Muscle training in ageing, denervation and beyond

Padova & Terme Euganee (Italy), November 23 - 25, 2012

Terme Eugane Conference Hall, Hotel Augustus, Viale Stazione 150 - 35136 Montegrotto Terme, Padova, Italy
Phone +39 049 793 200 - Fax +39 049 793518 - http://www.hotelaugustus.com/english/pages/hotel_augustus.php - E-mail: info@hotelaugustus.com

Organizers: H. Kern and U. Carraro

Friday November 23, 2012
Department of Biomedical Sciences, University of Padua, Viale G. Colombo 3, Padova, Italy - Room F-PR
14.30 Lecture High-resolution respirometry in permeabilized fibers from small biopsies of human muscle
Prof. Erich Gnaiger, Innsbruck Medical University, Austria

15.15 Lecture Malignant hyperthermia and exertional/environmental heat stroke: understand the molecular mechanisms to develop therapeutic interventions
Prof. Feliciano Protasi, Chieti University, Italy

Terme Eugane Conference Hall, Hotel Augustus, Montegrotto Terme, Padova, Italy
18.00 Workshop Rise2-Italy – S. Masiiero, Winfried Mayr, Chairman
Muscle reinnervation on a difficult-to-stimulate case of Conus-Cauda Syndrome, Emiliania Bizzarini, et al., Udine, Italy
*Long-term Effectiveness of h-b FES for denervated muscle is related to compliance. A case study by 3D Color Tomography, Paolo Gargiulo, et al., Reykjavik, Iceland *by Skype
*Muscle Density Changes after Electrical Stimulation Therapy with Neuroporthesis for Finger Rehabilitation, Thordur Helgason, et al., Reykjavik, Iceland *by Skype
Macrophages and muscle regeneration: a multi-faceted interaction that could lead to therapeutic tools. Eva Galletta et al., Padova, Italy

Program outlooks, Ugo Carraro, Padova, Italy
21.00 Organizing Meeting of the 2013 Spring Padua Muscle Days, March 15-17 & future collaborations

Saturday November 24, 2012
Terme Eugane Conference Hall, Hotel Augustus, Montegrotto Terme, Padova, Italy
09.00 Workshop Interreg IVa “Mobility in elderly” – H. Kern, Chairman
10 min Program overview, H. Kern, Vienna, Austria
20 min Morphometry of skeletal muscle in sedentary elderly and senior sportsmen, Sandra Zampieri et al., Padua, Italy
20 min Progressive uncoupling of mitochondria from calcium release units in ageing: implications for muscle performance, Feliciano Protasi, Simona Boncompagni, Chieti, Italy
10 min Training groups in Bratislava, Jan Cvecka, Milan Sedliak, Dusan Hamar, Bratislava, Slovakia
10 min Potential Fatigue responses in senior sportsmen, Nejc Sarabon, Koper, Slovenia
10 min *Assessment of total hip arthroplasty by 3D CT: evaluation of muscle and bone, Paolo Gargiulo, Reykjavik, Iceland *by Skype
10.30 Interreg IVa – Molecular Biology
30 min The molecular basis of muscle ageing, Antonio Musarò, Rome, Italy
30 min Atrophy/hypertrophy in healthy and diseased muscle and senior sportsmen, Marco Sandri, Padua, Italy
11.30 Interreg IVa – Perspectives in Rehabilitation of Oncologic Patients
20 min Strength of muscles and QoL in tumor bearing patients, Richard Crevenna, Helmut Kern, Vienna, Italy
20 min Myopathic features in patients affected with newly diagnosed colorectal cancer, Sandra Zampieri, et al., Padua, Italy
12.30 Lunch & Cycling Exercise
15.30 Papers in preparation: General Discussion
21.00 Interreg IVa: Future collaborations in ageing & oncology

Sunday November 25, 2012
Terme Eugane Conference Hall, Hotel Augustus, Montegrotto Terme, Padova, Italy
09.00 Interreg IVa “Mobility in elderly” Partner meeting, Outlook 2013-2015
High-resolution respirometry in permeabilized fibers from small biopsies of human muscle

Erich Gnaiger

D. Swarovski Research Laboratory (Mitochondrial Physiology), Dept. General, Visceral and Transplant Surgery, Medical University of Innsbruck; and OROBOROS INSTRUMENTS, Innsbruck, Austria.
E-mail: erich.gnaiger@oroboros.at

Abstracts

Aging implicates a progressive decline in muscle mass and strength (sarcopenia) which is counteracted by strength training, and a decline of aerobic performance (muscle fatigability, reduced aerobic capacity and loss of mitochondrial power or OXPHOS capacity in muscle tissue). The bioenergetic function of mitochondria in a tissue is evaluated by OXPHOS analysis and depends on mitochondrial structure and function [1-4]. Mitochondrial structure or mt-density is determined by measurement of mt-markers normalized for tissue mass or volume. Mitochondrial function per tissue mass (tissue mass-specific function), in turn, depends on mt-density and mt-specific function (normalized for a mt-marker). In contrast, respiratory control ratios are a normalization of flux independent of tissue mass or mt-content. Depending on normalization of OXPHOS capacity or metabolic fluxes in general, therefore, very different levels of integration are obtained: (i) Tissue mass-specific, (ii) mt-specific, and (iii) flux control ratios (FCR). Corresponding thermodynamic forces are intensive functional OXPHOS parameters, such as the chemiosmotic force, electrical mt-membrane potential or redox potentials. OXPHOS analysis has become possible using small biopsies of human muscle by combining high-resolution respirometry with preparations of permeabilized muscle fibres [1-4] or tissue homogenate [5]. When working with isolated mitochondria, the most commonly used mt-marker for normalization of OXPHOS capacity is mt-protein, but this marker is not accessible in permeabilized fibres or tissue homogenate. OXPHOS capacity per tissue mass is increased or maintained by endurance exercise and strength training [3,4]. Life style is modified from the age of 20-30 years to the elderly, but is subject to change and intervention at any age. Depending on group selection in cross-sectional studies, tissue OXPHOS capacity declines with age from 20-30 years onwards [6,7] or is independent up to 80 years [8,9]. In an aged population with a wide range of physical mobility, walking speed and VO2max are closely correlated with the bioenergetic function of mitochondria in a tissue which may be characteristic of a low-grade inflammatory state ("mitochondrial fever"). Onset of degenerative diseases (diabetes 2, neuromuscular degeneration, various cancers) and mitochondrial dysfunction interact in an amplification loop progressing slowly with age, such that cause and effect of mitochondrial dysfunction cannot be distinguished. Diminished antioxidant capacity at low mitochondrial density is an important mechanistic candidate in the state of mitochondrial fever. Novel perspectives emerge from the combination of different established techniques into a single experimental system, integrating high-resolution respirometry for measurement of mitochondrial respiration [1], and fluorometry for monitoring mt-membrane potential, Ca-retention capacity and H2O2 (and ROS) production [12] as a function of pro/antioxidant disposition, metabolic state, and oxygen level, providing new standards for OXPHOS analysis.

