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ABSTRACT

47th European Muscle Conference in Budapest, Hungary

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The 2018 European Muscle Conference was organized in the Basic Medical Science Center of Semmelweis University in Budapest, Hungary, between 30 August and 3 September. What follows below is the collection of abstracts that were submitted to the conference.

While focusing on a central theme of "motor-protein pharmacology", the meeting was organized in twelve thematic sessions: Skeletal muscle mechanics, Muscle cytoskeleton, Neuromuscular signaling and interaction, Cardiac contractility and failure, Molecular motors, Smooth muscle contraction and pathology, Thin filament and actin-binding proteins, Motor protein pharmacology, Muscle energetics, Contraction regulation and EC coupling, Muscle development, regeneration and disease, Integrative muscle biology. The plenary talk of the conference, following the central theme, was given by James Spudich of Stanford University. Besides the main thematic sessions, four workshops, hosted by instruments manufacturing companies, ran in parallel with the main program: Video-rate AFM of muscle proteins, Isolated cardiac myocyte experimentation, Mechanical manipulation of single molecules with optical tweezers, and Muscle fiber and myofibril mechanics. The abstracts were reviewed by the session organizers and chairs, and members of the Scientific Committee. The oral and poster presentations of the young investigators were evaluated by the members of the Young Investigator Awards Committee. I am truly grateful for their outstanding help.

Scientific Committee: Anders Arner, Karolinska Institutet, Stockholm, Sweden; Stefan Galler, Universität Salzburg, Salzburg, Austria; Michael Geeves, University of Kent, Canterbury, United Kingdom; Wolfgang Linke, Ruhr University, Bochum, Germany; Alf Mansson, Linnaeus University, Kalmar, Sweden; Maria Jolanta Redowicz, Nencki Institute, Warsaw, Poland.

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Young Investigator Award Committee: Joseph Chalovich (chairman), Brody School of Medicine at East Carolina University, Greenville, USA; Samantha Harris, University of Arizona, Tucson, USA; Christina Karatzaferi, University of St Mark and St John, Plymouth, UK; Alf Mansson, Linnaeus University, Kalmar, Sweden; Zsolt Mártonfalvi, Semmelweis University, Budapest, Hungary.

I gratefully acknowledge the help and support of the members of the committees, the session chairs and the keynote and invited speakers who contributed to a program of the highest quality. I particularly thank our sponsors for their support: Aurora Scientific, Auro-Science Consulting Ltd., IonOptix Ltd., Journal of General Physiology, Journal of Muscle Research and Cell Motility, Lumicks, MDE Co Ltd., Oxford Instruments—Asylum Research, and Semmelweis University. I acknowledge support provided by a grant from the Hungarian National Research, Development and Innovation Office (NKFIA; NVKP-16-1-2016-0017 National Heart Program). Finally, special thanks go to K&M Congress Ltd of Budapest, Hungary, for the impeccable logistics.

Miklós Kellermayer
Conference chairman
Semmelweis University, Budapest, Hungary

Opening Ceremony

OC-4

Plenary talk

The myosin mesa: on the underlying molecular basis of hypercontractility caused by hypertrophic cardiomyopathy mutations

James Spudich

Stanford University, Stanford, CA, USA

After 40 years of developing and utilizing assays to understand the molecular basis of energy transduction by the myosin family of molecular motors, all members of my laboratory are now focused on understanding the underlying biochemical and biophysical bases of human hypertrophic (HCM) and dilated (DCM) cardiomyopathies. Our primary focus is on HCM since these mutations cause the heart to be hypercontractile, and we hope to understand the molecular basis of this increased power output. HCM is most often a result of single missense mutations in one of several sarcomeric proteins, the sarcomere being the fundamental contractile unit of the cardiomyocyte. More than 40% of all HCM mutations occur in the motor domain of human β-cardiac myosin, while another ~ 40% occur in myosin binding protein-C. Associated with HCM worldwide are heart failure,

arrhythmias, and sudden cardiac death at any age. We are using in vitro molecular studies of biochemically reconstituted human sarcomeric protein complexes to lay the foundation for understanding the effects of HCM-causing mutations on power generation by the contractile apparatus of the sarcomere. With a detailed molecular understanding of the resultant increase in power output caused by HCM mutations, one should be able to exquisitely design appropriate small molecule therapies, which are desperately needed for treatment of these diseases.

Session 1: Skeletal Muscle Mechanics

S1-1

Keynote presentation

Myofilament length dependent activation: molecular mechanisms

Pieter de Tombe

Magdi Yacoub Institute, Harefield, UK

The Frank-Starling mechanism of the heart is due, in part, to modulation of myofilament Ca^{2+} sensitivity by sarcomere length (LDA). The molecular mechanism(s) that underlie LDA are unknown. Recent evidence has implicated the giant protein titin in this cellular process, possibly by positioning the myosin head closer to actin. To clarify the role of titin strain in LDA, we isolated myocardium from either wild-type (WT) or homozygous mutant (HM) rats that express a giant splice isoform of titin, and subjected the muscles to stretch from 2.0 to 2.4 μm sarcomere length. Upon stretch, HM compared to WT muscles displayed reduced passive force, twitch force, and myofilament LDA. Time-resolved small angle X-ray diffraction measurements of WT twitching muscles during diastole revealed stretch induced increases in the intensity of myosin (M2 & M6) and troponin (Tn3) reflections, as well as a reduction in cross-bridge radial spacing. Independent fluorescent probe analyses in relaxed permeabilized myocytes corroborated these findings. X-ray electron density reconstruction revealed increased mass/ordering in both thick- and thin-filaments. The sarcomere length dependent changes in structure observed in WT myocardium were absent in HM myocardium. Overall, our results reveal a correlation between titin strain and the Frank-Starling mechanism. The molecular basis underlying this phenomenon appears to not involve inter-filament spacing or movement of myosin towards actin, but rather, sarcomere stretch induced simultaneous structural rearrangements within both thin- and thick-filaments that correlate with titin strain and myofilament length dependent activation. In addition, we have recently demonstrated that Myosin Binding Protein C (MyoBPC) and its phosphorylation by PKA plays a pivotal role in modulation LDA independent of PKA mediated troponin-I phosphorylation. We propose that titin strain is transmitted via the N-terminus of MyoBPC to directly activate the thin-filament in a length-dependent manner, consistent with the unidentified electron density observed in our X-ray experiments. The Frank-Starling Law of the Heart represents a fundamental regulatory mechanism whereby cardiac pump performance is directly modulated by the extent of diastolic ventricular filling on a beat-to-beat as basis. Our findings provide novel insights into the molecular basis of the Frank-Starling regulatory mechanism.

S1-2

The relevance of form and function in the octopus arm hydrostatic limb

Letizia Zullo, Federica Maiole

IIT–Istituto Italiano di Tecnologia, Center for Synaptic Neuroscience and Technology (NSYN), Genova, Italy

Introduction: The Octopus vulgaris arm is a muscle hydrostat with extraordinary motor capabilities. The arm ‘bulk’ is composed mainly of transverse (T) and longitudinal (L) muscles acting synergistically. T and L are made by uninucleated striated cells sharing similar physiological properties and embedded in a dense connective matrix.
Objective: We aim at deciphering T and L structure, mechanics, mode of activation and their contribution to whole arm movements.
Methods: Confocal Microscopy was used to perform High-resolution morphometric study of muscles, connective tissues, and elastic fibers organization. Muscle biomechanics was investigated with a Dual-Mode Lever arm system on in-vitro preparations.
Results: L show a higher rate of elastic fibers organized in parallel to the main muscle force vector compared to T. T and L have different activation properties; T have a higher twitch to tetanus ratio than L and a force-frequency curve shifted to the right, hence they behave as slow muscles.
T and L have similar concentric force/velocity curve, suggesting an equivalent molecular motors dynamics.
Conclusion: T and L activation system and global mechanical output strongly depend on their architectural organization within the connective matrix. This significantly contributes to muscle performance and can serve the functional needs of body muscles during various movements.

S1-3

Effect of temperature on thick filament-based regulation of mammalian skeletal muscle

Massimo Reconditi[1], Marco Caremani[1], Elisabetta Brunello[1], Marco Linari[1], David Gore[2], Tom Irving[2], Gabriella Piazzesi[1], Malcolm Irving[3], Vincenzo Lombardi[1]

[1]PhysioLab, University of Florence, Italy; [2]BioCAT, Illinois Institute of Technology, Chicago, IL; USA; [3]King’s College London, London, United Kingdom

In the light of the new concepts of thick-filament based regulation (Linari et al. Nature 528, 276–279, 2015), here we analyze the changes in X-ray diffraction patterns from the fast skeletal muscle of the mouse (EDL) at rest and during isometric contraction in the temperature range 10–35 °C. In the skeletal muscle of the heterothermic frog increasing temperature from 0 to 17 °C increases the maximal isometric force (T0) by 40% and changes the intensity and fine structure of the third order myosin meridional reflection (M3), which is sensitive to the conformation of the actin-attached myosin motors, indicating a progression in the working stroke that accounts for a higher force per motor, with no change in the number of attached motors (Linari et al. J. Physiol. 567, 459–469, 2005). In the present study on mammalian muscle increasing temperature from 10 to 35 °C increases T0 by a factor of 3. The corresponding changes in the X-ray signals that report the fraction and the conformation of the actin-attached motors indicate a reduction of the number of attached motors at 10 °C to $\sim$ 50% of that at 35 °C. In the resting EDL muscle the X-ray reflections that signal the regulatory state of the thick filament indicate that the number of myosin motors in the OFF state at 10 °C is also $\sim$ 50% of that at 35 °C. The correspondence between these two fractions suggests that myosin motors that

leave the OFF state to accumulate in a disordered state at low temperature in mammalian muscle at rest are unavailable for actin interaction upon activation.

S1-4

Strain sensitivity of the elementary steps of the cross-bridge cycle, and the Le Chatelier principle

Masataka Kawai[1], Tarek Karam[1], Justin Kolb[2], Li Wang[3], Henk Granzier[2]

[1]University of Iowa, USA; [2]University of Arizona, USA; [3]Soochow University, China

The elementary steps of the cross-bridge (CB) cycle was studied before/after Tropomyosin (Tpm) (with Troponin) addition in bovine cardiac fibers, and before/after nebulin (Neb) addition in mouse slow-twitch soleus fibers. Muscle fibers were activated by Ca^{2+}, the CB kinetics were studied by using low amplitude sinusoidal length oscillations, and interpreted by the six state CB model. We found that active tension was 45% larger with these additions in both cases, but the attached CB number did not change when compared to the rigor state. The equilibrium and rate constants (K2, k2, k(-4)) which promote detached states became larger, and those (k(-2), k4, K4) which promote attached states became less with these additions. The results are consistent to the Le Chatelier Principle when applied to the muscle system: increased CB strain promotes less force-generating states, and decreased strain promotes more force-generating states. The Pi release step increased with +Tpm, but the effect was insignificant with +Neb. The ATP binding step did not change much. In conclusion, we observed expected changes in kinetic constants according to the strain on CBs, which justifies our six state CB model.

S1-5

Depletion of thick filaments in individual sarcomeres affects inter-sarcomere dynamics and force production by single myofibrils

Andrea C. Mendoza[1], Dilson E. Rassier[1]

[1]McGill University, Department of Kinesiology and Physical Education, Montreal, Canada

Background: The force produced by a myofibril should depend on its cross-sectional area, but not the number of active sarcomeres since they are arranged in series. However, a previous study performed in our laboratory (de Souza Leite et al., PNAS, 114:8794–8799, 2017) showed that depleting the thick filament of one sarcomere within an activated myofibril decreased the force production.
Purpose: In this study we examined how depletion of thick filaments in individual sarcomeres within a myofibril affects force production.
Methods: Myofibrils isolated from rabbit psoas were activated/relaxed using a perfusion system. An extra micro-perfusion needle filled with high ionic strength solution was used to erase thick filaments in real time before myofibril activation. Force sarcomere length non-uniformities were measured upon activation.
Results: The force produced by myofibrils with intact sarcomeres was higher than the force produced by myofibrils with one sarcomere lacking thick filaments ($p = 0.01$). Depleting the thick filaments of two sarcomeres decreased the force further ($p = 0.01$). The mechanism of the force decrease was associated with length adjustments developed by sarcomeres upon activation.
Conclusion: Our results suggest that the myofibril force is affected by intra-sarcomeres dynamics and the number of active sarcomeres in series.

S1-6

Varying stability of the immunoglobulin domains in titin's N2A region

Colleen Kelly, Matthew J. Gage

University of Massachusetts Lowell, USA

Titin is the largest known protein and is the primary source of passive elasticity in muscles. Recent work has also implicated titin in active muscle contraction, potentially through interactions between the skeletal muscle titin's N2A region and actin filaments. Experiments characterizing this interaction have shown that it is stabilized by Ca^{2+} through a currently undetermined mechanism. The N2A region is composed of four immunoglobulin domains (I80–I83). The purpose of this study is to determine the stability and folding kinetics of I81–I83. This was accomplished using chemical denaturation and chemical refolding studies. Our studies have shown that the I83 domain has the lowest stability while I81 and I82 have similar stabilities. Interestingly, the presence of calcium stabilizes the I83 domain, increasing the free energy of unfolding. These results demonstrate that the Ig domains in the N2A region have unique stabilities, with the I83 being the most unique of the three domains. This domain is partially deleted in mdm mice, which exhibit a loss of muscle function. These results suggest that stability of this domain could play an important functional role.

Session 2: Muscle Cytoskeleton

S2-1

Coordinators of mechanical signal transduction through sarcoglycans and archvillin

Chih-Hsuan Chou[1], Tara Smith[3], Georgios Vassilakos[1], Kay Son[3], Jason Puglise[1], Michael Matheny[2], Elizabeth Luna[3], Elisabeth Barton[2]

[1]Applied Physiology & Kinesiology, College of Health & Human Performance, University of Florida, Gainesville, FL, USA; [2]Pharmacology & Therapeutics, College of Medicine, University of Florida, Gainesville, FL, USA; [3]Department of Radiology, Division of Cell Biology & Imaging, University of Massachusetts Medical School, Worcester, MA, USA

An important load sensor in striated muscle is the sarcoglycan complex. Loss of gamma-sarcoglycan (g-SG) induces severe muscle degeneration and signaling defects in response to mechanical load. Archvillin (AV) is a muscle-specific isoform of the Supervillin family of proteins and interacts with g-SG to coordinate signal transduction after eccentric contraction, particularly by ERK1/2. The purpose of this study is to determine if loss of the AV region necessary for interaction with g-SG altered mechanical signal transduction pathways in skeletal muscle. We utilized a Svil mutant mouse in which the C-terminal region of Svil/AV was disrupted, and by extension, the domain known to interact with g-SG. With no stimulation, Svil mutant TAs displayed increased basal P-ERK1/2 levels. However, after eccentric contraction, there was a blunted response of P-ERK1/2 levels in TA muscles from Svil mutant mice, consistent with loss of AV/g-SG interaction. We are now pursuing identification and validation of additional partners in the SG/AV mechano-complex to expand upon proteins that coordinate mechano-sensing in muscle.

S2-2

Myosin X drives filopodia of mammalian myoblasts to promote cell fusion

H. Lee Sweeney[1,2], David W. Hammers[1,2], Cora C. Coker[1,2], Michael K. Matheny[1,2], Melissa Merscham-Banda[1,2], Zachary R. Wakefield[1,2], Ernest G. Heimsath[3], Hansol Kang[1,2], John A. Hammer[4], Richard E. Cheney[3,4]

[1]Department of Pharmacology & Therapeutics and [2]Myology Institute, University of Florida College of Medicine; Gainesville, FL 32610; [3]Department of Cell Biology & Physiology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill School of Medicine; Chapel Hill, NC 27599; [4]Cell Biology & Physiology Center, National Heart, Lung, and Blood Institute, National Institutes of Health; Bethesda, MD 20892

Formation and repair of multinucleated skeletal muscle requires the fusion of mononuclear myoblasts into muscle fibers. During vertebrate muscle formation, filopodia-like projections have been observed. Myosin X (Myo10) is an unconventional myosin motor thought to be required for filopodia formation, but it has not previously been described in skeletal muscle. This study demonstrates that Myo10 is expressed in mammalian muscle cells at high levels in development and post-natal growth and regeneration of skeletal muscle fibers and is present within filopodia of differentiating myoblast cultures. Loss of Myo10 prevents both filopodia formation and myoblast fusion in vitro. Conditional Myo10 ablation in muscle stem cells (satellite cells) of mice severely impairs postnatal muscle regeneration in vivo. Myo10-driven filopodia transport myomixer/myomerger, a muscle fusogenic peptide, to the tips of filopodia, likely for fusion initiation. Thus Myo10-driven filopodia formation promotes multi-nucleated mammalian muscle cell formation and repair.

S2-3

How does the fusion of myoblasts modulate their mechanics?

Céline Bruyère, Sylvain Gabriele

Mechanobiology & Soft Matter group, Interfaces and Complex Fluids Laboratory, Research Institute for Biosciences, University of Mons, Belgium

Myoblast fusion is a key cellular process to form and repair the multinucleated muscle fibers that make up the skeletal muscle. Despite its importance, the mechanisms underlying this process are still not well understood [1]. The improvement of the contractile forces generated by skeletal muscles requires to better understand the role of the myoblast morphology and the spatial distribution of the cytoskeleton during the fusion process.

To address this challenge, we imposed different geometries to individual C2C12 myoblasts using protein micropatterns deposited on soft hydroxy-polyacrylamide hydrogels [2]. The orientation of the actin network was quantified with confocal microscopy, whereas myoblasts contractile forces were determined with traction force microscopy (TFM).

The maximal traction force increases with the distance between the cell extremity and the center of mass, leading to higher mechanical outputs for elongated myoblasts. We form cell pairs on micropatterns for studying the evolution of the contractile forces during the myoblast fusion.

On the basis of these findings, we propose a conceptual framework for the mechanical regulation of myoblasts during their fusion.

References

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S2-4

Regulation of actin dynamics at thin filament pointed ends in the heart

Mert Colpan[1], Thu Ly[2], Alla S. Kostyukova[2], Carol C. Gregorio[1]

[1]University of Arizona; [2]Washington State University

The regulation of thin filament lengths is accomplished by modulation of actin polymerization from their pointed ends. Tmod1 and Lmod2 are proposed to be the exclusive thin filament length regulators at the pointed ends in cardiac muscle. Tmod1 shortens thin filaments, while Lmod2 elongates thin filaments. Recent findings suggest that cyclase-associated protein 2 (CAP2) localizes to the M-line (Peche et al., Cell Mol Life Sci 2007) and its deletion in mice results in dilated cardiomyopathy (Peche et al., Cell Mol Life Sci, 2013). Although CAP2 is essential for a viable heart, its function is largely unknown. Using super-resolution microscopy, we found that CAP2 co-localizes with Tmod1 and Lmod2 in cardiomyocytes, which suggests that it is a third molecule at the thin filament pointed ends. Our results show that CAP2 sequesters actin monomers and its assembly at the pointed end requires the availability of polymerization competent actin monomers. The function of CAP2 are linked to Lmod2 and Tmod1, since excess Lmod2 or Tmod1 in cardiomyocytes alters CAP2’s assembly. These findings provide essential links into our understanding of how thin filament lengths are regulated in the heart.

S2-6

Specific cleavage of the titin springs in situ uncovers titin’s role in active muscle contraction

Yong Li[1], Andreas Unger[1], Marion von Frieling-Salewsky[1], Jaime Andrés Rivas Pardo[2], Julio M. Fernandez[2], Wolfgang A. Linke[1]

[1]Institute of Physiology II, University of Münster, Germany; [2]Department of Biological Sciences, Columbia University, New York, USA

Background: The giant protein titin contributes to muscle force generation. However, no tool has been available to specifically cleave the titin springs in sarcomeres.
Methods: A HaloTag-TEV-protease cassette was cloned into elastic titin of a mouse model, allowing for in-situ imaging of titin, specific proteolysis during myofiber mechanics and visualization of successful cleavage on protein gels. Using permeabilized myofiber bundles, we measured passive force over the sarcomere-length (SL) range 2.2–3.4 µm and maximum Ca^{2+}-triggered force (pCa5) at 2.6 µm SL, in the absence or presence of TEV-enzyme.
Results: TEV-protease cleaved HaloTag-TEV titin in myofibers within $< 1/2$ h, but had no effect on wildtype titin or other proteins. Titin cleavage caused myosin-filament disarray and lowered passive tension by 50–70%, the remainder being attributable to extracellular-matrix proteins. Mean active force was reduced by $\sim 50\%$, with large inter-sample variability in the proportion of active-force reduction.

Conclusions: The HaloTag-TEV mouse enables direct quantitation of titin's contribution to passive and active forces in muscle. Intact titin springs are necessary for high active force.

Session 3: Neuromuscular Signaling and Interaction

S3-1

MicroRNAs in muscular dystrophy: modulators of molecular mechanisms involved in repairing muscle damage

Gregorio Valdez

Virginia Tech Carilion Research Institute

MicroRNAs (miRNAs) have been implicated in the biogenesis and maturation of muscle fibers. MiRNAs have also received significant attention as potential therapeutic agents to prevent and repair damages that accrue in muscles following injury and diseases. We recently examined the function of miR-133b, a muscle and synaptically-enriched miRNA, during the progression of Duchenne muscular dystrophy (DMD), in a mouse model for the disease (mdx mice). In the absence of miR-133b, the TA becomes populated with muscle fibers exhibiting a rather small cross-sectional area (CSA) and containing centralized myonuclei. Additionally, loss of miR-133b increases both the size of the interstitial space around muscle fibers and the number of mononucleated cells contained within it. Using RNA seq, we found a wide-range of genes altered in mdx muscle lacking miR-133b, including a number of previously identified miR-133b targets as well as several members of the TGF-β pathway. Combined, our data suggest that miR-133b functions to slow muscle degeneration in DMD.

S3-2

Canonical Wnt and Hippo regulators ensure proper synaptic gene transcription of acetylcholine receptors at the neuromuscular junction

Said Hashemolhosseini

Institut für Biochemie Friedrich-Alexander Universität Erlangen-Nürnberg, Fahrstr. 17 91054 Erlangen, Germany. e-mail: said.hashemolhosseini@fau.de

Wnts regulate processes such as development and differentiation by canonical Wnt/beta-catenin dependent, and non-canonical signaling pathways. Another important pathway involved in the control of organ size, tissue regeneration and stem cell self-renewal is the Hippo pathway, with its signaling members YAP/Taz and transcription factors belonging to the Tead family. Recently, we elucidated the role of canonical Wnt activity in adult muscle fibers using Axin2-lacZ reporter mouse. In these mice, active canonical Wnt signaling is reflected by lacZ expression under control of the Axin2 promoter, which itself is a target gene and negative regulator of canonical Wnt signaling. Apart from other subcellular expression sites in muscle cells, we detected active canonical Wnt signaling at neuromuscular junctions. Interestingly, we showed for the first time that YAP/Taz/Tead1-mediated signaling accompanied canonical Wnt signaling in adult muscle fibers. Importantly, we now demonstrate that specific canonical Wnt and Hippo regulators ensure proper synaptic gene transcription and thereby influence aggregation of acetylcholine receptors at the neuromuscular junction.

S3-3

Postnatal development of the interaction between sympathetic neurons and NMJs

Tatjana Straka, Veronica Vita, Kaltrina Prokshi, Muzamil Majid Khan, Sarah Janice Hörner, Tania Zaglia

Heidelberg University, Heidelberg, Germany

Recent experimental and clinical data suggest a relevant functional interaction between sympathetic neurons and NMJs. Immunofluorescence staining against tyrosine hydroxylase, a sympathetic neuron marker, on muscle cross- and longitudinal sections of EDL and diaphragm muscles showed the enrichment of fluorescence signals at NMJs in adult mice. However, the distribution of sympathetic innervation in whole muscle and the postnatal development of the interaction between sympathetic neurons and NMJs have been unknown. Here, we set up tissue clearing and staining protocols to visualize sympathetic innervation in different muscle whole mounts and characterized the enrichment of tyrosine hydroxylase at the NMJ during the postnatal period. We show an ample distribution of sympathetic neurons in hindleg and diaphragm muscles, that appears to increase in complexity in the months following birth. Plaque-like enrichment of tyrosine hydroxylase immunofluorescence at NMJs was found in several muscle types and augmented in the postnatal period in EDL.

S3-5

Characterizing the agrin-dependent internalisation of muscle specific kinase

Laura Geid, Maria Graeber, Ruth Herbst

Medical University of Vienna, Center for Pathophysiology, Infectiology & Immunology

Muscle specific kinase [MuSK] is a receptor tyrosine kinase [RTK] absolutely required for neuromuscular junction [NMJ] formation. MuSK is activated by binding of motoneuron-derived agrin to Lrp4, which forms a complex with MuSK. RTKs are commonly internalized upon ligand binding and crosstalk between endocytosis and signaling has been implicated. The aim of this project is to characterize agrin-dependent MuSK endocytosis and its role in NMJ formation. We are using biochemical analysis of MuSK localisation, inhibition of endocytic components as well as imaging approaches to study MuSK internalization.

Inhibition of endocytosis led to an accumulation of activated MuSK. Surprisingly, this did not result in enhanced acetylcholine receptor [AChR] clustering even though MuSK activation and AChR clustering are directly linked. Furthermore, agrin stimulation did not alter the surface expression of MuSK. Ongoing experiments aim to show that MuSK signaling occurs independent of its endocytosis. Also, we will use muscle cells expressing MuSK tagged with a pH-sensitive fluorescent protein to track MuSK endocytosis. These studies will reveal the role and properties of MuSK internalisation.

S3-6

The role of the actin-binding protein CAP2 for mammalian skeletal muscle development and function

Lara-Jane Kepser[1], Fidan Damar[1], Christine Chaponnier[2], Axel Pagenstecher[3]Marco B. Rust[1]

[1]Institute of Physiological Chemistry, University of Marburg, Germany; [2]Department of Pathology and Immunology, University of Geneva, Switzerland; [3]Department of Neuropathology, University Hospital Giessen and Marburg, Marburg, Germany

Actin filaments (F-actin) are one of the main components of sarcomeres, the basic contractile units of striated muscles. One important feature of skeletal muscle development and differentiation during late embryonic and early postnatal development is the sequential exchange of α-actin isoforms from smooth and cardiac to skeletal muscle α-actin. The switch of α-actin isoforms requires the coordinated activity of actin regulatory proteins, because it is vital that sarcomere structure and contractility are maintained during differentiation. However, the molecular mechanism behind this exchange is not completely understood yet. Actin-binding proteins of the cyclase-associated protein (CAP) family are important regulators of actin dynamics, which can control assembly and disassembly of F-actin. We reported a broad expression of CAP2 during skeletal muscle development and a novel function in regulating the exchange of α-actin isoforms during myofibril differentiation. The observed delay in the switch of α-actin isoforms coincided with the onset of motor function deficits and histopathological changes including a high frequency of ring fibers, internalized nuclei and changes in mitochondrial distribution. Overall, our study for the first time unraveled an important role for CAP2 in skeletal muscle development and function in mammals.

Session 4: Cardiac Contractility and Failure

S4-2

Time-depending changes in expression patterns of genes, encoding Z-disk proteins, in aortic coarctation model of cardiac hypertrophy

Knyazeva Anastasia[1], Krutikov Aleksandr[1], Golovkin Aleksey[1], Mishanin Aleksandr[1], Pavlov Georgii[2], Smolina Natalia[1], Galagoudza Mikhail[1], Kostareva Anna[1]

[1]Almazov National Medical Research Centre; [2]St. Petersburg State Academy of Veterinary Medicine

Background: Mechanotransduction plays an important role in different type of mechanosensing cells, and its dysregulation may lead to progression of various diseases. On molecular level stretch-sensing cells, like cardiomyocytes, perceive external stimuli and transmit it into hypertrophic response through many signaling pathways, leading to altered protein turnover. In case of pressure overload, Z-disk area is widely investigated as a key member of mechanotransduction in cardiomyocytes.

