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Abstracts of oral presentations

Proteome analysis of human vastus lateralis muscle (hVLM) from climbers after mountaineering expedition to Himalayas

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We have investigated the molecular mechanism underlying changes in hVLM structure and metabolism during its adaptive response after exposure to extreme altitude. hVLM biopsies from six climbers, before and after trip, were grouped into a total number of six pools including subjects with the same age (for pre-expedition time 1st/ ± 27 years, 2nd/ ± 44 years, 3rd/ ± 55 years old and for post-expedition time 4th/ ± 27 years, 5th/ ± 44 years, 6th/ ± 55 years old respectively). The total protein extract for each lysated tissue was separated using a broad pH range no linear IPG strips (3-10) and 12% SDS gel. By MALDI-TOF MS analysis carried out on the common spots we identified several housekeeping proteins which were classified into six categories i.e. cytoskeleton and motility, mitochondrial activity, energy metabolism, cellular stress, transport and others. It is notable that the proteins of the metabolism, cytoskeleton and mitochondrial compartments were present in the highest number (36%, 27% and 33% respectively). To evaluate the changes in expression level we have compared the housekeeping proteins between the pre- and post-expedition time samples. After the post-exposure time, the major component of the hVLM proteome is represented by the mitochondrial protein mainly involved in aerobic and anaerobic pathway. A significant down-regulation of the glycolytic enzymes was also found.

Red blood cell neocytolysis in humans after high altitude de-acclimatization

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A selective lysis of relatively young erythrocytes (neocytolysis), together with a decrease of erythropoietin (EPO) production, has been described in polycythemic, high altitude acclimatized people, after descent to sea level, and in astronauts, early after exposure to weightlessness. To study neocytolysis, we analysed blood samples drawn from 4 mountain climbers at sea level before (control) and after 53 days of high altitude dwelling (> 4500 m). After return to sea level, EPO's plasma levels were lower than before high altitude dwelling (mean values: 2.5 ± 3.3 , versus 10 ± 4.5 mIU/ml, $p < 0.05$). Red blood cells (RBC) populations were

separated into low, middle and high density subsets, which, by physical and phenotypical criteria were regarded as young, middle aged and old. The expression of membrane molecules CD55 and CD59, that are partially lost during RBCs aging, and phosphatidylserine and CD47, involved in the recognition and triggering or inhibition of RBCs phagocytosis by macrophages, was investigated by immunofluorescence and flow cytometry analysis on each subset. The comparative analysis of cell counts revealed an almost complete disappearance of young and middle aged RBCs after descent from high altitude (from 4.50 % (± 3.10) and 66 % (± 6.90) to 0.19 % (± 0.07) and 1.90 % (± 0.50), respectively). A dramatic increase of the high density population (from 29.50 % (± 7) to 97.90 % (± 2.00)) was also observed. Furthermore, in young and middle aged RBCs a "senescent" like phenotype was observed, which may account for their targeting to phagocytosis.

Endocrine and metabolic response to extreme altitude and physical exercise in "elite" climbers

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The physiological adaptive response to chronic exposure to hypobaric hypoxia include variations in the homeostatic steady-state of several endocrine and metabolic functions. A comprehensive description of the endocrine and metabolic effects of prolonged exposure to hypobaric hypoxia is, however, lacking; particularly, the combined effects of high altitude and physical exercise have still to be elucidated. Aim of our study was to evaluate the endocrine pituitary function as well as glucose and lipid metabolism during prolonged physical exercise at high altitude in hypoxia conditions in well-trained climbers. To this goal, we evaluated spontaneous GH, ghrelin and leptin secretion (2-hour profile), IGF-I, IGFBP-3, ACTH, cortisol, DHEAS, progesterone, testosterone, PRL, FT3, FT4, TSH, glucose, FFA and insulin levels at the sea level before the expedition and after a period of 7 weeks of acclimatization and climbing at an altitude no lower than the 5200 meters of the base camp in 9 members (age [mean $\pm$ SEM]: 40.2 ± 1.4 years) of the Italian expedition "K2-2004 50 anni dopo" at the north face of Mt. Everest. Mean GH concentration over 2 hours recorded in altitude and after climbing Mt. Everest was clearly higher ($p < 0.01$) than that at the sea level (AUC: 755.6 ± 130.5 vs 284.2 ± 104.8 $\mu\text{g/l} \cdot \text{min}$). Accordingly, mean total IGF-I and IGFBP-3 levels in altitude were higher ($p < 0.05$) than those at the sea level (219.6 ± 31.1 vs 167.5 ± 22.7 $\mu\text{g/l}$ and 3.7 ± 0.3 vs 2.9 ± 0.2 mg/l, respectively). Despite of a clear and important weight loss (mean - 5 kg) any variation was observed in ghrelin levels. PRL levels showed a trend toward increase (7.8 ± 0.8



vs 6.0 ± 0.6 $\mu\text{g/l}$, $p=0.051$) while, conversely, testosterone levels were significantly reduced (3.6 ± 0.4 vs 5.5 ± 0.6 ng/ml , $p<0.01$). Progesterone levels showed a significant increase (1.8 ± 0.1 vs 1.4 ± 0.1 ng/ml , $p<0.05$). Again, plasma glucose levels were reduced in altitude after maximal physical exercise (70.1 ± 3.7 vs 80.0 ± 2.6 mg/dl , $p<0.05$) without any significant change in insulin levels (10.9 ± 0.8 vs 10.1 ± 0.8 mU/l) thus suggesting marked increase in insulin sensitivity. FFA levels were significantly increased (0.53 ± 0.11 vs 0.36 ± 0.05 mEq/L , $p<0.05$) while a trend toward a reduction in leptin levels ($\text{AUC } 60.1 \pm 6.1$ vs 90.5 ± 11.0 $\text{ng/ml} \cdot \text{min}$) was observed. HPA axis did not showed any significant changes in altitude. An increase of free T4 levels (13.7 ± 0.7 vs 10.4 ± 0.6 ng/l , $p<0.05$) and a concomitant reduction of free T3 (2.0 ± 0.1 vs 2.7 ± 0.1 ng/l , $p<0.01$) were apparent at HA, though TSH levels remained unchanged. The results of the present study from a unique experimental human model of maximal exposure to altitude and physical exercise show that extreme high altitude and strenuous physical exercise induce a condition of hyperactivity of GH/IGF-I axis together with an apparent low T3 syndrome. At the same time we did not observed any variation in ghrelin secretion, despite a significant body weight loss, concomitantly with an increase of lipolysis and a trend toward decrease of leptin levels. Moreover, an increase of PRL and a decrease in testosterone levels coupled with an increase of progesterone levels was apparent. Though the experimental models adopted so far can not provide an accurate description of the underlying mechanisms, nevertheless these data represent important information to better understand human endocrine and metabolic physiology in hypoxic conditions. This study was supported by IMONT and Novo Nordisk.

Neuroregenerative effect of the anabolic steroids

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Oxandrolone is a potent anabolic steroid used to preserve or restore muscle mass in different clinical conditions. Several studies have shown that gonadal steroids exert profound trophic influences on the brain and spinal cord, evidenced from the structural to the molecular level. The neurons within the brain and spinal cord, including most cranial and spinal motor neurons, contain gonadal steroid receptors. Therefore, it is reasonable to expect that anabolic steroids can have a neuroregenerative effect due to increased protein synthesis in the peripheral nervous system. In this case report, to a patient with a demyelinating disease was administrated a therapeutic dose of oxandrolone (20 mg/day) during a 3 month period of concomitant isotonic muscular resistance training. At the end of 3 months it was observed a significant increase of muscle mass, strength and motor control. A repeat muscle biopsy revealed a type grouping of muscle fibers that suggests an expression of the reinnervation of the muscles since type grouping is evidence for an increase in myelinated axons of

the reinnervated muscle. The increase in strength and muscle mass is also related to axon regeneration. These results support the concept that oxandrolone is a neuroactive steroid with potential neuroregenerative effect through increased protein synthesis in the peripheral nervous system leading to the activation of a remyelination process.

Study of muscular activation during whole body vibration training

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Whole body vibration (WBV) training is more and more utilized not only for fitness but also in physical therapy, rehabilitation and sport medicine. WBV training aims to mechanically activate muscles by eliciting stretch reflexes. Mechanical vibrations are usually delivered to the entire patient body. Effects depend on intensity, direction and frequency of vibration. Training frequency is one of the most important factors involved and preliminary vibratory session can be dedicated to optimize treatment estimating the frequency that maximize the evoked EMG activity. This work aims to highlight the relationship between actual muscle motion and electromyographic activity during whole body vibration treatment. Vibrations were delivered via a sinusoidal oscillating platform at different frequencies (10-45 Hz), with a constant amplitude (1.2 mm). Simultaneous recordings of quadriceps Rectus Femoris EMG and 3D muscle accelerations were collected. Fifteen subjects were involved in the study. Large EMG motion artifacts were found to be superimposed on the raw EMG recordings and filtered out. Muscle motion analysis revealed a resonant-like mechanical behavior. RMS values of artifact-free EMG were found well correlated to the actual muscle displacement. In particular, the maximum of EMG activity was found in correspondence to the muscle mechanical resonance, in accordance to the hypothesis of proprioceptive response to stretch. Mechanical stimulation should provide an alternative method to improve impaired biomechanical function of the musculoskeletal system via the use of the neurophysiological pathways of patient.

