resting potential. A transient outward-decaying 'tail' current reflecting deactivation of inward rectification was suggested to be present on stepping from -100 mV to more positive potentials. The inward rectification as well as the tail current, supposed to be responsible for a delay of anode break firing, are reduced by Ba^{2+} and H_2O_2 100 µM.

The ROS agent H_2O_2 was therefore able to mimic the Ba^{2+} effect reducing the inward rectification and blocking the delay for spike initiation, suggesting its role as a modulator of the inward rectifier currents.

Our data confirm that ROS produced either during muscle activity or under action of various biogenic substances like growth factors, could play a role in control of excitability of developing muscle cells.

A GENOMIC APPROACH TO STUDY THE GENE EXPRESSION OF SKELETAL MUSCLES AT SINGLE-FIBRE LEVEL

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Skeletal muscle is a complex, versatile tissue composed of a large variety of functionally diverse fibre types. The overall properties of a muscle largely result from a combination of the individual properties of its different fibre types and their proportion. The analysis of the expression profile of such complex tissue is further complicated by the presence of additional components of other tissues (blood vessels, connective, etc.). In this study we aimed to identify the transcriptional profiles of single fibres isolated from mouse muscles. This was made possible by the development of a protocol that allows genomic analysis at single-cell level in muscles (microgenomics). We used a "supervised" approach, based on the classification of each isolated myofibre according to the expression of the Myosin Heavy Chains (MyHC), prior the microarray analyses. We studied two types of myofibres: slow (MyHC-1) from soleus and fast-glycolytic (MyHC-2B) from EDL. The complete picture of genes co-expressed with the related MyHC isoforms emerged and novel genes that could be useful for fibre type characterization were revealed. Since myofibres have a large adaptive potential in response to a variety of physiological and pathological stimuli, a microgenomics approach in muscle would be of great interest to study muscle plasticity at the fibre level when muscles adapt to new functional demands.

ACTOMYOSIN BONDS, TENSION AND STIFFNESS ON THE TETANUS RISE AND IN BTS TREATED SINGLE INTACT FROG MUSCLE FIBRES

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Cross-bridge number in single frog muscle fibres was determined during tetanus rise in normal Ringer and during submaximal tetanic contractions in N-benzyl-p-toluene sulfonamide(BTS)-Ringer (1 µM) by measuring the tension P_c needed to induce the forced cross-bridge detachment by fast stretches at 5°C. The results were compared with fibre stiffness, another indicator of cross-bridge number measured with sinusoidal length oscillations (4 kHz frequency ~1 nm hs^{-1} peak to peak amplitude). Force was measured with a fast capacitance transducer (30-50 kHz resonance frequency) and sarcomere length was measured by a striation follower device. The stiffness-tension relation was the same both during tetanus rise and BTS-Ringer and showed the non-linearity expected from the presence of myofilament compliance. However, the fitting of data with a simple model, including cross-bridge and linear filament compliances in series, was not satisfactory. A good fit was obtained only by assuming that a fraction (14%) of attached bridges at tetanus plateau was generating no-force. Relative filament and cross-bridge compliances resulted ~0.4 and ~0.6 respectively. The stretch data, both on the tetanus rise and BTS-Ringer, showed a linear relation between P_c and tension with a slope confirming the presence of the non-force generating bridges suggested by stiffness data. These results show that the relation between cross-bridge number and fibre stiffness is more complex than usually assumed.

TO STEP OR NOT TO STEP: THE FIRST MILLISECOND OF THE MYOSIN WORKING STROKE UNDER CONSTANT LOAD

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Myosin II is the motor that drives muscle contraction through cyclical interactions with actin filament. In each cycle an ATP molecule is split and a filament displacement (or working stroke or WS) is generated. The WS produced by a single myosin head has been previously measured but the effects of the loads acting on myosin during contraction could not be investigated. In fact, current single molecule techniques apply force with a delay of few milliseconds after actin-myosin binding, when the WS of skeletal myosin has already been completed. Here we developed a novel single molecule technique in which a constant force is continuously applied to the actin filament, so that the delay between myosin binding and force application is abolished. This method is capable of resolving the myosin WS under different loads with a high time resolution and detecting events as short as 100 μs due to a very high signal-to-noise ratio. We found that under loads in the range 1 to 10 pN myosin can follow two distinct pathways in its interaction with actin: a population of very fast events (240 ± 23μs) in which myosin detaches from actin before producing any movement and a second population of events where myosin steps and remains bound to actin for a longer time. At low forces the lifetime of the second population of events linearly decreases with ATP concentration in the range 5-50μM. The mean amplitude of the myosin WS is found to be smaller at increasing loads and vanishes at the isometric force (5.7 ± 0.6pN). On the other hand, the rise time of the WS becomes longer as the force increases.

FUNTIONAL PARAMETERS OF SKELETAL MUSCLE CONTRACTION IN NG2 MICE STUDIED AT DIFFERENT AGES

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NG2 is a integral membrane chondroitin sulfate proteoglycan that is widely expressed on numerous cell types including chondroblasts, myoblasts, fibroblasts and O2A glial progenitor cells. This proteoglycan is also expressed in the sarcolemma and neuromuscular junction of postnatal human muscle and gradually declines with advancing age. Although the function of NG2 remains unclear, several studies have suggested that NG2 may connect the interior and exterior environments of the cell. Moreover NG2 expression and localization are sensitive to pathological changes in muscle.
In this study, functional parameters of skeletal muscle contraction were determined in NG2 null mice *in vivo* and *ex vivo* at different ages (three month and six month years old).
Force development was measured *in vivo* (grip test) and *ex vivo* (intact isolated muscles) and resistance to fatigue was examined *in vivo* (treadmill) and *ex vivo* (intact isolated muscles). We also investigate any changes in myosin heavy chain isoform composition with electrophoresis on that isolated muscle. In addition the excitation-contraction coupling, was studied by recording Ca^{2+} transient in single fibres of FDB.
The results obtained showed that the ability to develop force *in vivo* of NG2 null mice decreased with age, while resistance to fatigue was not affected. Interestingly, this was in contrast with the pronounced and significant reduction in the distance covered by the mice in wheel free run. Finally, no significant variations related with age were detectable in the measurements of force developed by isolated muscles *ex vivo*.

SESSIONE 2

E - C COUPLING

INITIAL CHARACTERIZATION OF CASQ1/CASQ2 KNOCKOUT (dCASQ-NULL) MICE

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Calsequestrin (CASQ) is the major Ca^{2+}-binding protein of the sarcoplasmic reticulum (SR) terminal cisternae. Calsequestrin is expressed in two different isoforms in muscle: CASQ1 is the only isoform present in adult fast-twitch fibers, whereas it is co-expressed with CASQ2 in slow twitch muscle. CASQ2 is the only isoform found in cardiac muscle. We recently described the phenotype of CASQ1 knockout mice: lack of CASQ1 causes significant structural and functional alterations (Paolini et al., 2007), and results in a higher rate of spontaneous mortality and in a malignant hyperthermia (MH) phenotype (Dainese et al., 2009). However, in CASQ1-null mice CASQ2 is still expressed in slow twitch fibers. We have now generated a mouse lacking both CASQ isoforms (doubleCASQ-null), by cross-breeding our mice with CASQ2-knockout animals (Knollmann et al., 2006). Lack of both CASQ isoform is confirmed by western blot and immunostaining analysis. The double-null mice are viable and breed normally, however present a higher rate of spontaneous mortality than single-null animals. Initial characterization based on structural approaches (electron and confocal microscopy), function ex-vivo (measurement of single twitch and tetanic force in EDL and Soleus), in vitro (Ca^{2+} measurements in single fibers), and *in vivo* (grip test) have been performed. Stress protocols (halothane, heat, and exercise) have been also employed. The results obtained indicates that lack of CASQ2 exacerbate the phenotype of CASQ1-null mice.

IMPACT OF CALSEQUESTRIN ON INTRA-SR CALCIUM CONCENTRATION IN SKELETAL MUSCLES FIBERS AT REST AND DURING ACTIVITY MONITORED WITH A GENETICALLY ENCODED FRET BASED INDICATOR

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Calsequestrin (CS), the major Ca^{2+}-binding protein in the sarcoplasmic reticulum (SR), is thought to play a dual role in excitation-contraction coupling: buffer free Ca^{2+} increasing SR capacity, and modulate the activity of the Ca^{2+} release channels (RyR). In this study, we determined the changes in Ca^{2+}- concentration inside the SR during single twitches and tetanic stimulation in muscle fibres from WT (wild type) mice and from mice with null mutation of the gene coding for the skeletal muscle isoform of CS (CS1-null) or of both genes coding for two isoforms of CS (CS1-CS2 null). Ca^{2+}-concentration inside the SR was measured using an SR-targeted cameleon and confocal microscopy in single living adult muscle fibres which had been enzymatically dissociated from FDB muscles and kept in culture for 24 hours. Ca^{2+}- concentration was obtained from the ratio of fluorescent emissions (R) of the two fluorofores YFP/CFP present in the cameleon molecule. The results obtained showed that the basal Ca^{2+} concentrations at rest were not significantly different between the three groups of fibres. During contraction the reduction in Ca^{2+}- concentration as estimated by R values was small and did not increase much with stimulation frequency in WT fibres. In contrast, in fibres from CS1 null and CS1-CS2 null animals, the decrease in SR Ca-concentration was much larger than in fibers from WT animals and became more pronounced at high stimulation frequency. Minor and not significant differences were observed between CS1 null and CS1-CS2 null animals.