Purpose: To evaluate the expression patterns of genes, encoding for Z-disk proteins, during progression of cardiac hypertrophy induced by pressure-overload.

Methods: SPF Wistar rats underwent aortic banding to perform hemodynamic pressure overload. All animals were divided into groups according to model duration—1, 2, 8, 10 week including intact and sham-operated groups. At the end of experimental period echocardiographic parameters were obtained using Vevo 2100. After sacrifice isolated hearts were weighted and divided into left (LV), right (RV) ventricles and interventricular septum (IVS) for separate analysis. Evaluation of cardiomyocytes size was accessed using immunohistochemistry. Real-Time PCR with hydrolysis probes was performed according to standard protocol using primer sets obtained from Applied Biosystems. Actin protein level in LV, RV and IVS tissue samples was measured by Western blotting.

Results: Cardiac hypertrophy progression was validated by echocardiographic measurement. Significant increase of left ventricular mass along with no increase of left ventricular internal dimension at end-diastole confirmed the development of concentric hypertrophy by week 10 of aortic banding. Estimation of cell diameter in LV revealed cardiomyocytes enlargement after 8 weeks of pressure overload. Additionally, we observed gradual increase of Nppa expression at each time point in LV and IVS. Expression of Actn2, Cmya5 and Ldb3 was downregulated in LV after 1 week with subsequent increase to normal level and further decline to 10 weeks of aortic constriction. In RV we observed upregulation of the same gene expression after 2, 8 and 10 weeks of pressure overload. Expression pattern of above genes in IVS also reflected upregulation: mRNA level of Actn2 increased after 1 week, Cmya5—after 10 weeks and Ldb3 after 1, 8 and 10 weeks. Other genes, selected for the study (Fhl1, Fhl2, Synpo2, Ilk, Myoz2, Csrp3), did not demonstrate clear change in expression pattern, which did not differ between time points and parts of myocardium. In contrast with mRNA level changes, immunoblotting analysis identified significant increase of Actn protein level after 10 weeks of aortic constriction in LV myocardium, whereas no changes were detected in RV and IVS.

Conclusion: We demonstrated that expression patterns of Z-disk-associated genes have different profile in LV and RV, and patterns in IVS are more similar to RV. The observed discrepancies between mRNA and protein levels suggest the altered protein turnover under LV pressure overload. Changes of Z-disk gene expression may be associated with regulatory processes, confirming the important role of Z-disk proteins in mechanotransduction under pressure overload conditions.

S4-3

Nanomechanical phenotypes in cardiac myosin-binding protein C mutants that cause hypertrophic cardiomyopathy

C. Suay-Corredera[1], E. Herrero-Galán[1], D. Velázquez-Carreras[1], M. R. Pricolo[1,2], I. Urrutia-Irazabal[1], D. García-Giustiniani[3], J. Delgado[4], G. Frisso[2], L. Serrano[4], P. García-Pavía[5], L. Monserrat[3], J. Alegre-Cebollada[1]

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; [2]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy; [3]Health in Code, A Coruña, Spain; [4]EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Barcelona, Spain; [5]Unidad de Cardiopatías Familiares, Servicio de Cardiología, Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain

Hypertrophic cardiomyopathy (HCM) is a disease that causes left ventricle thickening and diastolic impairment. HCM is caused by mutations in genes encoding structural proteins with mechanical roles in the sarcomere, but its underlying pathogenic mechanism remains unknown. Here, we focus on cardiac myosin-binding protein C (cMyBP-C), whose mutations account for many HCM cases. 50% of these mutations lead to truncated polypeptides that cause cMyBP-C haploinsufficiency. The remaining mutations are single amino-acid changes that induce the same phenotype as truncating mutations. In silico tools predict that the majority of mutations do not lead to alterations in RNA splicing or protein thermodynamical stability, the two major mechanisms that cause reduced protein levels. We have experimentally examined four different cMyBP-C pathogenic mutations and found that mutations preserve their overall fold and thermodynamical stability. Atomic Force Microscopy experiments detected differences in the mechanical stability and/or mechanical refolding rate of some mutants. We propose that nanomechanical phenotypes induced by cMyBP-C missense mutations can contribute to the development of HCM.

S4-5

Cellular mechanisms leading to cardiomyocyte diastolic dysfunction

Beáta Bódi[1], Balázs Horváth[2], Zoltán Papp[1]

[1]University of Debrecen, Faculty of Medicine, Division of Clinical Physiology, Department of Cardiology, Debrecen, Hungary; [2]University of Debrecen, Faculty of Medicine, Department of Physiology, Debrecen, Hungary

Here we attempted to reveal how titin isoform composition and oxidative insults (i.e. sulfhydryl (SH)-group oxidation or carbonylation) influence Fpassive of left ventricular (LV) cardiomyocytes during rat heart development. DTDP or Fenton reagents increased Fpassive in 0- and 7-day-old rats to relatively higher extents than in 21-day-old and adult animals. The degrees of SH-group oxidation or carbonylation declined with cardiomyocyte age to similar extents for both titin isoforms. Moreover, the above characteristics were mirrored by increasing levels of HSP27 and αB-crystallin expressions during cardiomyocyte development. Our data implicate a gradual build-up of a protective mechanism against titin oxidation through the upregulation of HSP27 and αB-crystallin expressions during postnatal cardiomyocyte development.

Omecamtiv mecarbil (OM) is a myosin activator agent developed for the treatment of heart failure. We set out to investigate the effects of OM on unloaded cell shortening and intracellular Ca^{2+} ($[Ca^{2+}]i$) transients as a function of pacing frequency. The results suggest that high concentrations of OM can limit contractile performance especially in tachycardic patients.

Session 5: Molecular Motors

S5-1

Keynote presentation

Allosteric tuning of myosin force generation: new avenues towards therapeutical treatment

Anne Houdusse

Institut Curie, UMR144 CNRS, Structural Motility Laboratory, Paris, France

Force production by myosin motors plays major roles in muscle contraction, intra-cellular trafficking and maintenance of critical cellular structures such as microvilli, stereocilia and invadopodia. Deficit in different myosin motors can lead to a number of serious disease, thus myosins are important potential targets for therapeutical treatment. Structures of myosins in complexes with small molecules reveal unsuspected allosteric sites and provide valuable insights for the design of specific modulators. These reveal the mechanistic control of motor transitions by inhibitors and activators and provide novel understanding of the rearrangements controlling the force producing lever arm swing. Current progress and outstanding questions regarding the important sequential rearrangements that lead to force production by myosins will be presented in light of recently solved X-ray structures of myosin/drug complexes. New insights into the mechanism of allosteric tuning of myosin force generation is thus anticipated to lead the way in the development of new myosin-directed therapeutics

S5-2

A synthetic nanomachine based on the fast myosin isoform of skeletal muscle

Irene Pertici[1], Lorenzo Bongini[1], Luca Melli[1], Giulia Falorsi[1], Dan Cojoc[2], Tamás Bozó[3], Miklós S. Z. Kellermayer[3], Vincenzo Lombardi[1]

[1]PhysioLab, University of Florence, Italy; [2]IOM-CNR, Trieste, Italy; [3]Dept. Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

The emergent properties of the array arrangement of the molecular motor myosin II are studied with a synthetic nanomachine, in which HMM fragments (100 μg/ml) of myosin II purified from fast skeletal muscle (rabbit psoas) are randomly dispersed on a functionalised glass fibre (diameter 4 μm) and brought to interact with a single actin filament attached to a bead trapped in the focus of a Dual Laser Optical Tweezers (DLOT, Bianco et al. Biophys J. 101:866, 2011). The mechanical output of the machine is measured by means of the DLOT, which acts as a force transducer (range 0–200 pN, compliance 3.7 nm/pN), and a piezoelectric nano-positioner carrying the support for the motors, which acts as a length transducer. Isometric and isotonic contractions are reproduced by the motor ensemble in 2 mM ATP switching the control from position to force feedback. Up to five force–velocity (F–V) points for each interaction can be determined, allowing the definition of the maximum power ($\sim$ 5 aW at F $\sim$ 0.3 F0, V $\sim$ 1 μm/s). The nanomachine offers an unprecedented tool for investigating muscle contractile-protein physiology, pathology and pharmacology without the effects of the cytoskeletal- and regulatory-proteins, the effects of which can then be selectively tested with different degrees of reconstitution. Supported by IIT-SEED, Genova (Italy) and Fondazione CR Firenze, 2015 (Italy).

S5-3

Role of myosin VI in myoblast function and differentiation into myotubes

Malgorzata Suszek, Damian Matysniak, Vira Chumak, Maria Jolanta Redowicz

Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

Myosin VI (MVI) is a motor protein which belongs to the group of unconventional myosins. The fact that it is the only myosin walking towards the minus end of microfilament makes MVI unique. MVI plays a role in many cellular processes such as endocytosis, cell migration, adhesion, maintenance of the Golgi apparatus, autophagy and gene transcription.

In skeletal muscle, MVI is present in the neuromuscular junction, sarcoplasmic reticulum and myofiber nuclei thus implying that it is important in proper functioning of muscle [1, 2]. Moreover, we have also postulated that MVI could be involved in myoblast differentiation and maturation [3].

In order to elucidate the role of MVI in myoblast differentiation, we derived myoblasts from hind limb muscles of SV mice (Snell's waltzer mice). These mice have spontaneous mutation within MYO6 gene, which prevents from MVI synthesis and therefore are considered as a natural MVI KO animals. We demonstrated that SV myoblasts differentiate in a different way than the cells from control littermates. What is more, we observed changes in mitochondrial activity and differences in Ca^{2+} concentration in SV cells. Furthermore, we found changes in a level of proteins engaged in several cellular processes such as cell adhesion, protein synthesis, and

surprisingly also in inflammation. Our data indicate the MVI plays important roles in myoblasts functions.

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S5-4

Myosin: isoforms and cardiomyopathies

Michael A Geeves

School of Biosciences, University of Kent, Canterbury UK

It is well known that the events and the order of events in the basic cross bridge cycle remain the same for all myosins studied to date. Different mechanical activities results from adapting the basic cycle for different functions. The kcat and maximum velocity of shortening, k0 (in a muscle fibre or in vitro motility) are characteristic of specific myosin isoforms, but parameters such as the velocity at which maximum power is developed and ATP economy also differ from isoform to isoform. We have analysed the ATPase cycle of pure samples of human α-cardiac, β-cardiac and embryonic myosin motor domains (expressed in mouse C2C12 cells) and compared these to the well-studied cycle of fast muscle 2A myosin S1 from rabbit. This reveals some of the key characteristics of how each cycle is adapted to produce different behaviours. Key events are the time taken for the recovery stroke (linked to the ATP hydrolysis step); the entry into the strongly attached force holding state (linked to Pi release); and the exit from the force holding state (linked to ADP release). The latter two events are expected to be dependent on the load—i.e. strain dependent. Strain will slow both of these events and hence slow the overall ATPase cycling and shortening velocity. However the effect of strain is different on each parameter—a large load will reduce velocity to zero while reducing the ATPase by a factor of three. Our modelling indicates how the balance between the events coupled to Pi release, ADP release and the hydrolysis step produce motors with different ensemble forces, velocities and ATP economy.

A similar approach for β-cardiac myosins carrying mutations linked to hypertrophic (HCM) or dilated cardiomyopathy (DCM) also indicate how the two types of mutations can alter the cycle in distinct ways. Four DCM mutations result in lower force-holding capacity & duty ratios and an increase in the economy of ATP usage, while classic HCM mutations, R403Q and R453C, have little effect or a small increase force-holding capacity while reducing the economy of ATP usage. The effects we report for DCM would be sufficient to cause a deterioration in the heart performance. For the HCM mutations, where a hypercontraction phenotype is reported, these effects could be causative of the disease or secondary to other effects such as the destabilisation of the relaxed sate of the thick filament.

S5-5

Paralog selective regulation of non-muscle myosin 2 filaments by S100 protein binding and C-terminal phosphorylation

Péter Ecsédi[1], Neil Billington[2], Gyula Pálfy[3], Gergő Gógl[1], Bence Kiss[1], Éva Bulyáki[1], Andrea Bodor[3], James R. Sellers[2], László Nyitray[1]

[1]Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary; [2]Laboratory of Physiology, National Heart, Lung, and Blood Institute, NIH, USA; [3]Laboratory of Struct. Chem. and Biology, Inst. Chemistry, Eötvös Loránd Univ., Budapest, Hungary

Non-muscle myosin 2 (NM2) has three paralogs in mammals: NM2A, NM2B and NM2C. They have unique and overlapping functions in cell migration, cell adhesions and generating cell polarity. Their assembly to form homo- and heterotypic bipolar filaments is primarily regulated by phosphorylation of the N-terminally bound regulatory light chain. Here we present experimental evidence that the equilibrium between these filaments and single NM2A and NM2B molecules can be controlled via S100A4 protein-protein interactions and phosphorylation at the C-terminal end of the heavy chains. Importantly, S100A4, and some other members of the S100 family, can mediate disassembly of not only homotypic NM2A filaments, but also able to selectively remove NM2A molecules from heterotypic filaments. On the other hand, we found that tail phosphorylation by CK2, PKC and TRPM7 sites downregulates filament assembly in an additive fashion. S100 binding and tail phosphorylation therefore preferentially disassemble NM2A and NM2B, respectively, and these regulatory mechanism likely to contribute to the temporal and spatial sorting of the two NM2 paralogs within heterotypic filaments.

S5-6

Blebbistatin reveals otherwise hidden state in ATP turnover and force-generation by actomyosin

Mohammad A. Rahman[1], Marko Usaj[1], Dilson E. Rassier[2], A. Månsson[1]

[1]Linnaeus University; [2]McGill University

Phosphate release from the myosin active site is central in energy transduction by actomyosin. Here, we elucidate this process using blebbistatin as a pharmacological tool applied to muscle fibres and to actin and heavy meromyosin (HMM) from fast skeletal muscle. Saturating blebbistatin concentrations (10-30 μM) reduced the actin filament sliding velocity in the in vitro motility assay to 5% of the control value with half-maximal inhibition between 1 and 5 μM (25–30 °C). The blebbistatin effect was independent of the number of available myosin heads per filament in the range 50–2000 but was substantially attenuated by lowering ionic strength from 130 to 60 mM or [MgATP] from 1 to 0.1 mM. The effect of blebbistatin (2–10 μM; 5 °C) on isometric force was proportionally lower than the effect on velocity but higher than the effect on the force during a stretch applied to the fibres. The data are explained by a model in which actomyosin force-generation is preceded by Pi release which, in turn, is preceded by two serial transitions after/coincident with cross-bridge attachment. Blebbistatin changes the rate limiting step from the first to the second of the latter transitions.

Session 6: Smooth Muscle Contraction and Pathology

S6-1

A role for RSK2 in the contraction of pressurized arteries through activation of smooth muscle myosin and the Na^+/H^+ exchanger

M. Artamonov, S. Sonkusare, M. Good, K. Momotani, M. Eto, B. Isakson, T. Le, E. Cope, Z. Derewenda, U. Derewenda, A. Somlyo

University of Virginia, USA

Smooth muscle (SM) contraction is triggered when myosin light chain kinase (MLCK) phosphorylates the myosin regulatory light chain

(RLC20). We report new regulatory mechanisms. p90 ribosomal S6 kinase 2 (RSK2) promoted SM contraction by phosphorylating RLC20. Active, phosphorylated RSK2 was present in resistance arteries under basal tone, and increased in response to intraluminal pressure or agonist stimulation. Resistance arteries from Rsk2KO were dilated and showed reduced myogenic tone and RLC20 phosphorylation. RSK2 also phosphorylated the Na^+/H^+ exchanger, NHE-1, in response to intraluminal pressure. NHE-1 activity increased upon myogenic constriction and the increase in pHi was suppressed in Rsk2KO mice. RSK2 dependent activation of NHE-1 was associated with increased Ca^{2+} in pressurized arteries and was blocked by the NHE-1 inhibitor, cariporide. This increase in Ca^{2+} augments MLCK activity and contributes to basal tone and the myogenic response. Rsk2KO mice had lower blood pressure than normal mice. In conclusion, RSK2 provides a new procontractile signaling pathway that contributes to the regulation of basal vascular tone, myogenic vasoconstriction and blood pressure.

S6-2

Integrating the circadian dimension—how the molecular clock regulates mechano-sensitive signalling in resistance artery smooth muscle cells

Steffen-Sebastian Bolz

Department of Physiology, University of Toronto and Translational Biology and Engineering Program (TBEP) at the Ted Rogers Heart Centre, Toronto, Canada

Many cellular processes display circadian rhythmicity, which allows anticipation of recurrent environmental changes and optimization of physiological functions. In the cardiovascular system, circadian rhythms generate anti-phasic oscillations of cardiac output (CO) and total peripheral resistance (TPR): specifically, CO peaks in the subjective day and is low during the heart's regenerative phase in the subjective night; TPR's anti-phase relationship provides low resistance when robust tissue perfusion is needed during the day and dampens the reduction in MAP when CO falls at night. Disrupting circadian rhythmicity profoundly affects cardiovascular function implying that all underlying cellular processes are orchestrated with precise timing to optimize energy efficiency and system performance. Our investigation focuses on the myogenic response as a mechanism that prominently regulates microvascular tone and hence, the variable portion of TPR. Since TPR displays diurnal fluctuations, skeletal muscle resistance artery myogenic reactivity is likely to be under circadian control. We find pronounced locally generated circadian rhythmicity in skeletal muscle resistance artery myogenic responsiveness and identify these microvascular circadian rhythms as a novel homeostatic control mechanism with widespread effects on blood pressure and blood distribution.

S6-3

Role of telokin in the vasoregulation of murine portal vein

Philipp Lake, Lubomir T. Lubomirov, Doris Metzler, Fabian Böde, Frank Stassen, Gabriele Pfitzer

Institute of Vegetative Physiology, University of Cologne (UoC), Germany

Background: Telokin, a PKG target was suggested to augment PKG-mediated relaxation by activating myosin light chain phosphatase (MLCP). It is expressed predominantly in visceral smooth muscle but also in portal vein (PV). Here we investigated telokin's role in regulating the spontaneous rhythmic contractions (SRC) and PKG mediated relaxation in PV.

Methods: Telokin KO (KO) mice kindly provided by A. V. Somlyo were bred in the animal facility of UoC. PVs from 10 to 16 week old mice, split in half, were mounted longitudinally in a myograph, and stretched to 150% of slack length. Experimental conditions for intact and alpha-toxin permeabilized PV were as in Eifinger et al., 2014.

Results: Ca^{2+}-sensitivity in permeabilized KO PV was higher than in WT PV (pCa50, KO 6.06 ± 0.05, WT 5.88 ± 0.05, n = 3–7; p = 0.04). Relaxation induced by 8-Br-cGMP (10-100 μM) of pre-constricted intact (1 μM U46619) or permeabilized (pCa 6.02 + 1 μM U46619) PV was not different between groups. KCl (80 mM) induced force was ~ 1.6-fold higher in KO ($p < 0.001$). The amplitude (A) but not the frequency of SRC were also higher in KO (A in KO 0.22 ± 0.03, in WT 0.12 ± 0.02 mN, $p < 0.01$); dF/dtmax/A (1/s) of SRC was 30%, and -F/dtmax/A (1/s) was 22% slower in KO than in WT ($p < 0.05$).

Conclusions: The effect of telokin depletion on force amplitude and relaxation kinetics suggests that it acts as brake on Ca^{2+}-activated force possibly by increasing MLCP activity.

S6-4

The remarkable cardiovascular system of giraffes

Christian Aalkjaer

Institute of Biomedicine, Aarhus University, Denmark

The cardiovascular system of a 6 m high giraffe is challenged by gravity and it is of interest to understand how giraffes are physiologically adapted to meet this challenge.

The mean blood pressure of giraffes is about 200 mmHg.

In the lecture I will discuss (1) how giraffes avoid dependent edema, (2) how the heart can work against 200 mmHg, (3) how the kidney copes with a high input pressure and (4) how the cerebral circulation can accommodate the substantial changes in hydrostatic pressure when the head is abruptly moved 6 meters down when drinking.

Dependent edema is avoided by a sphincter, thick arterial walls, a high interstitial pressure and venous valves. The cardiac output is low for an animal that size and the workload on the heart is therefore normal. The filtration pressure of the kidney is reduced consequent to a high interstitial pressure and presumably a high preglomerular hydrodynamic resistance. During lowering of the head venous filling in the neck increases. This reduces preload. There is also precapillary vasoconstriction. Combined with valves in the jugular vein this protects the head capillaries.

S6-5

Is "catch" force present during active contraction of mollusk catch muscles?

Sandra Kogler and Stefan Galler

Department of Biosciences, University of Salzburg, Austria

In skinned catch muscle preparations of mollusks, the catch releasing factor cAMP causes not only quick relaxation during the catch phase but also a decay of force during active contraction at submaximal Ca^{2+} activations. Therefore, it was assumed that a certain component of catch force exists during submaximal active contractions (Butler et al. Biophys J, 90:3193–3202, 2006). To test this hypothesis, we investigated the cAMP effect at different pHs, because catch is pronounced at acidic pH and absent at alkaline pH. We found that the cAMP induced force decay at submaximal Ca^{2+} activation was

largest at pH 6.8 and smaller or absent at pH 6.2 and pH 7.4. No effect of cAMP on force was observed at maximal Ca^{2+} activation of any pH. These observations are not compatible with the assumption that catch force is established during the phase of Ca^{2+} activation. The linkages causing catch may already exist during active contraction; however, they are not under tension at this condition. The cAMP induced force depression at submaximal Ca^{2+} activation is probably caused by an effect on the force-generating myosin heads.

S6-6

The catch contraction: biophysical and ultrastructural evidences of paramyosin interactions (in memory of my deceased colleagues, Fernand Baguet and Johan Caspar Rüegg)

Jacques Gilloteaux

[1]Department of Anatomical Sciences, St George's University School of Medicine, Newcastle upon Tyne, United Kingdom; [2]Unit of Research in Molecular Physiology (URPhyM, Narilis), Medicine, University of Namur, Namur Belgium

Introduction: The anterior byssus retractor muscle under active, catch, relaxation and contracture states with biophysical recordings demonstrated each a specific ultrastructure of the contractile filaments [1-3].
Objective: During the years, uncertainty remained about the catch because of diverse techniques used instead of synchronized ones.
Method: Ultrastructure of ABRM undergoing catch state were revisited using strain gauge recordings while other assays were also treated with α-amylase.
Results: Close associations between paramyosin filaments with interconnections formed by some electron dense material, undigested by α-amylase, are shown.
Conclusion: Synchronized tension recording along with electron microscopy processing shows that paramyosin interactions could also accompanied by another intervening protein (twitchin? [4]) detected in the catch state contraction. This mechanism allows the muscle to maintain long lasting contraction with a very low energetic expenditure during long interval periods thus survival of the species along seashores.

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Session 7: Thin Filament and Actin-binding Proteins

S7-1

A new twist on tropomyosin assembly and binding onto actin-based thin filaments

William Lehman[1], Farooq A. Kiani[1], Michael J. Rynkiewicz[1], Jeffrey R. Moore[2], Stefan Fischer[3]

[1]Department of Physiology & Biophysics, Boston University School of Medicine, 700 Albany Street, Boston, MA 02118, USA;
[2]Department of Biological Sciences, University of Massachusetts-Lowell, One University Avenue, Lowell, MA 01854, USA;
[3]Computational Biochemistry Group-IWR, Heidelberg University, Im Neuenheimer Feld 305, Heidelberg, D-69120, Germany

Often considered an archetypal dimeric coiled-coil, tropomyosin, nonetheless, exhibits a number of distinctive "non-canonical" core residues located at the hydrophobic interface between its component α-helices. Here, charged aspartate, D137, takes the place of non-polar residues normally present. Our molecular dynamics indicates that residue D137 is a locus for tropomyosin twist variation, which optimizes electrostatic side-chain contacts between tropomyosin and actin on the assembled thin filament, without dampening coiled-coil stiffness and with only minor local effects on coiled-coil flexural motion. We argue that form fitting particularly of the C-terminal half of tropomyosin to F-actin facilitates their initial binding interaction. We propose that localized twisting of unattached free tropomyosin ends capture unbound dimers while sponsoring nascent tropomyosin cable growth on F-actin, transforming an otherwise three-dimensional assembly process into a cooperative one-dimensional one. Describing intrinsic material properties of tropomyosin appears to be key to understanding both recruitment and binding of tropomyosin onto actin, two separate processes that are often conflated.

S7-2

Single molecule imaging reveals how cardiac myosin binding protein-C sensitizes thin filaments to calcium

Alessio V. Inchingolo[1], Samantha Beck Previs[2], Michael J. Previs[2], David M. Warshaw[2], Neil M. Kad[1]

[1]University of Kent; [2]University of Vermont

Here we report on how Cardiac myosin-binding protein C (cMyBP-C) modulates thin filament activity through interactions of its N-terminus with actin. To provide single-molecule level molecular details of this process, we studied interactions of fluorescently tagged myosin with thin filaments suspended between pedestals above a microscope slide surface. We find that the addition of the full-length N-terminal fragment C0C3 enhanced myosin binding to thin filaments at low calcium levels, and blocked myosin binding at high calcium. Imaging of a fluorescently-labelled N-terminal cMyBP-C fragment indicated this modulation was due to C0C3 directly binding to the thin filament. Furthermore, dynamic imaging revealed some of these interactions were randomly diffusive at low calcium. The physiological interpretation of these results is that cMyBP-C uses a weak-binding mode to scan the thin filament for association sites, and a tight-binding mode to partially activate it for myosin association. At high calcium, we also show cMyBP-C associates stably to the thin filament and uses proximal clustering to block the binding of myosin heads locally.

S7-3

Nebulin stiffens the thin filament and augments cross-bridge interaction in skeletal muscle

Balazs Kiss[1], Eun-Jeong Lee[1], Weikang Ma[2], Frank Li[1], Paola Tonino[1], Srboljub M. Mijailovich[2], Thomas Irving[2], Henk Granzier[1]

[1]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ 85724, USA; [2]Department of Biology, Illinois Institute of Technology, Chicago, IL 60616, USA

Nebulin is a giant sarcomeric protein that spans along the actin filament in skeletal muscle, from the Z-disk to near the thin filament pointed end. Mutations in nebulin cause muscle weakness in nemaline myopathy patients, suggesting that nebulin plays important roles in force generation, yet little is known about nebulin's influence on thin filament structure and function. Here we used small angle X-ray diffraction and compared intact muscles deficient in nebulin (using a conditional nebulin-knockout, Neb cKO) with nebulin expressing control muscle (Ctrl). When muscles were activated the spacing of the actin subunit repeat (27 Å) increased in both genotypes and when converted to thin filament stiffness the obtained value was 30.3 pN/nm in Ctrl muscle and 10.0 pN/nm in muscle from Neb cKO muscle, i.e., the thin filament is ~ 3-fold stiffer when nebulin is present. In contrast, the thick filament stiffness was not different between the genotypes. A significantly shorter left-handed (59 Å) thin filament helical pitch was found in passive and contracting Neb cKO muscles as well as impaired tropomyosin and troponin movement. Additionally, a reduced myosin mass transfer towards the thin filament in contracting Neb cKO muscle was found, suggesting reduced cross-bridge interaction. We conclude that nebulin is critically important for physiological force levels as it greatly stiffens the skeletal muscle thin filament, and contributes to thin filament activation and cross-bridge recruitment.