Contribution to K-Regio MitoCom Tyrol


*****

Malignant hyperthermia and exertional/environmental heat stroke: understand the molecular mechanisms to develop therapeutic interventions

Feliciano Protasi, Antonio Michelucci, Cecilia Paolini

CeSI - Center for Research on Ageing & DNI – Dept. Neuroscience and Imaging, University G. d’Annunzio of Chieti, Italy

E-mail: fprotasi@unich.it

Background. Several skeletal muscle diseases have been associated to mutations in proteins involved in the excitation contraction (EC) coupling, the mechanism that links the gene encoding for ryanodine receptor type-1 (RYR1), the SR Ca^2+ release channel, underlie several debilitating, life-threatening muscle disorders including malignant hyperthermia (MH). To date, MH is only seen as a clinical syndrome in which genetically predisposed individuals respond to volatile anesthetics in the operating room with episodic muscle stiffness, muscle pain, and increased serum levels of CK, hyperkalemia, etc. Interestingly, some patients who experienced heat/exertion-induced MH have already been successful in curing CASQ1-knockout mice suffering of MH and heat-susceptibility by administration of N-acetylcysteine, a potent anti-oxidant administered in drinking water.

*****

RISE2-Italy: home-based FES in denervated/reinnervating human muscles

Ugo Carraro (1), Sandra Zampieri (1,2), Libero Vitiello (3), Roberto Stramare (4); Paolo Gargiulo (5), Stefano Masiero (6), Helmut Kern (2)

(1) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria; (3) Dept. Biology, University of Padova, Padova, Italy; (4) Radiology, Dept. Medicine, University of Padua, Italy; (5) Dept. Science, Education and Innovation, Landspitali University Hospital, and Dept. Biomedical Engineering, University of Reykjavik, Iceland; (6) Physical Medicine and Rehabilitation, Dept. Neurorosciences, University of Padua, Italy.

E-mail: sanzamp@unipd.it

During the last decade we contributed to translational myology and rehabilitation studying effects of physical exercise induced by Functional Electrical Stimulation (FES) in the special case of Spinal Cord Injury patients affected by
complete injury of the Conus Cauda, a syndrome in which the denervated leg muscles are fully disconnected from the nervous system. Denervated human muscles become unexcitable with commercial electrical stimulators and undergo ultra structural disorganization within a few months from SCI, while severe atrophy with nuclear clumping, and fibro-fatty degeneration appear within 3 and 6 years, respectively [1-4]. To counteract these progressive changes a novel therapy concept for paraplegic patients with complete lower motor neuron denervation of the lower extremity was developed in Vienna: home-based functional electrical stimulation of long-term denervated muscles (h-b FES). New electrodes and stimulator have been designed to reverse severe atrophy by delivering high-intensity (up to 2,4 J) and long-duration impulses (up to 150 ms) able to elicit contractions of denervated skeletal muscle fibers in absence of nerves [5,6]. At the same time, specific clinical assessments and training strategies were developed at the Wilhelminenspital Wien, Austria [6], based on sound evidence from animal experiments [7]. Light and electron microscopy of muscle biopsies were performed in Italy at the Universities of Padova and of Chieti, respectively. Main results [9-11] of this clinical study (EU Program RISE, Contract No: QLG5-CT-2001-02191) on patients which completed the 2-year h-b FES training are: 1. significant increase of muscle mass and of myofiber size, with striking improvements of the ultra-structural organization; 2. recovery of tetanic contractility with significant increase in muscle force output during electrical stimulation; 3. capacity to perform FES-assisted stand-up and stepping-in-place exercise. The study demonstrated that h-b FES of permanent denervated muscle is an effective home therapy that results in rescue of muscle mass, function and perfusion. Additional benefits, important for the patients, were the improved cosmetic appearance of the legs and the enhanced cushioning effect for seating.

We are now extending our studies to application of h-b FES to the larger cohort of incomplete denervated human muscles (RISE2-Italy, PRIN 2008J4MRW). Based upon the sound results of the EU Project RISE, which demonstrated that progressive rehabilitation strategies using powerful electrical stimulation delivered by large surface electrodes induced sustained tetanic contractions [2-6,8-10], the short-term and long-term objectives of the research program “Rise2-Italy” were: 1. to extend this advantage to a larger group of patients; 2. to establish new methods to frequently monitor regression of denervation-induce muscle properties and recovery by home-based Functional Electrical Stimulation (h-b FES); 3. to recover some motor function; 4. to increase the venous return from lower legs (anti-oedema effect). To reach these goals, the University of Padova Interdepartmental Research Center of Myology implemented “Rise2-Italy” an international clinical research joint project of several Italian Rehabilitation Centers: Padua, Udine, Villanova sull’Arda (PC), Montecatone (Im), Modena, and Chieti/Pescara and with the key collaboration of dr. Helmut Kern, Ing. Winfried Mayr (Vienna, Austria) and of Ing. Paolo Gargiulo (Reykjavik, Iceland). Three patients were enrolled during the first year of the project. Inclusion/exclusion evaluations for enrolment and follow-up are those validated by the EU Project RISE, integrated by a non invasive functional imaging analysis, the “Functional Echomyography”, a protocol that has been established during the first year of the project. This non-invasive approach allow to safely and routinely analyze: 1) size of muscles; 2) their contraction kinetics in response to electrical stimulation, and 3) the arterial blood flow to muscles at rest and in response to electrical stimulation.

Furthermore, by joining Functional Electrical Stimulation and Regenerative Medicine we hoped to develop a new multidisciplinary rehabilitation strategy combining engineering and tissue engineering, by taking advantage of the spontaneous muscle fiber regeneration potential of denervated muscles [1,2] or enhanced-regeneration by autologous myoblasts derived from satellite cells or mesenchymal myogenic progenitor cells. This part of the project (Unit 2-Vitiello) focus on delivery of myogenic cells into denervated muscles, based upon the use of injectable, biocompatible matrices. We assessed the grafting of freshly isolated satellite cells, and their progeny expanded in vitro. As for the matrix, we used a novel type of hyaluronic-based hydrogels containing VEGF and HGF and/or macrophage-secreted factors to improve the regeneration process.