PROGRESSIVE TRIAD-MITOCHONDRIA UN-COUPLING IN AGING

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An impairment of the mechanisms controlling the release of Ca^{2+} from internal stores (excitation-contraction (EC) coupling) has been proposed to contribute to the age-related decline of muscle performance that accompanies aging (*EC un-coupling theory*). EC coupling in muscle fibers occurs at specialized intracellular junctions called *calcium release units* (CRUs) or triads, which are specifically placed at sarcomere's I-A band transition. We have recently shown: a) that CRUs are *tethered* to mitochondria placed at the I band; and b) that this association occurs progressively during post-natal maturation. We have studied the frequency, sarcomeric localization, and ultrastructure of CRUs and mitochondria in EDL from male mice using transmission electron microscopy (TEM). Preliminary results indicates that the number of CRUs in aging mice (n=3, 25-27 months of age) decreases compared to the adult mice (n=4, 7-12 months of age), whereas the percentage of abnormally shaped/positioned triads (dyads, pentads, longitudinally oriented, etc.) increases. In addition, the total number of mitochondria, and their volume at I band also decreases, whereas a higher percentage is misplaced at the A band. These changes result in a decreased percentage of mitochondria functionally tethered to Ca^{2+} release sites. Our observations suggest a age-related partial disarrangement and spatial re-organization of EC coupling/mitochondrial apparatuses, which tend to loose their functional localization. This could in part explain the decline of muscle performance associated to increasing age.

MOLECULAR BASIS OF PROTEIN LOCALIZATION TO THE JUNCTIONAL SARCOPLASMIC RETICULUM OF SKELETAL MUSCLE CELLS

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The sarcoplasmic reticulum (SR) of striated muscle cells is composed of a variety of tubules, vesicles and cisternae surrounding each myofibril. Two main subdomains of the SR can be clearly distinguished: the longitudinal tubules and the terminal cisternae. The former are enriched in the sarcoplasmic/endoplasmic reticulum Ca^{2+} pumps SERCA, whereas the terminal cisternae are dilated areas of the SR closely associated with the plasma membrane T tubules, forming typical structures called triads. Triads are key elements in the excitation-contraction coupling mechanism, allowing translation of an electrical event into release of calcium from the SR. Many proteins have been identified as structural/functional components of triadic junctions, including the ryanodine receptor, the dihydropyridine receptor, junctophilins, triadin and junctin and the Ca^{2+} binding protein calsequestrin. However, little is known about the mechanisms leading to protein targeting to either T tubules or SR junctional membranes. In order to verify the existence of putative targeting signals in triadic proteins, we expressed wild type and deletion mutants of triadic proteins tagged with GFP in rat primary myotubes and followed their localization during *in vitro* differentiation. We will present data concerning triadin and junctin, two intrinsic membrane proteins of the junctional SR. Deletion analysis showed that at least three different regions in the primary sequence of triadin may be involved in protein targeting to the triads. In parallel the dynamic properties of both triadin and junctin were investigated by Fluorescence Recovery After Photobleaching (FRAP) technique. The results obtained showed that some, but not all, of the regions involved in protein targeting may also contribute to the establishment of protein-protein interactions within the multi-molecular complex associated with the calcium release channel.

SURF-4-DEPENDENT TARGETING OF CALSEQUESTRIN TO SARCOPLASMIC RETICULUM DURING SKELETAL MUSCLE DEVELOPMENT

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Background: Calsequestrin (CS) is a low affinity high-capacity calcium-binding protein, whose functions rely upon precise sub-cellular localization at the junctional face of the sarcoplasmic reticulum (SR). Thus, it is relevant to define the sorting and targeting mechanisms of CS and to identify the specific domains of the molecule involved in the process. Correct CS targeting in skeletal muscle needs active ER exiting via budding of COPII vesicles (Nori et al., Biochem. J. 2004). Moreover, an "I-L-V-containing ER-export motif" exists in a soluble protein for the COPII cargo receptors Erv29p/Surf-4 (Otte et al., Nature Cell Biol. 2004).
Objectives: A mutant CS was developed, i.e., a CS with triple-alanine point mutations, I57A, L60A and V70A (CSΔILV), in order to verify whether the "I-L-V motif" and binding to Surf-4 are required for CS targeting to SR.
Results: a) Surf-4 was expressed in skeletal muscle and distributed at ERES and/or ERGIC structures; b) targeting of CSΔILV was monitored in two skeletal muscle systems, differentiating C2C12 myotubes and adult rat soleus muscle: CSΔILV was mis-targeted in developing myotubes whereas was correctly targeted in adult skeletal muscle; c) Surf-4 bound wild-type CS in a calcium-dependent manner; d) Surf-4 didn't bind CSΔILV.
Conclusions: Binding of CS to Surf-4 via the I-L-V motif could be required for CS targeting only during the development; other sorting and targeting mechanisms could operate at steady state in adult skeletal muscle.

EXPRESSION AND LOCALIZATION OF RAB28 IN SKELETAL MUSCLE CELLS

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Skeletal muscle cells have a peculiar internal organization determined by the regular arrangement of the contractile apparatus and of the sarcoplasmic reticulum. Nevertheless, skeletal muscle cells have the usual membrane traffic pathways that are necessary for targeting and organizing muscle-specific membrane structures as well as partitioning of newly synthesized molecules to them. As of today, little is known about these pathways in skeletal muscle cells. Recently, Rab28, a new member of the Rab-GTPases has been described. In mammalian tissues, Rab28 is present as two splice variants called Rab28L and Rab28S respectively. We found that Rab28L is specifically expressed in skeletal and cardiac muscle and in differentiated C2C12 cells. Here we report results on the expression during striated muscle development and on the subcellular localization of Rab28 isoforms.

SESSIONE 3

MUSCLE DISEASES AND THERAPIES

GENERATION OF MYOCYTES FROM MURINE IPS AND ES CELLS

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Adult somatic cells have recently been reprogrammed into induced pluripotent stem (iPS) cells by introducing pluripotency-associated transcription factors (Oct3/4, Sox2, c-Myc, and Klf4 or Oct3/4, Sox2, Nanog and Lin28) resembling unique features of embryonic stem (ES) cells (1). iPS cells represent a very promising tool for developmental, pharmacological, disease-specific studies and for regenerative medicine. However, there are reasons for caution. For one, ES and iPS cells can form tumors. A further problem, until now unsolved, is how to force iPS/ES cell fate into a specific lineage with a high rate. In previous studies, embryonic stem (ES) cells have been highly differentiated in skeletal muscle by inducible over-expression of Pax3, a gene that belongs to the paired box (PAX) family of transcription factors. In this study we induce the differentiation of iPS and ES cells into skeletal muscle lineage by transient over-expression of Pax3 and Pax7. Transient transfected cells are able to form embryod bodies that downregulate the expression of late cardiac markers (CNX43 and cTnI-C). By contrast, a relevant upregulation of MyoD master gene was observed at early embryoid body stages. By immunofluorescence analysis, iPS overexpressing Pax genes stained positive for MF20 and form myotubes after 10 days in differentiative medium. In conclusion, Pax3 and Pax7 genes were able to induce skeletal muscle differentiation in ES/iPS cells that, in principle, could offer a great advantage for autologous cell therapies in cardiac and muscular diseases.

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UBIQUITINATION OF α-SARCOGLYCAN MUTANTS

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Sarcoglycanopathies are a group of autosomal recessive muscle-wasting disorders caused by genetic defects of four cell membrane glycoproteins α-, β-, γ- and δ-sarcoglycan. Proper assembly, trafficking and targeting of the sarcoglycan complex is of vital importance, and mutations that severely perturb tetramer formation and localisation result in sarcoglycanopathy. The majority of the sarcoglycanopathies are associated with missense mutations that generate substitution of single residues that could lead to a misfolded protein. In general, misfolded proteins are identified by the ER quality control system and eliminated through proteasomal degradation, despite sequence analysis of sarcoglycans indicates that many disease-causing missense mutations might not have functional consequences. We have recently shown that misfolded 'functional' sarcoglycans could be rescued to the cell membrane by inhibiting their degradation. Essential for proteasomal degradation is that proteins must be tagged covalently with a chain of ubiquitin (Ub) molecules on lysine residues, through a sequential process involving E1 Ub-activating and E2 Ub-conjugating enzymes and ultimately E3 Ub ligases. Aim of our work was to identify which among the five lysine residues (K193, K199, K211, K252, and K321) is specifically involved in the degradation of α-sarcoglycan mutants. We used heterologous cells (HEK-293) constitutively expressing β-, γ- and δ-sarcoglycan in which the V247M α-sarcoglycan mutant was transiently transfected, a condition that leads to ubiquitination and degradation of the mutant protein. Site direct mutagenesis experiments to replace the lysine residues, individually or in combination, indicate that K199 and K211 are specifically ubiquitinated and that their substitution prevents degradation of the V247M α-sarcoglycan mutant.

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STEM CELLS, NANOCOMPOSITES AND THREE DIMENSIONAL MUSCLE STRUCTURES

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Skeletal myogenesis is a complex process, which is known to be intimately depending on an optimal outside-in substrate-cell signaling. Moreover, the paired box transcription factor Pax3 plays a crucial role in directing mouse mesoangioblasts toward skeletal myogenesis. We aimed at investigating skeletal myogenesis by the over-expression of Pax3 and PAx7 transcription factors on novel multifunctional polymeric nanocomposites composed by combination of synthetic biodegradable PCL [poly(3-caprolactone)], SWNTs (single-walled carbon nanotubes) and Ag (silver nanoparticles) suitable for this application.