S7-4

Atomic resolution structures of F-form actin: mutual switching between G/F transition and ATPase

S. Takeda[1], A. Narita[1], T. Oda[2], N. Watanabe[3], T. Nagae[3], M. Ota[4], R. Koike[4], K. Moritsugu[5], Y. Kanematsu[6], I. Fujiwara[7], K. Tanaka[1], Y. Maéda[1]

[1]Structural Biology Res.Inst. Nagoya Univ. Nagoya, Japan; [2]Tokai-gakuin University, Kakamigahara, Gifu, Japan; [3]Synchrotron Rad.Res.Ctr. Nagoya Univ. Nagoya, Japan; [4]Graduate School of Informatics, Nagoya Univ. Nagoya, Japan; [5]Graduate School of Medical Life Science, Yokohama City Univ. Yokohama, Japan; [6]Graduate School of Information Sciences, Hiroshima City Univ. Hiroshima. Japan; [7]Nagoya Institute of Technology, Nagoya, Japan

Actin polymerization is accompanied by a change of shape from G- to F-form, which triggers ATP hydrolysis (Oda, 2009). The conformational change was confirmed by the near-atomic resolution cryo-EM structure (von der Ecken, 2015). However, for further mechanistic studies of actin assembly and ATP hydrolysis, real atomic resolution structures are indispensable. Here we present crystal structures of F-form actin. In F2A4 structure, total 4 actin molecules, 2 being in the F-form, assemble like a 4-mer filament, which is stabilized by fragmin. This first crystal structure of F-actin yields insights into distinct properties of intra- and inter-strand contacts, explaining the mechanism of double stranded filament assembly through stabilization of energetically unfavorable F-form conformation. We also determined F1A structures, single molecule F-form actin at 1.2 Å resolutions, in complex with fragmin segment-1, with different nucleotide states, AMPPNP, ADPPi and ADP. Remarkably, the three states share almost identical structures. Our structures provide mechanistic insight into how the actin ATP hydrolysis is induced by the G/F transition and why the Pi release destabilizes the filament.

S7-5

Myopathy causing mutations A4V and R91C in tropomyosin Tpm3.12 affect actin polymerization at the pointed end

Joanna Moraczewska, Marta Czajkowska, Thu Ly, Alla Kostyukova

Department of Biochemistry and Cell Biology, Faculty of Natural Sciences, Kazimierz Wielki University in Bydgoszcz, Poland; Voiland School of Chemical Engineering and Bioengineering, University of Washington, Pullman WA, USA

Tropomodulin (Tmod) binds to the pointed end of actin filament to regulate the filament's length. Disease-causing mutations in tropomyosin (Tpm) were shown to interfere with this process. This might be caused by changes within the Tpm-Tmod interface.

We examined effects of myopathy-causing mutations in Tpm3.12 on the rate of the pointed end polymerization ± Tmod1 and Tmod1 binding. Two substitutions in Tpm3.12 were used: A4V located within the N-terminus interacting with Tmod1, and R91C located in the actin-binding consensus site.

Elongation of the barbed-end capped short filaments (seeds) was followed by the increase of pyrene-labeled actin fluorescence. Tmod1 added to the actin seeds in the presence of Tpm3.12 strongly inhibited the rate of G-actin polymerization at the pointed end. The mutation A4V had no effect, but R91C released the inhibition almost 2-fold. Western-Blot analysis revealed that R91C, but not A4V reduced Tmod1 affinity for the actin filament.

We concluded that long-range effects of R91C disturbed the regulation of pointed end elongation by decreasing Tmod1 binding to the filament. In contrast, A4V had much smaller effect on the pointed end regulation, because most probably it is located outside the direct Tmod1 binding site. Weak control of the filament length may contribute to the myopathic phenotype.

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S7-6

Measuring the biochemistry and biophysics of calcium-dependent interactions between titin and actin

Samrat Dutta[1], Christopher Tsiros[2], Sai Lavanyaa Sundar[3], Jeffrey Moore[3], Brent Nelson[4], Matthew J. Gage[2], Kiisa Nishikawa[1]

[1]Center for Bioengineering Innovation, Northern Arizona University; [2]Chemistry Department, University of Massachusetts at Lowell; [3]Biological Sciences Department, University of Massachusetts at Lowell; [4]Mechanical Engineering Department, Northern Arizona University

Titin mutations are responsible for a variety of cardiac and muscle diseases, but these diseases are unexplained by the current models of titin function. A variety of roles for titin within muscle function have been proposed, one of which involves Ca^{2+}-dependent interactions between titin and actin in active muscle. This study describes cosedimentation assays, dynamic force spectroscopy (DFS), and in vitro motility (IVM) assays to determine Ca^{2+}-dependent interactions occur between actin and the N2A region of titin, which was overlooked in previous studies. Co-sedimentation demonstrated increased binding with increasing protein and Ca^{2+} concentration, IVM demonstrated a Ca^{2+}-dependent reduction in actin motility in the presence of N2A, and DFS demonstrated increased rupture forces and decreased koff in the presence of Ca^{2+}. These results all indicate that the strength of N2A-actin interactions increase in the presence of Ca^{2+}, supporting the hypotheses that N2A-actin binding in active muscle increases titin stiffness and plays a regulatory role in muscle contraction, with impairment of this interaction leading to the phenotype in muscular dystrophy with myositis. Future studies are needed to observe and verify this binding in skeletal muscle sarcomeres in vivo.

Session 8: Motor Protein Pharmacology

S8-1

Keynote presentation

Motor Pharmacology: novel inhibitors for different myosin-2 isoforms

Mate Gyimesi[1], Sarad Kumar Suthar[2], Andras Szabo[3], Mate Penzes[1], Laszlo Vegner[1], Gyorgy Hegyi[1], Mihaly Kovacs[1], Andras Malnasi Csizmadia[1]

[1]Eotvos Lorand University; [2]Printnet Ltd.; [3]SONEAS Research Ltd.

We have designed and synthesized over 100 different compounds targeting the blebbistatin site of myosin 2 and a detailed SAR analysis was performed on six myosin-2 isoforms including skeletal, cardiac, smooth and NM2A/B/C. We found that the maximal ATPase inhibition can be modulated between 0 and 100% by the chemical structure of the drug. This unique property of the series of these drugs provides a great pharmacological advantage because the physiological effect can be modulated by the maximal ATPase inhibition and not only by the drug dosage. Besides the general characterization two disease indications are being elaborated. (1) A highly specific drug to skeletal muscle myosin 2 was found to be an efficient muscle relaxant without causing any effect on other physiological processes including heart function and smooth muscle related functions. (2) We developed a compound which can be administered into the ischemic focus of stroke in rat brain and significantly increased ischemic regeneration visualized by MRI, SPECT and PET-CT. The drug treatment drastically improved the general and focal symptoms of stroke compared to the control.

S8-2

Quantifying calcium and myosin contributions to thin filament activation in slow twitch human muscle fibres

Alfredo J. Lopez-Davila[1], Stefan Zittrich[2], Birgit Piep[1], Faramarz Matinmehr[3], Andras Malnasi[4], Anna Rauscher[4], Joseph M. Chalovich[5], Theresia Kraft[1], Bernhard Brenner[1], Robert Stehle[2]

[1]Hannover Medical School, Institute of Molecular and Cell Physiology; [2]Institute of Vegetative Physiology, University of Cologne; [3]Institute of Molecular and Cell Physiology, Hannover Medical School; [4]Department of Biochemistry, Eötvös Loránd University; [5]Department of Biochemistry & Molecular Biology, Brody School of Medicine at East Carolina University

A pharmacological approach was established to test the functional role of human cardiac myosin on thin filament activation.

The myosin inhibitor para-amino-blebbistatin (PAB) was applied to isometric contracting human soleus fibres, which express the same myosin isoform as the human heart. The effect of myosin inhibition on thin filament activation was probed by using a well characterized fluorescent reporter assay in which the endogenous troponin complex of the fibres was exchanged for a fluorescently-labelled fast skeletal troponin complex (fsTnIANBD).

50 μM PAB reduced force max. (pCa 4.5) to 7% $\pm$ 1% (N = 8) while reducing the active change of emission intensity of fsTnIANBD to about 55% of those without PAB, respectively. At all sub-maximally activating [Ca^{2+}], 50 μM PAB significantly reduced thin filament activation ($\approx$ 20 to 35% of the total (myosin + calcium) activation induced at the respective [Ca^{2+}] without PAB) probed by fsTnIANBD in human soleus fibres.

Human cardiac myosin significantly contributes to thin filament activation. Applying this assay to soleus muscle fibres from patients expressing cardiac myosin mutations could reveal novel insights of these mutations.

S8-3

Selective inhibition of myosins by halogenated carbazoles and arylindole derivatives

Falk K. Hartmann[1], Kerstin Elfrink[2], Juliane Kirst[3], Adrian Kishonti[3], Dmitrij Malcev[1], Peter Franz[1], Herwig O. Gutzeit[4], Dietmar J. Manstein[1], Martin Bähler[2], Matthias Preller[1], Hans-Joachim Knölker[3], Georgios Tsiavaliaris[1]

[1]Institute for Biophysical Chemistry, OE 4350, Hannover Medical School, Hannover, Germany; [2]Institute of Molecular Cell Biology, Westfalian Wilhelms University, Münster, Germany; [3]Department of Chemistry, TU Dresden, Germany; [4]Department of Biology, TU Dresden, Germany

There has been a considerable progress in the discovery and design of small chemical compounds that modulate the functional activities of myosins. Here, we introduce two new compound classes, namely halogenated carbazoles and arylindoles, as potent and specific inhibitors of myosins from class-1 and class-9. The compounds bind with micromolar affinities to their targets, thus reducing actomyosin ATPase and motor activity to a minimum level. Since the compounds display no inhibitory effect on the ATPase of several myosin-2 isoforms, including skeletal and cardiac myosin-2, they are even at higher concentrations specific and less cytotoxic than the previously reported myosin inhibitors PBP and PClP of the pseudilin compound family. Using homology modeling, molecular docking and molecular dynamics simulations, we predict an allosteric mechanism of inhibition underlying the increased inhibitory potency of the compounds for the Rho-regulator myosin-9b and certain myosin-1 isoforms involved in endocytosis. The high level of selectivity and the reversible nature recommend them as valuable tools for studying myosin-1 and myosin-9b dependent cellular processes in health and disease.

S8-4

Medicinal chemistry and use of myosin II inhibitor (S)-blebbistatin and its derivatives

Bart Roman

SynBioC Research Group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering

(S)-Blebbistatin (S)-1, a chiral tetrahydropyrroloquinolinone, is a widely used and well-characterized ATPase inhibitor selective for myosin II. The central role of myosin II in many normal and aberrant biological processes has been revealed with the aid of this small molecule.

Unfortunately, (S)-blebbistatin has severe physicochemical deficiencies that trouble its use in advanced biological systems: low solubility, fluorescence interference, (photo)toxicity and stability issues. We and others have developed a toolbox of (S)-blebbistatin analogs in which particular shortcomings have been addressed. This talk will provide a user's guide for their optimal application.

Given the multiple roles of myosin II in a diverse range of motility-based diseases, potent and drugable inhibitors of particular isoforms of this protein could be valuable pharmacological tools. The potency of (S)-blebbistatin is too low to serve this goal. We and others have strived for potency enhancement via modification of rings A, C and D of the molecule. We have also analyzed the resulting structure-activity relationships using in silico methods.

S8-5

Investigation of small molecules that reverse the uncoupling caused by HCM and DCM mutations in contractile proteins

Steven B. Marston, Andrew E. Messer, Juan Eiros-Zamora, Ian Gould, Maria Papadaki, Afnan Choudry, Alice Sheehan

NHLI and Chemistry Departments, Imperial College London, London, United Kingdom

Mutations in contractile proteins can cause familial hypertrophic cardiomyopathy (HCM) or familial dilated cardiomyopathy (DCM). HCM has been linked to a higher myofilament Ca^{2+} sensitivity. The effect of DCM mutations in contractile proteins on myofilament Ca^{2+}-sensitivity is variable with a trend towards deceased Ca^{2+}-sensitivity. In addition, it has been shown that HCM and DCM-related mutations in thin filament proteins usually abolish the coupled relationship between Ca^{2+}-sensitivity and troponin I (TnI) phosphorylation by PKA (uncoupling). In the literature there are reports of 13 DCM mutations and 23 HCM mutations in sarcomeric proteins that cause uncoupling (in TNNI3, TNNT2, TNNC1, TPM1, ACTC, MYL2, MHC7 and MYBPC3 genes). We have not found any instances where disease-related contractile protein mutations are associated with intact coupling.

In normal heart, phosphorylation of Ser22 and 23 of TnI by PKA leads to a 2-fold decrease in Ca^{2+}-sensitivity and a corresponding increase in the rate of Ca^{2+} release from TnC and is essential for the lusitropic response to adrenergic stimulation. Consequently, uncoupling has deleterious consequences: a blunted response to β1-adrenergic activation is commonly observed in animal models with HCM or DCM mutations and it has been demonstrated in a DCM mouse model that this blunting is sufficient to induce symptoms of heart failure under chronic stress.

We have identified compounds that can specifically reverse these abnormalities in vitro and therefore have potential for treatment. Based on our lead compound, Epigallocatechin-3-Gallate (EGCG), we examined 40 compounds: variants of EGCG lacking the pyrogallol ring, variants of EGCG lacking the galloyl ring, silybin, its variants and stereoisomers and unrelated Hsp90 inhibitors and Ca^{2+}-desensitisers. We found 23 compounds that reversed the uncoupling; many of these are pure recouplers. 3 compounds desensitized but did not recouple, one compound has the reverse effect (P-TnI had higher Ca^{2+}-sensitivity than unP TnI). Importantly, recoupling was complete, independent of the causative mutation and the nature of the compound.

Accordingly, we have proposed a 4-state model to account for coupling, uncoupling and recoupling.

We have mapped EGCG, Silybin A and Silybin B binding to whole troponin by molecular dynamics simulations and found that they are usually located between the N-terminal phosphorylatable peptide of TnI and the N-terminal Ca^{2+} regulatory domain of TnC and differentially alter troponin dynamics; further compounds docking on troponin are being assessed by computational chemistry methods to establish a common molecular motif for recouplers.

We have established a biological assay platform for screening EGCG and related analogues in intact cardiomyocytes to study their effects on contractile regulation in vivo, using an E99K ACTC heterozygous-mutant HCM mouse model. In the mutant mouse the lusitropic response to dobutamine (Δt90rel) is blunted. Addition of the recoupling compounds resveratrol and Silybin B restores the dobutamine response (re-couples); EGCG and quercetin may recouple but have additional off-target effects. Silybin A is ineffective as predicted. Further compounds are under investigation.

S8-6

Development of a highly specific skeletal muscle relaxant directly acting on the myosin motor domain

Máté Gyimesi[1], Sarad Kumar Suthar[2], András Szabó[3], László Végner[1], Mihály Kovács[1], András Málnási Csizmadia[1]

[1]Eotvos Lorand University; [2]Printnet Ltd.; [3]SONEAS Research Ltd.

Current muscle relaxants and spasmolytics act on neuromuscular junctions that can have severe side effects like heart failure and paralysis. A specific drug directly inhibiting the motor unit of skeletal muscles would be of high importance and of immediate clinical need. Thus, we designed, synthesized and purified a small molecule myosin inhibitor that is highly selective for skeletal muscle myosin 2 while influencing neither cardiac, smooth nor non-muscle myosin 2 activities. Moreover, this compound is highly soluble and non-mutagenic providing optimal properties for further drug development. The molecular background for this selectivity is a single amino acid change among myosin-2 isoforms that is uniquely Leu in skeletal muscles of most species and Phe in all other myosin-2 isoforms. The specific inhibitor has a morpholino group, which is in steric hindrance with the Phe in smooth, cardiac and non-muscle myosin 2 s while it can optimally fit to the binding pocket of skeletal muscle myosin 2. In vivo results demonstrated that this compound reduced isometric force production while respiratory and heart functions remained unchanged.

Session 9: Muscle Energetics

S9-1

Myoplasmic free [Ca^{2+}] and force during intermittent submaximal contractions of intact mouse single fibres

Brian R MacIntosh[1], Arthur J Cheng[2], Lisa D Glass[1]

[1]Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada; [2]Dept of Physiology and Pharmacology, Karolinska Institute, Stockholm Sweden

Introduction: Submaximal repeated stimulation of whole mammalian muscle in situ results in activity dependent force potentiation followed by a fatigue-induced decline in active force. The potentiation is thought to be due to increased sensitivity to Ca^{2+} whereas the decrease in active force is thought to be due to both decreased myoplasmic free [Ca^{2+}] ([Ca^{2+}] m) and decreased Ca^{2+} sensitivity.
Method: We measured [Ca^{2+}] m with indo-1 fluorescence during intermittent submaximal (40–50 Hz) stimulation of single mouse flexor digitorum brevis fibres at 32 °C and evaluated the change in the relationship between force and [Ca^{2+}] m with respect to the control (prefatigue) relationship.
Result: Active force began at 194–75 kPa (mean SD) and [Ca^{2+}] was 0.319–0.012 M. Initially, active force decreased to 182–48 kPa while [Ca^{2+}] m increased to 0.372–0.015 M, representing a decrease in Ca^{2+} sensitivity. Subsequently force potentiated to 221–34 kPa then fell to 69.3–27.6 kPa while [Ca^{2+}] m decreased, reaching 0.318–0.014 M at the final target force of 40% of initial.
Conclusion: At no time did the Ca^{2+} sensitivity increase beyond that seen in the control situation. The final [Ca^{2+}] m was not significantly different from the initial [Ca^{2+}] m. Calcium sensitivity recovered to the control value within 5 min and low-frequency fatigue persisted due to a post-fatigue development of persistent low [Ca^{2+}] m.

S9-2

In vivo and in vitro muscle metabolic profiles of TIEG1 KO muscle mice using spectroscopy techniques (MRS/NMR)

Malek Kammoun[1], Sandra Même[2], Lydie Nadal-Desbarats[3], William Même[2], Frederic Szeremeta[2], Malayannan Subramaniam[4], John R. Hawse[4], Sabine F. Bensamoun[1]

[1]Sorbonne University, Université de technologie de Compiègne CNRS, UMR 7338 Biomechanics and Bioengineering, Centre de Recherche de Royallieu, Compiègne, France; [2]Centre de Biophysique Moleculaire, CNRS UPR4301, Orleans, France; [3]UMR 1253 iBrain, Université de Tours, Inserm, Tours, France; [4]Department of Biochemistry and Molecular Biology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

Introduction: TGFbeta inducible early gene-1 (TIEG1) is a member of the Krüppel-like family of transcription factors (KLF10). Deletion of TIEG1 results in muscle fiber hypertrophy, texture profile changes, dysfunction of mitochondrial biogenesis and defects in functional properties.
Aim: To further analyze the effect of TIEG1 gene on muscle metabolism.
Methods and Results: 12 WT and 12 TIEG1 KO mice were used for in vivo spectroscopy acquisitions 9.4T (Bruker). A home built coil was developed. Resonance frequencies were 400 MHz for the proton and 162 MHz for the phosphorus. Localized 1H and 31P spectroscopy were performed with PRESS sequence providing quantification of different metabolites. While 1H-NMR spectra showed no significant difference for choline, creatine, taurine and extramyocellular lipids between WT and TIEG1 KO. 31P spectra revealed a significant difference for phosphocreatine and ATP. For metabolomics analysis 1H-NMR spectra were obtained from soleus (N = 18) and EDL (N = 18) muscles isolated from WT and TIEG1 KO with a 600 MHz spectrometer (Bruker, 14T). Heatmaps were generated to visually depict changes in metabolites ($p < 0.05$) as a function of mouse genotype. For both TIEG1 KO soleus and EDL muscles, there were more down regulated metabolites compared to WT muscles.
Conclusion: The present study has demonstrated a new role for TIEG1 in the homeostasis of the muscle metabolome and specifically in energetic metabolism.

S9-4

The missense E258 K-MyBP-C mutation increases the energy cost of tension generation in both ventricular and atrial tissue from HCM patients

Giulia Vitale, Nicoletta Piroddi, Beatrice Scellini, Josè Manuel Pioner, Cecilia Ferrantini, Chiara Tesi, Corrado Poggesi

Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Mutations in MYBPC3, the gene coding for cardiac myosin-binding protein-C (cMyBP-C) are the most common cause of Hypertrophic CardioMyopathy (HCM). The E258K-MyBP-C is a highly penetrant missense mutation with poorly understood molecular mechanisms. Mechanics and kinetics of contraction as well the energetic cost of tension generation were investigated using left ventricular (LV) and atrial tissue from three E258K HCM patients and compared to those from controls (donor hearts, aortic stenosis patients, and HCM patients negative for sarcomeric protein mutations). Kinetics of tension generation and relaxation were measured in single LV and atrial myofibrils mounted in a force recording apparatus (15 °C), maximally Ca^{2+}-activated (pCa 4.5) and fully relaxed (pCa 9.0) by rapid solution switching (< 10 ms). Maximal ATPase and isometric active tension were simultaneously measured in Triton-permeabilized LV strips. In E258K, maximal tension of atrial myofibrils was reduced compared to controls, while maximal tension of LV myofibrils was unchanged. The rate of tension generation following maximal Ca^{2+} activation (kACT) was faster in both ventricular and atrial E258K myofibrils compared to controls. The rate of isometric relaxation (slow kREL) was also faster in E258K myofibrils, suggesting faster cross-bridge detachment and increased energy cost of tension generation. Direct measurements in ventricular skinned strips confirmed that tension cost was higher in E258K preparations compared to controls. We conclude that the E258K mutation primarily alters apparent cross-bridge kinetics and impairs sarcomere energetics. In vitro, the mutation seems to induce similar kinetic and energetic effects in both atrial and LV sarcomeres. The smaller impact of the mutation on atrial muscle function compared to LV muscle in vivo is likely due to the different loading conditions of the two chambers.

S9-5

Role of PGC-1α associated mitochondrial biogenesis in statin-induced myotoxicity

Miljenko Panajatovic[1,2], François Singh[1,2], Urs Duthaler[1,2], Stephan Krähenbühl[1,2], Jamal Bouitbir[1,2]

[1]University Hospital Basel, Division of Clinical Pharmacology & Toxicology, Basel, Switzerland; [2]University of Basel, Department of Biomedicine, Basel, Switzerland

Background: Statins impair expression of PGC-1α in human and rat skeletal muscle, suggesting a role of PGC-1α in statin-induced myotoxicity.
Objective: This study aimed to investigate these effects in differentially expressed PGC-1α mouse models.
Methods: We used 3 mouse models: mice with muscle PGC-1α knockout (MKO), mice overexpressing PGC-1α (MCK), and wild-type (WT) mice. Mice treated for 3 weeks with water or simvastatin (5 mg/kg/d) by oral gavage, were assessed with grip test, metabolic treadmill and glucose tolerance test. We measured mitochondrial respiration and H_2O_2 production in fresh permeabilized muscle fibres.
Results: Simvastatin showed impairment in WT mice, manifested by decreased exercise capacity, glucose intolerance, and decreased mitochondrial respiration in the glycolytic muscle coupled with increased H_2O_2 production. MKO mice treated with simvastatin, showed decreased exercise capacity and mitochondrial respiration in oxidative and glycolytic muscle types. MCK mice showed no impairments of mitochondrial function and physical capacity.
Conclusion: Oxidative muscles are more resistant to simvastatin-associated toxicity than glycolytic muscles. PGC-1α seems to be a susceptibility factor and has an important role in mitigating of simvastatin induced myotoxicity.

S9-6

Single cell analysis reveals the involvement of the long non-coding RNA Pvt1 in myofiber metabolism modulation

Lisa Buson, Enrico Alessio, Francesco Chemello, Luca Scorrano, Gerolamo Lanfranchi, Francesca Grespi, Alessandro Bertoli, Maria Lina Massimino, Caterina Peggion, Beniamina Pacchioni, Caterina Millino, Stefano Cagnin

University of Padova, Department of Biology

Long non-coding RNAs (lncRNAs) are emerging as important players in the regulation of several aspects of cellular biology. For a better comprehension of their function it is fundamental to determine their

tissue or cell specificity and to identify their subcellular localization. In fact, the activity of lncRNAs may vary according to cell-type specific expression and subcellular localization. Myofibers are the motor units of skeletal muscles characterized by great metabolic plasticity. How lncRNAs are expressed in different myofibers, participate to metabolism regulation, and are compartmentalized within a single myofiber is still unknown. We compiled a complete and integrated catalogue of lncRNAs expressed in skeletal muscle, associating the fiber-type specificity and subcellular location to each of them, demonstrating that many are altered when muscles change myofiber composition and metabolism according to specific stimuli. We demonstrated that the lncRNA Pvt1, activated early during muscle atrophy, impacts mitochondrial respiration and morphology and affects mito/autophagy and myofiber size in vivo. This work corroborates the importance of lncRNAs in the regulation of metabolism and neuromuscular pathologies and offers a valuable resource to study the metabolism in single cells characterized by pronounced plasticity.

S9-7

Nucleosides block AICAR-stimulated activation of AMPK in skeletal muscle and cancer cells

Klemen Dolinar[1,2], Vid Jan[1], Mojca Pavlin[2,3], Alexander V. Chibalin[4], Sergej Pirkmajer[1]

[1]University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Ljubljana, Slovenia; [2]University of Ljubljana, Faculty of Electrical Engineering, Group for nano and biotechnological applications, Ljubljana, Slovenia; [3]University of Ljubljana, Faculty of Medicine, Institute of Biophysics, Ljubljana, Slovenia; [4]Karolinska Institutet, Integrative Physiology, Department of Molecular Medicine and Surgery, Stockholm, Sweden

Introduction: AMP-activated kinase (AMPK), a major regulator of energy metabolism, is a promising target for the treatment of type 2 diabetes and cancer. AICAR, an adenosine analogue, is the most widely used pharmacological AMPK activator in cell-based assays. Some broadly used cell culture media, such as MEMα, contain high concentrations of nucleosides. We examined whether such media alter AICAR actions in skeletal muscle and cancer cells.
Methods: We evaluated the effect of AICAR and nucleosides on AMPK activation, glucose uptake and cell proliferation.
Results: In nucleoside-free media AICAR activated AMPK, increased glucose uptake and suppressed cell proliferation. These effects were reduced in MEMα with nucleosides. Addition of adenosine to nucleoside-free media also suppressed AICAR actions. MEMα with nucleosides blocked AICAR-stimulated AMPK activation even in the presence of methotrexate, which normally enhances AICAR actions by reducing its intracellular clearance.
Conclusion: Our findings show that nucleosides in cell culture media reduce effects of the most widely used pharmacological AMPK activator AICAR. Results of cell-based assays in which AICAR is used for AMPK activation therefore critically depend on media formulation. Furthermore, our findings highlight a role for extracellular nucleosides and nucleoside transporters in regulation of AMPK activation.

Session 10: Contraction Regulation, EC Coupling

S10-1

The role of septins in skeletal muscle

Mónika Gönczi, Beatrix Dienes, Péter Szentesi, Karolina Cseri, Norbert Balogh, János Fodor

University of Debrecen, Department of Physiology, Debrecen, Hungary

Septins are 30–65 kDa, highly conserved GTP-binding proteins controlling different cellular processes by polymerizing into hetero-oligomeric complexes. All septin filaments include SEPT7, which occupies the ends of hexameric building blocks generating non-polarized filaments.

There are limited information about the expression and function of septins in skeletal muscle. We have identified several septin isoforms both in skeletal muscle samples and in cultured C2C12 cell line, where ontogenesis- and differentiation-dependent septin 7 expression was observed.

