Annual Meeting of Young Researchers in Physiology

Magnano in Riviera (UD)
5-7 May 2016

PROGRAMME AND ABSTRACTS

col patrocinio di

UNIVERSITÀ DEGLI STUDI DI UDINE

THE PROGRAMME AT A GLANCE

Thursday May 5th, afternoon session

15h00 – 15h20	Introductory remarks
15h30 – 17h30	Free communications, session 1: MUSCLE and NUTRITION
17h30 – 18h00	Coffee break
18h00 – 18h45	**Lecture 1 : Stefano Schiaffino, Padova**
	Skeletal muscle physiology: from whole body to single fibers and back to whole body

Friday May 6th, morning session

8h40 – 10h40	Free communications, session 2: CELL PHYSIOLOGY
10h40 – 11h15	Coffee break
11h15 – 12h00	**Lecture 2: Tommaso Pizzorusso, Pisa**
	Visual cortical development: role of epigenetic mechanisms
12h00 – 13h00	Free communications, session 3: NEUROSCIENCE

13h00 – 14h20 LUNCH AT THE CASTLE

Friday May 6th, afternoon session

14h30 – 16h10	Free communications, session 4: HEART PHYSIOLOGY
16h15 – 16h45	Coffee break
16h45 – 17h30	**Lecture 3: Giovanna Lippe (Udine) e Paolo Bernardi (Padova)**
	The Mitochondrial Permeability Transition Pore: Channel Formation and Integration in Signal Transduction.
17h30 – 18h30	Free communications, session 5: INTEGRATIVE PHYSIOLOGY

20h00 **social dinner at Hotel Centrale, Tarcento**

Saturday May 7th, morning session

9h00 – 10h20	Free communications, session 7: EXERCISE SCIENCE
10h20 – 11h00	Coffee break
11h00 – 12h00	Free communications, session 8: EXERCISE SCIENCE

12h00 – 14h00 LUNCH AT THE CASTLE

Prize delivery at 13h00, during lunch

FREE COMMUNICATIONS SESSION 1

MUSCLE AND NUTRITION

Thursday, May 5th, 15h30 – 17h30

CHAIR :

Eleonora Bardi, Pavia

Francesca Pinzauti, Firenze

15.30 – 15.50 Mariangela Marrone Chieti

Role of oxidative stress in sarcopenia and therapeutic approach by antioxidants delivered by targeted liposomes

15.50 – 16.10 Martina Di Maro Napoli

Hypolipidic and hypocaloric diet improves skin microvascular blood flow in hyperlipidemic obese females.

16.10 – 16.30 Valentina Percario Firenze

In situ characterization of slow and fast isoforms of skeletal muscle myosin

16.30 – 16.50 Irene Pertici Firenze

Force-velocity relation of an ensemble of myosin II from frog skeletal muscle

16.50 – 17.10 Letizia Rasica Milano

Nitrate infusion does not alter isolated in situ canine muscle oxidative metabolism during hypoxia with normal convective O_2 delivery

17.10 – 17.30 Desy Salvadego Udine

Muscle oxidative function in vivo following inactivity and hypoxic exposure: what is the role of mitochondria?

Mariangela Marrone **Ph.D. student**

ROLE OF OXIDATIVE STRESS IN SARCOPENIA AND THERAPEUTIC APPROACH BY ANTIOXIDANTS DELIVERED BY TARGETED LIPOSOMES

Mariangela Marrone[1], S. Guarnieri[1], A. Rotini[1], V. Moresi[2], C. Bombelli[3], S. Sennato[4], S. Fulle[1], R. Mancinelli[1]

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Sarcopenia is the age-related loss of muscle mass and function. At molecular level, sarcopenia is a complex condition characterized by insufficient antioxidant defense mechanism, increased oxidative stress (Mecocci 1999; Fanò 2001) and altered function of respiratory chain (Pietrangelo, 2009). It has been hypothesized that the accumulation of oxidative stress is also related to an impaired regeneration (Beccafico S., 2007). The mitochondria are the most important source of ROS and several studies showed an increased in oxidative damage of aged mitochondria (Barbieri and Sestili, 2012). To study the role of ROS during sarcopenia, and in particular at mithocondria level, we investigated the ROS production and its effect on $\Delta\psi$mit in satellite cells, the skeletal muscle stem cells, as myoblasts and myotubes collected by human Vastus Lateralis skeletal muscle of young and old subjects. NBT and H2DCF-DA assays were used to measure O2•- and ROS production, respectively. Data revealed that oxidant species are more concentrated in elderly myoblasts compared to young ones. To evaluate if mitochondria are affected by ROS using JC-1 assay we found that in elderly myoblasts mitochondrial transmembrane potential decreased much more than in young ones. Furthermore, MitoSOX™ Red reagent was used to measure directly O2•- in mitochondria. We found that in elderly myoblasts O2•- production is increased with respect to young ones and this condition is worsened in myotubes. In an attempt to ameliorate muscle regeneration in elderly mitochondrion-specific liposomes carrying antioxidant were synthetized. The toxicity of liposomes were tested on human satellite cells and C2C12 cells, a murine skeletal muscle cell line. Preliminary results demonstrated that liposomes made using DPPC 97.5%/BOLA 2.5% gave the lowest toxicity at 24-48-72 hours.Overall, up to day our data suggest that ROS and deriving oxidative stress, related to the difficulty of muscle regeneration in elderly subjects, would act through a mitochondrial impairment.

Martina Di Maro **Ph.D. student**

HYPOLIPIDIC AND HYPOCALORIC DIET IMPROVES SKIN MICROVASCULAR BLOOD FLOW IN HYPERLIPIDEMIC OBESE FEMALES.

Martina Di Maro[1],T. Mastantuono[1],M. Chiurazzi[1],G. Nasti[1],E. Marotta[1],L. Battiloro[1],N. Starita[1],E. Muscariello[1],L. Iuppariello[2],M. Cesarelli[2],G. D'Addio[3],A. Colantuoni[1]

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Background: Obesity is known to affect microvascular function contributing to arterial hypertension and insulin resistance able to impair cardiovascular risk. Furthermore, many studies indicate that hyperlipidemia significantly influence endothelial function and vascular reactivity. Therefore, the aim of the present study was to investigate the relationships between hyperlipidemia and obesity-related microvascular changes. **Methods**: 45 adult females attending the Outpatient Clinic of the Department of Clinical Medicine and Surgery, "Federico II" University of Naples were divided in three groups: NW group was constituted by 15 normal weight females, while O and HO groups included obese [body mass index (BMI) ≥ 30 Kg/m^2] and hyperlipidemic obese (total cholesterol>220 mg/dL, LDL cholesterol >130 mg/dL) females, respectively. Nutritional status and microvascular function were studied under baseline conditions and after three months of hypocaloric and hypolipidic diet. Nutritional status was evaluated by anthropometric measurements, bioimpedence analysis and serum lipid parameters. Microvascular blood flow evaluation was performed by laser Doppler perfusion monitoring (LDPM),while skin blood flow oscillations were analyzed by Wavelet transform. **Results**: In NW patients the frequency component in the range 0.052-0.15 Hz (myogenic-related) was the prevalent in the total Power spectral density. Conversely, in obese patients the frequency component in the range 0.021-0.052 Hz (neurogenic component) was the highest when compared to NW subjects. Moreover, in HO females myogenic component appeared lower in spectral density when compared to O subjects. **Conclusions**: Our data indicate that hypercholesterolemia could play a role in the impairment of vascular smooth muscle cell function present in obese subjects. A hypolipidic and hypocaloric diet may ameliorate nutritional status and vessel functions.

Valentina Percario **Ph.D. student**

IN SITU CHARACTERIZATION OF SLOW AND FAST ISOFORMS OF SKELETAL MUSCLE MYOSIN

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2 Department of Biomedical Sciences, University of Padua, Padua, Italy

Force and shortening in muscle are generated by ATP-driven cyclical interactions of the myosin II motors extending from the thick filament with the thin filament. This investigation aims at defining the functional differences between myosin II isoforms in situ by using high resolution (nanometer-microsecond) mechanical methods in skinned fibres (sarcomere length 2.4 μm, temperature 12°C, 4% dextran T-500) from slow and fast rabbit muscles. The fraction of actin-attached myosin heads (f) responsible for Ca^{2+}-activated isometric force (T_0) was determined by measuring the half-sarcomere compliance (Chs) in isometric contraction (39.5 ± 6.5 nm/MPa and 27.4 ± 2.5 nm/MPa respectively in soleus and psoas muscles) and in rigor (18.7 ± 0.3 and 26.8 ± 0.6 nm/MPa) and subtracting the contribution of myofilament compliance (15.5 nm/MPa): f is 0.46 ± 0.09 in the slow isoform and 0.29 ± 0.03 in the fast isoform. With these parameters it can be calculated that the stiffness of the myosin motor, e, is 0.55 ± 0.12 pN/nm for the slow myosin isoform, about 1/3 of that of the fast isoform (1.66 ± 0.17 pN/nm). Correspondingly, the force per motor (F) of the slow isoform is reduced to 1/3 (1.8 ± 0.4 pN in soleus versus 5.4±0.5 pN in psoas). These results suggest that the stiffness of the myosin motor could play a major role in determining the functional diversity of muscle expressing different MHC isoforms.