First experience of TES for difficult-to-stimulate paraplegics

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The Spinal Unit of the Department of Rehabilitation Medicine of Udine admits patients with Spinal Cord Injury (SCI) after discharge from Neurosurgery or Intensive Care Divisions. Demographic data, etiology, duration of hospitalization, bladder managements, and mobility at



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admission and discharge were revisited from January 2000 to March 2008. From 490 SCI patients at their first admission to our Spinal Unit we treated with an intensive rehabilitation program 49 complete Spinal Cord Injury patients (ASIA Impairment Scale A). The patients have an average age of 38.63 years (range 16 - 67 years). The ratio between Male and Female was 4 : 1, 40 men e 9 women. All the patients were classified complete respectively to the ASIA Impairment Scale, when no motor or sensory function was preserved in the sacral segment S4-S5. All patients were classified with the Ashworth scale (Ashworth, 1964) from score 0 to 4. All SCI patients have been treated with conventional rehabilitation strategies for enhancing lower limbs function. At the same time these patients had active exercise for upper limbs and training for daily mobility tasks (transferring, bed mobility and sitting) and functional electrical stimulation. Using commercial stimulator we rather found difficulties to electrically stimulate denervated muscles, but we believe this approach could give more chances to patients in their recovery, if the dedicated stimulation parameters and the electrodes of needed size are applied to the denervated leg muscles. The hope is to obtain secondary benefits of the walking FES-assisted exercises or a combination of this with treadmill and biofeedback training in flaccid paraplegia at least in a clinical ambit. This was a good opportunity to engage a long and wide road that led us to be partners in the Rise2-Italy Project.

Clues to the formation of cores in a mouse model of malignant hyperthermia

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Malignant hyperthermia (MH) and central core disease (CCD) are closely related skeletal muscle diseases often linked to mutations in the ryanodine receptor (RYR1) gene. Knock-in mice harbouring the RYR1Y522S/WT mutation, which in humans is associated to MH susceptibility (MHS) with cores, exhibit MH-like episodes in response to halogenated anesthetics and heat. Here we investigated whether or not RyR1Y522S/WT skeletal fibers also develop cores and identified possible steps leading their formation. Starting at 2 months of age, we found clear evidence of mitochondrial abnormalities (swelling and partial disruption) which are confined to some regions of the cell (presumptive cores). Mitochondria disruption eventually leads to damage

of nearby sarcoplasmic reticulum (SR), T-tubules, and of some contractile elements (early cores). At later stages (1 year of age) regions of delimited myofibril contractures, characterized by complete lack of mitochondria and SR (contracture cores), or regions lacking also contractile elements (unstructured cores) becomes very frequent. Other various types of alterations were also detected (Z line streaming, external membrane folding, etc.). We suggest that the initial triggering event in core formation is a local imbalance in Ca²⁺ release due to increased RyR1 leak from mutated channels. Mitochondria are apparently the first organelles damaged by the altered Ca²⁺-homeostasis, and their disruption eventually damages closely associated organelles, such as the SR. Loss of Ca²⁺ sequestration by SR and by mitochondria in these regions likely contributes to the formation of contractures initiated by calcium diffusing from the SR in the adjacent regions. It is not clear whether unstructured cores derive from contracture cores.

Disferlinopathies, pathogenesis and effects of physical activity

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Dysferlin is involved in the repair of sarcolemma. Dysferlinopathy presents either as disto-proximal myopathy or myalgia/hyperkemia; progression is highly variable even in the same family, implying epigenetic or environmental factors. We evaluated whether strenuous exercise before onset might influence the clinical progression of the disease. We have followed 20 adult patients with biochemically and molecularly proven LGMD2B. We conducted a retrospective study of the natural history, including muscle MRI and neuromuscular assessment using the modified Gardner-Medwin-Walton (GMW) scale. Our investigation was focused on evaluating the role of sport activity performed before the onset of muscle weakness in the progression of the disease. We have divided the patients into two groups: cases with past history of regular sport activity (at least 3 hours/week for 2 years) and cases without physical activity. Sportive patients presented a significant more rapid clinical progression than non-sportive patients. The time elapsing from onset to loss of Gowers (grade 4 of the GMW scale) was significantly lower (Cox test: $p=0.11$; Log rank: $p=0.09$) in sportive patients (5.75 years) than in non-sportive patients (10 years). All LGMD2B patients showed a progressive loss of muscle function but patients which performed sports until onset of the disease had a more rapid clinical evolution. Physical activity produces a regular mechanical stress, which could determine a more severe muscular injury and should be considered as a negative prognostic factor which might anticipate the onset of disease and influence its progression. The physician should suggest to limit competitive physical activity.



Cell therapy for muscle dystrophies: delivery of myogenic precursor cells in mdx mice via polymeric scaffolds

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Our project is focused on the use of biomaterial to improve the grafting efficiency of myogenic precursors in normal and dystrophic skeletal muscle. We had previously shown that implanting a long lasting, lactic acid-based polymer seeded with myoblasts could improve the efficiency of cell delivery in regenerating, non-dystrophic muscle. Here we initially used a collagen-based scaffold to deliver in vitro-expanded muscle precursor cells in the muscles of mdx mice. Our data confirmed that scaffold-mediated cell delivery was indeed more efficient than direct intramuscular injection, both in terms of cell survival and dissemination, and consequently of number of dystrophin-positive fibers produced. However, the overall efficiency did not reach clinical significance; for this reason we decided to switch to a different cell type, i.e., satellite cells that had not been expanded in vitro, which have been shown to hold very good potential for muscle regeneration. In particular, we combined the implant of single, isolated muscle fibers or satellite cells freshly dissociated from their parental myofibers, with an innovative hyaluronic acid-based Hydrogel. This particular polymer holds great potential for clinical applications, as it is injectable, it is photopolymerizable in situ with UVA light and forms a non-porous yet permeable scaffold. Our initial data indicate that transplanted cells do contribute to muscle regeneration, albeit not as efficiently as what we obtained in parallel experiments with wt animals. In conclusion, our data suggest that polymer-mediated delivery of a small amount of freshly isolated satellite cells is feasible and efficient and could be an interesting approach for clinical application.

Aging strength ability: a “strenght condition” program in over 60 years aged fall prevention

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Strength clearly shows a moderate but regular decrement from youth to 45 years of age. After this period it becomes more and more relevant: it achieves the 25% at about 65 years of age [1]. Strength decrement can be considered as the main cause of disability prevalence in the elderly [2]. Explosive strength is connected to daily movement ability, effectiveness and velocity [3]. Lower limbs strength decrement shows a 3,5% drop titre in the years of age from

65 to 84 [4]. The aim of the study was to evaluate the effects of adjusted rehabilitative motorial protocol (ASA) on drop prevention; we have recruited 25 over 65 years of age, not institutionalised subjects according to Guidelines for exercise testing and prescription (ACSM, p. 126, 1991). We have used up to 80% [5] of maximum voluntary contraction overloads with DAPRE (daily adjusted progressive resistive exercise) methodology, which allows a rehabilitative plan individual progression [6+]. We have evaluated Anterior Tibialis, Vastus Medialis, Rectus Femoris and Biceps Femoris using EMG surface spectral statistics parameters. The normalized values rate of MDF (median frequency) and CF (conduction velocity) referred to electrostimulated isometric fatigue plotter [7] showed an average values shift from low (<100 Hz) to high (120 Hz) frequencies, thus demonstrating a FII type muscular fibres prominent intervention. This increment ($p < 0,05$) was proved by the jump test [8] as well: it showed a mechanical potency increment from the start (16WKg-1) to the end (21WKg-1) of the protocol. Tinetti test showed a drop risk decrement from high (22 ± 1) to low [9]. We propose our rehabilitation protocol as a useful mean to enhance walking motorial control and to improve strength, to prevent drops in the elderly as first and firm aim.

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The expression of developmental markers in muscle fibers in diagnostic biopsies

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We investigated the expression of several developmental markers of myogenesis in diagnostic muscle biopsies, in



Abstracts

Lectures

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Rehabilitation strategies from infrequent paraplegic syndromes to common aging-related muscle weaknesses

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Human muscle in aging share with long-standing denervated muscle similar impairments of the excitation contraction (EC) apparatuses. On the other hand, long-term denervated human muscles recover by FES even when at beginning they hardly respond to electrical stimulation with twitch contractions. We demonstrated that mid-term lower motor neuron denervated muscles present less damaged EC-Coupling apparatus, and respond with tetanic contraction to direct electrical stimulation, while long-standing lower motor neuron denervated muscles need very long and intense impulses to perform twitch contractions, but even in the latter case months of repeated daily training by twitch contraction restored tetanic contractility and improved synthesis and ultrastructural organization of myofibrils and Ca²⁺ handling membranes [1-3]. Experiments in a rat model of permanent denervation show by Electron Microscopy that the EC-C mechanisms are present and, in part, functional, supporting the hypothesis that, even in absence of ES-induced external-work (that is, visible contraction), ES-induced cyclical Calcium concentration change may drive recovery of the long-term denervated muscles. Our aim is now to translate these results in elderly to shorten recovery time after cast immobilization and other muscle weakness.

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Muscle design strategies

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The lecture will cover all aspect of muscle structure and function: from myofibril to molecules to membranes asking the question of how muscles have adapted to highly variable functional demands through the animal kingdom.

Sarcomeric myosin genes: evolution and transcriptional control

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The first part of the lecture will focus on the identification of novel ancient myosins in mammalian striated muscle and on evolutionary tinkering leading to functional diversification of myosin genes. The second part deals with the mechanisms that control the coordinated expression of myosin genes.

Plasticity and adaptive change: lessons from a paradigm shift

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“Plasticity is the capacity of fully differentiated cells to undergo, in response to a stimulus, a long-lasting change in phenotype that modifies their properties or responsiveness to the same or other stimuli.” [1]. I proposed this definition of plasticity in an attempt to discourage its use for almost everything, including regulation, modulation, and simple cause-and-effect. “Plasticity” it seems, has become the new fashion word of physiology. The scenario was very different fifty years ago, when the notion of a terminally differentiated cell undergoing further change was by no means part of the general thinking. Even when Buller and his colleagues demonstrated the effects of cross-reinnervating fast and slow muscle, the changes were thought of as a quantitative response to some unidentified “trophic” factor in the motor



nerves. The notion that muscle genes could actually be re-expressed was sufficiently new to be strongly resisted in many quarters. Various arguments were put forward to explain the observations in terms of the existing paradigm. But paradigms shift; attention has now moved from the phenomenon to the mechanism, with interest focused on unravelling the intracellular signalling pathways that underlie the re-expression of muscle genes. All the same, it is worth revisiting some of the old counter-arguments, for they have a bearing on the experiments that we do today.