In C2C12 cells stable septin 7 knockdown (KD) clones were generated using shRNA gene silencing and marked changes in cell shape and size were observed. The average area and perimeter of the cells increased in KD clones, and cells appeared more circular/round. In control cells septin 7 is present as a long, filamentous structure throughout the cytoplasm mostly co-localized with actin filaments, while in KD cells this well organized structure was broken. In conclusion, septin 7 has a crucial role in skeletal muscle physiology, and it could have a potential function in muscle regeneration and/or different muscle diseases.

S10-2

Defective Ca^{2+} signaling in centronuclear myopathies

Candice Kutchukian[1], Colline Sanchez[1], Laszlo Csernoch[2], Peter Szentesi[2], Pankaj Agrawal[3], Marc Bitoun[4], Ana Buj-Bello[5], Vincent Jacquemond[1]

[1]NeuroMyoGene Institute, CNRS, INSERM, Univ. Lyon 1, France; [2]University of Debrecen, Hungary; [3]Boston Children's Hospital and Harvard Medical School, USA; [4]Research Center for Myology, Univ. Paris 06, France; [5]Généthon, INSERM Evry, France

Control of ryanodine receptor-mediated sarcoplasmic reticulum (SR) Ca^{2+} release by CaV1.1 channels in the transverse tubule membrane is the core mechanism of excitation-contraction (EC) coupling in skeletal muscle. Mutations in genes including MTM1, DNM2, BIN1, SPEG and RYR1 are responsible for centronuclear myopathies (CNMs). Besides common features of weakness and centralized nuclei, it is not clear if the several CNMs share similar pathogenic mechanisms. We showed that MTM1-deficiency in mouse muscle is associated with disruption of EC coupling. Detailed investigations using voltage-clamp and confocal microscopy in isolated MTM1-deficient muscle fibers have revealed an array of functional defects including depressed amplitude and altered kinetics and spatial uniformity of SR Ca^{2+} release, as well as spontaneous Ca^{2+} release at rest under the form of Ca^{2+} sparks. Strikingly, some of these features are reproduced in mouse models of DNM2 and SPEG-related CNMs, highlighting the respective importance and role of these proteins in the control of specific aspects of Ca^{2+} signaling and prompting similar studies in other models of CNMS (supported by AFM-Téléthon–MyoNeurAlp # 5.3.4.4).

S10-3

Transverse tubule plasticity drives the assembly of calcium entry units in muscle during exercise

Anonio Michelucci[1,2], Simona Boncompagni[1], Laura Pietrangelo[1], Robert T. Dirksen[2], and Feliciano Protasi[1]

[1]CeSI-MeT, Center for Research on Ageing and Translational Medicine, Univ. G. d'Annunzio, I-66100 Chieti; [2]Department of

Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Acute treadmill exercise in-vivo drives formation of new junctions between stacks of sarcoplasmic reticulum (SR) cisternae and transverse-tubules (TTs) at the I band of sarcomeres. We named these new junctions Calcium Entry Units (CEUs) as they contain STIM1 and Orai1, the two proteins that mediate store-operated Ca^{2+} entry (SOCE).

Using electron microscopy, we evaluated the time course of CEU disassembly in extensor digitorum longus (EDL) fibers from wild type (WT) mice subjected to 1 h of running at increasing speed (from 5 to 25 m/min) and sacrificed within one hour (1 h), or 6 and 24 h following the running protocol. The number of SR-stacks/100 mm^2 (from 2.0 $\pm$ 0.3 to 9.9 $\pm$ 0.7) and the TT extension/100 mm^2 (from 2.4 $\pm$ 0.8 to 6.1 $\pm$ 0.8 mm) increased significantly after a single bout of acute treadmill exercise. While the number of SR-stacks/area further increased after 6 h to return to control values only after 24 h of recovery, the extension of TTs returned to control values already at 6 h. To assess the correlation between structural findings and function of SOCE, we then determined: (i) the fatigue resistance of EDL muscles to a high-frequency stimulation protocol (40 $\times$ 500 ms 50 Hz pulses every 2.5 s) in presence of external Ca^{2+}; and (ii) the rate of Mn^{2+} quench of Fura-2 fluorescence in single flexor digitorum brevis (FDB) fibers. Both fatigue resistance and Mn^{2+} quench were significantly increased in samples from mice sacrificed within 1 h from treadmill exercise, but were not different from control after 6 and 24 h of recovery. These results indicate: (i) great structural plasticity of TTs, as after exercise their retraction from CEUs occur prior to SR-stack disassembly; and (ii) stacks of SR membranes at the I-band function as CEUs only while being coupled to extensions of TTs.

S10-4

Role of triadin mutations in inherited arrhythmia syndromes

Chiara Di Resta[1], Sara Benedetti[1], Gamberucci Alessandra[2], Lorenzini Stefania[2], Pierantozzi Enrico[2], Ferrari Maurizio[1], Vincenzo Sorrentino[2]

[1]University Vita Salute San Raffaele, Milan; [2]Department of Molecular and Developmental Medicine, University of Siena

Inherited arrhythmia syndromes are a heterogeneous group of disorders, characterized by occurrence of malignant alterations of the cardiac rhythm that can lead to sudden death. Genetic studies revealed that these disorders are usually monogenic and associated with mutations in genes coding for ion channels or ion channels regulatory proteins. However, depending on the disease subtype, a large proportion of patients remain without a genetic diagnosis. In addition, the high variability and incomplete penetrance observed in the disease, suggest that multiple factors may affect the clinical phenotype. We recently identified a novel homozygous missense mutation in the transmembrane domain of triadin in a patient affected by Long QT syndrome. All other known mutations in triadin identified so far in Long QT patients are frameshifts, causing a premature stop codon and functionally resulting in a triadin-null phenotype. A missense mutation in the transmembrane domain of triadin was previously identified in a patient with CPVT, but also in this case the mutation caused the protein to be extensively degraded, resulting in absence of functional triadin.

Preliminary functional characterization of the newly identified mutant protein will be presented. The results obtained suggest that the mutation affects protein function by altering the SR localization and calcium release through RyR2 channels.

S10-5

Phosphorylation/glycosylation states of MLC2 regulatory protein in skeletal muscle in disuse conditions

Marie Pourrier, Laetitia Cochon, Valerie Montel, Prof. Bruno Bastide

UREPSSS, APMS

Post-translational modifications such as phosphorylation and O-GlcNAcylation are involved in the physiopathology of several acquired diseases, such as muscle insulin resistance or muscle atrophy. In this study, we compared the effects of various durations of disuse conditions on Myosin Light Chain 2 (MLC2) post-translational modifications, i.e. after a short-term 3-day dry immersion (DI), mid-term (21 days) and long-term (60 days) bed rest (BR), and 15 day-rat hypodynamia-hypokinesia (HH). Muscle phenotype was identified by myosin heavy chain (MHC) isoform expression.

In both conditions, there was a shift from slow to fast Myosin Light Chain MLC2 isoform expression. The extent of the transition was identical in BR and DI conditions, and more important after two weeks of HH. In all conditions, MLC2 phosphorylation state was increased while MLC2 glycosylation was decreased. These results suggested an interplay between phosphorylation and O-GlcNAcylation of MLC2, which might be involved in the regulation of associated phenotype changes. The extent of the modulation by phosphorylation/glycosylation process was proportional to the disuse duration.

In conclusion, a short period of muscle disuse by DI was sufficient to significantly induce phenotype changes in MLC2 protein and in its post-translational regulation; the more important was the disuse, the more important the regulation. It is suggested that the O-GlcNAcylation level of the phosphoprotein MLC2 is crucial in the modulation of muscle contraction, and should be responsible for changes in muscle contractile properties observed in functional atrophy. This study also contributed to underline that muscle regulatory proteins such as MLC2 are early good molecular biomarkers of skeletal muscle dysfunction conditions.

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S10-6

New insights of intracellular calcium regulation mechanism in dystrophin-deficiency

Shin’ichi Takeda

National Center of Neurology and Psychiatry

Duchenne muscular dystrophy (DMD) and the less severe Becker muscular dystrophy (BMD) are caused by mutations in the DMD gene. Previous reports show that in-frame deletion of exons 45-55 produces an internally shorted, but functional dystrophin resulting in a very mild BMD phenotype. In order to elucidate the molecular mechanism leading to this phenotype, we generated exon 45-55 deleted dystrophin transgenic/mdx (Tg/mdx) mice. Muscle function of Tg/mdx mice was restored close to that of wild type (WT) mice, but the localization of the nNOS was changed from the sarcolemma to the cytosol. This led to hyper-nitrosylation of the RyR1 causing increased Ca^{2+} release from the sarcoplasmic reticulum. On the other hand, Ca^{2+} reuptake by SERCA was restored to the level of WT mice, suggesting that the Ca^{2+} dysregulation had been compensated by SERCA activation. In line with this, expression of sarcolipin (SLN), a SERCA-inhibitory peptide, was upregulated in mdx mice, but strongly reduced in Tg/mdx mice. Furthermore, knockdown of SLN ameliorated the cytosolic Ca^{2+} homeostasis and the dystrophic

phenotype in mdx mice. These findings suggest that SLN might be a novel target for DMD therapy.

Session 11: Muscle Development, Regeneration and Disease

S11-1

Expression of truncated obscurins leads to maladaptive responses in the heart

Alyssa Grogan, Li-Yen R. Hu, Christopher W. Ward

University of Maryland School of Medicine

Obscurin was discovered as binding partner of titin and Novex-3, a titin splice variant. Although their direct binding is known for > 15 years, the physiological relevance of their interaction has been elusive. To assess the effects of the obscurin/titin binding in vivo, we generated a deletion model, Obscn-ΔIg58/59, that carries truncated obscurin lacking the Ig58/Ig59 region that supports binding to both titin and Novex-3. Homozygous Obscn-ΔIg58/59 male mice develop left ventricular (LV) hypertrophy by 6 months, which progresses to LV dilation and severe arrhythmia by 1 year, while female mice present mild arrhythmia. Exertion of pathological and physiological stress in young mice via β-adrenergic stimulation and strenuous exercise, respectively, revealed electrical abnormalities and poorer running ability. Mutations in obscurin and titins, including ones that disrupt their binding, are linked to cardiac and skeletal myopathies. It is thus apparent that the obscurin/titin complex is essential for normal muscle structure and function, and that disruption of their binding is associated with muscle pathogenicity. Our findings using the Obscn-ΔIg58/59 model corroborate this notion.

S11-2

Myocardial overexpression of ANKRD1 affects developmental cardiac remodeling and leads to adult diastolic dysfunction

Nicoletta Piroddi[1], Beatrice Scellini[1], Chiara Tesi[1], Laura Monti[2], Ileana Badi[3], Federico Caicci[4], Lucia Manni[4], Simonetta Ausoni[5], Corrado Poggesi[1], Cinzia Parolini[6], Francesco Acquati[2], Marina Campione[7]

[1]Dept. of Experimental and Clinical Medicine/University of Florence, Italy; [2]Dept of Biotechnology and Life Science/University of Insubria, Italy; [3]Experimental Cardio-Oncology and Cardiovascular Aging Unit, Monzino-IRCCS, Milan, Italy; [4]Dept. Of Biology/ University of Padua, Italy; [5]Dept. Of Biomedical Sciences/University of Padua, Italy; [6]Dept. of Pharmacological and biomolecular sciences, University of Milan, Italy; [7]CNR-Neuroscience Institute, Padua, Italy

Aims: Increased Ankrd1 levels linked to genetic mutations have been correlated to congenital heart disease onset and adult cardiomyopathy occurrence in humans. The link between increased ANKRD1 level and cardiac structural and functional disease onset is not understood. To get insight into this problem, we have generated a ANKRD1 mouse model by overexpressing ANKRD1 in the myocardium.

Methods and Results: We show that ANKRD1 delineates discrete sub-compartments in the developing mouse heart. ANKRD1 transgenic mice present impaired cardiac remodeling, which strongly affects the developing sinoatrial region and leads to sinus venosus defects. Transgenic mice survive to adulthood but develop left atrial enlargement accompanied by severe diastolic dysfunction. Embryonic and neonatal transgenic cardiomyocytes present irregular shape and sarcomeric disorganization, which progresses into sarcomeric loss and mitochondrial damage in adult ventricular but not atrial cardiomyocytes. While isolated embryonic transgenic myofibrils show the same mechanical properties of wild type samples, neonatal transgenic myofibrils present higher passive tension and maximal force compared to wild type. This indicates the presence in ANKRD1 transgenic mice of a faster functional shift towards stiffer and hypercontractile cardiomyocytes, triggered by the increase in workload at birth. At the molecular level, these changes are accompanied by dynamic alterations in titin isoforms ratio. Interestingly, adult wild type and transgenic myofibrils show the same passive tension as transgenic neonatal myofibrils, with adult transgenic myofibrils showing a higher maximal force accompanied at this stage by a marked slowing down of the relaxation phase compatible with the overt diastolic disfunction of adult ANKRD1 transgenic mice.

Conclusions: Our data indicate that genetic mutations leading to increased ANKRD1 levels can lead both to congenital heart disease and adult cardiomyopathy via a common cellular mechanism, with ANKRD1 playing the role of a critical strain sensor-signaling molecule finely modulating cardiomyocyte function during development and postnatal life.

S11-3

Compartmentalization of titin & Novex 3 during sarcomere assembly in regenerating skeletal muscle

Andreas Unger[1], Bettina Baumgarten[2], Wolfgang A. Linke[1]

[1]Institute of Physiology II, University of Muenster; [2]Dept. of Cardiovascular Physiology, Ruhr University Bochum

The giant protein titin spans from the Z-disk to the M-line in the sarcomere of striated muscle cells, where it functions as a molecular spring during stretching and relaxation. In this study we used immuno electron microscopy and three-dimensional reconstruction to localize full-length titin and Novex-3 ("tiny titin") at different stages of myofibrillogenesis in regenerating rat soleus muscle after notexin-induced myofibril breakdown. Two days after intoxication with notexin we observed first single thick filaments in the cytosol colocalized with full length titin. In addition, we identified subcellular compartments containing Novex-3 titin as integral elements of emerging Z-bodies. Thick filaments aligned to build first premyofibrils containing titin, myosin and Z-bodies; three days after intoxication we found Z-bodies fusing to Z-disks, forming contracted sarcomeric structures, which later develop into mature myofibrils with I- and A-Band showing the typical striation pattern. Our results support a model in which titin acts as a molecular scaffold for the assembly of Z-discs and thick filaments during skeletal muscle regeneration.

S11-4

Myocardial regeneration: uncommon sense for common problems

Mark Sussman

SDSU Heart Institute, San Diego State University, Department of Biology, LS 426, 5500 Campanile Drive, San Diego, CA 92182

Myocardial regenerative research remains an area of intensive study despite over a decade of frustratingly slow progress and modest clinical efficacy. A fundamental limitation in myocardial regeneration is inherently poor reparative capacity of adult mammalian heart which declines over lifespan. Augmentation of repair requires unnatural solutions to overcome normal adult myocardial biology using Regeneration Associated Cellular Effectors (RACE) to deliver functionally competent therapeutic interventions. The logic and rationale

of four distinct RACE conceptual strategies will be presented including CardioEnhancers (genetic engineering), CardioChimeras (cell chimerism), CardioClusters (multi-cell three dimensional clustering), and CardioEvolvers (increased ploidy). Each RACE approach addresses a distinct biological limitation that impairs current cell-based treatments for myocardial damage, and different RACE approaches can be combined to promote synergism of biological potentiation. These next-generation approaches represent the future of myocardial regenerative research, ultimately translating into novel clinical treatments achieving desperately needed treatment of heart failure.

S11-5

Nebulin's C-terminus is necessary for proper sarcomeric structure and function

Frank Li[1], Justin Kolb[1], Dr. Elisabeth Barton[2], Dr. Henk Granzier[1]

[1]University of Arizona; [2]University of Florida

Nebulin is a massive structural protein in skeletal muscle that exists wrapped around the thin filaments, with its C-terminus embedded within the Z-disc and its N-terminus extending out towards the pointed ends. Through full nebulin knockout models, it has been reported that nebulin contributes to thin filament length regulation, force production, and the arrangement of Z-discs. Furthermore, unique sequences within the C-terminus have interesting implications from both a clinical and basic sciences standpoint. Mutations within nebulin are known to cause a disease called nemaline myopathy, which has no genotype-phenotype correlation. Phenotypes observed in the Z-disc suggest a loss of nebulin's C-terminus may be a similarity amongst various mutations. And while investigating actin proliferation in muscles, Takano et al (2010) proposed that nebulin's C-terminus is involved in an IGF-1 stimulated growth pathway that would allow for myofibrillar hypertrophy.

In order to study the biological function of nebulin's C-terminus, we created a mouse model that produces a truncated nebulin that is missing only its two unique C-terminal domains, the Serine-Rich Region and the SH3 domain. Characterization revealed that the truncation caused a moderate myopathy phenotype reminiscent of nemaline myopathy despite nebulin being localized properly in the thin filaments. This included muscle weight loss, changes in sarcomere structure, as well as a decrease in force production. GST pulldown experiments found novel binding partners with the unstudied Serine-Rich Region many of which are associated with myopathies, suggesting that loss of nebulin's C-terminus may disrupt signaling with those proteins and thereby amplifying the myopathy phenotype. Lastly, we investigated the possibility of hypertrophy in muscles lacking nebulin's C-terminus and found that muscles still appeared to undergo hypertrophy in a manner comparable to wild-type muscles. Overall, we conclude that the C-terminus of nebulin contributes to myopathy, but does not contribute to hypertrophy signaling from the IGF-1 pathway.

S11-6

Pathogenic troponin T mutations with opposite effects on myofilament Ca^{2+} sensitivity attenuate each other's cardiomyopathy phenotypes in mice

Jose Renato Pinto[1], Karissa D. Jones[1], Yeojung Koh[1], Rebecca S. Weller[3], Rajdeep S. Turna[1], Ferhaan Ahmad[4], Sabine Huke[5], Björn C. Knollmann[3], Hyun Seok Hwang[2]

[1]Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL; [2]Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, FL; [3]Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN; [4]Department of Internal Medicine University of Iowa, Iowa City, IA; [5]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL

Mutations in cardiac troponin T (cTnT) associated with hypertrophic cardiomyopathy (HCM) generally lead to an increase in the calcium (Ca^{2+}) sensitivity of contraction and susceptibility to arrhythmias. In contrast, cTnT mutations linked to dilated cardiomyopathy (DCM) decrease the Ca^{2+} sensitivity of contraction. Here we tested the hypothesis that two cTnT disease mutations with opposite effects on myofilament Ca^{2+} sensitivity can attenuate each other's phenotype. To test this hypothesis, we crossed transgenic mice expressing the HCM cTnT-I79N mutation (I79N) with a DCM knock-in (KI) mouse model carrying the heterozygous cTnT-R141W mutation (HET). The resulting I79N/HET mouse constituted the experimental group. The Ca^{2+} sensitivity of contraction was measured in skinned cardiac muscle preparations. The results of the Ca^{2+} sensitivity ranked from highest to lowest were as follow: I79N $>$ I79N/HET $>$ NTg $>$ HET. The increased cooperativity of thin filament activation found in HET skinned muscle preparations was normalized in I79N/HET. Echocardiographic measurements revealed an improvement in hemodynamic parameters in I79N/HET compared to I79N and normalization of left ventricular dimensions and volumes compared to both I79N and HET. Ex vivo testing showed that the I79N/HET mouse hearts had reduced arrhythmia susceptibility compared to I79N mice. These results suggest that two disease mutations in TnT that have opposite effects on the myofilament Ca^{2+} sensitivity can paradoxically rescue each other's disease phenotype. Normalizing myofilament Ca^{2+} sensitivity may be a promising new treatment approach for a variety of diseases.

Session 12: Integrative Muscle Biology

S12-1

Keynote presentation

From basic muscle research to applications in the clinic

Else Marie Bartels

Copenhagen University Hospital, Bispebjerg and Frederiksberg/ Department of Neurology and the Parker Institute

Background: Basic muscle research is criticized for being non-physiological and far from muscle function in vivo. This may be so, but the results from the perturbed systems will, when put together, form an image of what is going on. Added together with in vivo measurements, an overall understanding of functioning of healthy and diseased muscle will lead to better clinical assessments where muscle dysfunction is expected.

Objective: To describe the process from bench results to muscle assessments in the clinic.

Methods: Through examples from own studies in basic and clinical muscle research to describe applications of methods, both involving basic laboratory and non-invasive in vivo measurements, applicable when describing muscle conditions and function in the clinic.

Results: Musculoskeletal diseases as different as polymyositis, uremia-linked muscle function and some neurologically based conditions can be singled out by a set of electro-physiological methods.

Conclusion: Lateral thinking and a strong collaboration between bench workers and clinicians are asked for when trying to disentangle the outer signs and patient self-assessments of musculoskeletal diseases.

S12-2

Striated muscle tissue mechanosensors in health and disease

Frank Suhr

Exercise Physiology Research Group, Group of Biomedical Sciences, KU Leuven, Tervuursevest 101, 3001 Leuven, Belgium

Mechanical forces determine striated muscle physiology. Physiological volume loading improves cardiac muscle function, whereas pathological pressure loading results in disordered phenotypes with reduced cardiac functions. Skeletal muscles increase strength and fiber size upon loading, whereas mechanical cutback conditions cause muscle fiber atrophy and reduced strength. Consequently, mechanical forces are essential for striated muscle physiology. However, how are these adaptations regulated at the molecular level? Striated muscles express mechanosensors that translate mechanical cues into biochemical signals mediating defined phenotypic hallmarks of muscles. These mechanosensors comprise large protein complexes, e.g. the dystrophin-glycoprotein complex or costameres. These protein complexes connect with integrin heterodimers to physically link the extracellular matrix to signaling hotspots beneath striated muscle fiber membranes. The present study will describe novel advancements in cardiac and skeletal muscle mechanosensation highlighting novel, yet undiscovered players in this complex process. A connection between basic research and clinical research will also be provided.

S12-3

Hormonal responses following resistance exercise performed at maximum movement velocity

Anastassios Philippou[1], Ilias Smilios[2], Savvas P. Tokmakidis[2], Michael Koutsilieris[1], Roxane Tenta[3]

[1]Department of Experimental Physiology, Medical School, National and Kapodistrian University of Athens, Greece; [2]Department of Physical Education and Sport Science, Democritus University of Thrace, Greece; [3]Department of Nutrition & Dietetics, School of Health Science and Education, Harokopio University, Greece

Introduction: Mechanical overloading of skeletal muscle is a strong stimulus for eliciting acute hormonal changes potentially involved in muscle adaptation following resistance exercise.
Purpose: This study investigated the responses of thyrotropin (TSH), free thyroxine (fT4) and prolactin (PRL) in young volunteers after resistance exercise of the knee extensor muscles of both legs.
Methods: Nine healthy males (age: 22.5 ± 3.3 years, height: 181 ± 5 cm, body mass: 81.6 ± 5.6 kg) underwent a resistance exercise protocol (4 sets of squat and 4 sets of leg press, 8 repetitions/set at a load of 10-RM) with the velocity of movement during concentric contractions being maximum. Blood samples were collected before, immediately after and at 20 and 40 min post-exercise. Serum levels of TSH, fT4 and PRL were measured by ELISA.
Results: TSH showed a gradual, non-significant increase up to 43% at 40 min post exercise ($p > 0.05$). Serum fT4 levels exhibited also a gradual increase reaching significance ($p < 0.01$) at 40 min post exercise. PRL levels showed a slight decrease up to 19% 40 min post exercise ($p > 0.05$).
Conclusion: Our findings suggest that resistance exercise induces acute increases in serum levels of thyroid hormones, particularly of fT4. Further studies are needed to characterize the mechanisms by which these hormonal responses are triggered and regulated during recovery after resistance exercise.

S12-4

Disturbances of the homeostasis of the neuro-muscular-tendon tissue-complex in contractures of individuals with cerebral palsy

J. Pingel[1], I. T. Andersen[1], J. Dyrberg[2], L. Barber[3], J. Bulow[4], J. Wienecke[1], R. Feidenhansl[5], J. B. Nielsen[1]

[1]University of Copenhagen, Center for Neuroscience; [2]Department of Forensic Genetics, University of Copenhagen, 2200 Copenhagen, Denmark; [3]Queensland Cerebral Palsy and Rehabilitation Research Centre, Child Health Research Centre, Faculty of Medicine, The University of Queensland, Brisbane, Australia; [4]Frederiksberg Hospital Denmark, Department of Clinical Physiology; [5]XFEL Hamburg, Germany

Muscle contractures are common in individuals with cerebral palsy (CP), but the mechanisms responsible for the development of contractures are still unclear. Here we propose that changes in tissue homeostasis within the neuro-muscular-tendon tissue-complex are at the heart of the development of contractures. In order to unravel the neural, mechanical and metabolic factors, as well as genetic and transcriptional factors in muscle contractures, several different studies have been conducted.

Changes at tissue level: Our recent results reveal that some individuals might be genetically predisposed to become contractures. Furthermore, a significant correlation was observed between the passive stiffness of skeletal muscle and the expression of HSPG2, PRELP, RYR3, COL5A3, ASPH and COL4A6.

Systemic differences: When levels of CRP, TGF-β and IL-6 was measured in serum of children with CP, adults with CP and healthy adults, it was observed that Children with CP has significantly higher systemic levels of CRP and TGF-β. Whether inflammation affects the growth of the muscles or might have other negative adverse effects in children with CP needs further investigation

Effect of treatments: While micro-architectural analyses still are under investigation in humans, our animal studies have shown, that BoNT/A injections damages the microstructure of both the non-fibrillar and the fibrillar tissue and impairs the motor control of the gait in rats, and causes an increased collagen turnover in the muscle tissue.

In summary, the present results indicate that muscle contractures might be caused by multiple factors, and we therefore suggest that it is necessary to reconsider of how and why muscle contractures develop.

S11-5

The muscle clock regulates titin splicing and sarcomere length

Lance A. Riley, Xiping Zhang, Joseph R. Mijares, Karyn A. Esser,

University of Florida

Introduction: Disruption of the muscle clock leads to weakness with myofilament disruption. Titin's role as a sarcomeric scaffold and spring could potentially link myofilament disarray with muscle weakness.
Objective: We tested if titin isoforms change after loss of skeletal muscle Bmal1, a circadian factor. We also tested if loss of Bmal1 has an effect on sarcomere length.
Methods: iMSBmal1−/− and iMSBmal1+/+ mice were used. Titin isoforms were determined at the protein and RNA level. Sarcomere

lengths were measured with immunohistochemistry using an α-actinin antibody to demarcate Z-lines.
Results: The ratio of short to long isoforms of titin protein changed in the tibialis anterior muscle of iMSBmal1−/− mice. RNASeq data indicated this shift is due to inclusion of exons 51-89 within titin's spring region. While average sarcomere length was not different, sarcomere length variability increased after knockout. Ongoing studies are testing if altering titin splicing can change sarcomere length.
Conclusion: Our results suggest that titin plays a role in regulating sarcomere length homogeneity downstream of the molecular clock.