Supported by MIUR-PRIN 2010R8JK2X and Telethon GGP12282 (Italy).

Irene Pertici **Ph.D. student**

FORCE-VELOCITY RELATION OF AN ENSEMBLE OF MYOSIN II FROM FROG SKELETAL MUSCLE

Irene Pertici[1], L. Melli[1], G. Falorsi[1], D. Cojoc[2], T. Bozó[3], M. Kellermayer[3], V. Lombardi[1], P. Bianco[1]

1 Laboratory of Physiology, Dept Biology, Univ. of Florence, Italy

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3 Semmelweis Univ., Budapest, Hungary

The function of skeletal muscle myosin II as a collective motor in the muscle sarcomere can be studied in vitro with a synthetic bio-machine, consisting of an array of myosin motors interacting with a single actin filament attached with the correct polarity to a bead trapped in the focus of a Dual Laser Optical Tweezers (DLOT, Bianco et al. Biophys J 101:866-874, 2011). The mechanical outputs of the machine are measured by means of the DLOT (dynamic range 0-250 pN, resolution ~0.3 pN), and a piezoelectric nano-positioner carrying the support for the myosin motors (1-75000 nm, resolution ~1 nm).The preliminary version of the machine demonstrated here consists of an ensemble of motors randomly adsorbed on the lateral surface of etched single-mode optical fibres. Isometric and isotonic contractions are reproduced by the motor ensemble in solution with 2 mM ATP. The mechanical output of the ensemble of motors is first recorded under position feedback (corresponding to isometric "fixed ends" conditions in vivo) and, when the force developed by the motors reaches a preset value, the feedback is switched from position to force (corresponding to isotonic conditions in vivo), and the actin filament starts to slide at constant velocity. Dropping the force to lower values, the actin sliding velocity increases as expected from the in vivo force-velocity relation, demonstrating the ability of the synthetic bio-machine to define the power of native and engineered myosin II from striated muscle.

Supported by IIT-SEED, project MYOMAC (Genova) and PRIN-MIUR, Italy.

Letizia Rasica **Ph.D. student**

NITRATE INFUSION DOES NOT ALTER ISOLATED IN SITU CANINE MUSCLE OXIDATIVE METABOLISM DURING HYPOXIA WITH NORMAL CONVECTIVE O_2 DELIVERY

Letizia Rasica[1], S. Porcelli[1], B. S. Ferguson[2], A. N. Kavazis[3], J. R. McDonald[3], F. Mowry[3], Y. Sun[4], M. C. Hogan[5], B. Grassi[3,6], L. B. Gladden[5]

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Introduction. Previous studies have shown a decreased muscle oxygen consumption ($V'O_2$) during moderate intensity exercise in hypoxia after augmented NO bioavailability, which suggests an increased efficiency of oxidative metabolism. It is not clear if these effects are related to a NO-induced increase in convective O_2 delivery and/or to an enhanced mitochondrial coupling efficiency. Aim of this study was to examine skeletal muscle contraction economy during hypoxic exercise following nitrite infusion with unchanged convective O_2 delivery. **Methods.** Canine gastrocnemius muscles (n=8) were surgically isolated and electrically stimulated via the sciatic nerve generating 1 contraction every 3 seconds (≈35% $V'O_2$peak) for 3 minutes in hypoxic conditions (FIO_2=0.12). During contractions, sodium nitrite (NITR) or sodium chloride (SAL) was infused in the popliteal artery. Muscle blood flow was kept constant (531.9±137.5ml/kg/min) by a perfusion pump. Muscle force was measured and muscle $V'O_2$ was calculated from the Fick principle. Muscle biopsies were obtained before and after NITR and were used for to isolate mitochondria. Mitochondrial respiration rates were recorded by high-resolution respirometry. **Results.** $V'O_2$ was not significantly different between NITR (60.6±18.0 ml/kg/min) and SAL (61.7 ±18.3ml/kg/min). After normalizing $V'O_2$ per unit of developed force, the values were slightly but not significantly higher in NITR vs. SAL (p=0.10). No differences were found for ADP-stimulated mitochondrial respiration (both for complex I and complex II), leak respiration and oxidative phosphorylation coupling. **Conclusion.** In hypoxic conditions, but in the presence of constant and normal convective O_2 delivery, nitrite infusion did not affect canine skeletal muscle oxidative metabolism. These results suggest that the effects of increased NO availability on muscle contraction efficiency in hypoxia, if present, are likely not attributable to changes in mitochondrial respiratory efficiency.

Desy Salvadego **post doc**

MUSCLE OXIDATIVE FUNCTION IN VIVO FOLLOWING INACTIVITY AND HYPOXIC EXPOSURE: WHAT IS THE ROLE OF MITOCHONDRIA?

Desy Salvadego[1], M.E. Keramidas[2], J. Rittweger[3], I. Mavelli [1], O. Eiken[2], I.B. Mekjavic[4], B. Grassi[1]

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3 Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany

4 Dept. Automation, Biocybernetics and Robotics, Jožef Stefan Institute, Ljubljana, Slovenia

Background. Alterations in mitochondrial respiratory function can contribute to determine muscle dysfunction after prolonged inactivity and can have important implications on exercise tolerance. Whether (and to what extent) a mitochondria functional remodeling can modulate changes in muscle oxidative function in vivo under physiologic stressors is poorly clear. **Objectives** We studied, in healthy young men, the effects of prolonged muscle inactivity (by 10 and 21-d bed rest campaigns), alone and combined with hypoxic exposure (simulated altitude 4,000m above sea level), on mitochondrial respiration ex vivo and skeletal muscle oxidative function in vivo. **Methods** Mitochondrial respiration (by high-resolution respirometry) was assessed in permeabilized v. lateralis muscle fibers. v. lateralis muscle fractional O_2 extraction (by near-infrared spectroscopy) and pulmonary O_2 uptake ($V'O_2$) were assessed during incremental one-leg knee-extension (KE) exercise up to the limit of tolerance. **Results** Maximal ADP-stimulated mitochondrial respiration was not affected by bed rest, nor by hypoxia, nor by the combination of both stimuli in a 10-d time period, whereas it decreased significantly (by 15-20%) after all the interventions in a 21-d period. Peak muscle fractional O_2 extraction and peak $V'O_2$ during KE were significantly reduced by bed rest (by ~12% and 8%, respectively) after both 10 and 21 days; both variables were not affected by hypoxia, either after 10 or 21 days. **Conclusion** Prolonged inactivity impaired muscle oxidative function in vivo, irrespective from alterations in mitochondrial respiration ex vivo. Substantial constraints were localized at the level of the intramuscular matching between O_2 delivery and O_2 uptake and/or peripheral O_2 diffusion. Superposition of hypoxia on inactivity did not aggravate the impairment of muscle oxidative function induced by inactivity alone.

EU VII Framework Programme (PlanHab project, grant No. 284438).

FREE COMMUNICATIONS SESSION 2

CELL PHYSIOLOGY

Friday, May 6th, 8h40 – 10h40

CHAIR :

Sara Landi, Milano

Angelica Marrone, Chieti

8.40 – 9.00 Teresa Balbi Genova

Impact of estrogenic compounds on early embryo development in the marine bivalve Mytilus galloprovincialis: effects on gene transcription

9.00 – 9.20 Linda Benincasa Siena

In vitro models for the study of human placenta

9.20 – 9.40 Valentina Carlini Milano

Functional role of CLIC1 ion channel in different tumor cell lines

9.40 – 10.00 Vincenzo Migliaccio Napoli

Chronic exposure to persistent environmental pollutants: effect on hepatic mitochondrial bioenergetics and oxidative stress in rat liver.