Mean results of RISE2-Italy are: 1. Recovery of muscle mass and function (shortened twitch relaxation time and recovered tetanic contractility); 2. Demonstration that h-b FES do not prevent synaptogenesis (functional and electromyográfic evidence of reinnervation during the years of training despite the high energy of the electrical stimuli); 3. Extent of muscle recovery is compliance dependent, but the muscle tissue lost during months of discontinuation of the therapy could be regained; 4. Since the gain of muscle is reversible, it can not be only related to eventual reinnervation events; 5. Usefulness of Functional Echomyography in the follow-up (by ecogenicity, thickness, contraction/relaxation kinetics and perfusion of denervated/reinnervating human muscle before, during and after electrical stimulation).

In conclusion, our findings strongly support the RISE rehabilitation protocol as a home-based method to improve mass and contractility of denervated/reinnervating muscles.

Abstracts


Home-based FES of denervated muscle. Muscle reinnervation on a difficult-to-stimulate case of an ASIA A Conus-Cauda Syndrome

Emiliana Bizzarini (1), Chiara Pinzini (1), Giuliana De Maio (2), Agostino Zampa (1), Sandra Zampieri (3,4), Helmut Kern (4), Stefano Masiero (5), Ugo Carraro (3)

(1) Spinal Unit, Dept. Rehabilitation Medicine, ASS N° 4 “Medio Friuli”, Udine, Italy; (2) International School for Advanced Studies (SISSA) Trieste, SPINAL Lab, IMFR, Udine, Italy; (3) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (4) ; (5) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria; (5) Physical Medicine and Rehabilitation, Dept. Neurorosciences, University of Padua, Italy.
E-mail: emiliana.bizzarini@mediofriuli.it

We report a 41-year-old male patient (Rise2-Friuli-LS) who sustained a severe traumatic spinal cord injury in an accident at work (April 11, 2008). On clinical examination he presented with flaccid paraplegia (motor level D12). After discharge from Neurosurgery at 15 days from trauma he was admitted in our Spinal Unit. At the clinical examination was evaluated as A at the ASIA Impairment Scale (Motor level D12, anesthesia below D12). From an electrophysiological point of view somato-sensory evoked potentials and motor evoked potentials were silent. He had begun the rehabilitation program with the purpose to reach autonomy in wheelchair locomotion and activities of daily living (ADL). The physical rehabilitation was directed to the strengthening of upper limbs, trunk control and to preventing lower limb muscle atrophy with patterned Electrical Stimulation. Specific stimulation parameters (i.e., pulse width, train duration, between train intervals, method of application) were applied, but we could not obtain tetanic contraction of leg muscles (quadriceps and tibialis anterior). The training was completed by passive exercise at the cycloergometer and assisted body-weight support treadmill training. Gait over ground was not possible. At demission we verified autonomy in wheelchair locomotion and ADL, a good trunk control and an increment of aerobic performance (VO2max). Meantime some reinnervation of leg muscles occurred demonstrated by minimal spontaneous activity of right foot muscles, but voluntary or electrical stimulation-induced muscle contractions did not appeared. A few months later FES-induced twitch dorsiflexion of the ankle were achieved by surface electrical stimulation of the tibialis anterior using the “Denervated Muscle Stimulation” program of the STIWELL Med4 device of MED-EL (Innsbruck, Austria), that delivers triangular or bidirectional impulses of
Utilities of Ultra Sound Myography in a clinical case of monolateral proximal sciactetomy: ecogenousity, thickness, contraction/relaxation kinetics and perfusion of denervated/reinnervating human muscle before, during and after h-b FES

Riccardo Zanato (1), Roberto Stramare (1), Leonora Martino (1), Sandra Zampieri (2,3), Helmut Kern (3), Stefano Masiero (4), Ugo Carraro (2)

(1) Radiology, Dept. Medicine, University of Padua, Italy; (2) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (3) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminalispital Wien, Austria, (4) Physical Medicine and Rehabilitation, Dept. Neurosciences, University of Padua, Italy.

E-mail: roberto.stramare@unipd.it

Permanent denervated muscles were evaluated by ultra sound (US) to monitor changes in morphology, thickness, contraction-relaxation kinetics and perfusion due to the
electrical stimulation program of the Rise2-Italy project. In a case of monolateral lesion, morphology and ultrasonographic structure of the denervated muscles changed during the period of stimulation from a pattern typical of complete denervation-induced muscle atrophy to a pattern which might be considered “normal” when detected in an old patient. Thickness improved significantly more in the middle third of the denervated muscle, reaching the same value as the contralateral innervated muscle. Contraction-relaxation kinetics, measured by recording the muscle movements during electrical stimulation, showed an abnormal behavior of the chronically denervated muscle during the relaxation phase, which resulted to be significantly longer than in normal muscle. The long-term denervated muscles analyzed with Echo Doppler showed at rest a low resistance arterial flow that became pulsated during and after electrical stimulation. As expected, the ultra sound measured electrical stimulation-induced hyperemia lasted longer than the stimulation period. Despite the higher than normal energy of the delivered electrical stimuli of Vienna home-based Functional Electrical Stimulation (h-b FES) the muscles shown electromyografic signs of re-innervation during the one-year of training. In conclusion, this pilot study demonstrates the usefulness of US myography in the follow-up and the positive effects of h-b FES of denervated muscles.

*****

Long-term effectiveness of h-b FES for denervated muscle is related to compliance. A case study by 3D Color Tomography

Paolo Gargiulo (1,2), Páll Ingvarsson (3), Sigrún Knútsdóttir (3), Vilborg Gudmundsdóttir (3), Stefán Yngvason (3), Thórdur Helgason (1)

(1) Dept. Science, Education and Innovation, Landspitali University Hospital, Iceland; (2) Dept. Biomedical Engineering, University of Reykjavik; (3) Dept. Rehabilitation Medicine, Landspitali-University Hospital, Reykjavik, Iceland

E-mail: “Paolo Gargiulo” <paologar@landspitali.is>

Muscle tissue composition accounting for the relative content of muscle fibres and intramuscular adipose and fibrous tissues can be efficiently analyzed and quantified using images from spiral computed tomography (S-CT) technology and the associated distribution of Hounsfield unit (HU) values. Muscle density distribution, especially when including the whole muscle volume, provides remarkable information on the muscle condition. The study case reported here show the changes related to compliance in normal muscle fiber tissue, fat content, and loose and dense fibrous connective tissues in denervated muscle treated with h-b FES. Spiral CT data were taken before, during and after interruption of h-b FES. The observed period is 2 years: 6 months FES treatment, 4 months interruption because of Colitis ulcerosa and following 1 year of resumed FES treatment. HU based density analysis show a remarkable decrease in muscle density when the denervated muscle is not stimulated, however muscle mass is regained when the electrical stimulation is resumed and consistently performed. Using 3-D modelling and segmentation tools was possible to monitor changes in rectus femoris demonstrating the sensitivity of the denervated muscle to respond to electrical stimulation: the muscle density decreased of approximately 15% after 3 months of non stimulation. This and other cases of h-b FES for denervated muscle demonstrate that: 1. effectiveness is compliance dependent, but the muscle tissue lost during several months of discontinuation of the therapy could be regained; 2. since the gain of muscle function and mass is reversible, it can not be only related to eventual reinnervation events.