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Muscle transcriptional pathways with electrical stimulation

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A large number of transcriptional pathways have been implicated in the response of muscle to an altered activity pattern. This is not surprising in the sense that muscle plasticity involves changes in gene transcription and translation for multiple sub-cellular systems. The transformation of muscle from a fast fatigable type to a slower more fatigue-resistant type is a paradigm in which the time course and sensitivity to stimulus can be investigated in a systematic manner: by means of an implantable stimulating device the activity dose can be closely controlled. We are searching for thresholds of activity that cause step changes in transcription. The existence of such thresholds would correspond with the observation that muscle fibres fall into a number of classes rather than showing a continuous range of phenotype in vertebrate muscles. We have measured by Q-RT-PCR the transcript levels in rat muscle after periods of muscle stimulation having designed QPCR primer pairs to monitor the expression of key transcripts involved in the adaptive response. These include primers for the myosin heavy chains 1, 2A and 2B, IGF-1, MGF, FOXO1A, myostatin, PPARdelta and PGC1alpha. Our first conclusion is that transcript levels for transcription factor change more consistently after 3 hours of changed activity than transcript levels for contractile proteins. After one week of stimulation however, the changes in transcript levels for contractile proteins are also changed consistently. We will investigate the time course of these responses within the first week to identify the earliest time point at which a new stable transcriptional state is achieved and at which it would be appropriate to perform a comparative study of the graded effect on transcription of graded activity patterns.

Malignant Hyperthermia (MH) and Exertional/environmental Heat Stroke (EHS) in mice lacking Calsequestrin-1

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Malignant hyperthermia (MH) and exertional/environmental heat stroke (EHS) in humans present as similar life threatening crises triggered by volatile anesthetics and strenuous exercise and/or high temperature, respectively. Many families (70-80%) diagnosed with MH susceptibility (MHS), and a few with EHS, are linked to mutations in the ryanodine receptor type-1 gene (RYR1), the Ca²⁺ release channel of the sarcoplasmic reticulum (SR) of skeletal muscle and key protein in excitation-contraction (EC) coupling. However, mutations in the RYR1 gene are not found in all MH families, suggesting that alternative genes remain to be identified. In our laboratory we have recently characterized a novel knockout model lacking skeletal muscle calsequestrin (CASQ1), a SR Ca²⁺-binding protein that modulates RYR1 function, and investigated whether these mice present a MH/EHS-like phenotype. Ablation of CASQ1 results in remodelling of the EC coupling apparatus and functional changes, which in male mice causes a striking increase in the rate of spontaneous mortality and susceptibility to trigger MH-like lethal episodes in response to halothane- and heat-stress. The demonstration that ablation of CASQ1 result in MH- and EHS-like lethal episodes validates CASQ1 as a viable candidate gene for linkage analysis in MH and EHS families where mutations in RYR1 are excluded.

Histological characteristics of cultured human muscle tissue: Light- and electron microscopy

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Morphological characteristics of muscle tissue were analyzed by light – and electron microscopy, when cultured as primary muscle tissue culture. Primary muscle tissue cultures were established from diagnostic muscle biopsies. The studies were performed on samples, where the routine histology did



not discover pathological alterations. After one month culture period, the histological investigations were carried out on paraffin -, frozen sections, and electron microscopy. The cultured muscle tissues formed spheroid-like structures. The samples demonstrated myofiber destruction, which was more pronounced in the centre of the spheroids. The degenerating fibers showed ragged-red like alterations, when stained by Gomori-trichrom staining. Cell proliferation was found in the outer layers of the spheroids. The proliferating cells were able to grow and differentiate into myofibers, which could be demonstrated by immunological markers. The new myofibers formed ring like structures around the original, degenerating fibers. The ultrastructural investigation demonstrated damage of the sarcolemma, disarrangement of the myofibrillar structure, alterations of the mitochondria, as well as dividing cells and growing new myofibers around the original fibers. Conclusion: The small, 1-2 mm sized muscle tissue pieces form spheroids in vitro, and survive. The physically damaged and denervated muscle fibers degenerate in this system, which degeneration is accompanied by regenerative changes. The pathomechanism of the morphological changes is further discussed. These experiments demonstrate that it is possible to generate morphological changes in cultured muscle spheroids that are commonly found in diagnostic biopsies, and could help us to understand the pathomechanism of muscle disorders.

MYH16: the masticatory myosin unveiled

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In human genome, 16 distinct genes code for class II myosin heavy chain subunit (Berg et al. *Molecular Biology of the Cell* Vol. 12, 780–794, April 2001). Among such genes, MYH16 has been identified as the gene coding for the masticatory or M MyHC (Desjasardins et al. *Mol. Biol. Evol.* 19(4):375–393, 2002) and subsequently found transcribed but not translated in human masseter and temporalis muscles (Stedman et al. *Nature* 428, 415–418, 2004). In several mammalian species, and among them in carnivores, the gene is transcribed and translated in jaw closer muscles where myosin containing M-MyHC represents the most abundant isoform (Qin et al. *J Mol Evol* 55, 544–552, 2002). Since only sparse information was available on the contractile properties of muscle fibers expressing M-MyHC (M fibers), we decided to characterized M fibers isolated from the jaw closer muscles (temporalis and masseter) of two species of domestic carnivores, the cat and the dog, in comparison with fibers expressing slow or fast (2A, 2X and 2B) isoforms in trunk and limb skeletal muscles. In each fiber, during maximally calcium-activated contractions at 12°C, we determined

isometric specific tension (Po), unloaded shortening velocity (Vo) with the slack test protocol and the rate constant of tension redevelopment (kTR) after a fast shortening-relengthening cycle. At the end of the mechanical experiment, we identified MyHC isoform composition of each fiber with gel electrophoresis. Electrophoretic migration rate of M-MyHC was similar in both species. We found that in both species the kinetic parameters Vo and kTR of M fibers were similar to those of 2A fibers, whereas Po values were significantly greater than in any other fiber types. The similarity between 2A and M fibers and the greater tension development of M fibers were confirmed also in mechanical experiments performed at 24°C. Myosin concentration was determined in single fibers and found not different in M fibers compared to slow and fast fibers, suggesting that the higher tension developed by M fibers does not find an explanation in a greater number of force generators. The specific mechanical characteristics of M fibers was then attributed to a diversity in cross bridge kinetics. Initial study on actin-myosin interaction kinetics showed that the rate of ADP release which is a determinant of maximum shortening velocity (Vo), is a biphasic process with a fast phase comparable to that of fast myosin and a slow phase comparable to that of slow and cardiac myosin. This suggests that ADP release might be strain dependent and this could in turn explain the high tension development and the intermediate kinetics parameters observed in single fibers.

Some features of the adaptation of man to hypoxia: from the integrative to the molecular level

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Over the last century, studies on humans exposed to hypobaric hypoxia have been centered mainly on the adjustments necessary to assure homeostasis of O₂ delivery, both at rest and at high levels of energy turnover. As a result, a large body of information was collected on blood, respiratory gas exchange, cardiovascular parameters such as heart rate and cardiac output and, more recently, on muscle efficiency and maximum power. The available data mainly refer to lowlanders (including second generation altitude populations) undergoing acute (from seconds to hours), subacute (up to a few days), subchronic (up to several weeks) and chronic (over years) low PO₂ exposure, as well as to natives, mainly Himalayan and Andeans, born and living permanently at altitude or commuting to higher and/or lower elevations. Such data appear to be often affected by a large variability that is not justified by the characteristics of the research protocols and “state of the art” measuring procedures. The appearance of a new player, the multi-gene transcription protein Hypoxia Inducible Factor (HIF-1), i.e.



the master regulator of cell hypoxic signaling and of hundreds of other genes whose products play a large number of metabolic and transport functions, opens a new scenario for an updated interpretation of earlier results that have been often overlooked. Among the latter, the large scatter of the percentage loss of maximum aerobic power as a function of altitude, the increase in metabolic efficiency of locomotion in chronic hypoxia, the origin and significance of the so-called “lactate paradox” and the functional significance of the muscle mitochondrial mass reduction in both altitude natives and acclimatized lowlanders. This has been done based on work on cell hypoxic signaling by Semenza’s group and on metabolic players recently identified by muscle proteome analysis.

Electrophysiological evaluation of denervated skeletal muscle fibres

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Skeletal muscle inactivity as a consequence of muscle unloading, ageing or denervation, determines a progressive muscle atrophy characterized by reduced i) fibre diameter, ii) T-tubule surface area, iii) plasma-membrane stiffness, iv) expression of protein involved in the excitation-contraction coupling process, v) Ca^{2+} endingly and vi) loss of the sarcomeric proteins. In isolated single fibres the atrophic state related to above points i-v can be evaluated by using microelectrodes and recording ionic current in current- and voltage-clamp condition. In current-clamp condition it is possible to evaluate a) the resting membrane potential index of increased plasma-membrane leak current, b) the excitability of the fibre as evaluated by the voltage threshold of the action potential (AP) and c) the conduction velocity of the AP. In voltage-clamp condition it is possible to evaluate 1) the passive properties of the sarcolemma as the membrane resistance, the overall surface area of the fibre as well as that of the T-tubule, 2) the active voltage-dependent responsiveness of the fibre as the time course and voltage dependence of intramembrane charge movement, and of Na^{+} and L-type Ca^{2+} channel, 3) the reciprocal control between the orthograde action from L-type Ca^{2+} channel to RyR and the retrograde action from RyR to L-type Ca^{2+} channel by blocking the ryanodine receptor with ruthenium red, ryanodine or heptanol, 4) the stiffness and mechano sensitivity of the plasma membrane can be evaluated by comparing the data related to points a-c and 1-3 in muscle stretched with muscle at resting length. The observed effects on normal and denervated fibres could be useful to evaluated the atrophic state of the fibre, and for therapies that use the functional electrical stimulation and passive movement of the limbs to repair the damaged denervated human muscle.