10.00 – 10.20 Francesca Proietti Serafini Viterbo

The ciliated protozoan toxin climacostol reduces tumor progression in a mouse model of melanoma via the p53-dependent intrinsic apoptotic program

10.20 – 10.40 Daniele Zanella Varese

Muscle oxidative function in vivo following inactivity and hypoxic exposure: what is the role of mitochondria?

Teresa Balbi **post doc**

IMPACT OF ESTROGENIC COMPOUNDS ON EARLY EMBRYO DEVELOPMENT IN THE MARINE BIVALVE MYTILUS GALLOPROVINCIALIS: EFFECTS ON GENE TRANSCRIPTION

Teresa Balbi[1], R. Fabbri[1], M. Montagna[1], E. Fabbri[2], L. Canesi[1], S. Franzellitti[2]

1 Department of Earth, Environmental and Life Sciences, University of Genoa

2 Department of Biological, Geological and Environmental Sciences, University of Bologna, Campus of Ravenna

Natural estrogens and estrogenic chemicals are widespread in aquatic environments, potentially affecting reproduction and development in wildlife. Although these compounds represent a potential concern for aquatic vertebrates, little is known in lower organisms. Studies involving exposure during early life stages, that are extremely sensitive to environmental perturbations, would greatly help identifying potential effects and underlying mechanisms of estrogenic chemicals in invertebrate species. In this work, the early developmental effects of the xenoestrogen Bisphenol A-BPA and of the natural estrogen 17β-estradiol-E2, were investigated in the bivalve mollusk *Mytilus galloprovincialis*. In *Mytilus*, early embryo development is characterized by a ciliated larva, the trocophora (24 h pf) and the D-shelled veliger (48 h pf); however, no information on physiological changes in gene transcription occurring during these stages is available. Environmental concentrations of BPA and E2 induced a delay in development, as well as alterations in shell formation. After selection of suitable reference transcripts for RT-q-PCR, expression of 13 genes involved in different physiological processes was evaluated in trocophorae and D-veligers compared with unfertilized eggs. Basal expressions showed a general up-regulation during development, with distinct transcript levels in trocophorae and D-veligers. BPA and E2 (10 μg/L) induced significant transcript down-regulations at 48 h pf, in particular of genes involved in biomineralization. These changes were associated with irregularities in shell formation, observed by scanning electron microscopy. The results indicate that the formation of the first calcified embryo, a key step in bivalve development, represents a sensitive target for estrogenic chemicals.

Linda Benincasa **Ph.D. student**

IN VITRO MODELS FOR THE STUDY OF HUMAN PLACENTA

Linda Benincasa, L. Ricci Paulesu, F. Ietta, C. Mannelli, R. Romagnoli

Department of Life Science, University of Siena, Italy

A successful pregnancy involves complex interactions between the mother and the fetus, which allow the embryo to develop in uterus. These interactions are mediated by the placenta, the highly specialized organ of pregnancy that supports the normal growth and development of the fetus. Activities of the placenta are precisely regulated and coordinated to ensure the exchange of nutrients and waste products between the maternal and fetal circulatory systems. The characterization of the mechanisms underlying maternal-fetal cross talk is a prerequisite for better understanding the physiological and pathological aspects of human pregnancy. Nevertheless, studying human placenta is challenging since in-vivo experiments are impractical and unethical, and studies in animal models, even though they represent an essential support, do not always translate well into humans. Trying to bypass these limits, we focused on setting up in vitro models of human placenta on two (2D) and three dimensions (3D). 2D cultures do not completely recapitulate the three-dimensional (3D) organization of cells and extracellular matrix (ECM) within tissues and organs. Consequently, there is a large gap between our detailed knowledge of sub cellular processes and our incomplete understanding of these mechanisms at the tissue level. The goal of reconstituting placental functions ex vivo led to the emergence of a wide range of techniques that are referred to as 3D culture, organotypic culture or organoid culture. In this work, we first provide an overview of the commonly used cellular inputs and culture formats, then, we provide an example of 3D culture technique that can mimic syncytium trophoblast tissue.

Valentina Carlini **Ph.D. student**

FUNCTIONAL ROLE OF CLIC1 ION CHANNEL IN DIFFERENT TUMOR CELL LINES

Valentina Carlini[1], M. Mazzanti[1]

Department of Bioscience, University of Milan, Milano, Italy

The Chloride Intracellular Channel 1 (CLIC1) is a peculiar metamorphic protein that shuttles between a cytoplasmic and a transmembrane form, the latter able to form a chloride selective ion channel. CLIC1 has been found to be overexpressed in different human solid tumors, but its exact function remains unclear. We have previously demonstrated that CLIC1 functional expression is important in human glioma stem cells proliferation and in cell motility. This led us to investigate the role played by CLIC1 in the neoplastic process of other tumour types. Immunofluorescence and western blot analysis were used to evaluate the level of CLIC1 expression in human prostate, colon and breast cancer cell lines, compared with normal epithelial cell lines. Growth curves were performed to establish the proliferation rate of these cells and to determine the effect of CLIC1 inhibition on cells proliferation and viability. The results showed that these cell lines have different proliferation rate as well as different sensibility to CLIC1 channel blocker (IAA94), probably correlated with the amount of CLIC1 expression. These data suggest that CLIC1 could be involved in the proliferation of different human cancer cells. Our experiments further demonstrated the potential role of CLIC1 as a novel pharmacological target.

Vincenzo Migliaccio **Ph.D. student**

CHRONIC EXPOSURE TO PERSISTENT ENVIRONMENTAL POLLUTANTS: EFFECT ON HEPATIC MITOCHONDRIAL BIOENERGETICS AND OXIDATIVE STRESS IN RAT LIVER.

Vincenzo Migliaccio, Rosaria Scudiero, Rosalba Putti, Lillà Lionetti

Dipartimento di Biologia, Università degli Studi di Napoli Federico II

Mitochondria adapt the energy production to cellular requirement in different physiological conditions. Given that liver is one of the main organ involved in response to toxic injury, the present work aimed to analyse the mitochondrial response to chronic exposure to persistent organic pollutant (POP) in rat liver. Two groups of eight rats were so treated for 4 weeks: 1- control rats (N rats); 2- rats treated with p,p´-diphenyldichloroethene (DDE, the DDT's major metabolite with the highest persistence) (10 mg/kg b.w.) (N+DDE rats). In isolated liver mitochondria, oxygen consumption rates were determined in presence of different substrates: a) succinate+rotenone, (FADH2-linked substrate), b) glutamate+malate (NADH-linked substrate), c) palmitoilcarnitine+malate (lipid substrate). Carnitine palimitoiltransferase (CPT) activities were determined. Oxidative stress was analyzed by determining H_2O_2, TBARs, SOD and UCP2 contents. N+DDE rats showed increased lipid oxidation rates and CPT activities as well as increased reactive oxygen species (ROS) production (higher H_2O_2 production, TBARs, and SOD contents) vs. N rats. DDE treatment also elicited an increase in mitochondrial uncoupling, as suggested by the higher succinate state 4 rate (rough index of mitochondrial uncoupling) and UCP2 content found in N+DDE vs. N rats. In conclusion, chronic exposure to low doses of DDE elicits an increase in lipid utilization useful to cater to energy request for detoxification processes. Given that the increased lipid mitochondrial utilization elicits high ROS production, the mild increase in mitochondrial uncoupling found in DDE-treated rats may be an adaptive physiologic mechanism useful to counteract further ROS production in response to toxic injury.

Francesca Proietti Serafini **Ph.D. student**

THE CILIATED PROTOZOAN TOXIN CLIMACOSTOL REDUCES TUMOR PROGRESSION IN A MOUSE MODEL OF MELANOMA VIA THE P53-DEPENDENT INTRINSIC PAPOPTOTIC PROGRAM

Francesca Proietti Serafini[1], F. Buonanno[2], C. Perrotta[3], S. Picchietti[1], E. Marcantoni[4], C. Ortenzi[2], E. Clementi[3], D. Cervia[1,3]

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Melanoma diagnoses have risen sharply over past decades, and despite new lines of therapy have been introduced the need for additional drugs is urgent. It has been previously shown that climacostol, an alkyl resorcinol produced by the ciliated protozoan Climacostomum virens, decreases the viability of five different mammalian cancer cell lines, while it was devoid of effects on endothelial cells. In order to expand these data and due to the recent availability of the synthetic toxin, we have further analyzed different tumoral and non-tumoral cell lines, observing that viability of human and rodent tumoral cells was negatively affected by climacostol with higher potency when compared to non-tumoral cells. We then focused on the B16-F10 mouse melanoma cells. In particular, in vivo melanoma progression was studied using a B16-F10 allograft transplantation tumor model. Climacostol rapidly formed adducts with DNA thus leading to DNA damage, reduction of viability and proliferation, and cell death. It also displayed additive effects in combination with the chemotherapeutic drug cisplatin. Of interest, we demonstrated that climacostol induced apoptotic cell death through the mitochondrial-caspase-dependent pathway, i.e. bax relocalization to the mitochondria, cytochrome c release from the mitochondria and activation of caspase 9 and 3. The apoptotic events were triggered by the up-regulation of p53 and p53 target genes such as noxa1 and puma. These results indicate that climacostol is a powerful and efficacious anti-cancer agent that it may be considered for the design of cytotoxic and pro-apoptotic new drugs for melanoma therapy.