*****

Muscle Density Changes after Electrical Stimulation Therapy with Neuroprosthesis for Finger Rehabilitation

Helgason T (1,2), Svanbjörnsdóttir D (2), Gudmundsdottir RA (1), Oskarsdottir A(2), Sigþórsson H (2), Gargiulo P (1,2), Gudmundsdottir V (3), Ingvarsson P (3)

(1) Department of Medical Engineering and IT, Landspitali-University Hospital; (2) Department of Science and Engineering, Reykjavik University; (3) Department of Rehabilitation at Grensas, Landspitali – University Hospital, Reykjavik, Iceland
E-mail: "Paolo Gargiulo" <paologar@landspitali.is>

Muscle tissue composition accounting for the relative content of muscle fibres and intramuscular adipose and fibrous tissues can be efficiently analyzed and quantified using images from spiral computed tomography (S-CT) technology and the associated distribution of Hounsfield unit (HU) values. Muscle density distribution, especially when including the whole muscle volume, provides remarkable information on the muscle condition. The study case reported here show the changes related to compliance in normal muscle fiber tissue, fat content, and loose and dense fibrous connective tissues in denervated muscle treated with h-b FES. Spiral CT data were taken before, during and after interruption of h-b FES. The observed period is 2 years: 6 months FES treatment, 4 months interruption because of Colitis ulcerosa and following 1 year of resumed FES treatment. HU based density analysis show a remarkable decrease in muscle density when the denervated muscle is not stimulated, however muscle mass is regained when the electrical stimulation is resumed and consistently performed. Using 3-D modelling and segmentation tools was possible to
Macrophages and muscle regeneration: a multi-faceted interaction that could lead to therapeutic tools

Eva Galletta (1), Chiara Saletti (1), Federico Galvagni (2), Chiara Bolego (3), Libero Vitiello (1)

(1) Dept. Biology, University of Padova, Padova, Italy; (2) Dept. Biotechnology, University of Siena, Italy; Department of Pharmacy, University of Padova, Italy
E-mail: eva.galletta@bio.unipd.it

Muscle regeneration is a complex process that involves many different types of cells, both myogenic and non-myogenic. In particular, macrophages play a fundamental role: they phagocyte debris, release cytokines to regulate inflammatory response and stimulate satellite cells proliferation. In vivo, there are two main types of macrophages: M1 (pro-inflammatory) macrophages, that can stimulate proliferation of myoblasts, and M2 (anti-inflammatory) macrophages, which can stimulate the differentiation of myoblasts. Inflammation is known to play a major role in the pathophysiology of Duchenne Muscular Dystrophy. To study the effects of macrophage-released factors onto myogenic cells we use the murine macrophage cell line J774 to obtain a serum-free, conditioned medium (mMCM). We previously found that mMCM enhance the proliferation rate and the appearance and enhanced cushioning effect for seating. Additional benefits are improved leg cosmetic

*****

Macrophages and muscle regeneration: a multi-faceted interaction that could lead to therapeutic tools

Eva Galletta (1), Chiara Saletti (1), Federico Galvagni (2), Chiara Bolego (3), Libero Vitiello (1)

(1) Dept. Biology, University of Padova, Padova, Italy; (2) Dept. Biotechnology, University of Siena, Italy; Department of Pharmacy, University of Padova, Italy
E-mail: eva.galletta@bio.unipd.it

Muscle regeneration is a complex process that involves many different types of cells, both myogenic and non-myogenic. In particular, macrophages play a fundamental role: they phagocyte debris, release cytokines to regulate inflammatory response and stimulate satellite cells proliferation. In vivo, there are two main types of macrophages: M1 (pro-inflammatory) macrophages, that can stimulate proliferation of myoblasts, and M2 (anti-inflammatory) macrophages, which can stimulate the differentiation of myoblasts. Inflammation is known to play a major role in the pathophysiology of Duchenne Muscular Dystrophy. To study the effects of macrophage-released factors onto myogenic cells we use the murine macrophage cell line J774 to obtain a serum-free, conditioned medium (mMCM). We previously found that mMCM enhance the proliferation rate and the appearance and enhanced cushioning effect for seating. Additional benefits are improved leg cosmetic

*****

Macrophages and muscle regeneration: a multi-faceted interaction that could lead to therapeutic tools

Eva Galletta (1), Chiara Saletti (1), Federico Galvagni (2), Chiara Bolego (3), Libero Vitiello (1)

(1) Dept. Biology, University of Padova, Padova, Italy; (2) Dept. Biotechnology, University of Siena, Italy; Department of Pharmacy, University of Padova, Italy
E-mail: eva.galletta@bio.unipd.it

Muscle regeneration is a complex process that involves many different types of cells, both myogenic and non-myogenic. In particular, macrophages play a fundamental role: they phagocyte debris, release cytokines to regulate inflammatory response and stimulate satellite cells proliferation. In vivo, there are two main types of macrophages: M1 (pro-inflammatory) macrophages, that can stimulate proliferation of myoblasts, and M2 (anti-inflammatory) macrophages, which can stimulate the differentiation of myoblasts. Inflammation is known to play a major role in the pathophysiology of Duchenne Muscular Dystrophy. To study the effects of macrophage-released factors onto myogenic cells we use the murine macrophage cell line J774 to obtain a serum-free, conditioned medium (mMCM). We previously found that mMCM enhance the proliferation rate and the appearance and enhanced cushioning effect for seating. Additional benefits are improved leg cosmetic

*****

Macrophages and muscle regeneration: a multi-faceted interaction that could lead to therapeutic tools

Eva Galletta (1), Chiara Saletti (1), Federico Galvagni (2), Chiara Bolego (3), Libero Vitiello (1)

(1) Dept. Biology, University of Padova, Padova, Italy; (2) Dept. Biotechnology, University of Siena, Italy; Department of Pharmacy, University of Padova, Italy
E-mail: eva.galletta@bio.unipd.it