The long-lasting denervated muscle: What we know from experimental animal research?

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Human muscles that have undergone atrophy as a result of persistent lower motor neuron lesion may experience considerable restitution of excitability, force and mass from home-based therapeutical electrical stimulation (TES), though initially they are unable to perform external work [1-3]. For ethical reasons we studied time course of post-denervation effects on the Excitation-Contraction Coupling (ECC), using a rat model as a substitute for human tissue [Squecco R, Carraro U, Kern H, Pond A, Adami N, Biral D, Vindigni V, Boncompagni S, Protasi F, Pietrangelo T, Bosco G, Fanò G, Marini M, Abruzzo PM, Germinario E, Danielli-Betto D, Francini F, Zampieri S. Despite lost contractility, a sub-population of rat muscle fibers maintains an assessable excitation-contraction coupling mechanism after long-standing denervation. *J Neuropath Exp Neurol* 2009, submitted.]. In this model, it is possible to demonstrate that in long term denervated muscle: i) muscle fibers having “voltage dependent Ca^{2+} channel activity” are twice those able to contract, ii) isolated muscle, unable to twitch by electrical stimulation, presents slow caffeine contracture, iii) the ECC mechanisms are still present and, in part, functional; and iv) ECC related gene expression is up-regulated in long-term denervated muscles. Importantly, we demonstrate that at any time-point there are muscle fibers that are more resistant than others to denervation atrophy and disorganization of the ECC apparatuses. In summary, our experimental evidence supports the hypothesis that, even in absence of electrical stimulation-induced external work, FES may induce internal calcium concentration alterations in muscle fibers that drive the early recovery of the long-term denervated human muscle.

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Abstracts of oral presentations

Proteome analysis of human vastus lateralis muscle (hVLM) from climbers after mountaineering expedition to Himalayas

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We have investigated the molecular mechanism underlying changes in hVLM structure and metabolism during its adaptive response after exposure to extreme altitude. hVLM biopsies from six climbers, before and after trip, were grouped into a total number of six pools including subjects with the same age (for pre-expedition time 1st/ ± 27 years, 2nd/ ± 44 years, 3rd/ ± 55 years old and for post-expedition time 4th/ ± 27 years, 5th/ ± 44 years, 6th/ ± 55 years old respectively). The total protein extract for each lysated tissue was separated using a broad pH range no linear IPG strips (3-10) and 12% SDS gel. By MALDI-TOF MS analysis carried out on the common spots we identified several housekeeping proteins which were classified into six categories i.e. cytoskeleton and motility, mitochondrial activity, energy metabolism, cellular stress, transport and others. It is notable that the proteins of the metabolism, cytoskeleton and mitochondrial compartments were present in the highest number (36%, 27% and 33% respectively). To evaluate the changes in expression level we have compared the housekeeping proteins between the pre- and post-expedition time samples. After the post-exposure time, the major component of the hVLM proteome is represented by the mitochondrial protein mainly involved in aerobic and anaerobic pathway. A significative down-regulation of the glycolytic enzymes was also found.

Red blood cell neocytolysis in humans after high altitude de-acclimatization

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A selective lysis of relatively young erythrocytes (neocytolysis), together with a decrease of erythropoietin (EPO) production, has been described in polycythemic, high altitude acclimatized people, after descent to sea level, and in astronauts, early after exposure to weightlessness. To study neocytolysis, we analysed blood samples drawn from 4 mountain climbers at sea level before (control) and after 53 days of high altitude dwelling (> 4500 m). After return to sea level, EPO's plasma levels were lower than before high altitude dwelling (mean values: 2.5 ± 3.3 , versus 10 ± 4.5 mIU/ml, $p < 0.05$). Red blood cells (RBC) populations were

separated into low, middle and high density subsets, which, by physical and phenotypical criteria were regarded as young, middle aged and old. The expression of membrane molecules CD55 and CD59, that are partially lost during RBCs aging, and phosphatidylserine and CD47, involved in the recognition and triggering or inhibition of RBCs phagocytosis by macrophages, was investigated by immunofluorescence and flow cytometry analysis on each subset. The comparative analysis of cell counts revealed an almost complete disappearance of young and middle aged RBCs after descent from high altitude (from 4.50 % (± 3.10) and 66 % (± 6.90) to 0.19 % (± 0.07) and 1.90 % (± 0.50), respectively). A dramatic increase of the high density population (from 29.50 % (± 7) to 97.90 % (± 2.00)) was also observed. Furthermore, in young and middle aged RBCs a "senescent" like phenotype was observed, which may account for their targeting to phagocytosis.

Endocrine and metabolic response to extreme altitude and physical exercise in "elite" climbers

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The physiological adaptive response to chronic exposure to hypobaric hypoxia include variations in the homeostatic steady-state of several endocrine and metabolic functions. A comprehensive description of the endocrine and metabolic effects of prolonged exposure to hypobaric hypoxia is, however, lacking; particularly, the combined effects of high altitude and physical exercise have still to be elucidated. Aim of our study was to evaluate the endocrine pituitary function as well as glucose and lipid metabolism during prolonged physical exercise at high altitude in hypoxia conditions in well-trained climbers. To this goal, we evaluated spontaneous GH, ghrelin and leptin secretion (2-hour profile), IGF-I, IGFBP-3, ACTH, cortisol, DHEAS, progesterone, testosterone, PRL, FT3, FT4, TSH, glucose, FFA and insulin levels at the sea level before the expedition and after a period of 7 weeks of acclimatization and climbing at an altitude no lower than the 5200 meters of the base camp in 9 members (age [mean $\pm$ SEM]: 40.2 ± 1.4 years) of the Italian expedition "K2-2004 50 anni dopo" at the north face of Mt. Everest. Mean GH concentration over 2 hours recorded in altitude and after climbing Mt. Everest was clearly higher ($p < 0.01$) than that at the sea level (AUC: 755.6 ± 130.5 vs 284.2 ± 104.8 $\mu\text{g/l} \cdot \text{min}$). Accordingly, mean total IGF-I and IGFBP-3 levels in altitude were higher ($p < 0.05$) than those at the sea level (219.6 ± 31.1 vs 167.5 ± 22.7 $\mu\text{g/l}$ and 3.7 ± 0.3 vs 2.9 ± 0.2 mg/l, respectively). Despite of a clear and important weight loss (mean - 5 kg) any variation was observed in ghrelin levels. PRL levels showed a trend toward increase (7.8 ± 0.8



vs 6.0 ± 0.6 $\mu\text{g/l}$, $p=0.051$) while, conversely, testosterone levels were significantly reduced (3.6 ± 0.4 vs 5.5 ± 0.6 ng/ml , $p<0.01$). Progesterone levels showed a significant increase (1.8 ± 0.1 vs 1.4 ± 0.1 ng/ml , $p<0.05$). Again, plasma glucose levels were reduced in altitude after maximal physical exercise (70.1 ± 3.7 vs 80.0 ± 2.6 mg/dl , $p<0.05$) without any significant change in insulin levels (10.9 ± 0.8 vs 10.1 ± 0.8 mU/l) thus suggesting marked increase in insulin sensitivity. FFA levels were significantly increased (0.53 ± 0.11 vs 0.36 ± 0.05 mEq/L , $p<0.05$) while a trend toward a reduction in leptin levels ($\text{AUC } 60.1 \pm 6.1$ vs 90.5 ± 11.0 $\text{ng/ml} \cdot \text{min}$) was observed. HPA axis did not showed any significant changes in altitude. An increase of free T4 levels (13.7 ± 0.7 vs 10.4 ± 0.6 ng/l , $p<0.05$) and a concomitant reduction of free T3 (2.0 ± 0.1 vs 2.7 ± 0.1 ng/l , $p<0.01$) were apparent at HA, though TSH levels remained unchanged. The results of the present study from a unique experimental human model of maximal exposure to altitude and physical exercise show that extreme high altitude and strenuous physical exercise induce a condition of hyperactivity of GH/IGF-I axis together with an apparent low T3 syndrome. At the same time we did not observed any variation in ghrelin secretion, despite a significant body weight loss, concomitantly with an increase of lipolysis and a trend toward decrease of leptin levels. Moreover, an increase of PRL and a decrease in testosterone levels coupled with an increase of progesterone levels was apparent. Though the experimental models adopted so far can not provide an accurate description of the underlying mechanisms, nevertheless these data represent important information to better understand human endocrine and metabolic physiology in hypoxic conditions. This study was supported by IMONT and Novo Nordisk.

Neuroregenerative effect of the anabolic steroids

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Oxandrolone is a potent anabolic steroid used to preserve or restore muscle mass in different clinical conditions. Several studies have shown that gonadal steroids exert profound trophic influences on the brain and spinal cord, evidenced from the structural to the molecular level. The neurons within the brain and spinal cord, including most cranial and spinal motor neurons, contain gonadal steroid receptors. Therefore, it is reasonable to expect that anabolic steroids can have a neuroregenerative effect due to increased protein synthesis in the peripheral nervous system. In this case report, to a patient with a demyelinating disease was administrated a therapeutic dose of oxandrolone (20 mg/day) during a 3 month period of concomitant isotonic muscular resistance training. At the end of 3 months it was observed a significant increase of muscle mass, strength and motor control. A repeat muscle biopsy revealed a type grouping of muscle fibers that suggests an expression of the reinnervation of the muscles since type grouping is evidence for an increase in myelinated axons of

the reinnervated muscle. The increase in strength and muscle mass is also related to axon regeneration. These results support the concept that oxandrolone is a neuroactive steroid with potential neuroregenerative effect through increased protein synthesis in the peripheral nervous system leading to the activation of a remyelination process.