S12-6

Denervation-related muscle atrophy is mitigated by photobiomodulation with no changes in autophagy

Jéssica Silva Ferreira, Maria Julia Marques, Humberto Santo Neto

Institute of Biology, Dept. of Structural and Functional Biology, University of Campinas (UNICAMP), 13083 970, Campinas, SP, Brazil

Background: Photobiomodulation (PBM) mitigates muscle atrophy induced by microgravity and ischemia-reperfusion but whether it affects denervation-related atrophy is unknown. Autophagy plays a central role in muscle atrophy and is affected by PBM in non-muscle tissue.
Objective: To examine the effects of PBM on denervation-muscle atrophy and autophagy.
Methods: Right tibialis anterior muscles were denervated by sciatic nerve section, in adult mice. Denervated right tibialis anterior muscles were treated with PBM during 5 or 14 days. Some denervated muscles were not treated. Controls were not denervated and did not receive PBM. Atrophy of the right tibialis anterior muscle was estimated by muscle fiber cross sectional area (CSA). Autophagy was evaluated by immunofluorescence, western blot and electron microscopy.
Results: CSA was higher ($p < 0.001$) in PBM than in untreated group at day 5 (1661.6 ± 139.6 vs 1094.8 ± 46.8 μm^2) and day 14 (1067.1 ± 46.8 vs 827.2 ± 63.5 μm^2). No differences were found between PBM and untreated in autophagy markers: (i) anti LC3 positive points, (ii) LC3II/LC3I ratio and (iii) autophagic figures at day 5 and 14.
Conclusion: Denervation-related muscle atrophy was mitigated by PBM without affecting autophagy. PBM emerges as a potential therapy to mitigate denervation-induced muscle atrophy in the clinical arena.
Supported by: FAPESP, CNPq, CAPES, Faepex-Unicamp

Poster Session 1: Skeletal Muscle Mechanics

P1-1

Hydrophobic surface unraveling in force-induced titin-domain unfolding

György G. Ferenczy, Miklós Kellermayer

Department of Biophysics and Radiation Biology, Semmelweis University

We performed constant velocity steered molecular dynamics simulations for various titin domains and in addition to the force-extension function, the number of optimal geometry hydrogen-bonds and the magnitude of apolar surface as a function of extension were also recorded and analyzed. Approximate energies and forces were assigned to both hydrogen-bond rupture and apolar surface unraveling. We found that both hydrogen bond rupture and apolar surface unraveling culminates near the force peaks. They both contribute to the force and to the energy barrier of unfolding and their relative importance depends on the secondary structure elements involved. Results suggest that the mechanical stability of beta proteins is partially due to the increased propensity of hydrophobic residues in beta-strands and to their resistance to exposure upon unfolding. These observations add new insight to surface unraveling in force-induced protein unfolding, a phenomenon known to have significance in physiological processes by exposing cryptic sites and influencing interactome networks.

P1-2

Monitoring sarcomeric titin unfolding by ANS-binding and two-photon microscopy

Kolos Turtóczki, Katalin Kispetik, Zsolt Mártonfalvi and Miklós Kellermayer

Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

Titin is responsible for muscle elasticity. In single-molecule experiments titin's domains unfold, some already at physiological forces. We aimed at detecting sarcomeric titin domain unfolding via the binding of anilino-naphthalenesulfonate (ANS), a dye that binds to hydrophobic protein regions. Conceivably, if titin domains become unfolded during stretch, the opening of the hydrophobic cores could be detected by an increased ANS binding.

Rabbit psoas muscle fibers were manipulated with a mechanics setup attached to a multi-photon microscope. The fibers were immersed in relaxing solution containing 20 μM ANS and stretched across a sarcomere length range of 2.5–4.5 μm. ANS was excited by multi-photon mechanism at 800 nm (effective excitation wavelength 400 nm). ANS fluorescence was uneven along the sarcomere, with greater intensities in the I-band which increased further upon stretching the fiber. Thus, upon sarcomere stretch there is an increment in the hydrophobic protein surfaces in the I-band, likely due to unfolding of titin's globular domains. Monitoring ANS fluorescence allows the exploration of the mechanics-dependent in situ conformational state of sarcomeric proteins.

P1-3

Skeletal muscle in renal insufficiency: is calcium sensitivity affected?

Georgia I. Mitrou[1], Giorgos K. Sakkas[1,2], Konstantina P. Poulianiti[1], Yiannis Koutedakis[1], Konstantinos Tepetes[3], Grigorios Christodoulidis[3], Michael A. Geeves[4], Giannis Giakas[1,5], Ioannis Stefanidis[3], Christina Karatzaferi[1,2]

[1]University of Thessaly, School of PE and Sport Science, Muscle Physiology & Mechanics, Trikala, Greece; [2]Plymouth Marjon University, Faculty of Sport, Health & Wellbeing, EMIP Cluster, Plymouth, United Kingdom; [3]University of Thessaly, Faculty of Medicine, Larissa, Greece; [4]University of Kent, School of Biosciences, Canterbury, United Kingdom; [5]Biomechanical Solutions, Karditsa, Greece

Introduction: Chronic renal insufficiency patients present with functional abnormalities of unknown etiology collectively described as uremic myopathy. Objective: We investigated possible differences in calcium sensitivity of uremic (UREM) and control (CON) muscle.

Method: We used psoas muscle from an approved rabbit model of UREM and sham-operated CON. Isometric tension (P0) was assessed in 128 CON and 195 UREM skinned fibers at 10 °C, 30 °C and at pH 7 and pH 6.2, in various $CaCl_2$ concentrations. Force data expressed as percentage of P0 at standard conditions (10 °C, pH 7) and free calcium expressed in pCa values, were fitted in the Hill equation ($p < 0.05$).
Results: At 10 °C pH 7, UREM and CON fibers presented with similar calcium sensitivity (pCa 50 UREM 6.12 ± 0.02 vs CON 6.20 ± 0.03) and cooperativity (nH UREM 2.11 ± 0.14 vs CON 2.36 ± 0.3). Acidosis (pH 6.2) at 10 °C caused a loss of calcium sensitivity, more so for UREM fibers (pCa50 UREM 5.32 ± 0.06 vs CON 5.58 ± 0.02). At 30 °C pH 7, UREM fibers showed lower sensitivity than CON (pCa 50 UREM 6.00 ± 0.25 vs CON 6.42 ± 0.19). At 30 °C pH 6.2 calcium sensitivity was similar for both groups (pCa50 UREM 5.71 ± 0.13 vs CON 5.80 ± 0.05). Changes in cooperativity followed a similar pattern.
Conclusion: In uremic muscle, calcium sensitivity may be depressed, even in resting conditions.
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P1-4

Nitrosative stress generates an impaired function of myosin to form force-generating cross-bridges

Malin Persson[1], Maarten M. Steinz[1], Håkan Westerblad[1], Johanna T. Lanner[2], Dilson E. Rassier[3]

[1]Karolinska Institutet, Department of Physiology and Pharmacology, Stockholm, Sweden; [2]Karolinska Institutet, Department of Physiology and Pharmacology, Stockholm, Sweden; [3]McGill University, Department of Kinesiology and Physical Education, Montreal, Canada

Introduction: Oxidative and nitrosative stress in muscle cells have been associated with muscle weakness observed in many diseases. Although there is indirect evidence to suggest that oxidation affects skeletal muscle myosin, the detailed acute effects of nitrosative modifications on myosin-actin interactions are not known.
Objective: In this study we examined the effects of peroxynitrite (ONOO)-derived nitrosative stress on the contractile properties of individual skeletal muscle myofibrils.
Method: Nitrosative stress were induced by adding the ONOO-generator SIN-1 or ONOO directly to myofibrils. To evaluate cross-bridge properties, forces and force development rates were measured by monitoring myofibril-induced displacements of an atomic force cantilever upon activation (pCa 4.5) and relaxation (pCa 9.0).
Results: The isometric force decreased by 50% in myofibrils treated with 10 mM SIN-1 while rates of force activation and redevelopment were unchanged. The rate of the slow linear phase of relaxation increased during nitrosative stress while the duration of this phase was not altered. Similar results were seen for myofibrils treated directly with ONOO.
Conclusion: Our results suggest that the decrease in force after acute nitrosative stress is linked to an impaired function of myosin to form force-generating cross-bridges and not from cross-bridge kinetics.

P1-5

Experimental testing and numerical modelling of passive behavior in muscle fibers and bundles

Lorenzo Marcucci, Piero Pavan, Giulia Randazzo, Michela Bondí, Arturo Natali, Carlo Reggiani

Padova University, Italy

Muscle forces can be divided into active, generated by the cyclical interaction of single myosin motors with the actin filaments, and passive, generated by intra-sarcomeric proteins like titin, and the extra-cellular matrix (ECM) which form the connective tissue from muscle fibers to muscle bundles and from muscle bundles to tendons. Historically, the characterization of active forces properties have received much more attention than the passive counterparts, both experimentally and theoretically, leading to an almost phenomenological approach in the definition of the constitutive properties of passive elements in the macroscopic finite elements models. In this work, we characterized these properties for a finite element model of a human bundle, for both the intra-sarcomeric protein and ECM. We compared the experimental data for single fiber under passive stretches to the bundle one, proposing an exponential characterization of the former and deducing the rigidity of the sole ECM by subtraction.

We observed the high rigidity of the ECM and obtained a quantitative characterization for it. This can be used for macroscopic models to study the importance of the ECM in the transmission of forces in physiological situations as well as in aging degradation or even pathologies like Duchenne muscular dystrophy.

P1-6

Titin's role in Skeletal Muscle Function; Sarcogenesis and Passive Tension

Ambjorn Brynell, Yaeren Hernandez, Maya Adler, Justin Kolb, Balazs Kiss, Jochen Gohlke, Henk Granzier

University of Arizona Department of Cellular and Molecular Medicine

Titin is a protein in striated muscle that provides passive tension in response to sarcomere stretch. How much titin-based passive tension contributes to skeletal muscle remains uncertain. This study focuses on titin's contribution to muscle passive tension at the sarcomere and whole muscle levels. A novel mouse model was created with a large portion of titin's PEVK-spring region (TtnΔex112-158) removed. This resulted in a mouse model with stiffer titin allowing us to study how altered titin-based passive tension affects overall muscle stiffness. Whole muscle tension was directly measured using skinned and intact muscle mechanics by comparing diaphragm, soleus and EDL muscles of TtnΔex112-158 to wild type mice. Mechanical results showed that titin provides most of the passive stiffness within the physiological sarcomere length range of both genotypes. Furthermore, all studied muscle types showed a 30–40% increase of sarcomeres in series in TtnΔ112-158 mice. This suggests that titin-based tension is a driver of sarcomerogenesis. Our study demonstrates that titin is the main contributor to muscle stiffness and provides evidence that titin is driver for sarcomerogenesis.

P1-7

Contractile function of vastus intermedius fibres from young rats on a high-fat, high-sucrose diet

Ian C. Smith, Graham Z. MacDonald, Curtis A. Ostertag, Walter Herzog

University of Calgary, Faculty of Kinesiology, Calgary, Canada

Introduction/background: How diet-induced obesity affects skeletal muscle contractile function is not well established.
Objective/purpose: We examined how a high-fat, high-sucrose (HFS) diet affects contractile properties of skinned rat vastus intermedius fibres.

Methods: Male rats aged 3 weeks began either a chow diet or a HFS diet. After 14 weeks on the diets, body composition was assessed with dual-energy X-ray absorptiometry. Muscles were then harvested, and contractile properties of skinned fibres were assessed at 19 °C.
Results: Fat comprised 27 ± 4% and 14 ± 2% of body mass in HFS and chow diet group animals, respectively. Maximum force, peak shortening rate, resting stiffness, and the ratio of force to stiffness due to cross-bridges did not differ between diet groups. However, the calcium-sensitivity of force production was higher in fibres of HFS-fed rats than fibres of chow-fed rats. This effect was more pronounced in type I than type IIa fibres.
Conclusion: The HSF diet used in this study caused a 2-fold increase in body fat, but aside from increasing the calcium-sensitivity of cross-bridge formation, contractile properties of skinned vastus intermedius fibres were not affected.

P1-8

Equatorial and meridional x-ray reflections after active stretch and shortening in skeletal muscle

Venus Joumaa, Ian C. Smith, Atsuki Fukutani, Timothy R. Leonard, Weikang Ma, Thomas C. Irving, Walter Herzog

University of Calgary, Human Performance Laboratory, Calgary, Canada; Illinois Institute of Technology, The Biophysics Collaborative Access Team (BioCAT), Chicago, USA

Background: The steady-state force achieved after a skeletal muscle is actively stretched or shortened is always greater or smaller than a pure isometric force obtained at the same final muscle length. The precise mechanism of these properties, termed residual force enhancement (RFE) and force depression (FD), is not well understood.
Objective: Our aim was to gain insight into the possible mechanism(s) of RFE and FD by examining the structural changes in the sarcomere after active stretch and shortening, using small angle x-ray diffraction.
Methods: We examined stiffness and the equatorial 1.0 and 1.1 and meridional M3 and M6 X-ray reflections for steady-state conditions after pure isometric and active stretch and shortening contractions in skinned rabbit psoas bundles.
Results and Conclusions: Active stretch did not affect stiffness or I1.1/I1.0 but it increased M3 and M6 spacings and decreased M3 peak intensity, compared to pure isometric contractions. Active shortening reduced stiffness, I1.1/I1.0, M3 and M6 spacings and M3 peak intensity. This suggests that the proportion of attached cross-bridges seems unaffected after stretch but decreases after shortening. Moreover, RFE and FD are likely accompanied by an increase in cross-bridge disorder and a change in their conformation.

P1-9

Effects of S-glutathionylation on passive force in human and rat skeletal muscle fibres

Daiki Watanabe[1], Cedric R. Lamboley[2], Graham D. Lamb[3]

[1]Department of Engineering Science, The University of Electro-Communications, Tokyo, Japan; [2]School of Biomedical Sciences, The University of Queensland, Brisbane, Australia; [3]Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, Australia

The stiffness of cardiac muscle is markedly decreased by *S*-glutathionylation of titin, which involves the formation of a mixed disulfide between a protein sulfhydryl residue and glutathione (Alegre-Cebollada et al. 2014). However, it is unknown if this also occurs in skeletal muscle fibres. Here, we investigated the effects of *S*-glutathionylation on passive force in mechanically-skinned fibres from freshly obtained muscle from rat and human, setting sarcomere length (SL) by laser diffraction. Fibres were stretched to produce ~ 20% of maximal Ca^{2+}-activated force and treated with 20 mM glutathione disulfide (GSSG) for 15 min, which resulted in a significant decrease in passive force across all SL in both type I and type II fibre of rat and human (e.g., the passive force at ~ 20% of maximal Ca^{2+}-activated force was reduced by 25 ± 4%, 12 ± 4%, 15 ± 4% and 14 ± 4% in rat type II, rat type I, human type II and human type I, respectively); this decrease was fully reversed by subsequent treatment with dithiothreitol (DTT; 10 mM for 10 min). If freshly skinned fibres were instead initially treated with DTT, there was a small increase in the passive force in type II fibres (e.g., the passive force at ~ 20% of maximal Ca^{2+}-activated force was increased by 10 ± 3% and 9 ± 2% in rat and human, respectively), but not in type I fibres. Interestingly, the passive length-force relationship was significantly different between rat and human fibres: human fibres had to be stretched to a longer SL before they started to produce force and passive force then increased with a less steep length-force relationship than in rat fibres. These results suggest that (1) S glutathionylation of titin does cause a decrease in passive force in skeletal muscle fibres, but the reduction is relatively smaller than that in cardiac muscle, and (2) there appears to be some level of reversible oxidative modification, probably involving *S*-glutathionylation of titin, in type II fibre, but not in type I fibre, in rested muscle in situ.

Poster Session 2: Muscle Cytoskeleton

P2-1

Structural insight into the myotilin-actin interaction

Vid Puž[1], Sibylle Molt[2], Friedel Drepper[3], Thomas Schwarz[4], Miha Pavšič[1], Brigita Lenarčič[1], Peter F. M. van der Ven[2], Bettina Warscheid[3], Robert Konrat[4], Dieter O. Fürst[2], Kristina Djinović-Carugo[4]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia; [2]Institute for Cell Biology, University of Bonn, Germany; [3]Faculty of Biology and BIOSS Centre for Biological Signalling Studies, University of Freiburg, Germany; [4]Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Austria

Z-discs are intricate webs of various proteins including α-actinin, actin and myotilin. Myotilin consists of two Ig-like domains (Ig1, Ig2) flanked by disordered N- and C-terminal tails and interacts with actin, α-actinin, ZASP, FATZ and filamin C. Here we investigated myotilin's actin-binding properties. First, we determined binding affinities for different myotilin fragments with actin, and found that Ig2 represents the main point of interaction, while Ig1 and regions flanking both Ig domains play supplementary roles. NMR and XL-MS experiments were performed as well, allowing us to further map the binding sites of actin on myotilin. Subsequent mutagenesis of single residues at one or more of these binding sites diminished binding to actin in a dose-dependent manner. In vivo FRAP experiments using C2C12 cells showed increased dynamics of mutant myotilins in Z-discs. Based on our data, we constructed an integrative model of myotilin-actin complex. Using our model and experimental data we showed that myotilin modulates binding of tropomyosin to actin, which in concert with α-actinin could explain absence of tropomyosin within Z-discs.

P2-2

Subcellular spatial control of non-muscle myosin 2 redistribution and stress fiber strain by Molecular Tattoo

Adam Istvan Horvath, Mate Gyimesi, Boglarka H. Varkuti, Miklos Kepiro, Gabor Szegvari, Istvan Lorincz, Gyorgy Hegyi, Mihaly Kovacs, Andras Malnasi Csizmadia

Eotvos Lorand University

The subcellular distribution of the motor protein non-muscle myosin 2 (NM2) leads to different forms of intracellular strain driving cell motility, cytokinesis and axonal growth. It remains elusive how these cellular processes are governed by the dynamic changes in NM2 localization. We determined the effect of NM2 inhibition on the dynamics of stress fibers and unloaded cytoplasmic NM2 structures in HeLa cells. We followed NM2 diffusion via FRAP, applied also in combination with Molecular Tattoo, which allows subcellular confinement of drug effects. The inhibition of NM2 by para-nitroblebbistatin or locally by tattooed azidoblebbistatin, in the stress fibers a significant acceleration and suppression of NM2 diffusion was detected at moderate and high inhibitor concentrations, respectively. The observed effects were local and specific for load-bearing peripheral stress fibers, implying the role of mechanical load in NM2 redistribution. These results highlight that variations in the localization and pharmacological mechanism of NM2 inhibition can produce distinct effects on intracellular strain and morphogenesis.

P2-3

Possible functional role of titin amyloid aggregation

Elmira Yakupova[1], Sergey Shumeyko[1], Liya Bobyleva[1], Ivan Vikhlyantsev[1,2], Alexander Bobylev[1,2]

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia; [2]Pushchino State Institute of Natural Sciences, Pushchino, Moscow Region 142290, Russia

Titin is a giant elastic muscle protein. In sarcomeres of cardiac and skeletal muscles, the amount of titin is third beside the number of actin and myosin filaments. Molecular simulations carried out in 2015 showed that 2 titin domains I27–I28/I27–I27 were able to form amyloid-like aggregates, called "intramolecular amyloids". Amyloids are protein aggregates with a cross-β-structures. The high ability of the formation of amyloid aggregates by full-length molecules of smooth muscle titin was demonstrated in our recently studies. Unlike most proteins forming amyloids under extreme conditions in vitro (at 37–100 °C, acidic pH for 24 h or longer), titin formed amyloid aggregates for 20–30 min in solutions with physiological ionic strength at a temperature of 4–24 °C (pH 7.0–7.4).

It is unknown whether the above mentioned aggregation of titin can occur in vivo. But if it is so, titin molecules forming the intracellular cytoskeletal extensible carcass would determines mechanical properties of muscle tissue. Perhaps, the aggregation of titin molecules in the sarcomere I-band may play a functional role—to contribute to increasing muscle stiffness. At the same time, this aggregation may play a protective role counteracting overextension of sarcomeres with unfavorable consequences for the muscle.

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P2-4

Elucidating the role of vinculin and its splice isoform metavinculin in cells and mice

Verena Kanoldt[1], Carleen Kluger[2], Christiane Barz[1], Anna Chrostek-Grashoff[1], Stefan Engelhardt[3], Carsten Grashoff[4]

[1]Max Planck Institute of Biochemistry; [2]Lehrstuhl für Angewandte Physik and Center for NanoScience (CeNS), Ludwig-Maximilians-Universität München, Max Planck Institute of Biochemistry; [3]Institute of Pharmacology and Toxicology, Technical University of Munich; [4]Institute of Molecular Cellbiology, University Münster, Max Planck Institute of Biochemistry

Vinculin is an ubiquitously expressed cell adhesion molecule that modulates force propagation in cell-matrix and cell-cell adhesion complexes. Interestingly, muscle tissues express a vinculin splice-isoform, called metavinculin, and mutations or deletion of metavinculin have been associated with idiopathic dilated cardiomyopathy. The function of metavinculin, however, is still unclear. We therefore established a series of FRET-based biosensors to evaluate vinculin and metavinculin function in cells. Our live cell FLIM experiments revealed a difference in molecular tension across the two isoforms, which is also reflected in differential force propagation across their common binding partner talin. To test the physiological role of metavinculin, we have generated metavinculin-deficient mice, which are currently being analyzed in transverse aortic constriction models. Together, our work contributes to a better understanding of vinculin-dependent force transduction in muscle tissues but also reveals how cells tune their mechanosensitivity through the expression of distinct splice isoforms.

P2-5

Calcium dependent elasticity of native titin filaments

Zsolt Mártonfalvi, Miklós Kellermayer

Semmelweis University, Department of Biophysics and Radiation Biology

Titin, the sarcomeric giant protein, is one of the main determinants of muscle's elastic properties. While it mainly contributes to the development of passive tension upon muscle stretch, activation of the contractile apparatus may also have an impact on titin's mechanics. It has been suggested that sarcomeric calcium induces structural changes in titin by binding to glutamate rich motifs in its PEVK domain. To test how such calcium-responsive elements might alter the elastic properties of titin, we have manipulated individual full-length titin molecules in optical tweezers experiments using laminar-flow microfluidic system. The experimental setup allowed the efficient and rapid control of calcium concentrations during repetitive stretch-release cycles. When molecules were manipulated at pCa 3, titin's apparent persistence length became reduced. As a consequence, titin molecules contracted into a more compact conformation that resulted in the shortening of the polymer chain stretched by a given force. Our findings support that titin may act as a calcium sensitive, elastic parallel element of the sarcomere, that may contribute to sarcomeric force generation.

P2-6

The topology of interactions between titin and the thick filament

Miklós Kellermayer, Dominik Sziklai, Zsombor Papp, Brennan Decker, Eszter Lakatos and Zsolt Mártonfalvi

Department of Biophysics and Radiation Biology, Semmelweis University, Budapest H1094 Hungary

Titin associates strongly with the myosin thick filament in the A-band. It has been speculated that titin might provide a template that determines thick-filament length. We tested the titin ruler hypothesis by mixing titin and myosin at in situ stoichiometric ratios (300 myosins per 12 titins) in buffers of different ionic strength ([KCl] 100–300 mM). The topology of the complexes was investigated with atomic force microscopy. We found distinct, segregated populations of titin and thick filaments. We were unable to identify complexes in which myosin molecules were regularly associated to either mono- or oligomeric titin in mechanically relaxed or stretched states of titin. Thus, self-association is stronger in both myosin and titin than their binding to each other, and it is unlikely that titin functions as a geometrical template for thick-filament formation. However, when allowed to equilibrate configurationally, long myosin filaments appeared with titin oligomers attached to their surface. The titin meshwork on the thick-filament surface may control thick-filament length by regulating the structural dynamics of myosin molecules and placing a mechanical limit on its length.

P2-7

Myosin binding protein-C slow function, regulation, and disease implications

Janelle Geist[1], Janis Stavusus[2], Baiba Lace[2,3], Nathan Wright[4], Carsten Bonnenman[5], Chris Ward[1], Aikaterini Kontrogianni-Konstantopoulos[1]

[1]University of Maryland, Baltimore, USA; [2]Latvian Biomedical Research and Study Center, Latvia; [3]Centre Hospitalier Universitaire de Quebec, Canada; [4]James Madison University, USA; [5]NINDS, NIH, USA

Myosin Binding Protein-C (MyBP-C) comprises a family of proteins with structural and regulatory roles in striated muscle. The slow (s) skeletal isoform is understudied, yet has been linked to severe and lethal forms of distal arthrogryposis.

One goal of my project is to examine the roles of sMyBP-C in skeletal muscles. To do so, I used in vivo gene transfer and electroporation to deliver control or sMyBP-C-targeting CRISPR plasmids into different muscles. sMyBP-C knockdown resulted in significantly decreased levels of thick filament proteins, selectively disorganized A-bands, and reduced sarcomere length. Examination of contractile activity showed that knockdown muscles developed decreased twitch and tetanic force and decelerated velocity of contraction.

Another goal of my project is to study the effects of an autosomal dominant mutation, E248K, linked to a novel myopathy accompanied by tremor. In vitro work showed that the E248K mutation significantly increased binding to myosin. Our knock-in (KI) mouse model reveals that homozygous KI mice are neonatally lethal, while heterozygous KI mice are significantly smaller and develop severe tremor. Currently, I am characterizing the morphological and functional phenotype of the model.

My studies indicate that alteration of sMyBP-C expression or the presence of mutations is associated with muscle pathogenicity and disease development.

P2-8

Impact of O-GlcNAcylation changes on desmin behavior in differentiated myotubes

Charlotte Claeyssen, Bruno Bastide, Caroline Cieniewski-Bernard

University of Lille, URePSSS-EA7369 Physical Activity, Muscle, Health

O-GlcNAcylation is an atypical glycosylation akin to phosphorylation. Dynamic and reversible, the O-GlcNAcylation modifies a plethora of myofibrillar proteins. We have previously demonstrated that O-GlcNAcylation regulated sarcomeric cytoskeleton since the sarcomere morphometry is modified consecutively to O-GlcNAcylation changes, in correlation with modification of the O-GlcNAcylation level of myofibrillar proteins. Moreover, these structural changes partly involved desmin, a key protein of intermediate filaments in striated muscle, and its molecular chaperone, the alphaB-crystallin.

We focused herein on the effect of O-GlcNAcylation changes on the desmin behavior in differentiated myotubes. The modulation of O-GlcNAcylation level on myotubes led to changes of O-GlcNAcylation and phosphorylation levels of desmin, associated with a modulation of the interaction between desmin and its molecular chaperone alphaB-crystallin. Interestingly, the partition of desmin between soluble and insoluble protein materials is also modulated, while the desmin filaments are remodeled consecutively to O-GlcNAcylation changes. Altogether, our data support the key role of O-GlcNAcylation in the organization and reorganization of sarcomeric cytoskeleton.

Poster Session 3: Neuromuscular Signaling and Interaction

P3-1

Distribution of sympathetic innervation in skeletal muscles

Tatjana Straka[1], Kaltrina Prokshi[1], Sarah Janice Hörner[1], Marion Patrick Ivey Williams[1], Muzamil Majid Khan[1], Rüdiger Rudolf[1,2,3]

[1]Institute of Molecular and Cell Biology, University of Applied Sciences Mannheim; [2]Interdisciplinary Center for Neurosciences, Heidelberg University; [3]Institute of Toxicology and Genetics, KIT

Recent studies proposed a relevant functional interaction between sympathetic neurones and neuromuscular junctions (NMJs). Apart from investigations on the consequences of sympathetic innervation at the NMJ, a detailed description of the distribution of sympathetic innervation in skeletal muscles is required. Previous studies focused on staining of tyrosine hydroxylase, a sympathetic neurone marker in sections of hindlimb muscles of adult mice. However, ramifications of a neuronal network, like the sympathetic nervous system, is impossible to appreciate in 2D. Therefore, we set up tissue clearing and staining protocols to visualise sympathetic innervation in muscle whole mounts. In addition, we characterised the enrichment of tyrosine hydroxylase at the NMJ during the postnatal period, to address a potential role of sympathetic innervation for NMJ development. We found an elaborate sympathetic plexus which seems to be partially ready at birth and increases in complexity and interaction rate with NMJs during postnatal development. These findings are consistent with a role of the sympathetic nervous system in NMJ development and they are asking for further studies in that direction.