SWNTs are very attractive as additives in nanocomposite, due to their aspect-ratio, high conductivity and superior strength. The combination of different nanostructures (SWNTs and Ag) in the same hybrid system is particularly useful to integrate the properties of the different components in the materials, in order to realize multifunctional nanocomposites, for use in nanobiotechnology. We focused on the in vitro behavior of mouse myoblasts (C2C12) and embryonic stem cell lines (ESCLs) on PCL films and PCL nanocomposite based on 1% SWNTs and 15%Ag. We transfected ESCLs with Pax 3, either alone or in combination with Pax7 to improve skeletal muscle differentiation on PCL, PCL+SWNTs, PCL+SWNTs+Ag substrates and on tissue culture polystirene (TCP) as control. C2C12 and ESCLs seeded on the scaffolds preserved growth rate and multi-differentiation properties, suggesting that the nanocomposite biomaterials were biocompatible and suitable for stem cell cultures.

Moreover, ESCLs trasfected with Pax3 and Pax7 cultivated on TCP after 12 days of serum starvation formed a larger number myosin heavy chain positive myotubes with respect to untrasfected cells. In conclusion, this model system for skeletal muscle maturation supports the potentiality of developing 3d muscle structures for use in tissue engineering and regenerative medicine applications.

MORPHO-FUNCTIONAL INTERACTION BETWEEN MUSCLE AND TENDON IN DYSTROPHIC MOUSE MODELS

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To investigate the relationship between muscles with a compromised functionality and the morpho-functional properties of their tendons we made use of a pathological animal model, the dystrophin-deficient mouse (*mdx*), that is commonly used as a model for human Duchenne muscular dystrophy. Without the dystrophin complex to tether the actin cytoskeleton inside the muscle cell to the extracellular matrix, forces generated by the muscle fibres result in tears of the sarcolemma and lead to muscle damage. In this study we hypothesized that muscular dystrophy would also involve alterations in the morphological and mechanical properties of tendons. To test our hypothesis, an evaluation of alive and necrotic cells has been performed on tendons deriving from two different muscle types. Furthermore, tendon's elastic and viscous response were evaluated separately measuring tissue complex compliance.

To in-depth correlate muscle functionality with tendon morpho-functional properties, we referred to *mdx/mIgf-1* mouse model, which expresses the local form of mIgf-1 selectively in skeletal muscle. It has been demonstrated that *mIgf-1* counteracts muscle wasting and reduces the fibrotic index associated with the lack of dystrophy.

Confocal microscopy and mechanical properties measurements revealed that *mdx* tendons, compared to WT ones, have an increase in the number of dead cells and a significant reduction in tissue elasticity, indicating a reduced quality of the tissue. Moreover, *mdx* tendons have an increase in the viscous response, pointing out that during dynamic loading, they dissipate more energy. On the contrary, tendons from *mdx/mIgf-1* mice show a complete recover in the morpho-functional characteristics that are now comparable with control ones.

Our results demonstrate that muscular dystrophy involves not only muscle wasting, but also alteration in the viscoelastic properties of tendons, and strongly suggest a paracrine effect of altered skeletal muscle on tendinous tissue.

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ANALYSIS OF REGENERATIVE POTENTIAL AND HOMEOSTASIS IN CACHECTIC MUSCLES: THE EFFECTS OF PHYSICAL ACTIVITY

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Cachexia, a muscle wasting syndrome associated to many chronic diseases, is a bad prognostic factor, interferes with therapy and worsens quality of life. With the aim to investigate the mechanisms underlying the loss of muscle homeostasis and to test countermeasures against cachexia, we investigated the effects of physical activity on mice bearing a colon carcinoma (C26). We find that while muscle of C26-bearing mice show increased muscle fiber damage, wheel running does not exacerbate damage in the majority of the muscles. Exercise has beneficial effects in cachectic mice including rescued muscle homeostasis and increased life span. Satellite cells activation in cachexia fails to maintain muscle homeostasis, possibly due to disregulation of Pax7 expression, rescued to control level by exercise. In addition, cytokines hamper muscle regeneration by activating non apoptotic caspase pathways in a population of PW1 Interstitial Cells (PICs), a phenomenon which ultimately results in loss of myogenic potential. PICs express the stem cell markers Sca-1, CD34 and PW1, can be rescued to myofiber formation by gene delivery (dominant negative form of PW1 or Hsp70 overexpression), pharmacological (caspase inhibitors) or hormonal (i.m. injection of the myogenic factor Arg^8-Vasopressin) approaches: these are all aimed at reducing caspase activation, highlighting caspase role in myogenic differentiation. Exercise increases the circulating levels of Vasopressin, suggesting it as a possible mediator of the beneficial effects of exercise on cachexia. All together our data highlight the importance of physical activity for an integrated approach aimed against cancer cachexia.

MUSCLE MODIFICATIONS IN HAPTOGLOBIN KNOCKOUT MICE

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Background and aim*:* During obesity it is been observed a condition of muscle hypotrophy in part due to insulin-resistance frequently associated to this condition. Moreover, recently obesity has been defined as a low chronic inflammatory state because of a cytokine and inflammatory proteins increasing in tissues and at systemic level. This could contribute to the muscle hypotrophy described in this condition. In fact, the expression of two muscle-specific ubiquitin ligases, atrogin-1/MAFbx and MuRF1, whose induction occurs before the onset of muscle weight loss and which is necessary for rapid atrophy, is modulated by different cytokines and inflammatory proteins.

Haptoglobin is an acute phase protein with elevated serum concentrations during inflammation.

Recently an increased haptoglobin serum concentration has been described during obesity.

We investigated the modifications of the muscular trophism in obese and control mice, and the possible modifications produced from the lacked expression of haptoglobin (Hp-/-).

Materials and Methods*:* 20 obese mice Hp-/- and Hp +/+ and 17 control littermate mice Hp-/- and Hp+/+ were investigated. We evaluated the cross-sectional areas of muscles, the expression of the E-3 ubiquitin-conjugating enzymes atrogin-1/MAFbx and MuRF1 and the PI3K/Akt pathway to study signals that could activate muscle proteolysis or synthesis.

Results*:* data obtained show muscle hypotrophy in the obese mice, wild type and knockout both.

In Hp-/- obese mice hypotrophy was significantly higher. The Atrogin1/MAFbx and MuRF1 expression was increased in the obese mice, wild type and knockout both, while the level of phosphorylated Akt was reduced in the obese mice, especially in Hp-/- mice.

Conclusion*:* these preliminary data ruled out a protective capacity of haptoglobin deficiency on the muscle hypotrophy, in obesity conditions. Moreover, it seems to exist a synergistic mechanism between obesity and haptoglobin deficiency in programs activation of atrogenes induction.

THE MISSENSE CAV-3/P104L MUTATION, INVOLVED IN THE ONSET OF LIMB GIRDLE MUSCULAR DYSTROPHY 1-C, CONFERS AN IMMATURE SIGNATURE TO C2C12 MYOBLASTS

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Abstract

Mutations in the Cav-3 gene are associated with a subset of skeletal muscle disorders, as Limb Girdle Muscular Dystrophy, Rippling Muscle Disease, Distal Myopathy and HyperCKemia. In this work we demonstrated that the expression of the dominant-negative Cav-3/P104L form, which is involved in the onset of Limb Girdle Muscular Dystrophy 1-C, impairs the *in vitro* myogenesis of C2C12 cells by reducing the levels of MyoD and causing persistent Myf-5 protein levels. The administration of Insulin-like Growth Factor 1 (IGF-1) or the increase of follistatin, as obtained after treatment with the histone deacetylase inhibitor Trichostatin A (TSA), significantly improved the differentiation of myoblasts regardless of Cav-3/P104L mislocalization, as demonstrated by partial restoration of proper MyoD and Myf-5 levels.

In summary, these data suggest that the expression of Cav-3/P104L confers a molecular signature typically recognized for immature myoblasts and that IGF-1 or TSA treatments might be effective to counteract the cell damage induced by Cav-3 loss of function.

MICROTRANSPLANTATION OF ACETYLCHOLINE RECEPTORS AND CA^{2+} CHANNELS FROM NORMAL OR DENERVATED RAT SKELETAL MUSCLES TO FROG OOCYTES

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Cell membranes, carrying neurotransmitter receptors and ion channels, can be "microtransplanted" into frog oocytes. This technique allows a direct functional characterization of the original membrane proteins, together with any associated molecules they may have and still embedded in their natural lipidic environment. This approach has been demonstrated to be very useful to study neurotransmitter receptors and ion channels contained in cell membranes isolated from human brains. In this work we examined the possibility of using the microtransplantation method to study acetylcholine receptors and calcium channels from normal and denervated rat skeletal muscles. We found that transcription of the subunits that make the acetylcholine receptors was increased 10-100 times after denervation. Accordingly the acetylcholine-currents generated by oocytes injected with denervated muscle membranes increased and, after 4 weeks denervation, they were about 50 times greater than the currents elicited by oocytes injected with membranes from innervated muscles. The oocytes also incorporated Ca^{2+}channels from the same tissues. Since our results show that oocytes injected with skeletal muscle membranes efficiently incorporate functional acetylcholine receptors and Ca^{2+}channels, we conclude that the microtransplantation approach make it possible to investigate further the receptors and ion channels of human muscle diseases.