Study of muscular activation during whole body vibration training

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Whole body vibration (WBV) training is more and more utilized not only for fitness but also in physical therapy, rehabilitation and sport medicine. WBV training aims to mechanically activate muscles by eliciting stretch reflexes. Mechanical vibrations are usually delivered to the entire patient body. Effects depend on intensity, direction and frequency of vibration. Training frequency is one of the most important factors involved and preliminary vibratory session can be dedicated to optimize treatment estimating the frequency that maximize the evoked EMG activity. This work aims to highlight the relationship between actual muscle motion and electromyographic activity during whole body vibration treatment. Vibrations were delivered via a sinusoidal oscillating platform at different frequencies (10-45 Hz), with a constant amplitude (1.2 mm). Simultaneous recordings of quadriceps Rectus Femoris EMG and 3D muscle accelerations were collected. Fifteen subjects were involved in the study. Large EMG motion artifacts were found to be superimposed on the raw EMG recordings and filtered out. Muscle motion analysis revealed a resonant-like mechanical behavior. RMS values of artifact-free EMG were found well correlated to the actual muscle displacement. In particular, the maximum of EMG activity was found in correspondence to the muscle mechanical resonance, in accordance to the hypothesis of proprioceptive response to stretch. Mechanical stimulation should provide an alternative method to improve impaired biomechanical function of the musculoskeletal system via the use of the neurophysiological pathways of patient.

First experience of TES for difficult-to-stimulate paraplegics

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The Spinal Unit of the Department of Rehabilitation Medicine of Udine admits patients with Spinal Cord Injury (SCI) after discharge from Neurosurgery or Intensive Care Divisions. Demographic data, etiology, duration of hospitalization, bladder managements, and mobility at



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admission and discharge were revisited from January 2000 to March 2008. From 490 SCI patients at their first admission to our Spinal Unit we treated with an intensive rehabilitation program 49 complete Spinal Cord Injury patients (ASIA Impairment Scale A). The patients have an average age of 38.63 years (range 16 - 67 years). The ratio between Male and Female was 4 : 1, 40 men e 9 women. All the patients were classified complete respectively to the ASIA Impairment Scale, when no motor or sensory function was preserved in the sacral segment S4-S5. All patients were classified with the Ashworth scale (Ashworth, 1964) from score 0 to 4. All SCI patients have been treated with conventional rehabilitation strategies for enhancing lower limbs function. At the same time these patients had active exercise for upper limbs and training for daily mobility tasks (transferring, bed mobility and sitting) and functional electrical stimulation. Using commercial stimulator we rather found difficulties to electrically stimulate denervated muscles, but we believe this approach could give more chances to patients in their recovery, if the dedicated stimulation parameters and the electrodes of needed size are applied to the denervated leg muscles. The hope is to obtain secondary benefits of the walking FES-assisted exercises or a combination of this with treadmill and biofeedback training in flaccid paraplegia at least in a clinical ambit. This was a good opportunity to engage a long and wide road that led us to be partners in the Rise2-Italy Project.

Clues to the formation of cores in a mouse model of malignant hyperthermia

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Malignant hyperthermia (MH) and central core disease (CCD) are closely related skeletal muscle diseases often linked to mutations in the ryanodine receptor (RYR1) gene. Knock-in mice harbouring the RYR1Y522S/WT mutation, which in humans is associated to MH susceptibility (MHS) with cores, exhibit MH-like episodes in response to halogenated anesthetics and heat. Here we investigated whether or not RyR1Y522S/WT skeletal fibers also develop cores and identified possible steps leading their formation. Starting at 2 months of age, we found clear evidence of mitochondrial abnormalities (swelling and partial disruption) which are confined to some regions of the cell (presumptive cores). Mitochondria disruption eventually leads to damage

of nearby sarcoplasmic reticulum (SR), T-tubules, and of some contractile elements (early cores). At later stages (1 year of age) regions of delimited myofibril contractures, characterized by complete lack of mitochondria and SR (contracture cores), or regions lacking also contractile elements (unstructured cores) becomes very frequent. Other various types of alterations were also detected (Z line streaming, external membrane folding, etc.). We suggest that the initial triggering event in core formation is a local imbalance in Ca²⁺ release due to increased RyR1 leak from mutated channels. Mitochondria are apparently the first organelles damaged by the altered Ca²⁺-homeostasis, and their disruption eventually damages closely associated organelles, such as the SR. Loss of Ca²⁺ sequestration by SR and by mitochondria in these regions likely contributes to the formation of contractures initiated by calcium diffusing from the SR in the adjacent regions. It is not clear whether unstructured cores derive from contracture cores.

Disferlinopathies, pathogenesis and effects of physical activity

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Dysferlin is involved in the repair of sarcolemma. Dysferlinopathy presents either as disto-proximal myopathy or myalgia/hyperkalemia; progression is highly variable even in the same family, implying epigenetic or environmental factors. We evaluated whether strenuous exercise before onset might influence the clinical progression of the disease. We have followed 20 adult patients with biochemically and molecularly proven LGMD2B. We conducted a retrospective study of the natural history, including muscle MRI and neuromuscular assessment using the modified Gardner-Medwin-Walton (GMW) scale. Our investigation was focused on evaluating the role of sport activity performed before the onset of muscle weakness in the progression of the disease. We have divided the patients into two groups: cases with past history of regular sport activity (at least 3 hours/week for 2 years) and cases without physical activity. Sportive patients presented a significant more rapid clinical progression than non-sportive patients. The time elapsing from onset to loss of Gowers (grade 4 of the GMW scale) was significantly lower (Cox test: $p=0.11$; Log rank: $p=0.09$) in sportive patients (5.75 years) than in non-sportive patients (10 years). All LGMD2B patients showed a progressive loss of muscle function but patients which performed sports until onset of the disease had a more rapid clinical evolution. Physical activity produces a regular mechanical stress, which could determine a more severe muscular injury and should be considered as a negative prognostic factor which might anticipate the onset of disease and influence its progression. The physician should suggest to limit competitive physical activity.



Cell therapy for muscle dystrophies: delivery of myogenic precursor cells in mdx mice via polymeric scaffolds

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Our project is focused on the use of biomaterial to improve the grafting efficiency of myogenic precursors in normal and dystrophic skeletal muscle. We had previously shown that implanting a long lasting, lactic acid-based polymer seeded with myoblasts could improve the efficiency of cell delivery in regenerating, non-dystrophic muscle. Here we initially used a collagen-based scaffold to deliver in vitro-expanded muscle precursor cells in the muscles of mdx mice. Our data confirmed that scaffold-mediated cell delivery was indeed more efficient than direct intramuscular injection, both in terms of cell survival and dissemination, and consequently of number of dystrophin-positive fibers produced. However, the overall efficiency did not reach clinical significance; for this reason we decided to switch to a different cell type, i.e., satellite cells that had not been expanded in vitro, which have been shown to hold very good potential for muscle regeneration. In particular, we combined the implant of single, isolated muscle fibers or satellite cells freshly dissociated from their parental myofibers, with an innovative hyaluronic acid-based Hydrogel. This particular polymer holds great potential for clinical applications, as it is injectable, it is photopolymerizable in situ with UVA light and forms a non-porous yet permeable scaffold. Our initial data indicate that transplanted cells do contribute to muscle regeneration, albeit not as efficiently as what we obtained in parallel experiments with wt animals. In conclusion, our data suggest that polymer-mediated delivery of a small amount of freshly isolated satellite cells is feasible and efficient and could be an interesting approach for clinical application.

Aging strength ability: a “strenght condition” program in over 60 years aged fall prevention

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Strength clearly shows a moderate but regular decrement from youth to 45 years of age. After this period it becomes more and more relevant: it achieves the 25% at about 65 years of age [1]. Strength decrement can be considered as the main cause of disability prevalence in the elderly [2]. Explosive strength is connected to daily movement ability, effectiveness and velocity [3]. Lower limbs strength decrement shows a 3,5% drop titre in the years of age from

65 to 84 [4]. The aim of the study was to evaluate the effects of adjusted rehabilitative motorial protocol (ASA) on drop prevention; we have recruited 25 over 65 years of age, not institutionalised subjects according to Guidelines for exercise testing and prescription (ACSM, p. 126, 1991). We have used up to 80% [5] of maximum voluntary contraction overloads with DAPRE (daily adjusted progressive resistive exercise) methodology, which allows a rehabilitative plan individual progression [6+]. We have evaluated Anterior Tibialis, Vastus Medialis, Rectus Femoris and Biceps Femoris using EMG surface spectral statistics parameters. The normalized values rate of MDF (median frequency) and CF (conduction velocity) referred to electrostimulated isometric fatigue plotter [7] showed an average values shift from low (<100 Hz) to high (120 Hz) frequencies, thus demonstrating a FII type muscular fibres prominent intervention. This increment ($p < 0,05$) was proved by the jump test [8] as well: it showed a mechanical potency increment from the start (16WKg-1) to the end (21WKg-1) of the protocol. Tinetti test showed a drop risk decrement from high (22 ± 1) to low [9]. We propose our rehabilitation protocol as a useful mean to enhance walking motorial control and to improve strength, to prevent drops in the elderly as first and firm aim.