P3-2

Role of ceramide in lipid raft disturbance in short-term hindlimb suspension

Maria Shalagina[1], Alexei Sekunov[1], Alexei Petrov[2], Irina Bryndina[1]

[1]Izhevsk State Medial Academy, Department of Pathological Physiology and Immunology, Izhevsk, Russia; [2]Kazan State Medial

University, Department of Normal Physiology, Kazan, Russia Kazan State Medial University

Introduction: Atrophy and dysfunction of skeletal muscle developing during space flight are related to changes in cell signaling mechanisms. One of the signaling pathways is associated with sphingolipids, mainly ceramide (Cer). It is known that Cer can displace cholesterol (Chol) from a lipid raft and it causes the destabilization of its structure.
Purpose: To study the role of Cer in lipid raft disturbance caused by 12-h hindlimb suspension (HS) of soleus muscle. Method: We used Morey's tail-suspension model to simulate the microgravity effect in muscle. In some experiments rats were pretreated with the inhibitor of acid sphingomyelinase (aSMase), clomipramine. We studied the lipid profile of rat soleus using HPTLC. Chol was detected by colorimetric enzymatic method. By confocal microscopy, we visualized lipid rafts and nAChR after the staining with cholera toxin B subunit and rhodamine-conjugated α-bungarotoxin, respectively.
Results: The amount of Cer increased and sphingomyelin and Chol decreased in suspended soleus muscle. Unloading also led to the decrease in raft labeling, indicating the disturbance in their structure. Clomipramine abolished these effects.

Conclusion: aSMase inhibitor prevents Cer accumulation and lipid raft disruption in rat soleus muscle during 12 h of HS.

This work is partially supported by the Russian Scientific Foundation (grant no. 16-15-10220).

P3-3

Clomipramine prevents GLUT4 and NADPH oxidase alterations in rat soleus muscle during 4 days of hindlimb suspension

Vladimir Protopopov, Alexey Yakovlev, Alexey Sekunov

Izhevsk State Medical Academy

Currently, the important part in the development of skeletal muscle atrophy and dysfunction is given to sphingolipids including ceramide (Cer) which is known to induce insulin resistance and oxidative stress. The role of Cer in muscle dysfunction during microgravity is not entirely studied.

The aim of this work was to investigate the effect of the inhibitor of Cer formation, clomipramine on GLUT4 and NOX2 (a component of membrane bound NADPH-oxidase) in soleus muscle during its functional unloading.

The work was performed in rats subjected to hindlimb suspension (HS) during 4 days. Some of the animals were pretreated with the inhibitor of acid sphingomyelinase (aSMase), clomipramine.

Using immunohistochemistry, we found the decrease in GLUT4 and the increase in NOX2 immunofluorescence in the membrane region of muscle fibers. The parallel Cer and aSMase enhancement in muscle homogenates was detected. Clomipramine prevented these changes, restoring immunofluorescence of both GLUT4 and NOX2 to the levels comparable with the control animals.

Based on this study, we conclude that aSMase and Cer may be possibly involved in the development of insulin signaling abnormalities and oxidative stress in skeletal muscle during short-term HS.

The study was supported by RFBR (grant no 16-04-01370)

P3-7

Innervation of cultured human myotubes leads to isoform-specific upregulation of Na^{+}, K^{+}-ATPase subunits

Vid Jan[1], Katarina Miš[1], Matej Podbregar[1,2], Tomaž Marš[1], Alexander V. Chibalin[3], Sergej Pirkmajer[1]

[1]University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Ljubljana, Slovenia; [2]University Medical Centre Ljubljana, Coronary Care Unit, Ljubljana, Slovenia; [3]Karolinska Institutet, Integrative Physiology, Department of Molecular Medicine and Surgery, Stockholm, Sweden

Introduction: Na^{+}, K^{+}-ATPase (NKA), a heterodimer comprising an α (α1-3) and a β (β1-3) subunit, is fundamental to skeletal muscle ion homeostasis and contractility. NKA is regulated by small transmembrane proteins from the FXYD family (FXYD1-7). In skeletal muscle, the most prominent among these are FXYD1 (phospholemman) and FXYD5 (dysadherin).
Objective: We determined whether innervation by motor neurons alters the expression of NKA subunits, FXYD1 and FXYD5 in cultured human myotubes.
Methods: To establish innervation, human myotubes were co-cultured with explants of embryonic rat spinal cord.
Results: Once innervated by motor neurons, myotubes contracted spontaneously. Aneural myotubes were quiescent and did not contract. Using qPCR and species-specific primers, mRNA levels of the NKA α3 and β2 subunits, respectively, were 3-fold and more than 100-fold higher in innervated, contracting myotubes than in aneural myotubes. Expression of other NKA subunits, FXYD1 and FXYD5 was unaltered. In co-cultures which failed to display contractile activity expression of α3 and β2 subunits was not significantly increased, highlighting the role of contractions in regulation of NKA expression.
Conclusion: Innervation leads to isoform-specific changes in the expression of NKA subunits in human myotubes. These changes are likely induced by motor neuron-driven contractions of myotubes.

Poster Session 4: Cardiac Contractility and Failure

P4-2

Chronic stimulation of the NO/sGC/cGMP/PKG signalling pathway improves diastolic function in a rat model of HFpEF

Árpád Kovács[1], Andreas Gevaert[2], Arthur J. Leloup[2], Paul Fransen[2], Melissa Herwig[1], Detmar Kolijn[1], Gábor Á. Fülöp[3], Mária Lódi[3], Dániel Czuriga[3], Zoltán F. Kisvárday[4], Peter Sandner[5], Sophie van Linthout[6], Carsten Tschöpe[6], Zoltán Papp[3], Gilles W. De Keulenaer[2], Wolfgang A. Linke[7], Nazha Hamdani[1]

[1]Department of Cardiovascular Physiology, Ruhr University Bochum, Bochum, Germany; [2]Department of Pharmaceutical Sciences, Laboratory of Physiopharmacology, University of Antwerp, Antwerp; [3]Division of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [4]Laboratory for Cortical Systems Neuroscience, Department of Anatomy, Histology and Embryology, University of Debrecen, Debrecen; [5]Bayer AG, Drug Discovery Cardiology, Wuppertal, Germany; [6]Department of Internal Medicine and Cardiology, Charité-Universitätsmedizin Berlin, Centre for Regenerative Therapies and DZHK, partner site Berlin, Germany; [7]Institute of Physiology II, University Hospital Münster, University of Münster, Münster, Germany

Background: Here we investigated the role of enhancing the NO/sGC/cGMP/PKG signalling pathway in the modulation of diastolic function in a model of heart failure (HF) with preserved ejection fraction (pEF).
Purpose: To study the effect of sGC stimulation on left ventricular (LV) diastolic dysfunction, and its action on myocardial stiffness and oxidative stress.
Methods: Chronic (4 weeks) stimulation of sGC (with BAY 41-8543) was studied on 15-week-old male Dahl/SS (HFpEF) and SS-13 (CTRL) rats (n = 8–12/group).

Results: LV diastolic dysfunction (E/A; IVRT; Tau), high LV end-diastolic pressure and stiffness parameters were improved in HFpEF compared CTRL upon sGC stimulation. Impaired arterial elastance, arterial stiffening and endothelial dysfunction in HFpEF were corrected upon sGC stimulation. Immunohistochemistry showed increased expression level of cardiac sGC after stimulation. Cardiac fibrosis/collagen gene expression and high oxidative stress/inflammation were reduced upon treatment, which in turn corrected the low NO level, [cGMP] and PKG activity observed in HFpEF. PKG-mediated hypophosphorylation of titin in HFpEF was greatly improved upon sGC stimulation. Accordingly, increased cardiomyocyte stiffness was reduced upon sGC stimulation in HFpEF.
Conclusion: Our data suggest that chronic stimulation of sGC may be a promising treatment option for HFpEF patients.

P4-3

Design of muscle contraction assist devices by liquid crystalline elastomers

Cecilia Ferrantini[#1,2], José Manuel Pioner[#1], Daniele Martella[2,3,4], Raffaele Coppini[5], Nicoletta Piroddi[1], Francesco Saverio Pavone[2,4,6], Diederik Sybolt Wiersma[2,4,6,7], Chiara Tesi[1], Elisabetta Cerbai[5], Corrado Poggesi[1,2], Leonardo Sacconi[2,4], Camilla Parmeggiani[2,3,4]

[1]Department of Experimental and Clinical Medicine, University of Florence, Italy; [2]European Laboratory for Non-linear Spectroscopy, Sesto Fiorentino, Italy; [3]Department of Chemistry "Ugo Schiff", University of Florence, Italy; [4]National Institute of Optics, National Research Council, Sesto Fiorentino, Italy; [5]Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Italy; [6]Department of Physics and Astronomy, University of Florence, Sesto Fiorentino, Italy; [7]Istituto Nazionale di Ricerca Metrologica INRiM, Strada delle Cacce, 91 10134 Torino, Italy. e-mail: cecilia.ferrantini@unifi.it; camilla.parmeggiani@lens.unifi.it

[#]Equally contributed

Aims: Loss of muscle contractility occurs in different, life-threatening diseases. Current treatments suggest the need for a new generation of contraction assist devices. Liquid Crystalline Elastomers (LCEs) can work as "artificial muscle", with particular focus on cardiac muscles.
Methods and Results: LCEs are biocompatible materials able to deform reversibly in response to given stimuli. Thin (20-μm) LCEs films were prepared and their light-response and mechanical properties measured from small strips (200–400 μm diameter, 3–4 mm length) isometrically mounted between a force transducer and a linear actuator. LCE film samples maximally activated and relaxed by a green light (200 mW/mm^2), showed a mechanical behavior similar to force responses of isolated human cardiac myofibrils. The nature of material composition and the stimulus intensity modulated mechanical and kinetic parameters.
Conclusions: LCEs are suitable to mimic cardiac muscles. We prepared light-responsive LCEs films, highlighting how different molecular parameters affect different aspects of mechanical functions. Our results open for a new generation of LCE-based contraction assist devices.

P4-4

Crucial role of protein kinase G in regulating Ca2(+)/calmodulin-dependent protein kinase-II phosphorylation and oxidation and thereby diastolic function

Melissa Herwig[1], Árpád Kovács[1], Detmar Kolijn[1], Sophie van Linthout[2,3], Carsten Tschöpe[2,3], Nazha Hamdani[1]

[1]Department of Systems Physiology, Ruhr University Bochum, Bochum, Germany; [2]Department of Internal Medicine and Cardiology, Charité-Universitätsmedizin Berlin; [3]Centre for Regenerative Therapies and DZHK, partner site Berlin, Germany

Rationale: Myocardial diastolic stiffness depends in part on signaling pathways and phosphorylation. Ca($^{2+}$)/calmodulin-dependent protein kinase-II (CaMKII) δ and protein kinase G (PKG) are known to target titin, but it is unknown if PKG phosphorylates CaMKIIδ.
Methods and results: CaMKIIδ phosphorylation by PKG was assessed in recombinant proteins and heart failure (HF) biopsies by autoradiography, immunoblotting and quantified in vivo by mass spectrometry (MS). Unchanged CaMKIIδ phosphorylation and increased oxidation was observed in HF biopsies. PKG-dependent phosphosites were identified within the CaMKIIδ by quantitative MS and confirmed in recombinant human CaMKIIδ. The most highly phosphorylated sites are located in the regulatory domain and the linker region. Acute intravenous injection of PKG stimulator in anaesthetized HF rats significantly improved diastolic function via increased PKG activity, reduced CaMKIIδ auto-phosphorylation and oxidation, and reduced oxidative stress and inflammation.
Conclusion: Our study shows that PKG plays a central role in regulating and maintaining the balance of CaMKIIδ activity and oxidative stress and thereby improving diastolic function.

P4-5

HCM mutation cardiac troponin C A8 V alters cardiomyocyte nucleus structure in a knock in mouse model

Carson L. Tougas[1], Adriano S. Martins[2], Kathryn M. Crotty[1], Katherine M. Ward[1], Jose R. Pinto[2], P. Bryant Chase[1]

[1]Florida State University, Department of Biological Science, Tallahassee, FL, USA; [2]Florida State University College of Medicine, Department of Biomedical Sciences, Tallahassee, FL, USA

Myopathy-associated mutations in myofilament proteins are most commonly characterized by their effects on Ca^{2+}-sensitivity of muscle function, but also affect other aspects of myocyte structure and function. Considering that thin filament proteins are found not only in the sarcomeres but also in the myocyte nucleus (Asumda and Chase, 2012, Differentiation), we hypothesized that HCM mutations in troponin could alter nuclear structure. To test this possibility, we used a mouse model with Ca^{2+}-sensitizing mutation cTnC A8V (Martins et al., 2015, Circ Cardiovasc Gene) that has been associated in humans with HCM (Landstrom et al., 2008, J Mol Cell Cardiol). We first examined cardiomyocyte nuclei in H&E stained, fixed sections from 18 mo old mice; nucleus area in A8 V heterozygotes was ~ 66% of WT. We next used confocal microscopy to examine nuclei in living cardiomyocytes, isolated from 3 mo old mice and stained with NucBlue and Fluo-5N AM; nucleus area in A8V homozygotes was ~ 66% of WT, and nucleus volume in A8V homozygotes was ~ 50% of WT. Analysis of myocyte contraction suggests that nuclei can resist longitudinal compression, but only up to a point after which they are compressed by sarcomere contraction. Conclusion: an HCM mutation in cTnC affects structure of nuclei in both homo- and heterozygous cardiomyocytes.

P4-7

Cardiomyocytes derived from induced pluripotent stem cells of patient with DiGeorge syndrome show altered beating frequency and irregularity

Tünde Berecz[1,2], Dr. Andrea Molnár[2], Brigitta Szabó[1,2], Dr. Irén Haltrich[3], Dr. László Homolya[1], Dr. Sian Harding[4], Dr. János

Réthelyi[5], Dr. Béla Merkely[2], Dr. Gábor Földes[2,4], Dr. Ágota Apáti[1]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary; [2]Heart and Vascular Center, Semmelweis University, Budapest, Hungary; [3]2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary; [4]National Heart and Lung Institute, Imperial College London, United Kingdom; [5]Department of Psychiatry and Psychotherapy, Semmelweis University, Budapest, Hungary

For in vitro modelling of DiGeorge syndrome (22q11.2 microdeletion syndrome) we generated human induced pluripotent stem cells (hiPSCs) from peripheral blood of members of a family where the disease is present in three generations. Grandfather and mother have milder symptoms (minimal facial dysmorphia, hypocalcaemia) and progeny had severe symptoms (pulmonary atresia, ventricular and atrial septal defect, hypoparathyroidism). hiPSCs were differentiated into cardiomyocytes to compare disease-affected and control cells. Beating started between day 7 and 12 of differentiation. Metabolic selection was performed between days 12 and 16 resulting in pure cardiomyocyte culture. Cells were characterised by expression of cardiac progenitor (Nkx2.5), cardiac (TNNT2, TNNI3) and cell type specific (MYL2, MYL7, HCN4) markers. For functional analysis our results show increased frequency and higher beating rhythm irregularity index in case of progeny compared to umbilical cord blood-derived healthy hiPSC line XCL1. We are planning contractility assays, electrophysiological measurements and analysis of calcium transients for further functional characterisation.

P4-8

Myofilament Ca^{2+} sensitivity correlates with alterations in cardiac contractility during the progression of pressure overload-induced left ventricular myocardial hypertrophy

Mihály Ruppert[1], Beáta Bódi[2], Sevil Korkmaz-Icöz[3], Sivakkanan Loganathan[3], Attila Oláh[1], Bálint András Barta[1], Béla Merkely[1], Matthias Karck[3], Zoltán Papp[2], Gábor Szabó[3], Tamás Radovits[1]

[1]Heart and Vascular Center, Semmelweis University; [2]Division of Clinical Physiology, University of Debrecen; [3]Department of Cardiac Surgery, University of Heidelberg

We aimed at investigating the dynamic alterations in left ventricular (LV) contractility as well as in sarcomere function during the progression of pressure overload (PO)-induced LV myocardial hypertrophy (LVH).

PO was evoked by abdominal aortic banding in rats for 6, 12 or 18 weeks. Age-matched, sham operated animals served as controls. The temporal development of LVH was detected by serial echocardiography. At the end of the experimental period (6, 12 or 18 weeks, respectively) LV pressure-volume analysis and force measurement in permeabilized LV cardiomyocytes were performed.

At week 6, PO-induced LVH was characterized by preserved LV ejection fraction, increased LV contractility and increased sarcomeric Ca^{2+} sensitivity. In contrast, in the AB groups at week 12 and week 18, LV ejection fraction decreased, while augmentation in LV contractility and Ca^{2+} sensitivity regressed back to the control's level. Alterations in LV contractility and sarcomeric Ca^{2+} sensitivity showed strong correlations among the study groups.

Alterations in sarcomeric Ca^{2+} sensitivity may contribute to the dynamic alterations in LV contractility during the progression of PO-induced LVH.

P4-10

Effects of phosphorylation of myosin regulatory light chain on the actin-myosin interaction in ventricle and atria

Daniil V. Shchepkin[1], Galina V. Kopylova[1], Valentina Y. Oshchepkova[1,2], Larisa V. Nikitina[1], Salavat R. Nabiev[1], Sergey Y. Bershisky[1]

[1]Institute of Immunology and Physiology, the Russian Academy of Sciences, Yekaterinburg, Russia; [2]Ural Federal University, Yekaterinburg, Russia

Myosin regulatory light chain (RLC) in the human myocardium is phosphorylated by $\sim$ 30 to 40% that is required for physiological cardiac performance. In heart failure, RLC phosphorylation is decreased by 30–40% and associated with a fall of cardiac contractile activity [Warren et al., 2012]. We compared the effect of RLC phosphorylation on the actin-myosin interaction in atria and ventricles using an in vitro motility assay and an optical trap.

We analyzed calcium dependence of the sliding velocity of thin filaments containing F-actin, troponin, and tropomyosin over pig atrial and ventricular myosin. Using NEM-modified myosin, we assessed the effect of RLC phosphorylation on force generation of myosin at pCa 4. In the optical trap, we measured a step size and duration of the actin-myosin interaction.

We found that RLC phosphorylation prolongs the myosin interaction with the thin filament at pCa 4, slightly decreases the maximal sliding velocity of the filament but does not affect its calcium sensitivity. The phosphorylation increased the force generated by ventricular myosin but did not affect the force of atrial myosin. This agrees with the results of Morano et al. [1990] who did not detect an effect of RLC phosphorylation on the tension of human atrial fibers. Thus, RLC phosphorylation differently affects the actin-myosin interaction in atria and ventricles. Supported by RSF grant 16-14-10044.

P4-11

Role of human cardiac RLC in modulating the super-relaxed state of myosin: A cardiomyopathy perspective.

Na Sa*, Ivan Tomasic*, Sampath Gollapudi, Ferdinand Evangelista, Kristina Green, Suman Nag

Dept. of Biology, MyoKardia, Inc; South San Francisco, CA

Myosin regulatory light chain (RLC) is a major regulatory subunit of the myosin molecule, the role of which has been well-characterized in non-striated muscle. However, in striated muscle, its purpose is less well defined, and it is only in the past decade that researchers have started unraveling the function of RLC and its phosphorylation in muscle contraction. Mutations in this protein result in $\sim$ 2% of total familial cardiomyopathy, an autosomal dominant disorder, manifested by ventricular and septal hypertrophy and myofibrillar disarray that can lead to sudden cardiac death. In this study, we report the role of human cardiac RLC in forming the super-relaxed state (SRX) of myosin in reconstituted full-length cardiac myosin thick filaments. We show that the presence of RLC fine-tunes the ability of myosin to form the SRX state and that its removal depopulates the SRX state. A similar reduction in SRX population is also achieved by phosphorylating the RLC with MLCK. The second mechanism of RLC-mediated regulation in muscle is thought to be by the binding of either Mg^{2+} or Ca^{2+} to the N-terminal EF-hand domain. Mg^{2+} did not affect myosin SRX population, but increasing Ca^{2+} enhanced the population of the myosin SRX state. Preliminary SRX studies on cardiomyopathy-causing RLC mutants R58Q (HCM), K104E (HCM) and D94A (DCM) show that none of the mutants affects the SRX

population in the dephosphorylated state of the RLC; however, these mutants also do not affect the SRX population when RLC is phosphorylated, unlike the wild-type. Additionally, in micro-scale thermophoresis binding experiments, only the dephosphorylated form of the DCM-causing D94A RLC mutant exhibits weaker binding to the myosin lever arm as compared to WT RLC. Binding of the mutants resembles WT upon phosphorylation. Altogether, these observations demonstrate that either RLC phosphorylation or Ca^{2+} binding to RLC can alter the number of accessible myosin heads for contraction and cardiomyopathy-causing RLC mutants can modify this mechanism to varying extents.

P4-12

Contractility of ventricular myofibrils from patients with dilated cardiomyopathy associated mutations

Petr G. Vikhorev[1], Waichun Yeung[1], Amy Li[2], Sean Lal[2], Cristobal G. dos Remedios[2], Cheavar A. Blair[3], Maya Guglin[3], Kenneth S. Campbell[3], Steven B. Marston[1]

[1]National Heart and Lung Institute, Imperial College London, London, W12 0NN, United Kingdom; [2]Discipline of Anatomy and Histology, Bosch Institute, University of Sydney, Sydney, NSW 2006, Australia; [3]Division of Cardiovascular Medicine, Department of Physiology, University of Kentucky, Lexington, Kentucky, USA

DCM mutations in cardiac troponin and myosin heavy chain slow down myofibril relaxation while myofibrils with TTN truncating mutations do not change contractility. Passive stiffness of myofibrils from all DCM samples decreased by 38%. We isolated myofibrils from donor and DCM hearts, changed phosphorylation levels of TnI and measured myofilament Ca^{2+}-sensitivity of force and the length dependence of Ca^{2+}-sensitivity. While myofibrils with mutation in Tn were contracting at lower Ca^{2+} concentrations, we showed that Ca^{2+}-sensitivities of myofibrils carrying truncated TTN mutations were the same as in myofibrils from donor heart. The modulation of the Ca^{2+}-sensitivity by TnI phosphorylation was unaffected in patients with TTN truncating mutations. The EC50 ratios of phosphorylated to unphosphorylated myofibrils were 2.1 and 2.4–2.6 for donor and patient hearts, respectively. To estimate the impact of mutations on myofibrils dynamics and cardiac output we further used a simulation of cardiac dynamic by cyclic changes in myofibril length and Ca^{2+} concentration (Supported by BHF, PG/17/5/32705).

P4-13

Structural and functional changes in HFpEF patients primarily associated with women and inflammation

Detmar Kolijn[1], Árpád Kovács[1], Melissa Herwig[1], Yi Lyu[1], Sophie van Linthout[2], Carsten Tschöpe[2], Nazha Hamdani[1]

[1]Department of Systems Physiology, Ruhr University Bochum, Bochum, Germany; [2]Charité-Universitätsmedizin Berlin, Centre for Regenerative Therapies and DZHK, partner site Berlin, Germany

Background: Heart Failure with preserved Ejection Fraction (pEF) is poorly understood and predominantly present in women. Inflammation and oxidative stress levels may differ between genders resulting in distinct signalling alteration.
Objective: Here we studied how oxidative stress/inflammation affects the pEF pathophysiology by modulation of LV stiffness in distinct manners-based gender.
Methods: We subdivided patients to four groups; men/women, with more (pEF+) or less (pEF−) inflammation.
Results: pEF+ women showed higher cardiomyocyte Fpassive, which was accompanied by lowest sGC, PKG activity, and global Titin hypophosphorylation compared to pEF− women and pEF+/− men. Myofilament Ca^{2+}-sensitivity and force generation capacity were lower in pEF+ compared to pEF− women and pEF +/− men, along with decreased phosphorylation of MyBP-C, Tnl and MLC2. Morphological changes were observed, pEF+ woman had higher collagen volume fraction and increased myocyte diameter.
Conclusion: Because of more inflammation and oxidative stress, women showed sever signalling pathway alterations and diastolic dysfunction. Understanding gender differences in pEF may help developing novel treatment options.

P4-14

Exercise-induced alterations of myocardial sarcomerdynamics are associated with hypophosphorylation of cardiac troponin I

Attila Oláh[1], Beáta Bódi[2], Judit Tamás[2], Marianna Török[1], Alex Ali Sayour[1], Bálint Barta[1], Dalma Kellermayer[1], Mihály Ruppert[1], Béla Merkely[1], Zoltán Papp[2], Tamás Radovits[1]

[1]Heart and Vascular Center, Semmelweis University, Budapest; [2]Division of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen

We aimed at determining left and right ventricular (LV and RV) cardiac sarcomeric modifications at cellular and molecular levels in a rat model of athlete's heart to understand processes leading to physiological hypertrophy.

Trained rats swam 200 min/day for 12 weeks and were compared to control ones. Hemodynamic properties were provided by LV pressure-volume analysis. Force assessments on isolated permeabilized cardiomyocytes and molecular biological measurements were applied to reveal underlying mechanisms.

Echocardiographic data confirmed training-induced cardiac hypertrophy, while pressure-volume analysis revealed increased LV contractility in exercised hearts. Cardiomyocyte Ca^{2+}-activated force production was improved along with increased Ca^{2+} sensitivity in trained rats. Cardiac troponin I phosphorylation was decreased, whereas the phosphorylation of titin and cardiac myosin binding protein-C was not altered in physiological hypertrophy.

Exercise-induced hypertrophy is associated with increased Ca^{2+}-activated force and Ca^{2+} sensitivity of LV and RV cardiomyocytes, which might be associated with hypophosphorylation of cardiac troponin I.

P4-15

Mechanisms of cardiotoxicity associated with tyrosine kinase inhibitors in H9c2 cells and mice

Abdallah Alshaikhali, Miljenko Panajatovic, Vanessa Abegg, Franziska Paech, Stephan Krähenbühl, Jamal Bouitbir

Division of Clinical Pharmacology & Toxicology, University Hospital, Basel, Switzerland

Introduction: The treatment with tyrosine kinase inhibitors (TKI) shows an increase in progression-free survival in cancer. However, TKIs are susceptible to develop cardiac toxicity in patients.
Objective: The aim of our study was to investigate the mechanisms of cardiotoxicity for imatinib, sorafenib and sunitinib in cardiac H9c2 cells and for sunitinib in mice.
Methods: For the in vitro experiments, we exposed H9c2 cells for 24 h with increased concentrations of TKI (from 1 to 100 μM). We also treated mice with sunitinib for 2 weeks at 7.5 mg/kg/d.

Results: In H9c2 cells, sorafenib and sunitinib showed a higher cytotoxicity profile in the presence of galactose (favoring mitochondrial metabolism) compared to glucose (favoring glycolysis). TKIs reduced the mitochondrial complex activities of the electron transport chain in cardiomyocytes after 24 h exposure. These compounds increased superoxide accumulation and decreased the cellular GSH pool leading to oxidative stress. Electron microscopy showed swollen mitochondria with loss of cristae leading to apoptosis. In mice, the treatment with sunitinib corroborated the same deleterious effects in hearts than observed in cells.
Conclusion: Mitochondrial dysfunction may represent a toxicological mechanism of cardiotoxicity associated with sunitinib.