CLUES TO THE FORMATION OF CORES IN MOUSE MODELS OF MALIGNANT HYPERTHERMIA AND CENTRAL CORE DISEASE

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Malignant hyperthermia (MH) and central core disease (CCD) are closely related diseases of skeletal muscle linked to mutations in the ryanodine receptor (RYR1) gene, the Ca^{2+} release channel of sarcoplasmic reticulum (SR). Here we characterized the structural and temporal aspects involved in core formation in skeletal muscle of two heterozygous knock-in mice harbouring human mutations: the $RYR1^{Y522S/wt}$ mutation, which is linked to MH susceptibility (MHS) with cores, and the $RYR1^{I4895T/wt}$ mutation, associated with CCD. The former is part of one class of mutations referred to as *leaky mutations*, whereas the latter is an *EC uncoupling mutation*. We discovered that in $RYR1^{Y522S/wt}$ fibers, focal mitochondrial damage is the first detectable change observed in muscle fibers (at 2 months), which is followed by disruption of nearby SR, T-tubules, and which will finally lead to extended regions of myofibril contractures (*contracture cores*) and complete disarrangement (*unstructured cores*). On the contrary, in $RyR1^{I4895T/wt}$ mice the first detectable alteration involves directly the myofibrils (Z line streaming). We suggest that mitochondrial disruption in $RYR1^{Y522S/wt}$ fibers is initiated by abnormal Ca^{2+} leak through the mutated RYR1 channels, whereas the mechanism of core formation in case of *uncoupling mutation* is still obscure.

AUTOPHAGY IS DEFECTIVE IN COLLAGEN VI MYOPATHIES AND ITS INDUCTION PROTECTS AGAINST MUSCLE DEGENERATION

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Autophagy is an evolutionarily conserved process which is crucial in the turnover of cell components both in constitutive conditions and in response to starvation. Clearance of damaged organelles is important to preserve cell function, and removal of dysfunctional mitochondria by autophagy prevents cell death. Alteration of the lysosomal degradative process with excess of autophagic vesicles plays a causative role in certain storage diseases and vacuolar myopathies. However, no causal link between defective autophagy and muscular dystrophy was reported so far. Here we show that collagen VI deficiency, which causes Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD), displays a defect in autophagosome formation. Muscles deficient in collagen VI are characterized by the presence of dysfunctional mitochondria and spontaneous apoptosis, which ultimately to myofiber degeneration[11,12]. We found that the persistence of abnormal organelles and the ensuing apoptosis are caused by impaired autophagy. Indeed, skeletal muscles of collagen VI knockout (*Col6a1*$^{-/-}$) mice display decreased LC3 lipidation, which matches the lower induction of Beclin1 and Bnip3 and the lack of autophagosomes after starvation. Forced overexpression of Beclin1 in *Col6a1*$^{-/-}$ muscle was able to trigger autophagy and decrease apoptosis. Moreover, activation of autophagy by prolonged starvation or by low-protein diet led to a marked recovery of the dystrophic phenotype. Analysis of human muscle biopsies showed reduced levels of Beclin1 and Bnip3 in UCMD and BM patients. Thus we have identified the first muscular dystrophy whose pathogenic mechanism involves a failure in the autophagic machinery.

FLUOXETINE BLOCKS MYOTONIC RUNS AND REVERTS ABNORMAL sEMG PATTERN IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

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Objectives

To verify the effects of a muscular injection of fluoxetine both on needle EMG 'myotonic runs' and on the surface EMG pattern in patients affected by Myotonic Dystrophy.

Methods

Needle EMG recording

We performed needle EMG recordings on the tibialis anterior or opponent thumb muscle in 3 patients. The resting electrical activity and the myotonic discharge were detected before and after the local injection of 100 µl of fluoxetine.

Surface EMG recording

A motor point stimulation protocol was carried out on the tibialis anterior of 3 patients. Stimulation consisted of 10-s, 15 Hz pulse train, 0.1 msec in duration. A supramaximal stimulation was applied and the surface myoelectric signal was recorded. The averaged rectified value of the amplitude was evaluated before and after the intramuscular injection of 300 µl of fluoxetine.

Results

Needle EMG

The injection of fluoxetine induced a clear-cut reduction of the basal electrical activity and made it impossible to evoke 'myotonic runs' in all the patients tested. The reversibility of the effect of the drug was checked in 2 patients who exhibited a partial recovery of myotonic EMG activity 40 minutes after the administration.

Surface EMG

The patients showed the typical decreasing ARV pattern before the drug administration; the fluoxetine injection consistently provoked a clear and complete recovery of the normal increasing ARV curve.

Conclusions

We showed, for the first time, that the local application of fluoxetine produces functional modifications in DM muscle electrical properties. The relevance of this study consists in the introduction of fluoxetine, a well-known and largely used drug, as a tool for investigating further therapeutical approaches in this disease.

SUBCLINICAL MYOPATHY IN PATIENTS AFFECTED WITH EARLY STAGE COLORECTAL CANCER

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We evaluated skeletal muscle biopsies from asymptomatic patients affected with early stage colorectal cancer, in order to identify pathologic features which may be indicative of tumor associated muscle disorders.
Morphometry, histochemichemistry, and immunohistochemistry with anti-NCAM and anti-MHCemb antibodies were performed on biopsies of the *rectus abdominis* muscle obtained from 10 patients during laparoscopic tumor resection and 10 healthy subjects as controls. In patients' biopsies, we observed a surprising high percentage of myofibers with internal nuclei compared to controls (9.15±8.9 vs 0.19±0.37, mean±SD $p<0.001$), together with 18.6% of muscle atrophy compared to controls ($p<0.001$). In the 30% of patients, myofibers expressing MHCemb have been identified (0.4±0.5 fibers/mm^2, mean±SD), while in the 50% of patients NCAM positive fibers have also been detected (0.7±1.1 fibers/mm^2, mean±SD) suggesting that investigated muscle biopsies exhibit evidence of muscle injury/regeneration and/or denervation. In control biopsies none MHCemb and only one NCAM positive muscle fibers have been detected.
These findings indicate that patients' biopsies display early signs of myopathy. Follow-up studies of this patients' cohort will elucidate the clinical relevance of our observation, and further analyses investigating the molecular mechanism underlying this early cancer-associated myopathy, will hopefully provide some pathogenetic clues leading to the identification of potential therapeutic targets to prevent tumor cachexia.

FUNCTIONAL ECHOMYOGRAPHY AND QUANTITATIVE COLOR 3D CT IMAGING OF HUMAN LONG-TERM DENERVATED MUSCLE. MONITORING MUSCLE DENERVATION-ATROPHY AND RECOVERY BY FES

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New non-invasive, repeatible methods were developed to monitor bed-side contractile and structural characteristics of human muscle by ultrasonography and by computer tomography images and special computational tools. Ultrasonography of human denervated muscle demonstrates that the changes in relaxation time and in the blood perfusion are pathognomonic of long term denervation. Changes in tissue composition within the muscle were visualised associating Hounsfield Unit values of normal or atrophic muscle, fat and connective tissue to different colors. The minimal volumetric element (voxel) is ten times smaller than the volume analyzed by needle muscle biopsy. The results of this microstructural analysis are presented as the percentage of different tissues (muscle, loose and fibrous connective tissue, fat) in the total volume of the rectus muscle and displaying the first cortical layer of voxels that describe the muscle perimysium directly on the muscle 3D reconstruction.

SESSIONE 4

MUSCLE PLASTICITY AND EXERCISE

CHANGES IN SKELETAL MUSCLE ELECTRO-MECHANICAL CHARACTERISTICS DURING FATIGUING ISOMETRIC CONTRACTIONS IN TRAINED AND UNTRAINED MEN

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The differences in finger flexor (FF) muscles motor control were determined in eleven trained athletes (climbers) and ten sedentary controls. Maximal voluntary contraction (MVC) was assessed and after 10 min of rest, an isometric contraction at 80% MVC was performed until exhaustion. During contraction, the surface electromyographic (EMG), mechanomyographic (MMG) and force signals were recorded, from which the root mean square (RMS), mean frequency (MF) and variation coefficient (VC) as an index of force control accuracy were then calculated. Athletes showed significantly higher MVC (+46%, $P<0.05$) and MVC per unit of muscle-plus-bone area (MVC/MBA +13%; $P<0.05$). Endurance time at 80% MVC was 43% longer in athletes than in controls (33±4 vs 23±5 s, respectively; $P<0.05$). VC throughout the first 20 s of contraction was lower in athletes ($P<0.05$), which showed also significantly higher values of EMG RMS, MMG MF and MMG RMS throughout the whole fatiguing exercise. Maximum force results and EMG, MMG and force signals analysis during constant-load contraction at 80% MVC revealed significant differences in muscle characteristics and motor control between the two groups. In particular, athletes showed stronger and more efficient FF muscles, which were capable to sustain the requested effort (80% MVC up to exhaustion) for a longer period of time and with a steadier force output. These results suggest a shift of athletes (climbers) muscles toward faster and more resistant MUs due to years of high-intensity isometric contractions typical of this sport activity.

FREEDIVING PERFORMANCE ENHANCEMENT THANKS TO ACUPUNCTURE AND TRADITIONAL CHINESE MEDICINE

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Aim

To verify the improvement in apnea times, if any, induced by acupuncture and other traditional chinese medicine (TCM) methods among elite breath-hold divers.