Muscle regeneration is a complex process that involves many different types of cells, both myogenic and non-myogenic. In particular, macrophages play a fundamental role: they phagocyte debris, release cytokines to regulate inflammatory response and stimulate satellite cells proliferation. In vivo, there are two main types of macrophages: M1 (pro-inflammatory) macrophages, that can stimulate proliferation of myoblasts, and M2 (anti-inflammatory) macrophages, which can stimulate the differentiation of myoblasts. Inflammation is known to play a major role in the pathophysiology of Duchenne Muscular Dystrophy. To study the effects of macrophage-released factors onto myogenic cells we use the murine macrophage cell line J774 to obtain a serum-free, conditioned medium (mMCM). We previously found that mMCM enhance the proliferation rate and the appearance and enhanced cushioning effect for seating. Additional benefits are improved leg cosmetic

*****
the effects of exercise on aging rehabilitation, we are analyzing by morphometric light and electron microscopy and molecular biology quadriceps muscle biopsies from young (23 years) [12] and senior male subjects: sedentary elderly and senior sportsmen (a peculiar group of subjects that performed life-long sport activities) with a mean age of 70 years. The group of sedentary seniors was also exercised for 10 weeks with two different types of training (leg press or electrical stimulation) and the analyses performed before and after the training period. Preliminary results confirm the effectiveness of h-b FES.

Based on our recent observation of the presence of a subclinical myopathy in patients affected with newly diagnosed colorectal cancer [13], we are now extending our approaches to oncologic rehabilitation. The factors associated with a subclinical myopathy at this stage of disease are unknown. A comprehensive study on the potential molecular mechanisms that are responsible for this cancer-associated myopathy could possibly provide new diagnostic and prognostic markers and new therapeutic targets to prevent the severe loss of muscle tissue which characterizes late-onset cancer cachexia.


*****
Morphometry of skeletal muscle in sedentary elderly and senior sportsmen

Sandra Zampieri (1,2), Katia Rossini (1), Ugo Carraro (1), Helmut Kern (2)

(1) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria, Italy
E-mail: “Sandra Zampieri” <sanzamp@unipd.it>

Aging is a multifactorial process that is characterized by decline in muscle mass and performance. Several factors, including reduced exercise, poor nutrition and modified hormonal metabolism, are responsible for changes in the rates of protein synthesis and degradation that drive skeletal muscle mass reduction with a consequent decline of force generation. In order to assess histology and morphometry of skeletal muscle in elderly people, and the effect of exercise on rehabilitation of aged muscle, we analyzed quadriceps muscle biopsies from senior male subjects: twelve sedentary elderly and sixteen senior sportsmen (a peculiar group of subjects that performed life-long sport activities) with a mean age of 69.8 ± 5.5 years and 70.2 ± 4.0, respectively (p = n.s). The group of sedentary seniors was also exercised for 10 weeks with different type of training (leg press, LP or electrical stimulation, ES) and the analyses were performed before and at the end of the training period. Similar histological features were observed in all muscle biopsies from sedentary and sportsmen seniors (no signs of degeneration, and/or of inflammatory reactions). Main differences were observed in the mean myofiber diameter in sportsmen compared to the pre training biopsies of sedentary seniors (61.22±17.11 vs 51.41±15.43, p < 0.0001), for both fast and slow fiber types. Accordingly, in the post training biopsies from the sedentary seniors, we observed a significant increase of the mean myofiber diameter compared to the pre training value (from 51.4±15.43 to 56.20±16.67, p < 0.0001), with no significant differences between the two type of training (LP or ES). Interestingly, the significant increase of the mean myofiber diameter was observed together with the presence in post training biopsies of numerous N-CAM positive cells, presumably activated satellite cells due to their localization beneath the basal lamina, but externally to the sarcolemma of the myofiber.

In senior sportsmen a significant increase of slow type fibers (69%) was observed in association with events of fiber type grouping, while in the sedentary seniors, the percentage of the slow type fiber decreased after the training, even though not significantly (55% vs 49%, p = 0.22).

Measurements of force development and functional tests (10-m walking tests, SPPB, and TUGT tests) of senior sportsmen and sedentary elderly, both before and after training, are in line with morphological analyses performed on muscle biopsies. Based on these findings, regular physical activity may represent a good strategy to attenuate and/or reverse skeletal muscle mass and strength decline as well as abnormalities associated with aging.

*****

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

Laura Pietrangelo, Alessandra D’Incecco, Simona Boncompagni, Feliciano Protasi
CeSI - Center for Research on Ageing & DNI – Dept. Neuroscience and Imaging, University G. d’Annunzio of Chieti, Italy
E-mail: fprotasi@unicht.it

At the most basic level, skeletal muscle contraction requires Ca\(^{2+}\) and ATP and, thus, is under direct control of two important intracellular organelles: Ca\(^{2+}\) release unit (CRU) - specialized intracellular junctions, also named triads, which are involved in excitation-contraction (EC) - and mitochondria. It is now becoming clear that: a) CRUs and mitochondria interact functionally and structurally because entry of Ca\(^{2+}\) into the mitochondrial matrix is required to stimulate the respiratory chain, and increase ATP production; b) efficient Ca\(^{2+}\) uptake into mitochondria may strongly depend on their location with respect to sites of Ca\(^{2+}\) release. Recent studies from our laboratory have shown that many mitochondria in skeletal fibers are connected to CRUs by small structures, called tethers, electron dense linkers which keeps mitochondria in proximity of CRUs.

Miss-function of mitochondria and impairment of the EC coupling mechanism have been both proposed to contribute to the age-related decline of skeletal muscle performance. We have studied the morphology, frequency, and sarcomeric-localization of both CRUs and mitochondria in: a) Extensor Digitorum Longus (EDL) muscle from ageing WT mice (25 to 35 months of age); and b) in human biopsies from elderly subject (65 to 75 years of age) using light, confocal, and electron microscopy (EM) to determine how EC coupling and mitochondrial apparatuses are affected by age and exercise.

Results. A - Studies in mice revealed that: a) the number of CRUs/100μm\(^2\) (measured in longitudinal EM sections) in aging mice decreases significantly compared to adult mice: 93 ± 9 vs. 76 ± 8, respectively (p<0.01); b) the number of mitochondria-profiles/100μm\(^2\) also decreases with age: 54 ± 7 vs. 43 ± 6, respectively (p<0.01); c) in ageing fibers mitochondria are more frequently found at the A band of the sarcomere (2.0 ± 0.0 vs. 0.4 ± 0.0), away from CRUs. The
miss-placement of mitochondria is likely the results of the
decreased frequency of tethers: in ageing fibers their number
decrease with age from 14/100μm² in adult vs. 6/100μm² in
ageing mice. The above changes taken together cause a
significant decrease in the number of functional CRUs-
mitochondria couples: 39 ± 5 vs. 26 ± 5 (a decrease of about
33%). This considerable reduction of CRU/mitochondria
couples may significantly contribute to the decrease of
specific force and endurance of skeletal muscle associated to
ageing.