Daniele Zanella **Ph.D. student**

METAL OXIDE NANOPARTICLES CAN ENTER INSIDE THE CELLS BY CROSSING PLASMA MEMBRANES

Daniele Zanella[1,2], E. Bossi[1,3], R. Gornati[1,3], G. Bernardini[1,3]

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2 Experimental and Translational Medicine PhD Course, University of Insubria; Varese, Italy.

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The ability of nanoparticles (NPs) to be uptaken by the cells makes them both dangerous and useful to human health; some NPs might cross the plasma membrane also by a non-endocytotic pathway, gaining access to the cytoplasm. To verify this hypothesis, we have used the SOFA technique (Single Oocyte Fluorescence Assay, technique applied to Xenopus laevis oocytes) with the aim of detecting the presence of divalent metal ions in the cytoplasm after NPs exposure by the use of a fluorescent indicator (Calcein) which is strongly quenched by divalent metal ions. Cobalt oxide NPs (Co_3O_4), but not cobalt NPs (Co_0), is capable of inducing a significant quenching in Calcein-injected oocytes exposed, indicating the entrance of NPs and the presence cobalt ions in the cytosol deriving from their dissolution. Experiments in the presence of an endocytosis inhibitor (Dynasore) did not alter metal entry, i.e. Calcein quenching, indicating that endocytosis is not involved in the phenomena observed. The addition of BSA to the experimental solution abolished Calcein quenching caused by Co_3O_4 NPs (probably because of protein corona formation and consequently altered NPs-membrane interaction). Electrophysiological experiments (Two Electrodes Voltage Clamp) were also performed to verify the NPs dissolution (release of cobalt ions in solutions) by measuring the ability of the solution to generate transport currents in oocytes expressing metal transporter (rDMT1). In control oocytes the absence of endogenous divalent cation transporters was verify by TEVC and SOFA techniques. Furthermore, the results of preliminary experiments conducted on iron oxide NPs are in agreement with the data reported for NPs (Co_3O_4).

FREE COMMUNICATIONS SESSION 3

NEUROSCIENCE

Friday, May 6th, 12h00 – 13h00

CHAIR :

Teresa Balbi, Genova

Daniele Zanella, Varese

12.00 – 12.20 Silvia Eusebi Urbino

Maternal creatine supplementation affects the morpho-functional development of hippocampal neurons in rat offspring

12.20 – 12.40 Jasmine Reggiani Cambridge MA, USA

A functional and molecular approach to identifying Retinal Ganglion Cell types in the mouse retina

12.40 – 13.00 Marialuisa Tognolina Pavia

Cerebellar plasticity resolved by advanced multi-photon microscopy

Silvia Eusebi **Ph.D. student**

MATERNAL CREATINE SUPPLEMENTATION AFFECTS THE MORPHO-FUNCTIONAL DEVELOPMENT OF HIPPOCAMPAL NEURONS IN RAT OFFSPRING

Silvia Eusebi, P. Ambrogini, D. Lattanzi, C. Galati, M. di Palma, D. Savelli, R. Cuppini, S. Sartini

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Creatine is a guanidine compound primarily known for ergogenic and, more recently, antioxidant role. Creatine supplementation has been shown to protect neurons from oxidative damage. These features have led to the hypothesis of creatine supplementation use during pregnancy as prophylactic treatment to prevent CNS damage due to pre-perinatal hypoxic-ischemic events. Very little is known on the effects of creatine supplementation during neuron differentiation, while in vitro studies revealed an influence on neuron excitability, leaving the possibility of creatine supplementation during the CNS development an open question. Therefore, using a multiple approach, we studied the influence of maternal creatine supplementation in the morphological and functional development of hippocampal CA1 neurons in offspring rats. At P_{21} neonatal rats born by dams supplemented with 1% creatine in drinking water during pregnancy showed enhanced dendritic tree development, higher intrinsic excitability, larger evoked-synaptic responses and increased LTP maintenance, consistently with a higher maximum firing frequency in comparison to controls. Moreover, a faster repolarizing phase of action potential with the appearance of a hyperpolarization was recorded in neurons of the creatine-treated group. Interestingly, an increased LTP maintenance was shown also at P_{60}, two months after the end of supplementation. In summary, we found that creatine supplementation during pregnancy positively affects morphological and electrophysiological development of CA1 neurons in offspring rats.

Jasmine Reggiani **regular participant**

A FUNCTIONAL AND MOLECULAR APPROACH TO IDENTIFYING RETINAL GANGLION CELL TYPES IN THE MOUSE RETINA

Jasmine DS Reggiani, Arjun Krishnaswamy, Joshua R. Sanes

Department of Molecular and Cellular Biology, Harvard University, Cambridge (MA), USA.

More than 30 types of retinal ganglion cells (RGCs) have been described that analyze the visual scene, each detecting a particular feature such as color, motion or edges, which they send to the brain. Each type distributes itself uniformly along the surface of the retina in a mosaic-like pattern. Efforts to study how feature preferences arise from retinal circuitry have been limited by a lack of clear relationships between RGC identity and functional properties. To close this gap, we have developed a library of immunohistochemical markers to identify RGC types and used this library in concert with calcium imaging of RGCs. Our preliminary results indicate that multiple RGC types can be labeled using a small number of antibodies combinatorially. Multiple RGC types that express a single marker could be disentangled using an assay that models the composites of mosaic arrangements. By developing methods to register calcium-imaged fields of RGCs to post-stained fields we correspond molecular expression patterns to functional responses. Our preliminary experiments indicate that this union of molecular and functional methods can clearly relate RGC molecular identity to feature preference. We will now use this approach to learn how such preferences are built from the interconnections between RGC types and retinal interneurons.

Marialuisa Tognolina **Ph.D. student**

CEREBELLAR PLASTICITY RESOLVED BY ADVANCED MULTI-PHOTON MICROSCOPY

Marialuisa Tognolina[1], L. Mapelli[1], E. D'Angelo[1,2]

1 Unity of Neurophysiology, Dept of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

2 Laboratory of Neurophysiology, Brain Connectivity Center, C. Mondino National Neurological Institute, Pavia, Italy

The SLM-2PM is a two photon microscope using a spatial light modulator (SLM) to divide the laser beam into beamlets that can be configured in different patterns. The SLM-2PM allowed to record calcium signals from multiple neurons simultaneously resolving the spatiotemporal organization of activity in acute cerebellar slices (Gandolfi et al., 2014). However, it was unclear whether SLM-2PM could be used to investigate long-term synaptic plasticity. In this work, we optimized the SLM-2PM to investigate the effect of Theta Burst Stimulation (TBS), that is known to induce long-term synaptic potentiation and depression (LTP and LTD) in the cerebellum granular layer. We actually observed long-term changes of granule cells calcium responses (Ca-LTP and Ca-LTD) showing impressive variations in signal amplitude (Ca-LTP +401.71±161.74% n=15, Ca-LTD -64.12±5.78% n=7, 30 min after TBS). The observed trend between Ca-LTP and Ca-LTD in function of initial DF/F_0 was comparable to local field potential measurements (Mapelli and D'Angelo 2007); moreover, Ca-LTP amplitudes negatively correlates with the initial DF/F_0 value, in agreement with electrophysiological experiments (Sola et al., 2004), and are several times larger than in EPSC measurements (D'Errico et al., 2009). The amplitude observed in Ca-LTP could be explained by the increase in the probability of spike generation after LTP induction (Armano et al., 2000), suggesting that Ca-LTP and Ca-LTD most likely reflect a combination of changes in granule cells intrinsic excitability and synaptic transmission. Ongoing experiments in cell-attached patch-clamp are helping elucidating the underlying mechanisms, allowing to provide further insight into the understanding of neuronal plasticity.