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The expression of developmental markers in muscle fibers in diagnostic biopsies

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We investigated the expression of several developmental markers of myogenesis in diagnostic muscle biopsies, in



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order to relate their expression to pathological findings, and detect any obvious difference in expression patterns in the most common groups of neuromuscular disorders. Frozen sections of one adult and one child biopsy from each of the following pathologic groups were selected: 1) normal muscle, 2) neuropathy, 3) dystrophy and 4) dermatomyositis. After routine stainings, N-CAM, PAX7, MyoD1, myogenin, desmin, neonatal myosin heavy chain (nMyHC), developmental myosin heavy chain (dMyHC), utrophin, MHC-I and FAS immunostainings were performed. The frequency of positive muscle fibers and satellite cells was calculated, and the expression of developmental markers was related to pathological signs of necrosis and regeneration, denervation and reinnervation. We found a relatively high expression of resting satellite (PAX7 and N-CAM positive) cells in the normal and neuropathic muscles, and in dermatomyositis. The early developmental markers (MyoD1 and myogenin) and nMyHC and N-CAM were more frequently expressed in neuropathic muscle and dermatomyositis than in normal or dystrophic muscles, whereas the MHC-I, utrophin and FAS expression was high in dystrophies and dermatomyositis. The high expression of the resting satellite cells and early developmental markers in neuropathic and normal muscle suggest a resting regenerating potential. The higher MHC-I, utrophin and FAS expression in dystrophic muscle and dermatomyositis might suggest a process where muscle fibers are vulnerable to structural (necrotic) damage. The findings are further demonstrated and serial section with the above stainings will be shown.

Anaerobic capacity and isometric contraction strength in six Italian climbers selected for Himalaya expedition

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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. One of these possible deleterious effects: the modification of anaerobic muscle capacity as possible consequence of decline of muscle trophism, was analysed in six climbers after a 43-day period of exposition to hypobaric hypoxia (>5000 m). To obtain this goal, previous analyzed alterations observed in such key variables as resting body mass indexes BMI (kg/m²) and the waist-to-hip ratio (WHR), we measured the maximal anaerobic power, the total anaerobic capacity by the Wingate Anaerobic Test (WAnT) carried out using a mechanically braked cycle ergometer. Bilateral isometric torque developed by the knee extensor muscles was measured during maximal voluntary contractions using a Leg Extension machine equipped with a load cell. The highest value of torque attained was taken as the isometric contraction strength. In addition we have tried to determine if any relationship could exist between BMI/anaerobic power. Wingate test showed that peak and average power and fatigue index recorded both pre- and post-expedition were not

significantly different. Peak blood lactate concentration measured after the WAnT were (mmol L⁻¹): 11.0±2.4 (pre) and 8.6±2.3 (post). No significant differences in blood lactate accumulation were found. Also the bilateral isometric strength was not significantly different at both pre- and post-expedition. We report significance as per BMI (p value < 0.05). The fNRM reports obtained before and after the expedition will be also discussed.

Rise2-Italy Trial. Structural and psychological effects of home electrical stimulation at 6 months treatment in complete SCI

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Home-based electrical stimulation treatment was performed on a L1-complete-Spinal Cord Injury, 32-yr old, male patient, starting within 1 year from SCI. Preliminary clinical evaluation by a myologist and a physiatrist considered this a useful strategy to recover the atrophic gluteal and quadriceps muscles, ameliorate respiratory and global resistance and prevent pressure ulcers. Furthermore, a physiotherapist planned the treatment for the fibrotic component of the syndrome (hyperflexion of ischiocrural tendons) using water exercises and stretching programs. Electrical stimulation tests were programmed. The RISE Project was explained to patients and his family. The electrical stimulation program and the devices (large skin electrodes and a device developed in Vienna, Austria as part of the EU Project RISE) were provided to the patient after clinical and functional evaluation in Vienna, Austria that confirmed denervation of the leg muscles and effective muscle response to electrical stimulation. The patient performed his treatment 5 days for week. A questionnaire was performed by the physiatrist after treatment and a quantitative analysis of muscles will be performed by the radiologist. The questionnaire confirmed the absence of collateral and side effect of the treatment and a very good compliance of the patient. Clinical and instrumental examination showed a bigger muscle masses, the absence of pressure ulcers, the absence of skin ulcers. From "patient perspective" a very better personal body image was obtained by this home treatment and a visual analogue scale (VAS) about this was 8 (from 0 to 10 points, 0 was "no effect on body image", 10 was "total amelioration of personal body image"). Personal value of this specific change on general depression scale was high and patient evaluation VAS was 8/10. We think that this approach and this treatment is quite simple, low economic impact featured and



safe for this patient. A scientific collaboration among myologists and clinical professionals ameliorated the quality of life of this person with complete SCI. Most work must be done to eliminate the word “chronic patient” from our old and obsolete medical dictionary. Often personal human suffering after SCI is ignored: evidence based medicine seems not to take care of these “problems”.

Injectable, photocuring biomaterial shaping 3d cell delivery

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Cell delivery-based strategies in regenerative medicine often face low efficiency problems. Biomaterial-assisted cell delivery was found as an attractive procedure to overcome some of the major limitations of the simple cell injection. Among different possibilities, the use of photocuring biomaterials, and in particular hydrogels, possesses intrinsic properties related to the in vivo control of the cell microenvironment. The chemico-physical and the mechanical properties, the morphology and the in vitro degradation rate of the cross-linked hydrogel can be specifically designed for supporting cell delivery in vivo. All these parameters can be tuned by adjusting the light exposure time, the polymer concentration and the media properties. An example of the feasible use of a photocurable hydrogel as cell carrier was investigated through in vivo experiments on muscle satellite cells. The results not only confirmed the hydrogel biocompatibility and biodegradability but also showed its potential in highly improving tissue regeneration.

Direct and indirect effects on bone mineral density in paraplegic patients due to TES

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3D modelling techniques are used to analyse density changes due electrical stimulation treatment from different regions of interest on the femur and in the patella bone. The method is based on the acquisition of high resolution Spiral CT scans from patients who have long-term flaccid paraplegia and the use of special image processing tools allowing tissue discriminations and segmentation. Three patients were measured at different points of time during 4 years of electrical stimulation treatment. The bone mineral density is measured in different regions on the femur to monitor the direct structural changes induced by the current flow. Indirect effects from the electrical stimulation treatment on the bone density are also evaluated measuring the changes in bone mineral density on the patella. Indeed an increase of density in this region can only be assigned to the mechanical force

applied through the induced quadriceps contraction. Though the evidences are not yet statistically relevant a kind of structural change in the femur is measured as secondary effect from the muscle stimulation. Beside, the force applied on the patella through the elicited contraction seems also to have a beneficial effect on bone density. Indeed the mineral loss in the patella is remarkably slowed down.

Muscle density distribution of rectus femoris in Electrical Stimulation Therapy

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Spinal cord injured (SCI) patients with a lower motor neuron lesion suffer from degeneration of muscles below the injury. This is due to inactivity, as the muscles are never contracting. This degeneration can lead in the end to a total loss of contractile elements in the muscle turning him into inactive tissue. Additional consequences are thinner skin, lower capillary density and lower bone mineral density. Up to now there is no treatment for denervated and degenerated muscles (DDM) that restores the muscle mass or bone strength or reverses the process of degeneration. The only therapy aims at the side effects of the paralysis like decubitus ulcers and others. In the European RISE project a new method has been developed for DDM patients based on electrical stimulation. This therapy has shown that a DDM can regain some size and force. It consists of electrically stimulating the muscle fibres directly in daily sessions for several months or years. In this work we look at density distribution of a muscle rectus femoris of three subjects that participated in the RISE project and have been in electrical stimulation treatment for six years. The density is measured in Hounsfield units (Hu) and reflects the X-ray absorption of the tissue. Since each thigh is stimulated with one stimulation channel using two big electrodes placed on the skin the muscles beneath are not activated in a physiological manner. Therefore the question arises, is the growth of the muscle modified by the unphysiological activation? Can we see the activation on the muscle tissue density? The hypothesis is that it could be higher where the muscle was activated and lower at other places. We investigate the density distribution of muscle tissue. The mean density for each CT slice is calculated and is plotted as a graph along the length of the muscle [1]. The results suggest that the therapy is modulating the muscles density in the way that at locations where there is more frequent stimulation or higher stimulation intensity the muscle tissue is denser.

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Genomic analysis of skeletal muscle under severe hypoxic conditions and physical activity

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The gene expression profiles is a powerful tool to analyze gene up- and down-regulation. 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.

Oxygen supply and oxygen utilization in muscles after chronic exposure to hypobaric hypoxia

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Six male subjects ($40 \text{ yy} \pm 14$; $79.3 \text{ kg} \pm 14.7$; 171.7 ± 10.0) were studied before and upon the return from the Himalayan Expedition Shisha Pangma 2008 during which they were chronically exposed to hypoxia living for about 30 days at 5000 m above sea level. Breath-by-breath (BbB) O₂ uptake (O₂) was measured at sea level during: i) a maximal incremental cycling test and; ii) a constant-load exercise transition performed pedalling at 100 W on a cycle

ergometer. Oxygen deficit (DefO₂) accumulated at the onset of the constant-load exercise was calculated as the difference between the O₂ that would have been consumed if a steady state had been attained immediately at the beginning of the exercise minus the volume of O₂ actually consumed during the exercise. The first quantity was calculated by multiplying O₂ at steady state (O_{2ss}) times the duration of the exercise set equal to 6 minutes. The volume of O₂ consumed during exercise was calculated by summing progressively the volume of O₂ taken up during each breath from the onset to the end of exercise. Finally, mean response time (MRT) of the O₂ kinetics, i.e. the time taken to achieve 63% of the response at steady state, was calculated from the ratio of DefO₂ to the corresponding O_{2ss}. Body mass after the expedition turned out to be significantly lower than that prevailing before (73.0 ± 10.8 , $P < 0.05$). Maximal oxygen uptake (O_{2max}) after prolonged exposure to hypoxia ($40.8 \text{ ml O}_2 \text{ kg}^{-1} \text{ min}^{-1} \pm 6.2$) was not significantly different from that assessed in the control condition ($38.2 \text{ ml O}_2 \text{ kg}^{-1} \text{ min}^{-1} \pm 8.0$). This implied that the subjects exercised at a lower percentage of O_{2max} ($55.0 \% \pm 10$) in the post condition than before ($63.0 \% \pm 18$, $P < 0.05$). DefO₂ after the expedition was significantly ($425 \text{ ml O}_2 \pm 174$) than that found in the control condition ($667 \text{ ml O}_2 \pm 231$, $P < 0.05$). MRT of O₂ kinetics confirmed that gas exchange kinetics was accelerated after chronic exposure to hypoxia (before: $38.0 \text{ s} \pm 13.8$; after: $25.2 \text{ s} \pm 7.8$, $P < 0.05$). These findings indicate that the responses of gas exchanges and of muscular O₂ uptake were accelerated after prolonged exposure to hypoxia. This may be likely the consequence of a better fitness status achieved by the subjects during the staying at altitude in spite of the reduction of maximal aerobic exercise capacity due the lower partial pressure of O₂.