Methods

All subjects volunteered and signed an informed-consent form for the study.

Twenty volunteers were enrolled (15 males and 5 females), aged 23 to 56 yr (39.2 +/- 8.69, mean and SD differences), height 162 to 184 cm (173.31 +/- 6.49), weight 52.5 to 95 Kg (72.13 +/- 10.92), apnea experience: advance-elite.

The tests of static apnea were completed in Padua (Italy) from February to April '09.

Acupuncture-divers (QiDivers) received: guasha and cupping at Huatuojiaji acupoints, acutaping and hot capsicum patches at the 'lower warmer' area of the TH meridian and microstimulation for extended periods (up to 7 days) at Shenmen auricular point and at the following points of the TCM nomenclature: BL-13, CV-17, GB-41, GV-13, GV-19, KI-6, LU-6, LU-7, LU-9, PC-9, TH-3, TH-5, TH-7.

In our single-blind study we used an ABCD-like (Adjustable Biased Coin Design) randomization method between Control and QiDiver group.

Measurement comprised: Arterial Pressure, Heart Rate pre and post test, apnea times.

Eight subjects could not complete the test for a variety of causes, unrelated to acupuncture.

Complete data were collected in twelve subjects (8 males and 4 females)

QiDiver group n=7 and Control group n=5.

Results

In QiDivers, static apnea times increased by 19.78% +/-12.18% SD (ranging from 6.81 to 38.27 % improvement), while the control group recorded 0.14% +/- 2.15 SD, ranging from -3.38 to + 1.95%. The difference between the two groups was significant ($p<0.013$).

Discussion

Best results were obtained in QiDivers, especially up to 7 days after the initial treatment and at a time when at least ≥ 65% of long term acupuncture needles were still kept in place since the beginning of the acupuncture insertion.

MUSCLE SYNERGIES AFTER STROKE AND THE EMERGENCE OF NEW MOTOR STRATEGIES

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Muscle activation patterns characterizing a given motor task are almost invariant across subjects. This suggests the presence of underlying rules somehow guiding the selection of a particular muscle activation pattern to perform a given task. “Muscle synergies” intended as the coordinated recruitment of muscle groups with specific activation profiles have been recently proposed as a possible solution to the redundancy management issue. Synergies consist of basic muscle activation patterns under supraspinal surveillance. Thus any given muscle activation profile is dependent upon two terms: a synergy (S) describing the behavior of the so-called central pattern generator (CPG) and a control function (P) that cumulatively describe the integrated sensory-motor influence on the CPG. To this respect, locomotion is a good model to evaluate the relevance of the terms S and P. The aim of this study was to test the hypothesis that in stroke patient there is an invariance of the synergies during locomotion with respect to a control population: being stroke a central lesion, spinal movement modules would theoretically be intact in stroke patient.
10 early (<2 weeks) stroke patients and 10 healthy age matched control subjects participated to the study. Both subjects and patients performed a five 6 meters long walking trials while sEMG was recorded from 32 muscles all over the body.
We found that 4 muscle synergies could reconstruct more than 85% of sEMG variance in both controls (according to literature) and patients. No differences in extracted synergies were found between left and right side in healthy controls, and a difference was found between affected and unaffected side in patients. Patients were also different from controls in both affected and unaffected side.
These results indicated that in stroke patients both terms S and P are affected. These stands for a profound rearrangement of central motor command involving also the unaffected side.

EFFECTS OF EIGHT WEEKS OF VIBRATION TRAINING IN SENIOR SPORTSMEN

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Purpose of this study was to examine the effects of vibration training on maximal isometric torque and force development and on muscle biopsies in senior sportsmen. Isometric peak torque both at 1 Hz and 15 Hz vibration frequency increased.The results of force development showed a slight increase at the 1 Hz training in measured time frames from 0 to 50 ms, but without statistical significance. The 15 Hz training showed no significant changes of force development. Muscle biopsies show that the muscles of these well trained senior sportsmen contain muscle fibers 35 % larger than those of sedentary elderly and, unexpectedly, 10 % larger than those of young sportsmen. Despite one year of heavy resistance and vibration trainings no evidence of muscle damage or denervation/reinnervation could be observed by light microscopy analyses, ATPase histochemistry and immunohistochemistry using anti-N-CAM or anti-embMHC antibodies.

OXIDATIVE STRESS IN THE DENERVATED MUSCLE

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Partial or total denervation of skeletal muscles occurs in a number of congenital or acquired diseases and conditions, including ageing and traumas. In an experimental model of surgical hind limb denervation in rats, we demonstrate here that oxidative stress takes place. In fact: i) ROS are formed; ii) oxidation of membrane lipids occurs; iii) ion channels and pumps display a loss of activity, likely owing to oxidative damage; iv) all the above mentioned events increase with denervation time; v) mRNA abundance of cytoprotective and anti-oxidant proteins (Hsp70, Hsp27, Sod1, Catalase, Gpx1, Gstm1) is increased in 15-day and, to a lesser extent, in 3-month denervated muscle; vi) SOD1 enzymatic activity and HSP70i protein increased in the denervated muscle. Further data, obtained from mRNA and protein analysis, suggest that the mitochondria is the most likely candidate as cellular source of ROS, since denervation induces an unbalance in the amount of mitochondrial enzymes involved in the respiration process and electron transport, particularly a decrease in Complex I components. In conclusion, an anti-oxidant therapeutical strategy seems to be advisable in the many medical conditions where the nerve-muscle connection is impaired, to aid healing and regeneration processes.

SKELETAL MUSCLE ADAPTATIONS TO MODERATE TRAINING IN A RAT MODEL

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Skeletal muscles are deeply modified by physical exercise; however, most studies examined the effects of strong rather than moderate training.

Six young adult male Sprague Dowley rats were trained to run on a rodent treadmill, to reach ~ 60% VO_{2max} in 14 weeks. At the end of the training period the rats were sacrificed. Hind leg muscles were isolated, weighed, and quickly frozen before being studied. The study compared Soleus muscle – a posture muscle mostly made up of slow (red) fibers, and two muscles actively involved in the exercise, Extensor Digitorum Longum (EDL) and Tibialis Anterior (TA), mostly made up of fast (white) fibers.

Exercise training increased the muscle mass of only TA and EDL; it altered a number of pathways, including those related to metabolic adaptation, to oxidative stress response, to regenerative repair, and to the increase in the percent of slow-type fibers in TA and EDL.The Sarcoplasmic Membranes of the Soleus muscle were altered by oxidative stress, as a likely consequence of the higher amount of mitochondria and, hence, of ROS production, in these muscles. On the other hand, RT-PCR analysis of mRNA abundance showed that some small cytoplasmic inflammatory cytokines were expressed at a higher degree only in fast muscle cells of trained rats.

In the trained rats, both kind of muscles displayed an increase of the chaperon protein HSP70i.

Some of these modifications apparently include reactions to cell injuries or to oxidative stress, however they may endow the muscle with additional resistance towards more severe damage.

PROTEOME ANALYSIS OF SKELETAL MUSCLE ADAPTATIONS TO 8 and 35-DAYS BED REST

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Head down bed rest (BR) is the most widely used model of microgravity that mimes the adaptations occurring during spaceflight. In order to study the muscle protein adaptations to BR, ten healthy sedentary subjects were subjected to 35-days BR; needle biopsy samples of the vastus lateralis muscle were collected pre-BR, post-8-days and post-35-days BR. The biopsy samples were immediately frozen and used to determine myosin heavy chain (MHC) isoform distribution and to perform proteomic analysis by two-dimensional gel electrophoresis. MHC isoform distribution showed a shift in the direction MHC-1 → MHC-2A → MHC-2X as expected on the basis of previous findings in rat models of disuse. The proteomic analysis was performed comparing 2D-gels pre-BR with 2D-gel post 8 and 35 days; more than 800 protein spots on each gel were detected by fluorescent staining and several differentially expressed proteins were identified. Protein changes concerned antioxidant defence systems, energy metabolism, structural and transport proteins. The antioxidant defence systems were down-regulated at both 8 and 35 days suggesting that oxidative stress occurs in disused human muscle as previously showed in rat models. The adaptations of oxidative and glycolytic enzymes to BR suggests a general downsizing of energy metabolism. The down-regulation of seven structural proteins and three transport proteins indicates increased protein degradation. This study supports the idea of an increased rate of protein degradation caused by both oxidative stress and energy metabolism in disuse atrophy in humans, although the relationship between the two phenomena and their relative role are still unclear.

IN VIVO AND IN VITRO ANALYSIS OF HUMAN SKELETAL MUSCLE FOLLOWING NEUROMUSCULAR ELECTRICAL STIMULATION

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Neuro-muscular electrical stimulation (NMES) has been used as a tool for muscle strength improvement in athletes and in rehabilitation programs in geriatric, cardiovascular and orthopaedic medicine. NMES has shown to increase muscle mass, strength and exercise capacity (*in vivo* studies).
In this study 14 young (18-35 years of age) healthy, male subjects were subjected to 24, 18-min sessions of isometric (bilateral) NMES of the quadriceps muscle over a period of 8 weeks with 3 sessions per week. Needle biopsies were taken from the vastus lateralis muscles pre- and post-training. *In vivo*, analysis of MVC (maximal voluntary contraction), of the thickness of the vastus lateralis (VL) muscle by ecography and of muscle fibre conduction velocity and motor unit recruitment by EMG by an array of surface electrodes were performed. *In vitro*, CSA, specific force and unloaded shortening velocity of identified types of muscle fibres were determined: 420 fibres (on average 14 fibres per subjects) were analyzed both pre- and post- NMES. MVC contraction and VL thickness increased in all subjects post-NMES compared to pre-NMES of 16% and 14% respectively.
Muscle fibres CSA significantly increased post-NMES compared to pre-NMES. MHC isoform distribution shifted in the direction MHC-2X > MHC 2A > MHC-1. The analysis of specific force and unloaded shortening velocity of identified types of muscle fibres and of the results of the electromyographic analysis are ongoing and will be presented at the meeting.