B - Studies in human Vastus Lateralis biopsies confirmed
general findings collected in mice (decrease in frequency of
both CRUs and mitochondria and partial miss-placement of
mitochondria). However, in human studies we also had the
chance to compare samples from two groups of elderly
individuals of 65-75 years of age (all males), sedentary and
sportsmen, in order to determine if structural changes in
CRUs and mitochondria are just caused by ageing itself or if
inactivity plays also a central role. These studies revealed
that both CRUs and mitochondria increase with exercise,
imitochondria much more than CRUs. Number of CRUs/100μm²: 16.0 ± 8.8 in sedentary vs. 21.6 ± 10.8 in
sportsmen; number of mitochondria/100μm²: 38.8 ± 20.3 in
sedentary vs. 52.0 ± 21.3 in sportsmen. The combined
increase of both CRUs and mitochondria results in largely
increased frequency of CRU/mito pairs: 4.3 ± 4.5 in
sedentary vs. 11.1 ± 8.3 in sportsmen.

In conclusion, the results suggest that structure/organization
of both EC coupling and mitochondrial apparatus a) is
improved by physical exercise and b) age-related changes
affecting mitochondrial and EC coupling are, at least in part,
caused by inactivity due to changes in life style.

*****

Neuro-muscular potentiation and fatigue in senior
sportsmen: in search for additional explanations

Nejc Sarabon (1,2), Feliciano Protasi (3), Helmut Kern (1)

(1) Ludwig Boltzmann Institute of Electrical Stimulation and
Physical Rehabilitation, Dept. Physical Medicine and
Rehabilitation, Wilhelmsenspitzen Wien, Austria, Italy; (2)
UP, SRC, IKARUS, Koper, Slovenia; (3) CeSI - Center
for Research on Ageing & DNI – Dept. Neuroscience and
Imaging, University G. d’Annunzio of Chieti, Italy
E-mail: Nejc Sarabon <nejc.sarabon@zrs.upr.si>

Muscle twitch, defined as a mechanical response of a muscle
on a supra-maximal electrical pulse, has been used for
decades as a method for the evaluation of muscle contractile
properties. In kinesiology, the twitch has been primarily
interpreted as an indirect measure of skeletal muscle fiber
type structure. Furthermore, it has also been used in studies
of acute and chronic adaptations of the neuro-muscular
system. The general goal of this study was to test acute
neuro-muscular changes in the force:time curve of the muscle
twitch in specific population of senior athletes. An additional
specific aim was to test inter-relations between the above
mentioned acute changes of twitch on one side and the
electron microscopy tested status of the excitation-
contraction apparatus on the other side.

Eleven healthy senior sportsmen (70.0 ± 4.1 years, 176.2 ±
5.7 cm, 81.9 ± 9.4 kg) participated in the study. They signed
the informed consent prior to the enrolment. A
standardized warm-up protocol the subjects were seated on a
knee extension dynamometer (knee at 60°, hip at 90°) and
they exercised their right leg in two different protocols: (i)
potentiation protocol – five maximal voluntary contractions,
each lasting for 2 s, repetitions separated with a 10 s pause;
and (ii) fatigue protocol – repeated maximal voluntary
contractions, 5 s on followed by 5 s off, until the drop below
60% of the maximal value. Before and immediately after
each protocol muscle twitch responses of the quadriceps
muscle were acquired and the following twitch-related
parameters were measured: electromechanical delay,
maximal torque, contraction time, rate of force development,
and half relaxation time. Additionally, needle biopsy of the
vastuslateralis muscle was acquired. Standard procedure for
the muscle sample preparation was used and electron
microscopy was performed. The following subcellular
structures were observed: total number of mitochondria,
number of mitochondria at A-band, number of triads (i.e.
mitochondria + calcium release units). In case of both
exercise protocols, pre-post changes in the twitch parameters
and the electron microscopy parameters were analyzed using
Pearson correlation coefficient. Pre-post changes were tested
with a paired t-test. Statistically significant (p < 0.05) increase in twitch force and
rate of force and a decrease in twitch contraction time were
observed after the potentiation exercise protocol. On the
other hand, after the fatiguing exercise protocol, statistically
significant drop in twitch force and an increase in half
relaxation time were observed. Correlations between the
selected electron microscopy parameters and the pre-post
changes of the twitch were low (R2 = 0.1 to 0.3) in case of the
potentiation exercise protocol. On the contrary,
considerably higher correlations were found in case of the
fatiguing exercise protocol. They were highest between the
number of triads versus twitch maximal force and rate of
force pre-post changes (R2> 0.65).

To summarize, in senior sportsmen, the changes in muscle
twitch which appear as a result of neuro-muscular
potentiation/fatigue are of comparable type and size as
previously described in young healthy subjects. An important
outcome is that the fatigue related changes in twitch do
depend on the number of intact excitation-contraction
coupling units. The higher the number of the triads, the lower
the drop in twitch force and the drop in rate of force development after fatiguing.

*****

**Assessment of Total Hip Arthroplasty: bone and muscle density in patients receiving cemented and uncemented prosthesis**

Gargiulo P (1,2), Pétursson T (2), Magnússon B (2), Bifulco P (3), Cesarelli M (3), Izzo G (3), Magnúsdóttir G (4), Halldórsson G (4), Ludvigsdóttir GK(4), Tribel J (5), Jónsson H jr (5,6)

(1) Dept. Science, Education and Innovation, Landspítali University Hospital, Iceland; (2) Biomedical engineering department, Reykjavik University, Iceland; (3) Department of Biomedical, Electronic and Telecommunication Engineering, University Federico II of Naples, Italy; (4) Rehabilitation Clinic Grensá Landspítali Hospital, Iceland; (5) Orthopaedic Clinic, Landspítali Hospital, Iceland; (6) Medical Faculty, Iceland University

E-mail: "Paolo Gargiulo" <paologar@landspitali.is>

Total hip arthroplasty (THA) can be achieved by using cemented or non-cemented prosthesis. Besides patient’s age, weight and other clinical signs, quantified evaluation of bones and muscle quality could be crucial for orthopedic surgeons to base the choice between cemented and non-cemented THA. Although bone density generally decreases with age and for old subjects is preferred a cemented THA, the bone quality of a particular patient should be quantitatively evaluated. This study proposes a new method to quantitatively measure bone density and fracture risk by using 3D models extracted by a pre-operative CT scan of the patient. Also the anatomical structure and compactness of quadriceps muscle is computed to provide a more complete view. A spatial reconstruction of the tissues is obtained by means of CT image processing, then a detailed 3D model of bone mineral density of the femur is provided, by including quantitative CT density information (CT must be pre-calibrated). A Finite element analysis will provide a map of the strains around the proximal femur socket when solicited by typical stresses caused by an implant. The risk for structural failure due press-fitting and compressive stress during non cemented THA surgery was estimated by calculating a bone fracture risk index (ratio between actual compressive stress and estimated failure stress of the bone).