Ultrasonography of medium and small muscle vessels

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The perfusion of a denervated anterior tibialis muscle has been assessed in a patient one-year after right femoral nerve unilateral permanent lesion. Anterior tibialis artery and intramuscular arteries velocity curves were assessed with echocolordoppler and compared with arterial vessels of the unaffected controlateral muscle. The velocity curves of the anterior tibialis artery and those examined in the intramuscular arteries (1 mm) of the denervated muscle at rest showed the presence of continuous flow component that was absent, as expected, in the anterior tibialis and intramuscular arteries of the unaffected controlateral anterior tibialis muscle. In our opinion the finding of continuous flow in the velocity curve by echocolordoppler assessment is the typical expression of long-lasting denervation in the vascular



tree of a resting muscle, sustained mainly by the nerve lesion. Voluntary contractions of the healthy muscle increase the echodopler signals. Interestingly, electrical stimulation-induced contractions of the denervated muscle changed the flow characteristics of the intramuscular flow that was more similar to the high-resistance flow of the normal muscle, whether at rest or after a short series of voluntary tetanic contractions (foot dorsiflexion against a low resistance). The method is easy to perform and may be repeated during denervation/reinnervation periods or during progression of permanent denervation atrophy to degeneration. Further studies will assess if flow (at rest and after exercise) and muscle atrophy are related, a potential tool to study rehabilitation of denervated and immobilized muscles.

TES of denervated muscles after peripheral nerve lesion

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Aim of the Rise2-Italy Project is to identify subjects with incomplete permanent injuries of arm/leg skeletal muscles to extend results of the EU Project RISE. An high-power electrical stimulator associated with large surface electrodes induces, indeed, single or sustained contractions of long-term denervated human muscles, thus promising trophic and some functional recovery of denervated muscle in patients with peripheral nerve lesions. Our cohort of patients suffered traumatic injuries to plexus or single nerve (e.g., circumflexus or femoral nerve). At enrolment, shoulder or leg muscles did not respond to the clinical stimulation protocols for innervated muscle (twitch stimulation with 0.5 msec long impulse at 5-20 V or mAmp). Using an electrical stimulator for denervated muscle (i.e., with an adequate power) that discharges triangular or bidirectional waves 150-200 msec long at 20-80 mAmp to large surface wet electrodes (20x40cm, in lap case) the denervated muscle produces repeatedly single contractions (twitch training). Patients were reevaluated monthly to verify if the denervated muscles partially recovered their excitability to a sufficiently short stimulations (duration < 50msec), to be able to respond to short trains of impulse at 10-20 Hz frequency (tetanic contractions 2-3 sec long). Therapeutic electrical stimulation (5 times a week) will not replace, but complement passive and active rehabilitation. Change in thickness and tissue composition are measured by ultrasound scan before and every three months during the 12 months of programmed home treatment. Our first observations suggest that this pilot study of the Padua Rehabilitation Unit could be extended with clinically significant results.

The cellular basis of muscle regenerative capacity after physical activity under severe hypoxic conditions

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The skeletal muscle plastic process is associated with the ability to regenerate following injury or overuse due to the intervening satellite cells, undifferentiated quiescent mononucleated cells present in muscle (Charge and Rudnicki, 2004; Mauro, 1961), which have properties of stem cells and are activated as a consequence of strenuous muscle work and/or injury. In response to muscle damage, satellite cells are activated to proliferate as myogenic precursor cells. Proliferating myoblasts migrate to the damaged region of the fiber, where they differentiate and fuse to form myotubes via a similar process to that of myogenesis. Aim of this contribute is to define the role of oxygen lack due to hypoxia on the muscle capacity to regenerate after strenuous physical activity. Biopsies from vastus lateralis (VL) 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. Biopsies (muscle pieces 0.1-0.4g in weight) were performed using the procedure of Engel (1994), and immediately treated to obtain explants, as described by Decary (1996) and processed according to the procedure of Fulle et al. (2005). Myogenic purity was monitored by immunocytochemistry using desmin. The efficiency of differentiation process was determined by Fusion Index by staining fast and slow myosin heavy chains using the procedure described by Edom et al. (1994). Results have surprisingly pointed out that, with the exception of one subject, none of the observed volunteers were able to show regenerative activity after the expedition. Causes and possible explanations of this phenomenon will be discussed.

Enhanced myogenic potential of CD44+ low bone marrow murine cells and Pax7+ cells formation after their injection in mdx mice

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Our work focused on murine MSCs both in vitro and in vivo. In vitro: we isolated by plastic adhesion mouse MSCs from bone marrow of C57BL/6J mice and we cultivated them considering different medium, serum concentration and presence or absence of leukemia inhibitory factor (LIF);



secondly, through cytofluorimetric analysis we characterized the cells' phenotype. We confirmed the self-renewal capacity by limiting dilution assay and we proved the mesenchymal potential by means of adipogenic, osteogenic and myogenic differentiations. Mdx mice were used to study muscle engraftment. Fresh BM GFP+ cells were selected through FACS sorter; mice were injected with fresh with GFP+CD44+, GFP+CD44+ low, GFP+CD44+ high cells in left tibialis anterioris (TAL) muscles, only medium or MEF as control were injected in right tibialis anterioris (TAR) muscles. Animals were sacrificed after 10 days and 4 weeks. We compared in vivo myogenic potential of CD44+ cells to CD44+ high and CD44+ low cells all from male GFP transgenic mice. The exclusive use of female recipient mice and male mBM-MSCs donors allow to further demonstrate the origin of fibers by performing FISH analysis in treated muscles. In particular, CD44+ low shaped an increased number of myofibers dystrophin positive in respect to CD44+ high. Furthermore, although both groups developed GFP/Pax7+ cells (CD44+ low 35.64 ± 0.31 ; CD44+ high, only the CD44+ low population generated greater number of dystrophin/GFP+ fibers. In conclusion, CD44+ low bone marrow murine cells possess enhanced myogenic potential and are able to generate Pax7+ cells when injected in mdx mice. Ideally, subpopulations of human bone marrow cells could be relevant for transplantation and muscle engraftment.

FES-induced recovery of skeletal fibers lacking innervation

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The relative importance of muscle activity versus neurotrophic factors in the maintenance of muscle mass has been greatly debated. Five spinal cord injury (SCI) patients lacking any peripheral nerves in the lower extremities were trained for prolonged periods of time (2.4 to 9.3 years) with an innovative rehabilitation protocol of functional electrical stimulation (FES). Biopsies from these patients (plus five controls) offered the unique opportunity of studying structural recovery of human muscle fibers from severe atrophy in the total absence of motor and sensory innervation and of nerve-derived trophic factors. FES treatment induced surprising recovery of muscle structure mass and force in the patients that were effectively stimulated, even after prolonged denervation (up to 2 years). 90% (or more) of the fibers analyzed show a striking recovery of the ultrastructural organization of myofibrils and Ca²⁺ handling membranes.

Thus, despite the almost complete loss of muscle-specific structure, the long-term denervated fibers maintain the capability of a full differentiation program. Interestingly, this almost complete recovery follows a pattern that mimics in many aspects normal muscle differentiation and, most importantly, it occurs in complete absence of nerve endings, solely under the influence of muscle activity induced by electrical stimulation.

Different muscle stem cell sources delivered through photopolymerizable Hydrogel scaffold improve muscle regeneration

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In our work we combined the regeneration potential of different muscle stem cell sources with an innovative hyaluronic acid-based Hydrogel, injectable and photopolymerizable in situ with UVA light. We isolated single fibers from C57BL-GFP mice *flexor digitorum brevis* (FDB), *extensor digitorum longus* (EDL) and *soleus* muscles through digestion with type-I collagenase. Isolated muscle fibres, disaggregated satellite cells and expanded in culture satellite cells were resuspended in a solution of hyaluronic acid-based photopolymerizable Hydrogel. They were then delivered into damaged mice muscles. Experiments were performed on C57BL/6J mice. Part of TA muscle were surgically removed and injected with Hydrogel containing cells. We performed histochemical, immunofluorescence and physiological analyses of the engrafted muscles after 6 weeks. Samples were compared to muscles engrafted only with Hydrogel and sham controls. TA muscles engrafted with fibers and dissociated satellite cells presented increased muscle mass and significantly higher number of GFP+ve fibers and GFP+ve satellite cells under the basal lamina when compared to the ones injected with saline. Isolated fibers and stripped satellite cells delivered through photopolymerizable Hydrogel are able to greatly regenerate muscles with severe injury after 6 weeks. Newly regenerated fibres are hypertrophic than fibres from TA muscles of control mice. Future perspective is the use of isolated single fibres and stripped satellite cells combined with the hyaluronic Hydrogel in mouse model of muscle diseases, as Duchenne muscular dystrophy to promote regeneration.