FREE COMMUNICATIONS SESSION 4

HEART PHYSIOLOGY

Friday, May 6th, 14h30 – 16h30

CHAIR :

Nazzareno Fagoni, Brescia

Letizia Rasica, Milano

14.30 – 14.50 Joyce Bernardi Milano Bicocca

Modifier genes in the LQT1 syndrome: mechanistic analysis of NOS1AP polymorphism

14.50 – 15.10 Angelica Gualdoni Milano

Exercise modulates heart rhythm through miR-1 upregulation: a functional analysis in miR-1 overexpressing mouse embryonic stem cell-derived sinoatrial precursors.

15.10 – 15.30 Sara Landi Milano

The transcription factor NFIX: a novel modulator of cardiac rhythm in the adult heart

15.30 – 15.50 Francesca Pinzauti Firenze

The size and speed of the working stroke of cardiac myosin in situ

15.50 – 16.10 Eleonora Torre Milano Bicocca

Contribution of Late Sodium Current (INaL) on Ca^{2+} dynamics during acute ischemia in rat ventricular myocytes

Joyce Bernardi **Ph.D. student**

MODIFIER GENES IN THE LQT1 SYNDROME: MECHANISTIC ANALYSIS OF NOS1AP POLYMORPHISM

Joyce Bernardi, C. Ronchi, E. Torre, M. Rocchetti, A. Zaza

Laboratory of Cardiac Cellular Physiology, dept. of Biotechnologies and Biosciences, University of Milano-Bicocca

Background: Recently, minor SNP variants of the NOS1AP gene have been reported to be associated with QT prolongation and increased incidence of sudden death in LQT1 patients. The NOS1AP gene encodes for a protein that localizes NOS1 close to the sarcoplasmic reticulum (SR). NOS1 activity accounts for NO-mediated modulation of ICaL, RyR2 channels and SERCA, thus interfering with regulation of Ca^{2+} handling and SR stability. Therefore we hypothesize that NOS1AP SNPs might affect NOS1 localization/function to decrease SR stability. In this setting, mutation induced QT prolongation would induce Ca^{2+} overload whose proarrhythmic effect would be unveiled by abnormal NOS1 localization/function. **Aim**: To evaluate the effect of changes in NOS1 activity on SR functional stability, repolarization and arrhythmogenesis in the context of IKs deficiency (LQT1). **Methods**: In guinea-pig ventricular myocytes subjected to IKs blockade (to reproduce the LQT1 phenotype) and adrenergic stimulation (isoproterenol, ISO), we measured electrical activity and evaluated SR functional stability, in basal condition and under selective inhibition of NOS1 (SMTC 3μM). **Results**: Under basal conditions, NOS1 inhibition prolonged AP duration (APD) enhanced ICaL density and did not affect IKs and IKr. ISO (1nM) induced delayed afterdepolarizations (DADs), an index of SR instability, in SMTC treated cells, but not in control ones. **Conclusions**: These results indicate that NOS1 deficiency may contribute to APD prolongation and enhance Ca^{2+} influx; moreover, these effects may compromise SR stability in the presence of adrenergic stimulation. Therefore, the effects of NOS1 inhibition are such as to account for the arrhythmogenic effect of NOS1AP polymorphism.

Angelica Gualdoni **Ph.D. student**

EXERCISE MODULATES HEART RHYTHM THROUGH MIR-1 UPREGULATION: A FUNCTIONAL ANALYSIS IN MIR-1 OVEREXPRESSING MOUSE EMBRYONIC STEM CELL-DERIVED SINOATRIAL PRECURSORS.

Angelica Gualdoni[1], C. Vavassori[1], S. Landi[1], M. Bonzanni[1], A. D'Souza[2], M. Boyett[2], A. Bucchi[1], M. Baruscotti[1], D. DiFrancesco[1], A. Barbuti[1]

1 Department of Biosciences, Università degli Studi di Milano, Milano, Italy

2 Institute of Cardiovascular Sciences, University of Manchester, Manchester, UK

Intense endurance training induces electrical, structural and functional adaptations in the heart in order to sustain the increased cardiac output and metabolic needs. Recently, microRNAs (miRNAs) were discovered as novel modulators of patho-physiological remodelling of the heart. Endurance-trained mice, like humans, display sinus bradycardia caused by a down-regulation of the If current in sinoatrial (SAN) cells and an up-regulation of miR-1, one of the muscle-specific miRNAs. The aim of this work is to study the effect of miR-1 up regulation specifically in sinoatrial-like cells. To do this, we generated both a clone of mouse embryonic stem cells overexpressing miR-1 (mESC-miR-1) and the corresponding control. Undifferentiated mESC-miR-1 show a 200-fold overexpression of miR-1 compared to control mESC which affects neither pluripotency nor their capacity to differentiate into cardiomyocytes. We then selected CD166+ sinoatrial-like cells from differentiating mESC by flow cytometry. We found that the proportion of CD166+ SAN-like cells is significantly higher in mESC-miR-1 than that in control (20.9±3.8% n=10 vs. 10.6±2.4% n=9). Luciferase reporter assay demonstrates that miR-1 directly binds to HCN4 mRNA, the main isoform responsible for the native pacemaker If current. Electrophysiological analysis in sinoatrial-like cell aggregates shows a lower firing rate in SAN-like cells from mESC-miR-1 than in those from control cells (1,37±0,09 Hz n=7 vs. 2,05±0,41Hz n=3). This evidence is consistent with the idea that miR-1 plays an important role in regulating cardiac rhythm, by altering ion currents important for rhythm generation, thus participating in the establishment of endurance athletes' bradycardia.

Sara Landi **Ph.D. student**

THE TRANSCRIPTION FACTOR NFIX: A NOVEL MODULATOR OF CARDIAC RHYTHM IN THE ADULT HEART

Sara Landi, G. Campostrini, V. Fontana, G. Rossi, G. Messina, A. Bucchi, M. Baruscotti, D. DiFrancesco, A. Barbuti

Department of Biosciences, , University of Milano, Milano 20133, Italy

Nfix is a transcription factor involved in the development of various organs. In skeletal muscle Nfix induces the switch from embryonic to fetal myogenesis. Here we investigated the expression profile and role of Nfix in the heart. We discovered that the NFIX gene is highly expressed in the adult sinoatrial node (SAN), atria and ventricles, with a peak of expression after birth. To study the role of Nfix in electrical activity of the heart of wild-type and knockout mice, we recorded electrocardiograms in free-moving mice. Preliminary data show that knockout mice are tachycardic. In in vitro experiments, NFIX-silenced rat neonatal cardiomyocytes show a faster action potential rate than control cardiomyocytes. Comparing the expression levels of several genes by quantitative PCR, no alterations of the cardiac myosins (MYH6, MYH7, MLC2V), troponin (cTNI) and NKX2.5 were found between wild-type (n=3) and NFIX knockout (n=3) mice, suggesting that the sarcomeric structure is unaltered. We found instead a slight increase in the expression of the transcription factors Mef2a and Mef2c in the SAN. Since Mef2A interacts with Nfix and Mef2C is able to enhance HCN4 transcription, we evaluated the expression of HCN genes, encoding for ion channels important for generating cardiac automaticity. In agreement with the faster beating rate observed both in vitro and in vivo, compared to wild-type, knockout mice exhibit an upregulation of HCN4 in the SAN and atria and of HCN2 in ventricles. Our data suggest a role for Nfix in setting heart rate by affecting HCN channels expression.

Francesca Pinzauti **Ph.D. student**

THE SIZE AND SPEED OF THE WORKING STROKE OF CARDIAC MYOSIN IN SITU

F. Pinzauti[1], M. Caremani[1] M. Reconditi[1], G. Piazzesi[1], G. Stienen[2], V. Lombardi[1] and M. Linari[1]

1 Laboratory of Physiology, BIO, University of Florence, Florence, Italy

2 VU University Medical Center, Amsterdam, The Netherlands

The power in the myocardium sarcomere is generated by two bipolar arrays of the motor protein cardiac myosin II extending from the thick filament and pulling the thin, actin-containing filaments from the opposite sides of the sarcomere. Here sarcomere-level mechanics are applied for the first time to electrically stimulated (0.5 Hz) intact ventricular trabeculae from rat heart to record the isotonic velocity transient elicited by a stepwise drop in force from the isometric peak force Tp to a value T (0.8-0.2 Tp). The trabecula is attached between the levers of the force and motor-length transducers (Lombardi and Piazzesi J Physiol 431:141-171, 1990) and sarcomere shortening during force development is prevented with a feedforward method based on the changes in sarcomere length (SL) recorded by a striation follower in the preceding twitch. We find that the size and the speed of the early rapid shortening (the isotonic working stroke) increase with the reduction of T from ~3 nm per half-sarcomere (hs) and 1000 s-1 at high load to ~8 nm hs-1 and 6000 s-1 at low load and are not affected by either the increase of SL (1.9-2.2 μm) or external [Ca2+]o (1-2.5 mM). Thus length- and Ca2+-dependent increase of Tp and power in the heart can solely be explained by modulation of the number of myosin motors, an emergent property of their array arrangement.