A clinical trial was carried out including 36 volunteer patients (aging from 22 to 77) underwent unilateral THA surgery for the first time: 18 received a cemented implant and 18 received a non-cemented implant. CT scans are acquired before surgery, immediately after and after 12 months. Bone and quadriceps density resulted to be higher in the healthy leg in about the 80% of the cases. Bone and quadriceps density generally decrease with age but mineral density may vary significantly between patients.

*****

**The molecular basis of muscle ageing**

Antonio Musarò

Sapienza University of Rome; DAHFMO-unit of Histology and Medical Embryology, IIM, Rome, Italy

E-mail: "Antonio Musarò" <antonio.musaro@uniroma1.it>

Skeletal musculature is particularly susceptible to the effects of aging and diseases, undergoing a steady reduction in function and losing up to a third of its mass and strength. This decline in mass and functional performance is due to an overall decrease in muscle integrity as fibrotic invasions replace functional contractile tissue, and marked changes occur in muscle fiber composition, with a characteristic loss in the fastest most powerful fibers. Moreover, the alteration in the regenerative response represents one of the pathogenic features of muscle aging, leading to impairing physiological function and progression of sarcopenia. Despite numerous theories and intensive research, the principal molecular mechanisms underlying the process of muscle wasting are still unknown. Current data point out that the development of muscle wasting is a multifactorial process and believed to be the result of both intrinsic factors, involving changes in molecular and cellular levels, and extrinsic ones. Among these, we studied anabolic factors, such as transgenic mice expressing different isoforms of IGF-1, and catabolic factors (IL-6 and oxidative stress) that potentially modulate muscle mass and function. Preliminary evidences revealed a unique profile of actions for IGF-1Ea and IGF-1Eb isoforms, which was manifest at both structural and functional levels. The study underscores the significance of prepro-peptides in the biology of IGF-1, and emphasizes the importance of isoform choice in considering clinical applications. Moreover, localized accumulation of oxidative stress or systemic increased of IL-6 levels are sufficient to promote several signs of sarcopenia. Interestingly, stimuli that mimic physical exercise, such as electrical stimulation, counteract several sign of sarcopenia. All of this represents an important advance in our understanding for the treatment of muscle aging.

*****

**Signaling pathways that control protein breakdown and muscle loss**

Marco Sandri

Dulbecco Telethon Institute at Venetian Institute of Molecular Medicine, Padova, Italy and Dept. Biomedical Science, University of Padova, Italy

E-mail: marco.sandri@unipd.it

Skeletal muscle is the most abundant tissue in the human body accounting for almost 50% of the total body mass and it
is a major site of metabolic activity. Muscle performance is influenced by turnover of contractile proteins. Production of new myofibrils and degradation of existing proteins is a delicate balance which, depending on the condition, can promote muscle growth or loss. Protein synthesis and protein degradation are coordinately regulated by pathways that are influenced by mechanical stress, physical activity, availability of nutrients and growth factors. Understanding the signaling which regulates muscle mass may provide potential therapeutic targets for the prevention and treatment of muscle wasting in metabolic and neuromuscular diseases. I will present the last results about pathways that control muscle mass during disuse with particular emphasis on ubiquitin-proteasome and autophagy-lysosome systems.

*****

Strength of muscles and quality of life in tumor bearing patients

Richard Crevenna (1), Helmut Kern (2)

(1) Department of Physical Medicine and Rehabilitation, Vienna Medical University, Vienna, Austria; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria

E-mail: richard.crevenna@meduniwien.ac.at

Patients with cancer experience muscle wasting and weakness. Therapeutic exercise may be beneficial but is not always practical. An alternative approach may be neuromuscular electrical stimulation (NMES) of the quadriceps muscles [1]. We report studies of one of us, which are in line with results on the use of electrical Stimulation as a palliative treatment for cancer bearing patients. In a pilot study muscle strength and quality of life (QOL) were compared in cancer patients suffering from two different “typical male” cancer entities (prostate cancer and head and neck cancer) [2]. The Biodex System 3 device was used for isokinetic strength testing of thigh muscles in both groups. QOL was evaluated by using the SF-36 Health Survey. Surprisingly, prostate cancer patients showed significantly higher values for muscular strength of thigh muscles than significant younger patients with head and neck cancer. Furthermore, prostate cancer patients revealed significantly better values in QOL subscales “bodily pain” and “physical functioning”.

A 47-year-old female patient suffering from advanced lung cancer with metastatic bone and brain disease participated in a passive exercise program, consisting of NMES five times a week, carried out for 4 weeks [3]. After the training period, the results of the 6-min walk have improved by 44%, which demonstrates the increase of physical performance (mobility and endurance capacity). The results of the “Timed up and go” indicate an improvement of mobility and functional health of skeletal muscles. Furthermore, the quality of life (QOL)-scales (assessed by using the SF-36 health survey) “Physical functioning”, “Role-physical”, “Mental health”, “Role-emotional”, “Vitality”, “Bodily pain”, and “General health” showed improvements after the intervention period. Feasibility, safety, and beneficial effects of the NMES program were proven for the patient in this case study. These findings indicate that NMES, initiated and executed with appropriate care, may serve as a useful supportive means of palliative treatment in some patients with advanced cancer and metastatic disease, especially in cases of metastatic involvement of the brain and of the skeletal system with the risk of seizures and pathological fractures where volitional training is not allowed.