A defective SERCA1 protein is responsible for Congenital Pseudomyotonia in Chianina cattle

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Recently a muscular disorder defined as “congenital pseudomyotonia” has been described in Chianina cattle, one of the most important Italian breed for meat quality and leather. The clinical picture is characterized by an exercise-induced muscle contracture which prevents animals from performing muscular activities. On the basis of clinical symptoms Chianina pseudomyotonia was related to human Brody’s disease. Brody’s disease is a rare inherited disorder of skeletal muscle function, due to a sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA1) deficiency, resulting from a defect of ATP2A1 gene coding for SERCA1. The SERCA1 is involved in transporting calcium from cytosol into the lumen of sarcoplasmic reticulum. We have fully characterized the genetic defect underlying Chianina cattle pseudomyotonia. A missense mutation in exon 6 of the ATP2A1 gene, leading to a Arg 164 His substitution in the SERCA1 protein was found. We have provided also biochemical evidence of a selective deficiency of SERCA1 protein expression in sarcoplasmic reticulum membranes from affected muscles, although the mRNA levels result unchanged. The reduction of SERCA1 levels accounts for the reduced Ca^{2+} -ATPase activity, without any significant change in Ca^{2+} -dependency. The loss of SERCA1 is not compensated by the expression of the SERCA2 isoform. We believe that Chianina cattle pseudomyotonia might be the true counterpart of human Brody’s disease and that bovine species might be used as a suitable animal model.

Joint stability and balance training in injury prevention and rehabilitation protocols

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Proprioception, the combined functions of joint position sense and kinesthesia, has been identified as an important component for optimal joint function. Proprioception obviously involves different sensory systems of muscles, ligaments, tendons, joints and skin, and organs of vision and balance. Joint injury can affect proprioception, disrupting the normal neuromuscular function such as affected protection reflexes, impaired joint reposition sense, and other proprioceptive deficits. The contents of proprioceptive training are very effective, relatively safe, demand little energy and are at the same time very entertaining. Means of

such exercise include balance exercises on tilt and wobble boards and other unstable supporting surfaces, which cause dynamically unstable positions of joints or joint systems. As in motor abilities, it is reasonable to adhere to the principle of gradual progression in proprioceptive training. Each basic exercise can be performed in many different ways. And if we want to make a basic exercise more demanding, we can in addition perform a coordination exercise, or preliminarily disturb the balance organ, or eliminate the organ of sight, etc. According to the chosen exercise and corresponding geometry of the facility we can determine most suitable load for each individual. The effects of proprioceptive training are: the increase of muscular activation after the injury, the reduction of reflex-reaction times on stretching, the improvement of inter-muscular coordination, the improvement of poise and balance, the improvement of the awareness of one’s body in space and time and therefore, the reduced susceptibility to injuries. We are firmly convinced that health prevention contents like joint stability training should be a part of every physical conditioning and rehabilitation program.

Closing remarks from Manaslu project: perspectives for an interdisciplinary approach in hypoxic studies

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The tradition of scientific expedition has been well established in Italy from the middle of last century and the Manaslu 2008 project offered a new opportunity to further emphasize the advantages coming from mountain studies in the comprehension of the working capacity of human muscle apparatus. The outcomes obtained in this interdisciplinary research overcomes the scientific results to document the possibility of a positive collaboration among several different groups each of those contributing to the final positive conclusion. This coordination could advance by a greater extent with the actual availability of a new laboratory that in now operating in Rovereto at CeBiSM. In a large room there will be the possibility to re-create an artificial mountain environment with a level of oxygen from the sea side to 7000 msl and with a temperature ranging from 40 to -20 degrees. The room could also be equipped by any kind of ergometers as well as bio-physiological measurement tools and it can host some persons for hours or days. This new lab could represent a stimulating opportunity for a new development in the special environment study and it strongly support interdisciplinary studies.

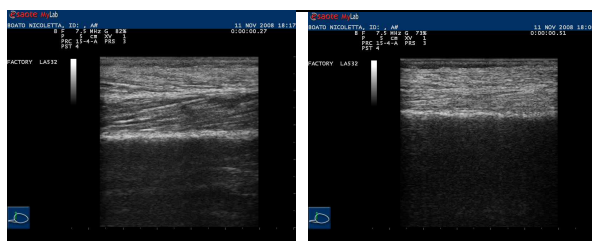


Functional EchoMyography of denervated muscle. Preliminary results

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The first goal of the Rise2-Italy Project is to identify among the clinical cases of the Physical Medicine and Rehabilitation Unit of the University of Padua a group of subjects with permanent injuries (complete or incomplete) of arm/leg skeletal muscles aimed at extending the results of the European Project RISE [1-3]. Demonstration that a high-power electrical stimulator associated with large surface electrodes induces single (twitch) or sustained (tetanus) contractions in long-term denervated human muscles of paraplegics, open the opportunity to obtain trophic effect and some functional recovery of denervated muscle in patients with severe muscle atrophy secondary to lower motor neuron lesions. To monitor changes in thickness and tissue composition of trained muscles ultrasound scan are performed before and every three months during the 12 months of programmed treatment.



The figure show in the left panel the left normal Tibialis Anterior (TA), and the right panel the right TA after one year of permanent denervation.

Here we show that thickness of skeletal muscle is easily and reliably measured by Echomyography. Extent and dynamics of the contraction-relaxation response to electrical stimulation is also consistently analyzed (see on-line materials to activate the movies). Combined with Echo-doppler of intramuscular arteries (see in this Issue: Martini R, Ostrowski M, Andreozzi GM, Stramare R, Carraro U. Ultrasonography of medium and small muscle vessels), Functional Echo-Myography is an ultrasound approach that allows non-invasive, repeated analyses of the muscle response to rehabilitation treatments. These may be tailored to the personal needs of each patient, the decisions being based, beside expert clinical evaluations, on instrumental results. This pilot study was performed as part of the Rise2-Italy Project, an institutional research activity of the University of Padova Interdepartmental Research Center of

Myology. We are confident that it will extended with clinically significant results the sound outcomes of the EU Project RISE.

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Molecular and cellular muscle adaptations to physical activity in hypobaric hypoxia

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Skeletal muscle is a very plastic tissue, able to adapt to new functional demands, such as determined by increased activity during training and by changed environmental conditions as reduced oxygen supply. A stay at high altitude during a climbing expeditions offers a good chance to study such adaptations. In this study we examined single muscle fibres taken from biopsy of vastus lateralis of five male volunteers (40 ± 14 years old) before and upon the return from the Himalayan Expedition (INTERAMNIA 8000 - MANASLU 2008) during which they were chronically exposed to hypoxia living for about 30 days above 5000 m. Fibre type distribution was changed in the second sampling compared to the first one as slow fibre proportion was increased. Cross sectional area was not significantly modified and no open sign of atrophy was detectable. In one subject, average cross sectional area was significantly increased. Mechanical parameters of muscle contraction did not show any significant variations. We can conclude that the stay in hypoxic conditions did not cause any adverse change in muscle fibre structure and function in substantial agreement with data obtained by testing the physical performance of the subjects as reported in other presentations at this meeting.



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University of Padua – *cirMYO Interdepartmental Research Center of Myology*
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Manaslu Expedition 2008: physiological adaptations to chronic altitude hypoxia

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High-altitude hypoxia is a condition characterized by reduction of partial O₂ pressure as a direct consequence of barometric pressure reduction. Cellular PO₂ is maintained within a narrow range and the oxygen-gradient diffusion of the capillary tissues level is essential for the cellular survival. High altitude hypoxia has been known to influence metabolic, ventilatory and muscular adaptations. Skeletal muscle intracellular PO₂ is important, oxygen pressure in maximally exercising muscle is close to zero determines a condition of intracellular hypoxia. In altitude skeletal muscle undergoes mass reduction with reduction of mitochondrial volume density. The decrease in maximum oxygen consumption with altitude is related to the low cell PO₂ and to the increase level of ROS and HIF (Hypoxic Inducible Factor) levels. Involvement of HIF on the regulation of adaptation processes in skeletal muscle tissue after training is under studies. Six members of the Manaslu (8163 m) Expedition in 2008 were examined before and after a stay of 43 days at an altitude ranging from 0 to 5800 m above sea level. Before and after the scientific expedition we have performed functional assessment measure, blood samples, semen samples collected, muscle biopsy, nuclear magnetic resonance of leg muscle, test, interviews and psychological profiles. The aim of the present study is to evaluate the effect of chronic altitude hypoxia on human metabolic, ventilatory, muscular, nervous and blood adaptations and in male reproductive functions.

Identification of cachexia predictive biomarkers in serum and muscle biopsies from patients bearing colon rectal cancer

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Cachexia is a severe debilitating condition which occurs in association to several diseases such as cancer, AIDS, diabetes, renal or heart failure and aging. The aetiology of this syndrome has been extensively studied, both in humans and in animal models, and several humoral and inflammatory markers have been identified so far mediating this condition. In cancer cachexia factors of tumor origin can induce muscle wasting, either alone or in combination with host-derived factors and cytokines. In particular, pro-inflammatory cytokines (such as TNF- α , IL-1 β , and IL-6) play a direct role in eliciting cachexia. Within the skeletal muscle, several signal transduction pathways are activated in response to secreted cytokines, primarily to TNF- α , which in turn mediate muscle atrophy and wasting. Muscle atrophy is characterized by reduced protein synthesis and enhanced protein catabolism. Ubiquitination of target proteins represents one of the main pathways activated for degradation, but also factors involved in signal transduction or gene transcription are involved. Interestingly, it has been reported that the myogenic transcription factor MyoD is negatively regulated in many experimental model of cachexia, such as in C26 mice bearing colorectal cancer. Induction of MyoD transcription is a hallmark of muscle precursor cell activation after muscle damage, and the blockade of this factor may result in the inhibition of muscle regeneration, but turnover of muscle nuclei (in the so called apoptotic muscle changes) may be also relevant thus facilitating muscle atrophy and wasting. The aim of our study is to verify whether in patients affected with colorectal cancer at disease onset and beyond, it is possible to detect, both locally within muscle tissue as well as circulating in the serum, the upregulation of early biomarkers which may predict the progression of the disease towards cachectic syndrome. Data obtained from these analyses will further elucidate molecular mechanism underlying cachexia, in particular during early cancer phases, providing potential specific targets for therapeutic intervention.