MOLECULAR AND CELLULAR ANALYSIS OF SKELETAL MUSCLE UNDER SEVERE HYPOXIC CONDITIONS AND PHYSICAL ACTIVITY

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The high altitude hypobaric hypoxic environment is known to induce in humans several metabolic, ventilatory and muscular adaptations depending on time and level of exposure. The gene expression profiles is a powerful tool to analyze the gene regulation under specific stimulus. The aim of this contribute is to analyse the skeletal muscle transcriptoma after strenuous physical activity under hypoxia condition. Biopsies from vastus lateralis muscles were obtained from six male volunteers (40 ± 14 years old) before and upon the return from the Himalayan Expedition during which they were chronically exposed to hypoxia living for about 30 days at 5000 m. Needle-biopsies (about 15 mg) were used for RNA isolation followed by amplification and labeling. A high-density oligonucleotide microarray technique was used. The human oligonucleotide gene set consisting of 21,329 (70-mer) oligonucleotides (Operon version 2.0), designed on the basis of the Human Unigene clusters. Arrays were scanned and recorded fluorescence intensities were subjected to LOWESS normalization. The expression of each gene was defined as the log base-2 of the ratio between the intensity of cyanine-coupled aaRNA from post-expedition and those from pre-expedition samples. Differentially expressed genes were selected using a permutation test procedure "Significance Analysis of Microarrays" which defines genes with a computed score larger than the threshold value. The false discovery rate associated with the given threshold was additionally calculated from permutation data. Genes that appeared to be significantly affected were further evaluated to elucidate the mechanisms by which hypoxia associated to strenuous physical activity could influence the skeletal muscle transcriptome. Different genes with well-known muscle function received particular attention and were classified as (i) genes of energy metabolism (ii) genes dealing with muscle plasticity.

EFFECT OF STRENGTH TRAINING ON CONTRACTILE PERFORMANCE AND MHC ISOFORM COMPOSITION IN HUMAN UPPER LIMB MUSCLE FIBRES

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Strength or resistance training is frequently used to improve muscle mass and performance. Although there are many studies on the relation between training protocols and fibre adaptations in lower limb muscles, there are relatively few data available on changes of fibre type, size and performance in upper limb muscles.

This study aimed to analyse at muscle fibre level the effect strength training of shoulder muscles in 18 healthy subjects (9 men and 9 women) who trained three times a week for 2 months. These subjects were further divided in 2 groups assuming different amount of proteins.

Needle biopsy samples were collected from upper limb muscle (Longissimus Dorsi) at the University of Pavia and small bundles were transferred to the Muscle Biophysics Laboratory in Padova. Single muscle fibres were dissected and the following parameters were determined: cross sectional area (CSA), isometric tension, i.e. isometric force/CSA during maximal activations (Po), and MHC isoforms composition.

The results obtained comparing pre- and post-training samples showed that 1) CSA increased significantly although large variations in CSA increase were detectable among different subjects, without any clear relation with the protein intake, 2) isometric tension (Po) increased significantly, i.e. the increase in force was greater than the increase in CSA, 3) a trend towards a MHC transition from fast to slow was also detectable.

PROTEIN KINASE C THETA IS INVOLVED IN THE CONTROL OF CLC-1 CHLORIDE CHANNEL CONDUCTANCE IN MOUSE FAST AND SLOW SKELETAL MUSCLES

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In skeletal muscle resting chloride conductance (gCl) sustained by the ClC-1 chloride channel controls the membrane electrical stability and its reduction can produce myotonia-like symptoms. We showed that gCl is negatively regulated *in vitro* by Protein Kinase C (PKC). Slow-twitch muscles are characterized by lower value of gCl compared to fast-twitch muscles, due in part to a higher basic activity of PKC (Pierno et al. J Physiol 584:983, 2007). In skeletal muscle different PKC isoforms have been found to be expressed, including PKCtheta (Serra et al. J Cell Physiol 196:89, 2003). To better characterize the modulation of gCl by PKC and the possible involvement in muscle diseases we measured resting gCl and muscle excitability in soleus and EDL muscles of PKCtheta-KO mice (Sun et al. Nature 404:402, 2000) by using the two intracellular microelectrode technique. We found a significant increase of gCl in soleus muscle of KO mice with respect to control, being 1876±53 □S/cm^2 (n=41) and 1356±37 □S/cm^2 (n=19), respectively. A slight increase of gCl was also found in EDL muscle. Muscle excitability was reduced accordingly to gCl increase. Chelerythrine, unspecific PKC inhibitor, further increase gCl (by 25%) in soleus muscle showing that other PKC isoforms are involved in the control of gCl. In contrast chelerythrine have slight effect on EDL of PKC-KO mice. Fluvastatin, which is able to activate PKC (Pierno et al. Br J Pharmacol 156:1206, 2009), reduce gCl in EDL muscle by 30% confirming that also in EDL other PKC isoforms contribute to ClC-1 channel modulation. (Supported by ASI-OSMA)

SESSIONE 5

MYOGENESIS, MUSCLE HOMEOSTASIS AND REGENERATION

MITOGENIC EFFECT OF CERAMIDE 1-PHOSPHATE IN MOUSE MYOBLASTS

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Bioactive sphingolipids are represented by a large family of compounds capable of eliciting a huge variety of biological responses. Most of the key elements of this lipid category such as sphingosine, sphingosine 1-phosphate, ceramide have been recently emerged as important regulators of fundamental skeletal muscle biological processes including myoblast proliferation and myogenesis (1). However, no information is presently available on the biological effects exerted in these cells by ceramide 1-phosphate (C1P), which has been more recently identified as cell mediator.

Here we provide the first experimental evidence that C1P acts as mitogen in C2C12 myoblasts. C1P (15 µM) was found to stimulate DNA synthesis measured by determining radioactive thymidine incorporation as well as by assaying MTT dye reduction. Moreover, analysis by FACS revealed that challenge of myoblasts with C1P accelerated cell cycle. Specific inhibitors of PI3K or ERK1/2 signaling pathways strongly attenuated the C1P action, whereas pertussis toxin treatment, or inhibition of p38 MAPK or JNK were uneffective. In agreement, C1P was responsible for a rapid phosphorylation of Akt and ERK1/2. Thus, in this study a novel important biological action of C1P in myoblasts has been highlighted, which possibly will be exploited in the future to enhance skeletal muscle regeneration.

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ELUCIDATING THE MECHANISM OF S100B-DEPENDENT REGULATION OF MYOBLAST DIFFERENTIATION

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Embryonic myogenesis and muscle regeneration are tightly controlled processes resulting from a balanced succession of proliferation/migration and differentiation of myoblasts and satellite cells (SCs), respectively. Some extracellular factors activate myoblasts/SCs and stimulate their proliferation/migration, while other factors stimulate myoblast/SC differentiation. S100B, a Ca^{2+}-binding protein of the EF-hand type, was shown to exert anti-myogenic and mitogenic effects in high-density (HD) myoblast cultures via inhibition of the myogenic p38 MAPK and activation of the mitogenic ERK1/2, respectively. However, the receptor mediating these effects was not identified. We show here that in HD myoblast cultures S100B binds bFGF thereby enhancing bFGF receptor 1 (FGFR1) mitogenic and anti-myogenic effects. S100B also binds RAGE (receptor for advanced glycation end products) and recruits it into a RAGE/S100B/bFGF/FGFR1 complex on apposed cells with resultant enhancement of FGFR1's mitogenic signaling and blockade of RAGE's promyogenic signaling. An S100B/bFGF/FGFR1 complex also forms in $RAGE^{-/-}$ myoblasts leading to enhanced proliferation and reduced differentiation. However, at an early differentiation stage of low-density (LD) myoblast cultures S100B cannot bind FGFR1-bound bFGF on the same cell; S100B engages RAGE leading to stimulation of proliferation by activating ERK1/2 and activation of the myogenic program by stimulating p38 MAPK. Yet, prolonging the treatment of LD myoblast cultures with S100B results in RAGE/S100B/bFGF/FGFR1 complex formation and stimulation of proliferation and inhibition of differentiation similar to HD myoblasts. These results suggest that S100B regulates myogenic differentiation by differentially activating RAGE and FGFR1 in a cell density- and bFGF-dependent manner.