Supported by MIUR-PRIN, Ente Cassa di Risparmio di Firenze and Telethon (Italy).

Eleonora Torre **regular participant**

CONTRIBUTION OF LATE SODIUM CURRENT (INaL) ON Ca^{2+} DYNAMICS DURING ACUTE ISCHEMIA IN RAT VENTRICULAR MYOCYTES

Eleonora Torre, C. Ronchi, J. Bernardi, G. Mostacciuolo, M. Rocchetti, A. Zaza

Dept. of Biotechnology and Biosciences, University of Milano–Bicocca

Background: Myocardial ischemia is characterized by an overproduction of toxic metabolites, well-known enhancers of the late sodium current (INaL). Ranolazine (RAN), a blocker of INaL, improves recovery of heart performance during reperfusion after global ischemia. In our previous results, INaL significantly increased, in spite of action potential waveform changes induced by ischemia. Moreover, INaL enhancement partially contributes to cytosolic Na^+ increment during ischemia. It is well know that Na^+ increment lead to cytosolic Ca^{2+} (Cai) accumulation, however the INaL contribution to this process is controversial during ischemia. **Aim**: To evaluate Cai dynamic associated with INaL enhancement in a single cell model of acute ischemia. **Methods**: Rat ventricular myocytes were exposed for 7 min to a (normoxic) ischemia-mimic solution (ISC); to evaluate the contribution of INaL, RAN (10μM) or TTX (1μM) were added throughout the protocol. Cai was monitored in field-stimulated myocytes (1Hz), using FLUO4AM. The sarcolemma (s) and mitochondrial (m) Na^+/Ca^{2+} exchanger (NCX) were blocked by SEA-0400 (1μM) and CGP37157 (1μM) respectively. **Results**: ISC increased Cai, which was partially prevented by RAN; SEA-0400 boosted cytosolic Cai and sarcoplasmic reticulum Ca^{2+} content (CaSR) during ISC. SEA-0400 effects were sharply curtailed by INaL blockers. CGP37157 shared RAN and TTX effects on Cai, reducing ISC-induced Cai accumulation in the presence of sNCX blockade. RAN, but not TTX and CGP-37157, blunted CaSR increment. **Conclusion**: Unexpectedly, sNCX blockade unrevealed RAN and TTX effects on Cai. These results suggest Na+-dependent, but sNCX-independent Ca^{2+} accumulation mechanisms during ischemia. Indeed, mNCX appears to be involved in the INaL-induced Cai accumulation.

FREE COMMUNICATIONS SESSION 5

INTEGRATIVE PHYSIOLOGY

Friday, May 6th, 17h30 – 18h30

CHAIR :

Angela Bisconti, Milano

Desy Salvadego, Udine

17.30 – 17.50 Nazzareno Fagoni Brescia

Cardiovascular and baroreflex responses during resting and exercise apnoeas

17.50 – 18.10 Alessandro Messere Torino

Vascular changes induced by repetitive mechanical compression of forearm muscles

18.10 – 18.30 Anna Taboni Brescia

Alveolar gas composition during maximal and interrupted apnoeas in ambient air and pure oxygen

Nazzareno Fagoni **Ph.D. student**

CARDIOVASCULAR AND BAROREFLEX RESPONSES DURING RESTING AND EXERCISE APNOEAS

Nazzareno Fagoni[1], Aurélien Bringard[2], Christian Moia[2], Anna Taboni[1], Guido Ferretti[1,2]

1 The Medical School, University di Brescia, Brescia, Italy

2 Departements APSI and NEUFO, University of Geneva, Geneva, Switzerland

Introduction. Analysis of baroreflex sensitivity responses (BRS) after prolonged bedrest showed an impaired arterial baroreflexes (Adami, 2013). This analysis was performed on series of three heart beats by application of an analogous of the sequence method proposed by Iellamo in 1997 (closed-loop approach). Currently, there are scanty data for longer series of consecutive beats. Apnoea is characterized by three phases (Sivieri, 2014); the first dynamic phase (φI) shows, due to Valsalva maneuver, a sudden decrease in MAP, counteracted by an increase in HR. The aim was to perform a closed-loop analysis of BRS over a longer number of consecutive beats, using apnoea φI as experimental model. **Methods**. Six divers performed maximal resting and exercise (30 W) apnoeas. Beat-by-beat MAP and HR were recorded by means of Portapres®. The slopes of linear MAP-HR relationship, before and after the minimum of MAP (minMAP), was computed in both conditions. Paired t test was performed to locate differences in BRS between rest and exercise. **Results**. BRS were -0.508±0.192 and -0.550±0.251 for rest and exercise, respectively ($p>0.05$), before attaining minMAP. After minMAP, BRS was -0.882±0.452; during exercise data were not available by the difficult of performing this computation. **Discussion**. HR increased during φI in both conditions, and tends to attenuate the MAP drop via baroreflex stimulation. At rest, after minMAP, the MAP-HR relationship showed a different pattern, implying a different origin than in the earlier of φI. This could be due to sympathetic activation after attainment of minMAP. The results correspond to those obtained in dynamic condition, after prolonged bed rest (Adami, 2013), and in steady-state conditions (Gallagher, 2006). Our speculation is that in the earlier φI, arterial baroreflexes attempt at controlling blood pressure; after minMAP at rest, the higher slope of the MAP-HR relationship suggests possible baroreflex resetting, as a consequence of sympathetic

Alessandro Messere **Ph.D. student**

VASCULAR CHANGES INDUCED BY REPETITIVE MECHANICAL COMPRESSION OF FOREARM MUSCLES

Alessandro Messere, G. Millo, M. Turturici, S. Roatta

Department of Neurosciences, University of Torino, Torino, Italy

Recent studies reported the attention on a particular characteristic of blood vessels, mostly prominent in skeletal muscles: the ability to develop a rapid hyperemic response to mechanical stimulation, e.g., muscle compression (MC). It has been hypothesized that this vascular mechano-sensitivity (VMS), may have an important role in the initial phase of functional hyperemia and may fade away upon repetitive stimulation. However, this 'adaptation' has not yet been described in humans. The present human study aims at verifying whether this VMS 1) undergoes inactivation upon repetitive MCs, as suggested by animal studies, and 2) has a role in the contraction-induced hyperemia. In 10 healthy subjects, brachial artery blood flow was measured in response to 1) single and repetitive compressions of the forearm (20 MCs, 1-s pause) 2) electrically-stimulated contraction of forearm muscles performed 2 min before and immediately after repetitive MCs. Local changes in blood volume and tissue oxygenation were also monitored by near infrared spectroscopy (NIRS). MC produces rapid increases in blood flow (peak= 70 ± 20 % of baseline), blood volume and tissue oxygenation. All parameters exhibiting a progressive decrease during repetitive MCs (peak blood flow= 22 ± 10 % at the end of the MCs train, $p<0.01$). In addition, muscle preconditioning by repetitive MC reduced the hyperaemic response to MC by 47% ($p<0.05$). The results support the idea that VMS contributes to the functional hyperemia at the beginning of exercise and that its role may subsequently subside due to inactivation upon continuous stimulation. Preliminary observations suggest that NIRS may be more adequate than classical ultrasound velocimetry to monitor these phenomena due to its capacity to focus on local perfusion of skeletal muscle.