*****

Myopathic features in patients affected with newly diagnosed colorectal cancer: identification of serologic and tissue-specific diagnostic and prognostic markers

Zampieri S (1,2), Adami N (1), Carraro U (1), Kern H (2), Masiero S (3), Polese L (4), Gruppo M (4), Merigliano S (4)

(1) Laboratory of Translational Myology, Dept. Biomedical Sciences, University of Padua, Italy; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Dept. Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Austria; (3) Physical Medicine and Rehabilitation, Dept. Neurosciences; (4) Dept. Surgical, Oncologic and Gastroenterologic Sciences, University of Padua, Italy

E-mail: “Sandra Zampieri” <sanzamp@unipd.it>

Skeletal muscle in patients with cancer undergoes many morphological changes due to immuno-inflammatory factors of tumor origin or as a consequence of therapy. The latest event is cachexia, which is characterized by severe muscle wasting associated to an increased protein turnover. The reduction in protein synthesis and the increase in protein degradation are associated with an increased synthesis of proteins of the acute phase response. On the other hand, some of these modifications appear early during the natural history of cancer disease. The aim of our study is to identify in weight stable patients with colorectal cancer at diagnosis, and without asthenia/muscle weakness/cachexia, in both serum
and skeletal muscle biopsies myopathic features which may provide new diagnostic and prognostic biomarkers of myopathy in association with the progression of cancer disease. Morphometric analyses (histopathology of skeletal muscle tissue and distribution of the mean myofibers diameter), immunohistochemical studies [evaluation of the fiber type distribution and identification of regenerating myofibers expressing neural cell adhesion molecule (N-CAM) or the embryonic isoform of myosin heavy chain (MHC-emb)] were performed on intraoperative muscle biopsies from patients with colorectal cancer and from weight stable patients undergoing surgery for benign non-inflammatory conditions. A rectus abdominis biopsy was taken in all patients and controls. Correlations between clinical characteristics, histopathologic findings, circulating inflammatory biomarkers (PCR), markers of muscle necrosis (CPK) and regeneration (soluble form of N-CAM) as well as of protein turnover (prealbumin, albumin), and cancer phenotype were investigated.

Forty four patients and 17 controls were studied. In cancer patients' biopsies, we observed a subclinical myopathy characterized by an abnormal distribution of myonuclei, which were localized inside the myofiber rather than at the periphery, and by the presence of regenerating muscle fibers. Regenerating muscle fibers expressing two classical markers of damage-induced muscle regeneration (MHC-emb and N-CAM) were detected in muscle biopsies from cancer patients. Control muscle biopsies were negative for the detection of regenerating myofibers and the number of internally nucleated myofibers was significantly lower compared to that observed in muscle biopsies from cancer patients. In patients we observed a significant correlation between the number of internally nucleated fibers and the presence of node metastasis. Our findings show for the first time the presence of a subclinical myopathy in patients affected with colorectal cancer at the early stage of the clinical onset of the disease [1-3]. The factors associated with a subclinical myopathy at this stage of disease are still unknown. An analytical study on the potential molecular mechanisms that are responsible for this cancer-associated myopathy could possibly provide new diagnostic and prognostic markers and new therapeutic targets in order to prevent the late-onset and severe loss of muscle tissue which is typical for late cachectic syndrome.

## Abstracts

### Index

<table>
<thead>
<tr>
<th>Author</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adami N</td>
<td>16</td>
</tr>
<tr>
<td>Bifulco P</td>
<td>15</td>
</tr>
<tr>
<td>Bizzarini E</td>
<td>8</td>
</tr>
<tr>
<td>Bolego C</td>
<td>11</td>
</tr>
<tr>
<td>Boncompagni S</td>
<td>13</td>
</tr>
<tr>
<td>Carraro U</td>
<td>6,8,9,11,13,16</td>
</tr>
<tr>
<td>Cesarelli M</td>
<td>15</td>
</tr>
<tr>
<td>Crevenna R</td>
<td>16</td>
</tr>
<tr>
<td>De Maio G</td>
<td>8</td>
</tr>
<tr>
<td>D'Incecco A</td>
<td>13</td>
</tr>
<tr>
<td>Galletta E</td>
<td>11</td>
</tr>
<tr>
<td>Galvagni F</td>
<td>11</td>
</tr>
<tr>
<td>Gargiulo P</td>
<td>6,10,15</td>
</tr>
<tr>
<td>Gnaiger E</td>
<td>5</td>
</tr>
<tr>
<td>Gruppo M</td>
<td>16</td>
</tr>
<tr>
<td>Gudmundsdottir DA</td>
<td>10</td>
</tr>
<tr>
<td>Gudmundsdottir V</td>
<td>10</td>
</tr>
<tr>
<td>Halldorsson G</td>
<td>15</td>
</tr>
<tr>
<td>Helgason T</td>
<td>10,10</td>
</tr>
<tr>
<td>Ingvarsson P</td>
<td>10</td>
</tr>
<tr>
<td>Ingvason S</td>
<td>10</td>
</tr>
<tr>
<td>Izzo G</td>
<td>15</td>
</tr>
<tr>
<td>Kern H</td>
<td>6,8,9,11,13,14,16,16</td>
</tr>
<tr>
<td>Jonsson jr H</td>
<td>15</td>
</tr>
<tr>
<td>Knutsdottir S</td>
<td>10</td>
</tr>
<tr>
<td>Ludvigsdottir GK</td>
<td>15</td>
</tr>
<tr>
<td>Magnusdottir G</td>
<td>15</td>
</tr>
<tr>
<td>Magnusson B</td>
<td>15</td>
</tr>
<tr>
<td>Martino L</td>
<td>9</td>
</tr>
<tr>
<td>Masiero S</td>
<td>6,8,9,16</td>
</tr>
<tr>
<td>Merigliano S</td>
<td>16</td>
</tr>
<tr>
<td>Michelucci A</td>
<td>6</td>
</tr>
<tr>
<td>Musarò A</td>
<td>15</td>
</tr>
<tr>
<td>Paolini C</td>
<td>6</td>
</tr>
<tr>
<td>Petursson T</td>
<td>15</td>
</tr>
<tr>
<td>Pietrangelo L</td>
<td>13</td>
</tr>
<tr>
<td>Pinzini C</td>
<td>8</td>
</tr>
<tr>
<td>Polese L</td>
<td>16</td>
</tr>
<tr>
<td>Protasi F</td>
<td>6,13,14</td>
</tr>
<tr>
<td>Rossini K</td>
<td>13</td>
</tr>
<tr>
<td>Saletti C</td>
<td>11</td>
</tr>
<tr>
<td>Sandri M</td>
<td>15</td>
</tr>
<tr>
<td>Sarabon N</td>
<td>14</td>
</tr>
<tr>
<td>Stramare R</td>
<td>6,9</td>
</tr>
<tr>
<td>Svanbjornsdottir D</td>
<td>10</td>
</tr>
<tr>
<td>TribeJ</td>
<td>15</td>
</tr>
<tr>
<td>Vitiello L</td>
<td>6,11</td>
</tr>
<tr>
<td>Yngvason S</td>
<td>10</td>
</tr>
<tr>
<td>Zampa A</td>
<td>8</td>
</tr>
<tr>
<td>Zampieri S</td>
<td>6,8,9,13,16</td>
</tr>
<tr>
<td>Zanato R</td>
<td>9</td>
</tr>
</tbody>
</table>