P4-16

Loss of cMyBP-C N′-terminal domains induces spontaneous oscillatory contractions (SPOC) in permeabilized myocytes from Spy-C mice

Samatha Harris

University of Arizona, Department of Cellular and Molecular Medicine

Background: Truncation mutations in MYBPC3 are a common cause of hypertrophic cardiomyopathy (HCM) leading to reduced cMyBP-C in cardiac sarcomeres, but functional effects of reduced cMyBP-C are not well understood.
Objective: We sought to determine acute effects of reduced cMyBP-C in permeabilized myocytes.
Method: We used a novel "cut and paste" approach to reduce and replace cMyBP-C N′-terminal domains (C0–C7) in myocytes from "Spy-C" mice that express a TEV protease site and a "SpyTag" embedded between domains C7 and C8. TEV protease was used to "cut" cMyBP-C and then recombinant C0–C7 was "pasted" using the split protein "SpyCatcher".
Results: Similar to genetic cMyBP-C knockout, acute loss of cMyBP-C increased apparent cross-bridge cycling rates (ktr). However, loss of cMyBP-C also dramatically increased the appearance of spontaneous auto-oscillatory contractions (SPOC) that were sustained during Ca^{2+} activation and eliminated by add back of C0–C7.
Conclusions: Results suggest a novel role for cMyBP-C to modulate oscillatory waves of contraction across sarcomere ensembles and provide new insights into mechanisms by which reduced cMyBP-C causes cardiac dysregulation.

Poster Session 5: Molecular Motors

P5-1

Autoregulatory functions of myosin 16 domains

András Kengyel, Elek Telek, Alexandra Holló, Miklós Nyitrai

Department of Biophysics, University of Pécs Medical School

Myosin 16b (Myo16b) is a lesser-known motor protein, which may have a role in the neuronal development. Recently it was connected to several human neurological disorders such as schizophrenia or autism. Myo16b contains a motor domain, a disordered tail (Myo16Tail) and an ankyrin-repeat containing N-terminal domain (Myo16Ank). We are attempting to characterize the Myo16Ank and Myo16Tail domains using skeletal myosin motor domain as a model system.

Based on spectroscopic experiments we found, that Myo16Ank is able to bind to the motor domain and increase its actin-activated ATPase activity. It can also enhance the motility speed of actin filaments during the in vitro motility assay. The prolin-rich Myo16-Tail is supposed to bind profilin, but we found no direct interaction between them using anisotropy measurements. Meanwhile, we also tested the interaction between Myo16Tail and Myo16Ank domains, which showed a moderate affinity (KD ~ 2.7 μM).

Our results suggest that one possible role of Myo16Ank is to regulate the motor function of Myo16, meanwhile the binding of Myo16Tail to Myo16Ank assumes a regulatory function by backfolding of the tail to the N-terminal of the Myo16.

P5-2

Presence of ATP during heavy meromyosin incubation reduces actin velocity in vitro

Mohammad A Rahman, Aseem Salhotra, Alf Månsson

Department of chemistry and biomedical sciences, Linnaeus University, Kalmar, Sweden

The in vitro motility assay (IVMA) allows studies of muscle contraction through observation of actin filament propulsion by surface-adsorbed myosin motors. However, motility is often compromised by nonfunctional, "dead", motors. Here we investigate the efficiency, and effects on motile function, of two approaches designed to remove dead motors. We first performed "dead heading" of heavy meromyosin (HMM), removing ATP-insensitive "dead" heads by pelleting them with actin at 1 mM MgATP. Alternatively, we preincubated with non-fluorescent, "blocking actin" (1 μM) to block the dead heads after surface adsorption, followed by rinse with 1 mM MgATP. Both dead heading and blocking actin increased the fraction of motile filaments compared to control conditions. However, surprisingly, dead heading, but not blocking actin, reduced the actin gliding velocity by 38 ± 9% (n = 4; mean ± 95% CI). The reduction in velocity was reproduced without dead heading if HMM was mixed with 1 mM MgATP before adsorption to a silanized surface. A similar but smaller effect was observed using nitrocellulose for HMM adsorption. Clearly, dead heading may produce unexpected effects on IVMA results.

P5-3

Phosphorylation of essential light chain of skeletal myosin is an on/off switch of the actin-myosin interaction

Salavat R. Nabiev[1], Daria S. Logvinova[1,2], Alexander M. Matyushenko[1,2], Larisa V. Nikitina[1], Galina V. Kopylova[1], Daniil V. Shchepkin[1], Sergey Y. Bershitsky[1]

[1]Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia; [2]A.N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow Russia

Background: In myosin essential light chain (ELC), there are two residues, Thr65 and Ser193 that can be phosphorylated. It is however unclear what functional role of the ELC phosphorylation is in skeletal muscle.
The aim was to study effects of the ELC phosphorylation on the interaction of skeletal myosin with actin.
Material and methods: Three recombinant human myosin ELC constructs with mutations T65D, S193D, and T65D/S193D, imitating natural ELC phosphorylation were produced, and the native ELC in rabbit skeletal myosin were replaced with these mutants. We measured the sliding velocity of F-actin or regulated thin filaments over myosin with all these ELC constructs and its pCa50 in an in vitro motility assay and an average step size of myosin in an optical trap.

Results: The T65D mutant significantly decreased the sliding velocity of thin filaments and its pCa50 as compared with non-mutated ELC construct. However, with the mutants S194D and T65D/S193D it was the same as that with the recombinant native ELC. None of the ELC mutants changed the myosin step size significantly.
We conclude that the phosphorylation of Thr65 and Ser193 of ELC renders differently directed effects on the actin-myosin interaction in skeletal muscle: the T65D mutation inhibits the interaction and the S193D mutation eliminates this inhibition.

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P5-4

Controlled surface silanization for actin-myosin based nanodevices

Frida W. Lindberg[1], Marlene Norrby[2], Mohammad A. Rahman[2], Hideyo Takatsuki[2], Sören Jeppesen[1], Heiner Linke[1], Alf Månsson[2]

[1]Lund University; [2]Linnaeus University

The surface hydrophobicity is critical for fine tuning heavy meromyosin (HMM) driven actin filament sliding velocity in nanodevices based on the in vitro motility assay. The suggested mechanism of HMM binding is via the motor domains to surfaces of low contact angle and via the tail domain to surfaces of high contact angle. Here, we tested these ideas further by varying the surface hydrophobicity of trimethylchlorosilane (TMCS) derivatised SiO_2, deposited by chemical vapour deposition. A wider range of contact angles (10°–85°) than in previous studies was obtained by varying the deposition chamber pressure and silanization duration.

Higher contact angles increased the actin filament sliding velocity at both 60 and 130 mM ionic strengths. Motility on surfaces with contact angles $< 50°$ was only detected in the presence of viscosity enhancing methylcellulose to prevent diffusion of actin filaments from the surface when only few myosin heads are accessible. Overall, our studies support previous hypotheses for HMM adsorption mechanisms on surfaces of different contact angles. The reported method will be valuable for tuning the surface hydrophobicity in acto-myosin nanodevices.

P5-5

Adaptation of mammalian myosin II sequences to body mass

Sarah T. Jeanfavre[1], Charlotte Henson[1], Michael P. Coghlan[1] Martin Ridout[2], Mark N. Wass[1], Anthony J. Baines[1], Michael A. Geeves[1]

[1]School of Biosciences, University of Kent, Canterbury, UK; [2]School of Mathematics, Statistics and Actuarial Science, University of Kent, Canterbury, UK

Contraction velocity is a property of the myosin isoform expressed in a muscle fiber and the velocity of contraction is also related to body size; muscles in larger species contract at a lower velocity (maximum shortening velocity or in vitro motility). This behaviour is most obvious in the slow muscle/β-cardiac isoform. There must be changes in the myosin sequence that underlie the relationship between velocity of contraction, body size and myosin isoform. A dependence of myosin motor domain sequence on size would not be expected in a non-muscle myosin II isoform.

We examined the sequence of > 650 myosin-2 motor domains for 12 different isoforms across all mammals from the mouse to the killer whale. Non-muscle isoforms, as predicted, show little variation in sequence (0.11–0.14% seq. change for each 10 fold change in mass). In contrast, β-cardiac myosin (MyH-7) shows a 5-fold greater sequence variation (0.73% seq change) and greater than any other myosin II. All adult fast muscle myosins show a change (0.24–0.30%) half of the β-cardiac value while, smooth (0.089) and developmental muscle isoforms (0.09) have a seq change as low as the non-muscle isoforms.

Poster Session 6: Smooth Muscle Contraction and Pathology

P6-1

Signal transduction pathways of the thromboxane prostanoid receptor in urinary bladder smooth muscle

Péter J. Molnár[1], Bálint Dér[1], Zsófia Borbás[1], Krisztina Molnár[1], Éva Ruisanchez[1], Stefan Offermanns[2], Zoltán Benyó[1]

[1]Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary; [2]Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

Prostanoids and isoprostanes are important mediators of the detrusor smooth muscle (SM) contraction and their effects are mainly mediated by the thromboxane prostanoid receptor (TP).

Therefore, we aimed to analyze the signaling pathways of TP in the urinary bladder SM.

Contraction force was measured by myograph on detrusor muscle strips prepared from wild type (C57BL/6) and knockout mice, deficient for the TP (TP KO) or the α-subunits of heterotrimeric G proteins (Gαq/11-KO, Gα12/13-KO).

The TP agonist U-46619 evoked contraction, which was decreased in Gα12/13-KO bladder strips. Correspondingly, the responses evoked by the U-46619 were reduced by the Rho-kinase (ROCK) inhibitor Y-27632. In the Gαq/11-KO strips, the responses were also decreased and in the presence of Y-27632 abolished completely.

In conclusion, the activation of the TP leads to SM contraction and is linked simultaneously to the Gαq/11 and to the Gα12/13-Rho-ROCK intracellular signaling pathways in the murine urinary bladder.

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Poster Session 7: Thin Filament and Actin-binding Proteins

P7-1

Comparison of structural and functional properties of different isoforms of skeletal muscle tropomyosin

Alexander Matyushenko[1], Daniil Shchepkin[2], Galina Kopylova[2], Sergey Y. Kleymenov[3], Sergey Y. Bershitsky[2]

[1]Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia; [2]Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia; [3]Koltzov Institute of Developmental Biology of the Russian Academy of Sciences, Moscow, Russia

The tropomyosin (Tpm) isoforms α (Tpm 1.1) and γ (Tpm 3.12) are expressed in fast and slow human skeletal muscles, respectively, while β-Tpm (Tpm 2.2) is expressed in both fast and slow muscles. This results in formation of αα- and γγ-homodimers (ββ-homodimers

are unstable and occur rarely) of dimeric Tpm molecules as well as αβ- and γβ-heterodimers. The properties of αα-homodimer are well studied, whereas nothing is known about the properties of γγ-homodimer and γβ-heterodimer. Using differential scanning calorimetry, we showed that the thermal stability of γγ-homodimer is much higher than that of αα-homodimer, and ββ-homodimer is the least stable Tpm. The stability of γβ-heterodimer is much lower than that of γγ-homodimer, and the thermal unfolding of αβ-heterodimer is similar to that of αα-homodimer. Sliding velocity of regulated thin filaments containing either Tpm γγ-homodimers or γβ-heterodimers moving over fast or slow skeletal myosin measured in an in vitro motility assay was significantly less than that of the filaments with αα- or ββ-homodimers, or αβ-heterodimers. Both γγ- and γβ-Tpm dimers significantly decreased the calcium sensitivity of the sliding velocity over fast myosin but increased it over slow myosin. We conclude that the Tpm γ-chain is one of essential factors that determine the properties of slow muscles.

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P7-2

Novel regulatory elements within the COOH-terminus of human cardiac troponin T

Dylan Johnson, William Angus, Li Zhu, Joseph M. Chalovich

Brody School of Medicine

Regulated actin filaments lacking the 14 terminal residues of troponin T cannot form the B state at low Ca^{2+} and more fully enter the M state at high Ca^{2+}. To understand this function we studied the D4, D6, D8, D10 and D14 deletion mutants of troponin T. The M state population at high Ca^{2+} was measured by ATPase rates relative to full activation. There were roughly linear increases in ATPase rate and M state as the COOH-terminal region was deleted. The population of the B state, at low Ca^{2+}, was estimated by acrylodan tropomyosin fluorescence, kinetics of S1 binding to excess regulated pyrene-actin and lag duration for binding excess of S1 to pyrene-actin. The acrylodan and lag measurements showed a linear loss of the B state as the COOH-terminal region of troponin T was removed. Pyrene-actin binding kinetics deviated from that pattern because the rate of binding of S1 to pyrene labeled actin was not at its maximum rate with wild type troponin at high Ca^{2+} as is normally assumed. We suggest that most of the terminal residues of troponin T are essential for forming the inactive B state and for inhibiting full activation upon Ca^{2+} binding to troponin. Our hypothesis is that the COOH-terminal region of troponin T limits activity at high Ca^{2+} allowing myosin-ADP to cooperatively activate contraction.

P7-3

Effects of myopathy-related mutations A4V and R91C on regulatory functions of tropomyosin Tpm3.12

Małgorzata Śliwinska[1], Katarzyna Robaszkiewicz[1], Wenjun Zheng[2], Joanna Moraczewska[1]

[1]Department of Biochemistry and Cell Biology, Faculty of Natural Sciences, Kazimierz Wielki University in Bydgoszcz, Poland;
[2]Department of Physics, University at Buffalo, SUNY, Buffalo, NY 14260

Point mutations in TPM3 encoding tropomyosin (Tpm) isoform Tpm3.12 cause congenital myopathies. Studies suggest that the severity of the disease depends on location of the substitution in Tpm. While the substitution A4V is located within the overlap region between Tpm molecules and troponin T, R91C is directly involved in actin binding.

The goal of this work was to examine structural basis of the regulatory activities of Tpm3.12 using in vitro biochemical assays and molecular dynamics (MD).

The A4V and R91C mutations reduced Tpm3.12 affinity for actin 2.5 and 1.6 fold, respectively. Reduced actin affinities of both Tpm mutants were also observed in the presence of troponin ± Ca^{2+}. The actin-myosin ATPase showed 2-fold lower activation in the presence of each mutant and Ca^{2+}, but the inhibition in the absence of Ca^{2+} was normal. Ca^{2+} sensitivity of the ATPase (pCa^{2+}) was decreased by R91C, but not A4V.

In agreement with the above experiments, our MD simulation found that both mutants had lower affinity for actin, as measured by the van der Waals energy, which could be attributed to different molecular mechanisms—increased Tpm-actin separation in R91C and increased structural fluctuations in A4V.

In conclusion, location of disease-causing mutation influences molecular mechanism of actin filament regulation by Tpm3.12.

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P7-4

Tropomyosin isoforms regulate cofilin 1 activity by modulating the conformation of actin filament

Zofia Ostrowska-Podhorodecka[1], Joanna Moraczewska[1], Emil Reisler[2]

[1]Department of Biochemistry and Cell Biology, Faculty of Natural Sciences, Kazimierz Wielki University in Bydgoszcz, Poland;
[2]Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles

Tropomyosin (Tpm) and cofilin (Cof) are at the cross-roads of actin filaments dynamic stability. Tpm1.8 protects, but Tpm3.4 enhances severing of the filament by Cof. This might be due to different actin conformations maintained by Tpm isoforms. To confirm this hypothesis we probed actin conformation in the presence of Cof-1 and both Tpm isoforms by using yeast actin mutants Q41C in D-loop and S265C in the H-loop. Dimer production via zero-length cross-linking between C41-C374 and C265-C374 was the measure of changes in longitudinal and lateral residues proximities, respectively.

Cof-1 inhibited the longitudinal cross-links by 50%. In the presence of Cof and Tpm1.8, but not Tpm3.4, these cross-links were further inhibited. Tpm3.4 and Cof-1 inhibited the lateral cross-link by 50%, but Tpm1.8 was less efficient. These reactions were almost completely inhibited by binding of both Tpms along with Cof-1. Fluorescence changes of acrylodan attached to C41 and C265 confirmed the opposite effects of Tm3.4 and Tpm1.8 on Cof-induced changes in F-actin conformation and proximities of the tested residues at the longitudinal and lateral inter-protomer interfaces.

We concluded that Cof-induced changes in D- and H-loop conformation are differently modulated by Tpm isoforms, which is the structural basis for Cof activity regulation.

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P7-6

Experimentally varying the number of super-repeats in the Neb gene of the mouse: assessing the role of nebulin in thin filament length regulation

Balazs Kiss, Paola Tonino, Justin Kolb, John E. Smith, Henk Granzier

Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ-85724, USA

Nebulin is a giant, modular actin binding protein that spans along the thin filament in skeletal muscle and consists of numerous tandem copies of a simple ~ 35 aa module. Most of these simple modules are organized in seven-module 'super-repeats' that correspond to the arrangement of troponin and tropomyosin on the thin filament. Besides being known as a thin filament stabilizer, nebulin has been speculated to act as a thin filament ruler by determining the length of actin in different skeletal muscle types, yet little is known about nebulin's direct influence on thin filament length or its mechanism of action. Here we developed two novel mouse models in which nebulin super-repeats 9-11 are deleted (NebΔSR9-11) or duplicated (NebdupSR9-11), respectively. Mice of either model are viable, born at Mendelian ratios and do not develop muscle weight deficits. Super-resolution structured illumination microscopy (SR-SIM) and immunoelectron microscopy (IEM) on extensor digitorum longus (EDL) muscle revealed that the N-terminus of nebulin, together with tropomodulin (Tmod) localizes at the pointed end of the thin filament. Furthermore, both the length of nebulin and thin filament length were found to be reduced by ~ 115 nm in NebΔSR9-11 and increased by ~ 115 nm in NebdupSR9-11 EDL. Compound heterozygous animals (NebΔSR9-11,dupSR9-11) express both the shorter and the longer nebulin close to a ratio of 1:1 and the difference in their lengths is ~ 230 nm. The same epitope distance difference found in Tmod localization suggests a bimodal thin filament length distribution according to the shorter and longer nebulin. Functional studies on skinned EDL fibers revealed shorter myofilament overlap in NebΔSR9-11 and longer in NebdupSR9-11 consistent with their altered thin filament lengths. We conclude that nebulin seems to fully cover the thin filaments in EDL muscle and is important in thin filament length regulation with each of nebulin's super-repeats being responsible for a quantal 38 nm thin filament length.

P7-7

Leiomodin3—more than just another thin filament pointed end protein?

Michaela Yuen[1,2], Lauren Schultz[3], Rachel Mayfield[3], Shen Shengy[3], Stefan Conijn[1], Irene De Vries[1], Carol Gregorio[3], Coen A. C. Ottenheijm[1,3]

[1]Department of Physiology, Institute for Cardiovascular Research, VU University Medical Centre, Amsterdam, the Netherlands; [2]Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, Sydney, Australia; [3]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, USA

Introduction: Leiomodin-3 (LMOD3), encoded by LMOD3, is a member of the tropomodulin protein family localising predominantly near striated muscle thin filament pointed ends. LMOD3 mutations cause a severe myopathy. The severity of disease and the presence of abnormal thin filaments in patient muscle suggest that LMOD3 is critical for muscle function likely by helping to form and maintain thin filaments.
Objective: To date, little is known about LMOD3s function and the cause of weakness in LMOD3 patients. We aim to determine whether LMOD3 is directly involved in skeletal muscle contraction as well as thin filament stability.
Method: We tested the structural integrity and contractile function of intact soleus muscle and permeabilised single myofibers of Lmod3 knock-out (KO) mice.
Results: Eccentric "damaging" contractions resulted in less force loss in KO mice, suggesting muscle integrity is not affected by LMOD3 loss. Permeabilised single myofibers showed a decrease in specific force in both fiber types. Interestingly, cross bridge cycling kinetics and active stiffness was decreased specifically in fast myofibres.
Conclusion: Our research suggests LMOD3 is directly involved in skeletal muscle contraction in fast myofibers, perhaps by regulating myosin-actin interactions. This represents a novel role for LMOD proteins and likely contributes to weakness in LMOD3 patients.

P7-8

The ATP hydrolysis mechanism of fibrous actin

Yusuke Kanematsu[1], Yu Takano[1], Shuichi Takeda[2], Ryotaro Koike[3], Motonori Ota[3], Yuichiro Maéda[2]

[1]Grad. Sch. Info. Sci., Hiroshima City Univ., Hiroshima, Japan; [2]Structural Biology Res. Inst. Nagoya Univ., Nagoya, Japan; [3]Grad. Sch. Info. Sci., Nagoya Univ., Nagoya, Japan

Introduction: The ATP hydrolysis of actin, which occurs only at the fibrous (F) form, is known to drive the unidirectional polymerization of the filaments. The molecular mechanism of actin ATPase was largely unknown due to the lack of the high resolution structure of F-form actin.
Purpose: To elucidate the detailed ATPase reaction mechanism of F-actin, a theoretical investigation was performed using the atomic resolution (1.2 Å) X-ray crystal structures of the F-form actin with various nucleotide states we have recently determined.
Method: We employed ONIOM (DFT:MM) calculation with electronic embedding.
Results: Our analyses revealed that the ATP hydrolysis of actin proceeds via a dissociative reaction pathway, which resembled that of myosin ATPase (F. A. Kiani and S. Fischer, 2014). The attack of lytic water was concerted with the double proton transfer among two waters and the metaphosphate. In the final product, a strong H-bond was found between ADP and Pi, which may contribute to the stability of the ADP/Pi state.
Conclusion: We achieved a full exploration of actin ATPase reaction, which reasonably explained the characteristic irreversibility of the reaction.

P7-9

The effect of the Gly126Arg mutation in Tpm1.1 on the interaction between myosin and actin in ATP hydrolysis cycle

Nikita A. Rysev[1], Ilya A. Nevzorov[2], Olga E. Karpicheva[1], Stanislava V. Avrova[1], Dmitrii Levitsky[3], Yurii S. Borovikov[1]

[1]Institute of Cytology RAS, St Petersburg, Russia; [2]Institute of Biotechnology, University of Helsinki, Helsinki, Finland; [3]A.N. Bach Institute of Biochemistry, Moscow, Russia

Muscle contraction is regulated by tropomyosin (Tpm) and Ca^{2+}-sensitive protein troponin which form together with F-actin thin filaments in muscle fibre. The non-canonical Gly126 residue in the central part of skeletal α-tropomyosin (Tpm1.1) destabilizes the structure of this protein. Replacing this amino acid with Arg stabilizes the central region of Tpm. In order to investigate how the Gly126Arg mutation affects the actin-myosin interaction, we incorporated the myosin subfragment-1 labeled with fluorescent probe into the ghost muscle fibre. Multistage changes in spatial organization of the myosin head during modeling of the ATP hydrolysis cycle were studied using polarized fluorescence microscopy. In the regulated thin filaments at high Ca^{2+} the Gly126Arg mutation increases the number of myosin heads strongly associated with actin at simulating strong-binding stage, which increases the efficiency of myosin cross-bridges. A

marked rise in the proportion of such myosin heads at low Ca^{2+} indicates a high Ca^{2+}-sensitivity of the thin filament induced by the mutation. Therefore, the effects of Tpm stabilization by the Gly126Arg mutation are realized through the abnormal behavior of Tpm and troponin that lead to a change in the nature of the interaction of myosin with actin and Tpm in the ATP hydrolysis cycle. This work is supported by Russian Science Foundation (grant no 17-14-01224).

P7-10

Effects of stabilization of flexible sites in the alpha-tropomyosin molecule

Daniil V. Shchepkin[1], Galina V. Kopylova[1], Alexander M. Matyushenko[2], Dmitrii I. Levitsky[2], and Sergey Y. Bershitsky[1]

[1]Institute of Immunology and Physiology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia; [2]Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia

In the structure of muscle tropomyosin (Tpm 1.1) molecule, except non-canonical residues Asp137 and Gly126, mostly responsible for its flexibility, there are two more residues, Glu218 and Ala134, which according to crystal structures and MD simulations, should possess an extra elasticity. However, their role in the Tpm structure is unknown.

We aimed to examine the functional effects of these residues on the actin-myosin interaction.

Using an in vitro motility assay, we studied how does the replacement of A134 or E218 in the Tpm molecule for A134L and E218L, respectively, affect the sliding velocity of reconstructed thin filaments moving over skeletal myosin, its Ca^{2+} sensitivity, and the maximal force they can develop. The force-generating ability of myosin we assessed with NEM-modified myosin.

We found that the replacements affect characteristics of the thin filaments movement appreciably though differently. The A134L mutant, compared to WT, considerably, 2.4 fold, decreased the maximal force and by 0.15 pCa, the Ca^{2+} sensitivity, but only tiny increased the velocity. The E218L mutant significantly, 1.6 fold, increased the sliding velocity and pCa50 (0.27 pCa) but did not affect the maximal force.

Thus, we for the first time experimentally shown functional meaning of Glu218 and Ala134 residues in alpha-Tpm.

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P7-11

Effect of point substitutions in tropomyosin on its bending stiffness probed by molecular dynamics

Natalia A. Koubassova[1], Alexander M. Matyushenko[2], Daniil V. Shchepkin[3], Galina V. Kopylova[3], Sergey Y. Bershitsky[3], Dmitrii I. Levitsky[2,4], Andrey K. Tsaturyan[1]

[1]Institute of Mechanics, Moscow State University, Russia; [2]A.N. Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia; [3]Institute of Immunology and Physiology, Russian Academy of Sciences, Yekaterinburg, Russia; [4]Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia

Tropomyosin (Tpm) is a long coiled-coil protein that participates in regulation of muscle contraction. In order to understand how point mutations in the Tpm molecule affect its mechanical properties and cause some cardiovascular diseases, molecular dynamic (MD) simulation was performed with GROMACS v. 2016. The initial models of different parts of the Tpm were taken from PDB (IDs 2b9c or 2g9j). The Tpm constructs carrying point mutations were built with the UCSF CHIMERA. The persistence length, a value reciprocal to the Tpm bending stiffness, was determined from the averaged over MD simulation time variation in the direction of unit vectors, tangent to the backbone of the Tpm at different positions along the molecule.

The results of the MD simulation explain some experimental data concerning effects of stabilizing point mutations in the central part of Tpm (G126R, M127A/I130A, D137L, M141A/Q144A) and cardiomyopathy-associated mutations in the 'head-to-tail' junction between two Tpm molecules (M8R, K15N, A277V, M281T, I284V).

Molecular dynamics shows that the studied substitutions in the central part of the molecule increase bending stiffness of the coiled-coil structure, probably due to closure of the interhelical gap.

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P7-12

The E173A substitution in γ-tropomyosin disturbs the transition of contractile system to relaxation

A. O. Simonyan[1,2], O. E. Karpicheva[1], V. V. Sirenko[1], C. S. Redwood[3], Y. S. Borovikov[1]

[1]Institute of Cytology, Russian Academy of Sciences, 4 Tikhoretsky Av., St. Petersburg 194064, Russia; [2]Saint Petersburg State University, 7/9 Universitetskaya emb., St. Petersburg 199034, Russia; [3]Radcliffe Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom

The E173A substitution in Tpm3.12 has been identified previously in a patient with congenital fiber type disproportion. In the present study we investigated the effects of this substitution on tropomyosin's (Tpm) ability in its complex with troponin to regulate actin-myosin binding in a ghost muscle fibre at various mimicked stages of ATPase cycle at different [Ca^{2+}]. SDS-PAGE showed that the E173A-Tpm retains the ability to incorporate into thin filaments of a fibre. Polarized fluorimetry technique revealed that during the ATPase cycle at low (10–8 M) and high (10–4 M) calcium E173A-Tpm showed a greater amplitude of change in its azimuthal positioning over actin than the wild-type Tpm. Such behavior of the mutant Tpm was accompanied by an increase in proportion of both the strongly bound to actin myosin heads and the switched ON actin monomers at almost all mimicked stages of the ATPase cycle. There was also an increase in Ca^{2+}-sensitivity of thin filaments in the presence of the E173A-Tpm. There are reasons to believe that the E173A substitution in Tpm disturbs the relaxation process of muscle tissue, which can lead to energy depletion of the latter and, as consequence, cause the development of muscle weakness.