Anna Taboni **regular participant**

ALVEOLAR GAS COMPOSITION DURING MAXIMAL AND INTERRUPTED APNOEAS IN AMBIENT AIR AND PURE OXYGEN

Anna Taboni[1], Nazzareno Fagoni[1], Giovanni Vinetti[1], Sara Bottarelli[1], Christian Moia[2], Aurélién Bringard[2], Guido Ferretti[1,2]

1 The Medical School, University di Brescia, Brescia, Italy

2 Departements APSI and NEUFO, University of Geneva, Geneva, Switzerland

Introduction. Cardiovascular responses during static apnoeas are characterised by three phases. It was proposed that the dynamic phase III (φ3), leading to volitional exhaustion, is characterised by the appearance of diaphragmatic contractions. Thus, we hypothesized that the transition between steady phase II (φ2) and φ3 corresponds to the attainment of the physiological breaking point. **Methods**. Twelve elite divers performed maximal apnoeas (MA) and apnoeas that were interrupted (IA) at a time corresponding to the end of φ2, as determined at the end of MA, both in air and pure oxygen. We recorded beat-by-beat cardiovascular variables and we measured the alveolar oxygen and carbon dioxide pressure (PAO2 and PACO2, respectively) at the end of apnoea. We also calculated the difference in PACO2 between the end and the beginning of apnoeas (ΔPACO2). **Results**. On an O2 - CO2 diagram, the PAO2 and PACO2 observed at the end of interrupted apnoeas lied around the physiological breaking point curve as determined by Lin et al. (J Appl Physiol 37: 291-296, 1974), In maximal and interrupted apnoeas, ΔPACO2 was higher in oxygen than in air. **Conclusions**. The tested hypothesis that the transition between φ2 and φ3 corresponds to the attainment of the physiological breaking point is confirmed by the present results. However, both in air and in oxygen, the onset of φ3 could be attributed more to the ΔPACO2, than to the absolute PACO2 values.

FREE COMMUNICATIONS SESSION 6

EXERCISE PHYSIOLOGY

Saturday, May 7th, 9h00 – 10h20

CHAIR :

Alessia Buso, Udine

Angelica Marrone, Chieti

9.00 – 9.20 Eleonora Bardi Pavia

Skeletal muscle deterioration in dilated cardiomyopathy: molecular mechanisms and effects of prolonged endurance training in a mice model

9.20 – 9.40 Angela Bisconti Milano

Local and systemic vascular hemodynamic response to passive static stretching in young healthy humans.

9.40 – 10.00 Alessia Buso Udine

Mitochondrial bioenergetics- and biogenesis-related proteins adaptation following two weeks of bed-rest and recovery in elderly and young men

10.00 – 10.20 Giovanni Vinetti Brescia

The three-parameter critical power model for the power output – time to exhaustion relationship in humans

Eleonora Bardi **Ph.D. student**

SKELETAL MUSCLE DETERIORATION IN DILATED CARDIOMYOPATHY: MOLECULAR MECHANISMS AND EFFECTS OF PROLONGED ENDURANCE TRAINING IN A MICE MODEL

Eleonora Bardi[1], Joanna Majerczak[2], Zenon Nieckarz[3], Stefan Chlopicki[4], Jerzy Zoladz[2], Roberto Bottinelli[1], Maria Antonietta Pellegrino[1]

1 Department of Molecular Medicine, Pavia University, Pavia, Italy

2 Department of Muscle Physiology, Faculty of Rehabilitation, University School of Physical Education, Kraków, Poland

3 Department of Biophysics, Institute of Physics, Jagiellonian University, Krakow, Poland

4 Jagiellonian Center for Experimental Therapeutics (JCET), Jagiellonian University, Kraków, Polan

Dilated cardiomyopathy (DCM) is a progressive disease that results in death from congestive heart failure or sudden cardiac death. Although the beneficial effects of exercise are recognized in cardiac patients rehabilitation, patients develop exercise intolerance (EI). To clarify the molecular mechanisms underlying EI in DCM we used the transgenic mouse model Tg αq*44h, characterized by a slow development of the disease. We performed functional and molecular analysis before and after 2 months of free wheel running. Tg mice are characterized by an impairment of in vivo functional performance concomitant to the onset of the disease. Before the development of the disease (age:6 months) Tg mice showed (i) lower PGC1α (regulator of mitochondrial biogenesis) and DRP1 levels (involved in mitochondrial fission) in comparison to control mice suggesting a mitochondrial dysfunction and (ii) lower SOD1 (antioxidant defense system protein) and AMPK levels (energy sensor) suggesting the presence of redox imbalance and energy impairment. At the onset and overt disease (age:12 and 14 months), mitochondrial and energetic alterations persisted and a higher level of protein oxidation was found in Tg mice according with the early SOD1 decrease. In addition, at 12 months the catabolic pathways basal levels, atrogin1 and MuRF1 (ubiquitine proteasome system), Cathepsin-L, and LC3 (autophagic system) were lower in Tg mice suggesting a degradation processes alteration. Our data demonstrated a positive effect of exercise both in vivo and at the molecular level showing an improvement of functional performance in Tg mice associated with a recovery of some mitochondrial and energetic parameters.

Angela Bisconti **Ph.D. student**

LOCAL AND SYSTEMIC VASCULAR HEMODYNAMIC RESPONSE TO PASSIVE STATIC STRETCHING IN YOUNG HEALTHY HUMANS.

Angela Valentina Bisconti , M. Venturelli, S. Rampichini, E. Cè, E. Limonta, A. Fantauzzi, F. Esposito.

Department of Biomedical Sciences for Health, University of Milan

The aim of the present study was to determine the acute effects of passive static stretching (PSS) on femoral blood flow (FBF) in a stretched and non-stretched limb. Our hypothesis was that PSS would increase FBF in the stretched limb mainly through local vasodilator mechanisms. PSS effects may be expected also in the non-stretched limb possibly through an imbalance between the systemic hemodynamic control and the local vasodilator response. To this purpose, eight young healthy individuals (age: 22±3 yrs) underwent PSS (5 cycles of 45 s stretch/15 s rest) of the knee extensors of the dominant limb. Femoral artery blood velocity and diameter were taken from both limbs by ultrasound. FBF was then calculated. PSS increased FBF by ~78% in the stretched limb (from 495±110 to 882±121 ml/min; $P<0.05$). FBF returned to baseline within the end of the 45 s stretch. Conversely, FBF decreased transitory by ~71% (from 334±155 to 138±17 ml/min; $P<0.05$) in the non-stretched limb during PSS maneuver. In conclusion, PSS increased FBF in the stretched limb, and induced a FBF decrease in the contralateral limb. These findings may suggest the predominance of a local vasodilator mechanism in the stretched limb during PSS maneuver, probably induced by nitric oxide release. On the contrary, a possible systemic vasoconstriction, likely mediated by an elevation of sympathetic nerve activity, may prevail in the contralateral limb.

Alessia Buso **Ph.D. student**

MITOCHONDRIAL BIOENERGETICS- AND BIOGENESIS-RELATED PROTEINS ADAPTATION FOLLOWING TWO WEEKS OF BED-REST AND RECOVERY IN ELDERLY AND YOUNG MEN

Alessia Buso, M. Comelli, M. Isola, B. Grassi, I. Mavelli

Department of Medical and Biological Sciences and MATI Centre of Excellence, University of Udine, Udine, Italy

The aim of the study is to evaluate the expression levels of proteins related to mitochondrial biogenesis regulation and bioenergetics in vastus lateralis muscle biopsies from 16 elderly and 7 young people subjected to 14 days of bed-rest, causing hypotrophy, and subsequent rehabilitation via moderate exercise training. Based on quantitative immunoblot analyses, in both groups there is a reduction in abundance of the mitochondrial biogenesis regulator PGC1α and of the mitochondrial activity regulator Sirt3 during bed-rest, with a subsequent up-regulation after rehabilitation, indicating a major involvement of PGC1α-Sirt3 axis in the response to our treatments. A difference is observed comparing the young and elderly subjects, as for both proteins the abundance in the elderly is more affected by immobility and less responsive to exercise. The expression levels of TOM20, assayed as a marker of outer mitochondrial membrane and mitochondrial mass, show a greater sensitivity in the elderly group, where they are affected by bed-rest and rehabilitation recalling the pattern of PGC1α. Unexpectedly, TOM20 protein remains unchanged in young subjects. Single OXPHOS complexes show peculiar patterns which are, in general, different from PGC1α and suggest different imbalance between protein biogenesis and degradation. Overall, exercise is capable to counteract the effect of immobility, when present, except for complex V, which is markedly down-regulated by bed-rest, but remains unaffected after rehabilitation. We may suppose that in this case degradation should be likely prevalent over biogenesis down-regulation in atrophic muscle. Finally, the phosphorylation extent of AMPK and its upstream activator LKB1 does not change during bed-rest and rehabilitation, indicating that the energy-sensing LKB1-AMPK signalling pathway is not triggered in either young or elderly subjects.