HMGB1/RAGE MODULATES PAX7 EXPRESSION IN MYOBLASTS VIA P38 MAPKDEPENDENT UPREGULATION OF MYOGENIN

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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 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 either the blockade of HMGB1/RAGE activity or inactivation of p38 MAPK results in downregulation of myogenin expression and upregulation of Pax7 expression in differentiating myoblasts, suggesting that RAGE might repress Pax7 expression via p38 MAPK-dependent induction of myogenin. Also, whereas myogenin binds to 3 out of the 6 myogenin recognition sites in the Pax7 promoter in GM, it binds to all of them in differentiation medium (DM), as investigated by chromatin immunoprecipitation. Consistently, transient transfection of myoblasts with myogenin results in repression of Pax7 (mRNA and protein) expression in GM, and, conversely, knocking down myogenin results in upregulation of Pax7 (mRNA and protein) expression in DM. RAGE is expressed in myoblasts in GM and its expression levels increase in DM, and knocking down Pax7 results in enhanced RAGE expression. Our data suggest that: 1. RAGE has a role in repression of Pax7 expression via p38 MAPK-dependent induction of myogenin, a prerequisite for satellite cell differentiation; 2. Pax7 modulates RAGE expression; and 3. repression of RAGE expression and/or function in SCs might contribute to Pax7-dependent rhabdomyosarcomagenesis.

EFFECTS OF DELETION OF RAGE IN MUSCLE REGENERATION: PRELIMINARY OBSERVATIONS

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RAGE (receptor for advanced glycation end products), a multiligand receptor of the immunoglobulin superfamily, plays an important role in innate immunity and in endothelial cell activation and vascular smooth muscle proliferation in atherosclerosis and inflammation (*J Clin Invest* 108:949-55, 2001; *J Mol Med* 83:876-86, 2005). RAGE is expressed in several cell types during development, repressed at completion of development and re-expressed in the course of certain pathological conditions (*J Clin Invest* 108:949-55, 2001; *J Mol Med* 83:876-86, 2005). The expression of RAGE in several cell types during development suggests that RAGE might not be regarded simply as a transducer of inflammatory cues. RAGE is expressed in skeletal muscle fibers during prenatal and postnatal development being repressed thereafter (*Mol Cell Biol* 24:4880-94, 2004). Also, RAGE is expressed in proliferating and differentiating myoblasts, and once activated by its ligand, HMGB1, it transduces a promyogenic, pro-apoptotic and anti-proliferative signal in myoblasts and rhabdomyosarcoma cell lines (*Mol Cell Biol* 24:4880-94, 2004; *J Biol Chem* 281:8242-53, 2006; *Am J Pathol* 171:947-61, 2007). We show that following damage, RAGE becomes expressed in satellite cells (SCs) and in regenerating myofibers (likely as a result of fusion of RAGE-expressing SCs), becoming repressed at completion of regeneration, and that deletion of *RAGE* results in an elevated SC basal number, a strong infiltration of undamaged tissue with activated SCs at early regeneration phases, and retarded muscle regeneration. Also, primary $RAGE^{-/-}$ myoblasts exhibit enhanced proliferation and defective differentiation compared to wild-type myoblasts supporting the possibility that RAGE might physiologically contribute to muscle regeneration.

FUNCTIONAL ANTAGONISM BETWEEN P38 AND MYOSTATIN PATHWAYS IN RHABDOMYOSARCOMA CELLS

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Rhabdomyosarcoma (RMS) tumors are believed to arise from mesenchimal cells, which are genetically committed to myogenic lineage, but fail to complete the differentiation program. Among the molecular hallmarks identified in RMS cells, elevated myostatin expression and reduced p38 phosphorylation contribute to the delay of myogenic program in a common subsets of RMSs. In this work, we have investigated the molecular relationship between the p38 signalling and the myostatin pathway during physiological myogenesis and rhabdomyosarcomagenesis. Delivery of a dominant-negative form of Activin receptor type IIb (dnActRIIb), which has been reported to induce muscle doubling in mice by antagonizing mtyostatin, reduced cell proliferation and promoted terminal differentiation in RMS embryonal RD cells. This effect was underlined by abolition of the canonical Smad-2/3 phosphorylation pathway and inactivation of myostatin signalling, and was paralleled by remarkable increase of p38 phosphorylation. Block of p38 activity, as obtained either via pharmacological blockade or via expression of the upstream dominant-negative MKK6 kinase form, completely reversed the ability of dnActRIIb to enhance differentiation, whereas induction of p38 activation, as obtained via TPA administration or expression of the constitutively activated MKK6 kinase form, further improved the dnActRIIb induced RD differentiation. Overall, our findings uncover that both myostatin and p38 pathways are not mutually exclusive but instead converge to each other to control rhabdomyosarcoma differentiation.

CARDIOMYOCYTES DIFFERENTIATION IS IMPROVED IN COCULTURE WITH SKELETAL MYOBLASTS OVEREXPRESSING RELAXIN

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Exogenous stem cell transplantation into the post-infarcted heart has been suggested to be a new therapy for damaged hearth. We studied if differentiation of mouse neonatal cardiomyocytes was affected in vitro by the presence of mouse skeletal myoblasts C2C12, wild type or engineered to produce the cardiotropic hormone relaxin.. After 24 h in culture, the cardiomyocytes spontaneously formed beating clusters. The cardiomyocytes in the cluster were connected by connexin-43 gap junction (GJ) and expressed distinctive electrical cardiac features: typical action potential and inward voltage-dependent Na+, T- and L-type Ca2+ currents, outward and inward rectifier K+ currents, If current. The cardiomycyte clusters were connected by less differentiated stripe-like cardiac cells. These immature cells may represent a pathway allowing a synchronized functionality between surrounding clusters. The copresence of the myoblasts greatly increased cardiomyocytes differentiation, as estimated by the changes of the action potential, resting potential, beating rate and ionic channels kinetics voltage dependence. The functionality of the GJ between cardiomyocytes of the cluster was improved as well as of those between cardiomyocyte and stripe-like cells. The secretion of relaxin by the engineered myoblasts enhanced the cardiomyogenic differentiation towards mature cells. These findings highlight the possibility that grafted myoblasts and cardiotropic factors such as relaxin may regulate the regeneration and communication of resident immature cardiac cells improving their differentiation.

SKELETAL MUSCLE ENGINEERING: SCAFFOLDS AND RECOMBINANT PROTEINS

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Abstract

Hepatocyte Growth Factor (HGF) is a pleiotropic cytokine of mesenchymal origin that mediates cell proliferation, survival, motility and morphogenesis acting with its high affinity receptor, the tyrosine kinase Met. Following muscle injury, autocrine HGF-Met stimulation promotes activation and early division of satellite cells expressing both HGF and Met. Many attempts have been made so far to construct bioartificial muscles; in this context, developing polymeric scaffolds revealed promising results. One of the suitable biomaterial for these studies is DegraPol, a biodegradable block copolymer processed by electrospinning. DegraPol scaffold slides were characterized with reference to their morphological, degradative and mechanical properties. C2C12, L6 and human primary satellite cells adhered, proliferated and fused on differently coated and uncoated DegraPol slides [1]. Magic-F1 (Met-Activating Genetically Improved Chimeric Factor-1) is an HGF-derived, engineered protein that contains two Met-binding domains and protects myogenic precursors against apoptosis, increasing their fusion ability and enhancing muscular differentiation. Hemizygous and homozygous Magic-F1 transgenic mice displayed constitutive muscular hypertrophy and accelerated muscle regeneration following injury [2]. Preliminary data show a possible implication of the engineered protein in development of the vascular network, increasing the capillary vessel number, a phenomenon that could guarantee a possible application of Magic F1 in muscular tissue engineering. The membranes did not show any toxic residuals and exhibited satisfactory mechanical properties. Together the results of this study provide significant evidence of the suitability of electrospun DegraPol membranes as scaffolds for skeletal muscle tissue engineering using Magic F1 expressing myoblasts.

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DEVELOPMENT AND CHARACTERIZATION OF AN EX VIVO- MUSCLE ENGINEERED TISSUE (X-MET) FOR CELL/GENE THERAPY

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Cell-based therapies for severe muscle disease still struggle by the difficulty in obtaining a sufficient number of autologous myoblasts and by their inefficient incorporation into the host muscle. Poor survival of injected cells, minimal migration from injection site and rapid senescence of the surviving population have failed to produce satisfactory protocols of muscle regeneration that might be considered for therapeutic purposes. A role for stem cells in adult mammalian regeneration has been implicated by recent studies, demonstrating the homing of bone marrow-derived haematopoietic stem cells to sites of injury and subsequent differentiation into multiple tissue types. However, transplantation of bone marrow-derived stem cells into injured/pathological cardiac and skeletal muscle had so far a limited impact on muscle cell replacement.

To overcome this problem, remarkable progress have been recently done in tissue engineering technology toward the goal of creating organoids in vitro from cells and cellular scaffolding. Tissue engineering represents, therefore, the novel scientific approach that attempts to mimic neo-organogenesis to “produce” ex-vivo living tissue.

Conventional muscle-tissue engineering techniques employ artificial scaffolds, which interfere with the ability to measure and control contractile properties during tissue growth and do not mimic the complex architecture that characterizes skeletal muscle tissue.

In contrast, here we propose the generation of a 3-dimensional skeletal muscle tissue construct, named Engineered Tissue Muscle (X-MET), without the support of any scaffold and of any specific chemical layer.

The final goal of the project is to transplant the X-MET in recipient pathological models of both skeletal and cardiac muscle and verify whether the transplanted organoid is able to work in vivo and to rescue the muscle function.