Giovanni Vinetti **regular participant**

THE THREE-PARAMETER CRITICAL POWER MODEL FOR THE POWER OUTPUT – TIME TO EXHAUSTION RELATIONSHIP IN HUMANS

Giovanni Vinetti, A. Taboni, S. Camelio, N. Fagoni, G. Ferretti

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The two-parameter (2p) critical power model provides a hyperbolic relationship between power output (P) and time-to-exhaustion (T_{lim}), where the horizontal asymptote corresponds to the critical power (CP) and the curvature corresponds to the energy store component (W') (Jones et al. 2010). However, the domain over which this model applies is limited to T_{lim}>2' (Bishop et al. 1998), since the vertical asymptote (time constant, k_t) is constrained to 0 s. Letting k_t vary generates the three-parameter (3p) model, whereby the hyperbole is shifted leftward. Therefore, the y-axis intercept takes a definite value, which we hypothesize corresponds to the maximal instantaneous alactic power (Ferretti et al, 1987). To test this hypothesis, 5 healthy subjects (29.8 ± 2.6 years) performed eleven different exercise trials on a cycle ergometer (Ergoline, Germany). Powers ranged between 75% and 250% of the maximal aerobic power (W'_{max}) and were carried out until volitional exhaustion, in order to determine T_{lim}. W'_{max} was calculated by extrapolation of the $V'O_2$-P relationship up to $V'O_2max$ during an incremental test with step duration of 2' and power increment of 25 W. Pulmonary gas exchange at the mouth was determined breath-by-breath (Cosmed, Italy). Nonlinear regression analysis was used to obtain CP, W' and k_t as parameters of the P- Tlim relationship. Student's T-test was used to detect significant differences. The 3p model provided a CP of 202 ± 55 W, a W' of 17 ± 5.1 kJ, a k_t of -22.4 ± 8.5 s and a mean y-axis intercept of 995 ± 97 W, with a R^2 of 0.99 ± 0.001. In contrast, application of the 2p model provided significantly ($p<0.05$) higher values of CP (224 ± 65 W) and lower values of W' (8.6 ± 3.2 kJ), with a R^2 of 0.91 ± 0.07. Restricting the analysis to T>110 s, the 2p model predicted a CP of 203 ± 52 W and a W' of 15.5 ± 3.7 kJ with a R^2 of 0.94 ± 0.03. These values were not significantly different from the corresponding values obtained with the 3p model. Only the 3p model can be reasonably applied when T<2', reliably detecting CP and W'. However, its y-axis intercept seems to correspond to the average, rather than the instantaneous, maximal alactic power.

Jones AM et al. (2010) Med Sci Sports Exerc 42:1876–90

Bishop D et al. (1998) Int J Sports Med 19:125–9

Ferretti G et al. (1987) J Appl Physiol 62:2288–2294

FREE COMMUNICATIONS SESSION 7

EXERCISE PHYSIOLOGY

Saturday, May 7th, 11h00 – 12h00

CHAIR :

Angela Bisconti, Milano

Martina Di Maro, Napoli

11.00 – 11.20	Hailu Kinfu Alemayehu	Udine

Correlation of heart rate and anthropometric parameters with performance scores obtained from IAAF tables in elite Ethiopian middle distance runners

11.20 – 11.40	Lea Biasutti	Udine

Oxidative metabolism during a wheelchair propulsion test in patients with spinal cord injury: effects of lesion level

11.40 – 12.00	Luca Festa	Verona

Effects of flywheel strength training in running economy of recreational endurance runners

Hailu Kinfu Alemayehu **Ph.D. student**

CORRELATION OF HEART RATE AND ANTHROPOMETRIC PARAMETERS WITH PERFORMANCE SCORES OBTAINED FROM IAAF TABLES IN ELITE ETHIOPIAN MIDDLE DISTANCE RUNNERS

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The level of athletic performances is commonly rated with the International Association of Athletics Federations (IAAF) score value. For running events, this scoring system proved to be strongly based on the total power output corresponding to a given performance. The aim of this study was to develop a semi-objective rating system for competitors in 800-1500m races based on the correlation between IAAF scores and anthropometric parameters. The study was carried out in top Ethiopian athletes, with IAAF score ranging 976-1113 for men and 1008-1093 for women. Significant regressions with negative slope were found in both sexes by plotting individual IAAF score vs sum of skinfold (biceps, triceps, subscapular, and suprailiac) as well as vs BMI/sum of skinfold ratio. We also related the increase in heart rate (HR) to the mechanical power output during an exercise test on a variable inclination treadmill through an exponential regression of the type Y=Y0+A*exp(B*X), where Y0 represents the asymptotic value of HR at the highest work load. Significant relationships with negative slope were found by plotting IAAF score vs Y0 in both sexes indicating that a lower cardiac burden at any mechanical load corresponds to a higher athletic fitness.

Lea Biasutti **Ph.D. student**

OXIDATIVE METABOLISM DURING A WHEELCHAIR PROPULSION TEST IN PATIENTS WITH SPINAL CORD INJURY: EFFECTS OF LESION LEVEL

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A functional evaluation of oxidative metabolism during exercise was carried out on 10 patients (age 34.9 ±6.5 yr [range 24-46 yr], BMI 23.1 ±5.6 kg/m^2) with spinal cord injury (SCI). Patients (6 paraplegic (P), lesion level D4-D10; 4 tetraplegic (T), lesion level C4-C8) were tested 10.6 ±6.7 yr [range 1-23 yr] after the event (n=8 traumatic, n=2 non-traumatic). Two 4-min exercises were performed in the patient's everyday-wheelchair, one at the self-selected speed (SSS) during habitual activity, and the second at the maximal speed sustainable during an "all-out" effort (MS). Wheelchair propulsion was performed on a computer-controlled ergometer, with no resistance set on rollers. Heart rate (HR), pulmonary O_2 uptake ($V'O_2$) and biceps brachii oxygenation levels (concentration changes in deoxy hemoglobin + myoglobin, Δ[deoxy(Hb+Mb)], taken as an index of fractional O_2 extraction; concentration changes in deoxy + oxy Hb+Mb, Δ[tot(Hb+Mb)], taken as an index of muscle vasodilation; near infrared spectroscopy, NIRS) were determined. $V'O_2$, HR and the speed during MS were inversely and linearly correlated with the lesion level, and were higher in P vs T. $V'O_2$ during MS corresponded to 4.9 and to 2.3 metabolic equivalents of task (METs), respectively, in P and in T. No significant relationship was observed between Δ[deoxy(Hb+Mb)] and Δ[tot(Hb+Mb)] and the lesion level. No differences between P and T were observed for any variable during SSS. In conclusion, a higher level of lesion in people with SCI was found to be associated with a lower performance, likely due to cardiovascular and muscle recruitment limitations.

Luca Festa **Ph.D. student**

EFFECTS OF FLYWHEEL STRENGTH TRAINING IN RUNNING ECONOMY OF RECREATIONAL ENDURANCE RUNNERS

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Introduction. Several studies showed the effects of combined strength and endurance training on endurance performance and running economy (RE). Aim of this study was to measure variation of RE after a combined flywheel strength-endurance training versus low or high intensity endurance training programs. **Methods.** Twenty-nine recreational runners (avg age 35-55y; V'O_2max 50 ml-1min-1kg-1) were randomized in 3 training programs: combined flywheel strength-endurance (EST), low intensity endurance (LIT), high intensity training (HIT). Each group performed 8w of training, 3/w; training load was accurately equalized among the groups. Reference Velocity (RV) as the avg speed between the first and the second ventilatory threshold was used to identify individual training intensity. RV ranged 95-140% for HIT and 70-105% for LIT and EST groups respectively. EST group, in addition to LIT program, performed 1/w, 4 sets of 7 repetitions of Yo-Yo Leg Press. Before and after training each runner underwent metabolic measurements on a treadmill for determination of V'O2max and RE at three submax steady state speeds. Lower Limb Free Fat Mass (LLFFM) were measured by DXA and leg-press 1RM. Data were analysed by two way ANOVA. **Results.** The EST group show a significant improvements in RE (pre 4.5±0.3 Jm-1, post 4.3±0.4 Jm-1 ($p \leq .05$)) and 1RM leg press (pre 143.8±35.8 kg, post 162.4±37.3kg ($p \leq .05$) while no change in RE was found in HIT (pre: 4.6±0.3, post 4.6±0.4 Jm-1) and in LIT (pre: 4.2±0.5, post 4.2±0.3). V'O_2max and LLFFM. Did not show significant modification in any groups. **Discussion.** The present study show a significant increase of muscle strength with associated reduction of RE in EST group with no changes in the V'O_2max and in the lower limb muscle mass. These adaptations seem to indicate that short duration eccentric training produces changes in structural muscle characteristics and intra and inter muscular coordination allowing a reduction in RE recreational runners.