PHARMACOLOGICAL RE-PROGRAMMING OF MUSCLE-DERIVED PRIMARY CELLS

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Satellite cells are considered to be the main source of myogenic progenitors in post-natal skeletal muscle. However, their use in cell therapy for muscle disorders is limited because these cells cannot be delivered through circulation and they are rapidly exhausted in severe myopathies. Pharmacological compounds used to modify cell surface expression have been exploited to develop innovative cell therapy strategies for muscular dystrophies for their ability to induce a reversible blockade of the differentiation program, thus allowing treated cells to re-differentiate when placed in the right microenvironment. We have previously shown that Bisperoxovanadium (BpV), a phospho-tyrosine phosphatase inhibitor, induces myogenic cells to acquire a gene expression profile and a differentiation potential typical of circulating precursors, while maintaining their myogenic potential. This suggests that BpV can be considered a valuable tool to obtain multi-potent stem cells from committed cell populations. We are currently comparing gene expression profiles following BpV treatment of freshly FACS-purified muscle-derived cells in order to analyze the reversible molecular changes in treated cells features and to compare the induced phenotype to the one of known stem cell populations. Molecular pathways known to be affected by BpV treatment, such as PTEN-PI3K-Akt, NFkB, AP1 and NFAT is also being dissected in order to identify the molecular players responsible for BpV's de-differentiating effect on myoblasts and the effect of BpV-induced signaling on muscle cell phenotype.

MOLECULAR MECHANISMS REGULATING SKELETAL MUSCLE HOMEOSTASIS: EFFECTS OF V1a AVP RECEPTOR OVER-EXPRESSION

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The mantainance a working skeletal musculature is conferred by its remarkable capacity to regenerate after mechanical or pathological injury. Most muscle pathologies are characterized by the progressive loss of muscle tissue due to chronic degeneration combined with the inability of the regeneration machinery to replace damaged myofibers. In particular, cachexia or muscle wasting is characterized by a dramatic loss of adipose and muscle mass associated with a compromised muscle 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*. 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.

Here we investigated the role of V1aR over-expression in animals undergoing cachexia as a result of muscle over-expression of a specific cytokine (TNF-□). 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.
The positive effects of V1aR on muscle homeostasis are due to the promotion of the calcinuerin-IL-4 pathway and to the inhibition of atrophic genes expression mediated by FOXO phosphorilation.
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.

ERK INHIBITION PREVENTS MUSCLE ATROPHY IN EXPERIMENTAL CANCER CACHEXIA

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Cancer cachexia is a syndrome characterized by loss of skeletal muscle protein, depletion of lipid stores and hormonal perturbations. The role of MAP/ERK kinase in the regulation of muscle mass is still debated. ERK inhibition has been shown to prevent IGF-I-induced hypertrophy in rat muscles, but also to induce a hypertrophic phenotype similar to that elicited by IGF-1 treatment. Aim of the present study was to evaluate the effects of ERK inhibition in two different experimental models of cancer cachexia (AH-130-bearing rats and Colon26-bearing mice). At the end of the experimental period muscle depletion is associated with increased ERK phosphorylation. Administration of the ERK inhibitor PD98059 improves body and muscle weight loss. To investigate if proinflammatory cytokines may contribute to modulate ERK phosphorylation, TNFα-treated C2C12 cells were used. Indeed, exposure to TNFα increased the levels of phosphorylated ERK and reduced those of myosin heavy chain (MyHC) in C2C12 myotubes. In addition, in differentiating cells TNFα induced loss of the muscle-specific transcription factor MyoD. All these changes were prevented by treatment with PD98059, suggesting that ERK likely plays a crucial role in the pathogenesis of molecular alterations associated with muscle wasting. In addition, these observations point to ERK as a target for the development of new therapeutic approaches.

THE CHEMOTHERAPIC COMPOUND CISPLATIN CAUSES MYOTUBE ATROPHY BY INCREMENT OF PROTEASOME ACTIVATION

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In patients with cancer, muscle cachexia often occurs as a consequence of presence of circulating factors that increment the muscle wasting. On the other side, although chemotherapic agents provide a benefit in terms of reduced tumor invasiveness, often produce a remarkable side effect on muscle mass.
On this basis, the aim of this study was to investigate whether the chemotherapic agent cisplatin (cisPt) might directly influence the integrity of myofibers. To this purpose, treatment of terminally differentiated C2C12 myotubes with 50 μM cisPt up to 24 hours triggered atrophy. Under these conditions, RT-PCR analysis showed a down-regulation of different specific myogenic markers along with a significant increment of Atrogin-1, an E3 ubiquitin ligase that is predictive of proteasome machinery activation. By using a fluorometric assay, we evaluated that cisPt treatment significantly increases the activity of proteasome in atrophic myotubes compared to control, suggesting that the oxidative damage produced by cisPt stimulates increased protein breakdown. Accordingly, refeeding cisPt-treated myotubes with insulin partially rescued the myofiber size and reverted the increase of Atrogin-1; additionally, delivery of a constitutively activated Akt form significantly prevented the activation of proteasome upon cisPt administration, suggesting therefore that this chemoterapic agent directly impairs the sarcomere architecture presumably by interfering with the Akt pathway, leading at least to activation of proteasomal pathway.

AUTOPHAGY IS REQUIRED TO MAINTAIN MUSCLE MASS

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The ubiquitin-proteasome and autophagy-lysosome pathways are the two major routes for protein and organelle clearance in eukaryotic cells1. In skeletal muscle both systems are under Akt-FoxO regulation and their excessive activation induces severe muscle loss 2-4. Conversely altered autophagy has been observed in various myopathies with accumulation of inclusions and vacuoles5. However the role of autophagy in skeletal muscle has not been determined by specific loss-of-function approaches. Here we specifically deleted Atg7 gene in skeletal muscle and we analysed the contribution of autophagy to homeostasis of organelle and proteins and its role in muscle wasting. Genetic ablation of Atg7 resulted in profound muscle atrophy because of increased expression of atrophy-related genes. Physiological studies revealed an important decrease in absolute and specific force which is age-dependent. Morphological analysis showed accumulation of abnormal mitochondria, sarcoplasmic reticulum distension, disorganization of sarcomere and formation of aberrant concentric membranous structure. Autophagy inhibition exacerbated muscle loss during denervation and fasting and induced sarcolemmal instability which resulted in myofiber death. Thus maintenance of autophagy flux is important to preserve muscle mass and to maintain myofiber integrity. Our results suggest that inhibition or alteration of autophagy can contribute to muscle degeneration in some muscular dystrophies characterized by accumulation of abnormal mitochondria and inclusions program.

ROLE OF SFINGOSINE 1-PHOSPHATE RECEPTORS IN MUSCLE REGENERATION

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The molecular basis of the regenerative process has been widely studied under *in vitro* conditions but it is almost unexplored *in vivo*.This study is aimed at elucidate the role of the bioactive lipid sphingosine 1-phosphate (S1P), and of related specific receptors ($S1P_{1-3}$), in the regeneration of skeletal muscle *in vivo*. The possible role of S1P as a muscle factor is supported by recent findings demonstrating a mitogenic action of S1P on mesangioblasts and on cultured satellite cells.

Regeneration was induced in rat and mouse soleus muscle by injecting the myotoxic drugs bupivacaine or notexin, respectively. The action of S1P was investigated by the exogenous addition of the lipid at the moment of degeneration. The expression level and localization of $S1P_{1-3}$ receptors was evaluated during the early regeneration phases. To establish the role of $S1P_{1-3}$ receptors, regeneration was carried out in the presence of specific modulators of receptors, as well as in the transgenic mouse knock out for $S1P_2$. The exogenous addition of S1P (50 µM) during regeneration caused a significant increase of the mean cross sectional area of regenerating fibers and of activated Akt level. $S1P_1$ and $S1P_3$ receptors seem to modulate regeneration by exerting opposite functions. Interestingly, the apparent trophic action of S1P was lost in the $S1P_2$ knock out mice. These results indicate that S1P signalling participates in the regenerative processes of skeletal muscle.

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LACK OF PKCθ IMPAIRS SKELETAL MUSCLE GROWTH AND REGENERATION

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Skeletal muscle is a dynamic tissue able to respond to several stimuli, such as atrophy and hypertrophy and to regenerate following muscle damage. Following muscle damage, satellite cells (SCs) are able to give rise to myoblasts, which then merge together to form new muscle fiber or fuse to pre-existing myofibers. An initial immunological response is required to remove damaged fibers and to contribute to a favorable microenvironment for SCs activation/differentiation; finally, extra-cellular matrix remodeling is required to restore muscle functionality. PKCθ is the predominantly expressed Protein kinase C isoform in skeletal muscle. We and other have previously shown that PKCθ activity is involved in myofiber development, neuromuscular cross talk and muscle metabolism. In this study we investigated the role of PKCθ in skeletal muscle regeneration and satellite cells activity in Tibialis anterior (TA) at different periods of time after freeze injury damage, comparing WT to PKCθ$^{-/-}$ mice. Histological analysis of Hematoxylin/Eosin-stained TA transverse sections, showed an impaired reorganization of regenerating muscle at 4 and 7 day post injury, and a reduced Cross-sectional Area (CSA) of regenerating, eMyHC (embryonic Myosin) positive, fibers in PKCθ mice, as compared to WT. Accordingly, immunofluorescence and western blot analyses showed a reduction in eMyHC expression in mice lacking of PKCθ. Semi-quantitative RT-PCR analysis showed a delay in the expression of SCs activated markers (MyoD, myogenin, Myf5) but not of the early marker, Pax7. Indeed, cultured PKCθ SCs give rise to thinner, oligonucleated myotubes, when compared to WT. Whether PKCθ activity is required for SCs activation/differentiation, for the inflammatory response or for extra-cellular matrix remodeling, is under investigation.