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P7-13

Actin filament multiplication for biocomputation

Venukumar Vemula[1], Tamas Huber[2], Beata Bugyi[2], Alf Månsson[1]

[1]The Molecular motor and bionano-group, Dept. of Chemistry and Biomedical Sciences, Linnaeus University, Kalmar, Sweden; [2]Department of Biophysics, Medical School, University of Pécs, Pécs, Hungary

'Network based biocomputation (NBC)' relies on molecular-motor-driven filaments, e.g. myosin propelled actin filaments, exploring

nanostructured networks that encode mathematical problems. The scale-up of this parallel, energy efficient approach requires multiplication of the filaments during computation by rapid severing and regrowth. Here, we tested lowered ionic strength and actin severing proteins for fast severing of myosin propelled actin filaments. In 60 mM ionic strength solution, heavy meromyosin propelled actin filament sliding in the in vitro motility assay led to doubling of the number of actin filaments within 60 s due to motility induced severing. By lowering the ionic strength to 35 mM and 25 mM the increase was instead ~ 3-fold and ~ 4-5-fold, respectively without changes in velocity. Another strategy involves use of the actin severing protein gelsolin. In the presence of 2 μM free Ca^{2+}, gelsolin (0.85 nM) increased the number of actin filaments ~ 5 to 7-fold within 60 s. Our results demonstrate the capacity for several-fold rapid increase in the number of actin filaments, supporting the usefulness of the described approaches as basis for upscaling of NBC devices.

P7-14

The function of two tropomyosin isoforms in regulating the contraction of insect flight muscle

Demetris Koutalianos, Konstantinos Drousiotis, Kate English and Belinda Bullard

University of York, Department of Biology, York, UK

Introduction: High frequency contractions of insect flight muscle (IFM) power wing beats. Opposing muscles act reciprocally to produce resonant distortions of the thorax and rapid stretches activate the IFM, suggesting there is a length sensor. Bridges between troponin on thin filaments and the thick filaments are candidates.
Objective: The aim was to identify components of troponin bridges, to measure the binding of the tropomyosin-troponin complex (Tm-Tn) to thick filaments and the affinity of an association with myosin.
Methods: Tm-Tn and tropomyosin isoforms Tm1 and Tm2 were isolated from Lethocerus IFM or expressed in E. coli. Association with thick filaments and myosin was determined by pulldown experiments, overlay blotting and electron microscopy (EM). The affinity of Tm-Tn and Tms for myosin-S1 (S1) was measured by MST and ITC.
Results: Tm-Tn bound to thick filaments and myosin. Unexpectedly, Tm1 bound to S1 with high affinity (Kd ~ 65 nM) but Tm2 did not bind. EMs of Tm1-Tn associated with thick filaments showed binding with a periodicity of ~ 40 nm. Tm1 crosslinked thick filaments in a ladder-like structure with rungs ~ 40 nm apart.
Conclusion: Tn bridges are midway between the target sites on actin that bind force-producing crossbridges (39 nm apart). We suggest non-force producing bridges bind to Tm1 at the position of Tn, and Tm1 is directly pulled from an inhibitory position on actin.

P7-15

Thin filament-based impaired muscle relaxation kinetics in KBTBD13-related NEM (NEM6)

Josine M. de Winter[1], Joery P. Molenaar[2], Michaela Yuen[3], Robbert van der Pijl[4], Shen Shengyi[4], Stefan Conijn[1], Menne Willigenburg[1], Sylvia Bogaards[1], Esmee van Kleef[2], Saskia Lassche[2], Malin Persson[5], Dilson Rassier[6], Tamar E. Sztal[7], Avnika A. Ruparelia[7], Robert J. Bryson-Richardson[7], Christopher N. Johnson[9], Manuela Marabita[8], Bert Blaauw[8], Richard J. Rodenburg[10], Benno Küsters[11], Alan H. Beggs[12], Ken Campbell[13], Weikang Ma[14], Thomas Irving[14], Edoardo Malfatti[15], Norma Romero[15], Jonne Doorduin[16], Baziel van Engelen[16], Nicol Voermans[16], Coen A. C. Ottenheijm[1]

[1]Dept. of Physiology, VU University Medical Center, Amsterdam, The Netherlands; [2]Dept. of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands; [3]Muscle Research, Faculty of Medicine, University of Sydney, Sydney, Australia; [4]Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ; [5]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; [6]Department of Kinesiology and Physical Education, McGill University, Montreal, Canada; [7]School of Biological Sciences, Monash University, Melbourne, Australia; [8]Venetian Institute of Molecular Medicine, University of Padova, Italia; [9]Division of Clinical Pharmacology, Center for Arrhythmia Research and Therapeutics, & Center for Structural Biology, Vanderbilt University Medical Center, Nashville, TN, USA; [10]Dept. of Pediatrics, Translational Metabolic Laboratory, Radboud University Medical Centre, Nijmegen, The Netherlands; [11]Dept. of Pathology, Radboud University Medical Centre, Nijmegen, The Netherlands; [12]Division of Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA; [13]Department of Physiology and Division of Cardiovascular Medicine, University of Kentucky, Lexington, KY, USA; [14]BioCat, Illinois Institute of Technology, Chicago, IL, USA; [15]Sorbonne Universités, Institut de Myologie, Paris, France; [16]Dept. of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands

Background: The mechanisms that modulate the kinetics of muscle relaxation are understudied despite their importance for muscle contractility. A prime example of the impact of impaired relaxation kinetics is nemaline myopathy caused by mutations in KBTBD13 (NEM6): in addition to weakness, patients have impaired muscle relaxation, compromising contractility and daily-life activities. The role of KBTBD13 in muscle is unknown, let alone its role in NEM6 pathology.
Methods and Results: With the use of transcranial magnetic stimulation, we established that the origin of impaired muscle relaxation kinetics in NEM6 myopathy is myogenic. The pathomechanism underlying these impaired muscle relaxation kinetics was studied using contractility assays in permeabilized muscle fibers and myofibrils isolated from NEM6 patient biopsies. We discovered that impaired muscle relaxation is sarcomere-based. By applying a combination of low-angle X-ray diffraction, super-resolution microscopy, modeling of muscle kinetics, binding- and contractility assays with bacterially-expressed KBTBD13, novel Kbtbd13-deficient and Kbtbd13R408C-knockin mouse models and a transgenic zebrafish model we showed that KBTBD13—the protein implicated in NEM6—is an actin binding protein.
Interpretation: Mutations in KBTBD13 slow relaxation kinetics of muscle through direct, structural effects on the actin-based thin filament. We propose that this pathomechanism is central to NEM6 pathology.

P7-16

Mutations in slow skeletal troponin I (TNNI1) cause contractile dysfunction

Stefan Conijn[1], Martijn van de Locht[1], Josine de Winter[1], Michiel Helmes[2], Weikang Ma[3], Tom Irving[3], Osorio Neto[4], Carsten Bönnemann[4], Coen Ottenheijm[1]

[1]VU University Medical Center, Department of Physiology, Amsterdam, The Netherlands; [2]IonOptix Llc., Milton, MA, USA; [3]Illinois Institute of Technology, Biophysics Collaborative Access Team, Center for Synchrotron Radiation, Chicago, USA; [4]National

Institutes of Health, Neuromuscular and Neurogenetics Disorders of Childhood Section, Neurogenetics Branch, National Institutes of Neurological Disorders and Stroke, Bethesda, USA

Nemaline myopathy (NEM) is a rare muscle disease caused by mutations in genes encoding proteins associated with the sarcomeric thin filament. To date, troponin I has not been implicated in NEM. Here, we investigated a muscle biopsy of a patient (P1) with a compound heterozygous mutation in the gene encoding slow skeletal TnI (TNNI1). To investigate the mechanism underlying muscle weakness, we performed calcium induced contractility measurements on permeabilized single muscle fibers (n = 8) isolated from P1, as well as on those isolated from biopsies of control subjects (CTRL) (n = 5; 10–15 fibers per subject). Type 1 fibers from P1 were severely atrophied, whereas type 2 fibers were not. Contractile force, both absolute and normalized to fiber cross sectional area, were decreased in type 1 fibers of P1 compared to CTRL, and was unaltered in type 2 fibers. Type 1 and 2 fibers of P1 did not show a change in the calcium-sensitivity of force. To study the effect of the mutation on thin filament structure, we performed low angle X-ray diffraction on isolated fibers. The periodicity of ALL6 was reduced in P1 fibers, suggesting a tighter thin filament helix. Myosin reflections showed a decrease in periodicity. Thus, the mutation in TNNI alters thin filament structure. We propose that this structural alteration contributes to the reduced maximal active tension of P1 fibers.

P7-17

Mutations in Fast Skeletal Troponin C (TNNC2) cause contractile dysfunction

Martijn van de Locht[1], Josine de Winter[1], Stefan Conijn[1], Weikang Ma[2], Michiel Helmes[3], Tom Irving[2], Sandra Donkervoort[4], Payam Mohassel[4], Livija Medne[5], Colin Quinn[6], Osorio Neto[4], Steven Moore[4], Reghan Foley[4], Nicol Voermans[7], Carsten Bönnemann[4], Coen Ottenheijm[1]

[1]VU University Medical Center, Department of Physiology, Amsterdam, The Netherlands; [2]Illinois Institute of Technology, Biophysics Collaborative Access Team, Center for Synchrotron Radiation, Chigago, USA; [3]IonOptix Llc.,Milton, MA, USA; [4]National Institutes of Health, Neuromuscular and Neurogenetics Disorders of Childhood Section, Neurogenetics Branch, National Institutes of Neurological Disorders and Stroke, Bethesda, USA; [5]Children's Hospital of Philadelphia, Roberts Individualized Medical Genetics Center, Philadelphia, USA; [6]University of Pennsylvania, Department of Neurology, Philadelphia, USA; [7]Radboud University Medical Centre, Department of Neurology, Nijmegen, Netherlands

Nemaline myopathy is a rare muscle disease caused by mutations in genes encoding proteins associated with the sarcomeric thin filament. To date, fast skeletal (fs)TnC has not been implicated in disease. Here, we investigate muscle biopsies of 2 patients with heterozygous mutations in the gene encoding fsTnC (TNNC2). Patient 1 (P1; 27 yrs) presented with a more severe phenotype, whereas patient 2 (P2; 19 yrs) presented with a milder phenotype. Calcium-induced contractility measurements were performed on permeabilized single muscle fibers isolated from patient (N = 2) and control (N = 5) biopsies. P1 showed atrophied type 2 and hypertrophied type 1 fibers. Maximal force and calcium-sensitivity of force (pCa50) were decreased in type 2 and were increased in type 1 fibers. P2 showed similar results but less pronounced: a smaller decrease in pCa50 in type 2 fibers, no change in maximal force and in trophicity in type 1 and 2 fibers. In P1, low angle X-ray diffraction data suggested a tightened thin filament in both fiber types, indicated by a reduced ALL6 reflection. In P2, structural changes were less pronounced. Based on these findings, we propose that TNNC2 mutations impair contractile function, presumably due to changes in thin filament structure. The more severe clinical phenotype of P1 versus P2 is reflected at the myofiber level, both structurally and functionally.

P7-18

A nebulin-dendra2 mouse model to localize individual nebulin molecules in sarcomeres.

Sylvia Bogaards[1], Robbert van der Pijl[1,2], Shengyi Shen[2], Paola Tonino[2], Kees Jalink[3], Michaela Yuen[1], and Coen Ottenheijm[1,2]

[1]VU University Medical Center, Department of Physiology, Amsterdam, the Netherlands; [2]University of Arizona, Cellular and Molecular Medicine, Tucson, USA; [3]Van Leeuwenhoek Centre for Advanced Microscopy, Amsterdam, the Netherlands

Background: Nebulin spans the length of the thin filament, with its C-terminus located in the z-disc and its N-terminus near the thin filament pointed-end. Mutations in nebulin, as well as in proteins that bind to nebulin cause myopathy. The pathophysiological mechanisms are incompletely understood, in part because of a lack of tools to precisely locate nebulin on the thin filament.
Objective: Develop a tool for the localization of individual nebulin molecules.
Methods: We generated a mouse in which dendra2, a photoconvertible protein, is incorporated by gene targeting into nebulin's N-terminus. Individual nebulin molecules were visualized by photoactivated localization microscopy (PALM). In PALM, dendra2 switches between fluorescent and dark states so that in every snapshot, only a small, optically resolvable fraction of dendra2 is detected.
Results: Nebulin-dendra2 mice have normal muscle weights. Western blot showed the presence of full length nebulin. IEM showed that incorporation of dendra2 does not affect nebulin's N-terminus position in sarcomeres. PALM localized individual nebulin N-termini with a resolution of 25 nm. Within a myofibril, individual nebulin N-termini show a gaussian distribution with a width at half-maximum of 90 nm.
Conclusion: These pilot results indicate the successful generation of a tool to localize individual nebulin molecules.

Poster Session 8. Motor Protein Pharmacology

P8-1

In vivo neural regeneration induced by non-muscle myosin-2 inhibition

Demeter Turos, Adam Istvan Horvath, Aron Zsigmond, Mate Varga, Andras Malnasi Csizmadia

Eotvos Lorand University

Overriding the inhibitory factors which hinder the central nervous system (CNS) regeneration in the adult CNS could provide novel alternatives to the treatment options in neurodegenerative diseases and neural traumas.

During development and regeneration, growth cones propel the advancing neurites using the actomyosin machinery while sampling the environment for external cues. Non-muscle myosin 2s (NM2) are essential, since these motor proteins regulate the protrusions in the growth cone. Using aminoblebbistatin (a highly soluble, non-fluorescent, non-phototoxic derivative of blebbistatin), it becomes possible to selectively inhibit NM2s in vivo, resulting in neural outgrowth, even in the presence of inhibitory cues.

With the help of two-photon microscopy we followed the changes caused by aminoblebbistatin during neural development and regeneration in zebrafish in vivo. The treatment induced neural growth and changed the neurite fluctuation patterns in the tectum opticum. We conducted laser axotomy to investigate the drug effects on neural regeneration. The labelled Mauthner cell axons recovered three times faster compared to the untreated larvae during the regeneration process.

P8-2

Influence of omecamtiv mecarbil on the actin-myosin interaction in ventricle and atria

Galina V. Kopylova[1], Valentina Y. Oshchepkova[1,2], Maxim R. Sharipov[1,2], Olga I. Koshcheeva[1,2], Daniil V. Shchepkin[1]

[1]Institute of Immunology and Physiology, the Russian Academy of Sciences, Yekaterinburg, Russia; [2]Ural Federal University, Yekaterinburg, Russia

Heart failure is accompanied by a decrease in the contractile function of the ventricles associated with the inhibition of the actin-myosin interaction. A pharmaceutical activator of myosin, omecamtiv mecarbil (OM), aimed to compensate for a reduction in the ventricle contractility in heart failure has been recently developed by Cytokinetics.

We studied the molecular mechanisms underlying the modulation of the myosin function in the ventricles and atria with OM using an in vitro motility assay. The force was measured as a fraction of NEM-modified myosin mixed with native myosin that stops the filament movement. Myosin was obtained from pig left atrium and ventricle. Troponin was extracted from pig left ventricle. Human Tpm1.1 was expressed in *E. coli*.

We found that OM reduced in a dose-dependent manner the sliding velocity of F-actin and thin filaments reconstructed from F-actin, troponin, and tropomyosin at pCa 4. In concentration 0.1 μM, it increased the force of both ventricular and atrial myosin at maximal calcium concentration. Thus, using myosin isolated from atria and ventricle, we revealed that OM affects the force-generating ability of myosin. Supported by RFBR (No 18-015-00252) and State Program AAAA-A18-118020590135-3.

P8-3

Modulation of cardiac myosin dynamics by omecamtiv mecarbil

Shaima Hashem, Matteo Tiberti and Arianna Fornili

Queen Mary University of London, School of Biological and Chemical Sciences, London, United Kingdom

New promising avenues for the pharmacological treatment of skeletal and heart muscle diseases rely on direct sarcomeric modulators, which are molecules that can directly bind to sarcomeric proteins and either inhibit or enhance their activity. A recent breakthrough has been the discovery of the myosin modulator Omecamtiv Mecarbil (OM), which is currently in clinical trials for the treatment of heart failure. While the overall effect of OM is an increased contractility of the cardiac muscle, its molecular mechanism of action is still elusive.

We present here an in silico study of the motor domain of cardiac myosin bound to OM, where the effects of the drug on the dynamical properties of the protein are investigated for the first time with atomistic resolution using Molecular Dynamics simulations.

We found that OM increases the coupling of the converter and lever arm subdomains to the rest of the protein, leading to a strong reduction in the amplitude of their motions. This finding is consistent with recent experimental observations that indicate an OM-induced inhibition of the power stroke. The identification of the interactions mostly responsible for this effect could be used for the future development of improved drugs.

This research is supported by the British Heart Foundation and the UK High-End Computing Consortium for Biomolecular Simulation, HECBioSim.

P8-4

Mavacamten stabilizes the super-relaxed state of β-cardiac myosin: deciphering the mode of action from myosin molecules to muscle fibers

Darshan V. Trivedi[1], Robert L. Anderson[2], Saswata S. Sarkar[1], Marcus Henze[2], Weikang Ma[3], Henry Gong[3], Christopher S. Rogers[4], Fiona L. Wong[2], Makenna M. Morck[1], Jonathan G. Seidman[5] Kathleen M. Ruppel[1], Thomas C. Irving[3], Roger Cooke[6], Eric M. Green[2], James A. Spudich[1]

[1]Stanford University; [2]MyoKardia Inc.; [3]BioCAT, Illinois Institute of Technology; [4]Exemplar Genetics; [5]Harvard University; [6]University of California, San Francisco

Mutations in β-cardiac myosin, the predominant motor protein for human heart contraction, can alter power output and cause cardiomyopathy. However, measurements of the intrinsic force, velocity and ATPase activity of myosin have not provided a consistent mechanism to link mutations to muscle pathology. An alternative model posits that mutations in myosin affect the stability of a sequestered, super-relaxed state (SRX) of the protein with very slow ATP hydrolysis and thereby change the number of myosin heads accessible to actin. Here, using a combination of biochemical and structural approaches, we show that purified myosin enters a SRX that corresponds to a folded-back conformation, which in muscle fibers results in sequestration of heads around the thick filament backbone. Mutations that cause HCM destabilize this state, while the small molecule mavacamten promotes it. These findings provide a biochemical and structural link between the genetics and physiology of cardiomyopathy with implications for therapeutic strategies.

P8-6

Fast skeletal muscle troponin activator tirasemtiv improves in vitro muscle function in the Tg.ACTA1D286G nemaline myopathy mouse model

J. M. de Winter[1], T. C. Borsboom[1], E. Minardi[2], C. Gineste[3], D. T. Hwee[4], F. I. Malik[4], K. J. Nowak[5,6], J. Gondin[3,6], M. A. Pellegrino[2], R. Bottinelli[2], C. A. C. Ottenheijm[1]

[1]VU University medical center, Amsterdam, The Netherlands; [2]Department of Molecular Medicine, Pavia University, Pavia, Italy; [3]Aix Marseille Univ, CNRS, CRMBM, Marseille, France; [4]Research and Early Development, Cytokinetics, Inc., South San Francisco, California, United States; [5]Centre for Medical Research, Faculty of Health and Medical Sciences, The University of Western Australia, and Harry Perkins Institute of Medical Research, Nedlands, Western Australia; [6]Office of Population Health Genomics, Division of Public and Aboriginal Health, Department of Health, Western Australia; [7]Institut NeuroMyoGène, UMR CNRS 5310, INSERM U1217, Université Claude Bernard Lyon 1, Villeurbanne, France

*contributed equally

Nemaline myopathy (NM) results in muscle weakness and a poor quality of life. Here, we evaluated the acute effect of tirasemtiv—a fast skeletal muscle troponin activator—on in vitro extensor

digitorum longus (EDL) muscle mechanics in the skeletal muscle α-actin-based NM mouse model (Tg.ACTA1D286G, n = 14) and wildtype mice (Wt, n = 14).

Intact muscle preparations were stimulated at incremental frequencies in both the absence and presence of tirasemtiv (3 μM). Fiber cross-sectional area (CSA) was determined by histology.

Tg.ACTA1D286G mice had lower EDL muscle weights (Tg.ACTA1D286G: 9 ± 0.3 mg vs. Wt: 12 ± 0.4 mg, $P < 0.001$) and muscle fiber CSA (Tg.ACTA1D286G: 1251 ± 45 μm^2 vs. Wt: 1650 ± 116 μm^2, P = 0.03). Absolute force production was lower in Tg.ACTA1D286G mice. Administration of tirasemtiv (3 μM) restored absolute force levels at submaximal stimulation to those of Wt mice (20 Hz: Tg.ACTA1D286G-vehicle: 25 ± 3 mN, Wt-vehicle: 35 ± 3 mN (P = 0.03), Tg.ACTA1D286G-tirasemtiv: 42 ± 5 mN; 40 Hz: Tg.ACTA1D286G-vehicle: 40 ± 5 mN, Wt-vehicle: 64 ± 8 mN (P = 0.02), Tg.ACTA1D286G-tirasemtiv: 76 ± 10 mN).

These findings are pivotal steps towards a therapeutic strategy to combat muscle weakness in NM.

P8-7

Piperine binding destabilizes the myosin neck via interactions with the regulatory light chain

Dmitri Tolkatchev[1], Daniel Elnatan[2], Leonardo Nogara[2,3], Thu Ly[1], Nariman Naber[2], Kenny Haak[1], Ryan Meech[1], Roger Cooke[2], Alla S. Kostyukova[1]

[1]Voiland School of Chemical Engineering & Bioengineering, Washington State University, Pullman, WA 99164-6515, USA; [2]Department of Biochemistry/Biophysics, Univ. California, San Francisco, CA 94143, USA; [3]Dipartimento di Scienze Biomediche, University of Padua, 35122 Padua PD, Italy

Piperine, an alkaloid from black pepper, was found to inhibit super-relaxed state (SRX) of skeletal myosin. To test our hypothesis that piperine binds in the neck region of myosin, we studied interactions between piperine and a complex consisting of the full-length regulatory light chain (RLC) and a fragment of the heavy chain (HCF). The sequence of HCF was designed to bind RLC and to dimerize via formation of a stable coiled coil, thus producing a well-folded heterotetrameric complex (RLC/HCF)2. Both polypeptides were co-expressed in Escherichia coli, the RLC/HCF complex was purified and tested for stability, composition and binding to piperine using circular dichroism, nuclear magnetic resonance and small-angle X-ray scattering. RLC and HCF chains formed a stable heterotetrameric complex, which was found to bind piperine. We also demonstrated that piperine binds isolated RLC and heavy meromyosin that contains RLC, whereas it does not interact with RLC-free S1. Piperine binding destabilized and reduced compactness of the (RLC/HCF)2 complex, suggesting that the mechanism of SRX inhibition by piperine is based on reducing the "stiffness" of the myosin neck upon binding to RLC.

Poster Session 9: Muscle Energetics

P9-1

Basal metabolism is increased in mice susceptible to malignant hyperthermia and heat stroke.

Flávia Guarnier

CeSI-MeT, Center for Research on Ageing and Translational Medicine; Univ. G. d'Annunzio, I-66100 Chieti Italy; Dept. of General Pathology; Univ. Estadual de Londrina, 86057-970 Londrina Brazil

Introduction: Malignant hyperthermia susceptibility (MHS) leads to lethal episodes under exposure to anesthetics, heat and strenuous exercise. The hyperthermic crises involves abnormal Ca^{2+} leak from SR and heat production. We recently discovered that Calsequestrin-1 knockout (CASQ1-null) mice develop a phenotype similar to MHS.
Objective: Demonstrate an association between heat generation and increased basal oxygen consumption in CASQ1-null mice.
Methods and results: In comparison with age-matched controls, 2 months old CASQ1-null mice showed increased food consumption (29.7%) and higher basal core temperature (mean of 0.7 °C), although no differences were found in body weight. These evidences of increased metabolism were supported by: (a) enhanced basal oxygen consumption during the awake period; (b) elevated levels of SERCA protein and activity; and (c) increased mitochondrial volume and percentage of damaged mitochondria.
Conclusion: MHS in CASQ1-null mice is associated to increased oxygen consumption and basal metabolism, possibly as a result of increased ATP demand and mitochondrial activity to support the enhanced SERCA activity, needed to reuptake the excess of cytosolic Ca^{2+}.

P9-2

Effect of prior knowledge of acceleration increase on oxygen uptake and oxygenation during running

Takashi Migita

Department of Sports Medicine and Science, Faculty of Human Health, Kurume University

During long-distance races, an abrupt increase in acceleration results in a sudden energy requirement. This study investigated whether prior knowledge of increase of acceleration affected oxygen uptake (VO_2) and tissue oxygenation during running.

Subjects were divided into two groups: those who had prior knowledge of acceleration increase and those who did not. They performed a velocity-incremental maximal test and two 10-min transient submaximal running tests. Submaximal tests were performed pre-acceleration (4 min) and post-acceleration (6 min). Pre-acceleration velocities corresponded with the ventilation threshold (VT); post-acceleration velocities corresponded with Δ40% ($VT + (VO_{2peak} - VT) \times 0.4$).

During submaximal tests, pulmonary gas exchange parameters were determined breath-by-breath; a non-linear regression technique analyzed the variables. The oxygenation status of the vastus lateralis muscle was monitored using a near- infrared spectroscopy (NIRS) system.

There was no significant difference in variables of VO_2 kinetic response between both groups. NIRS-derived deoxygenated hemoglobin was similar for both groups.

These results suggest that prior knowledge of acceleration increase did not affect energy requirement during running.

P9-3

Physical exercise combined to corticoid/omega-3 therapy improved muscle function and respiratory performance in old mdx mouse

Paula Andrea Saenz Suarez, Marcos Maciel Jr, Samara Camaçari de Carvalho, Humberto Santo Neto, Maria Julia Marques

Institute of Biology, Dept. of Structural and Functional Biology, University of Campinas (UNICAMP), 13083 970, Campinas, SP, Brazil

Background: In the mdx mouse, dystrophin deficiency leads to muscle degeneration. Corticoids and omega-3 alleviate dystrophy progression. In the young mdx mice, treadmill exercise can worsen dystrophy. Objectives: To verify the effects of treadmill exercise in the old mdx (12 months of age) and the effects of exercise associated to deflazacort (DFZ) alone or combined to omega-3.
Methods: Exercised (exe)-mdx run on a horizontal treadmill (12.4 mts/min, 15 min), 2 times/week, from 13 to 14 months of age. Exe-mdx were treated from 12 to 14 months of age with DFZ alone or combined to omega-3. Controls sedentary (sed) mdx, sed-C57BL/10 and some exe-mdx were not treated. Respiratory performance was quantified during exercise.
Results: Exercise alone or associated to therapy improved maximal oxygen consumption (VO_{2max}: sed-mdx: 22.6 ml/kg/h; exe-mdx: 66.2*; exe-DFZ/O3: 63.3*; C57BL/10: 61.6*; *$p < 0.05$ compared to sed), total energy expenditure and the time to exhaustion (sed-mdx: 8 min; exe-DFZ: 14 min). Sed-mdx showed reduced performance in functional evaluations (grip strength, open field, rotarod). Exe + therapy improved functional performance (grip strength sed-mdx: 0.7 ± 0.1 gr/gr; exe-mdx: 0.9 ± 0.1*; exe-DFZ: 1.6 ± 0.1*; *$p < 0.05$ compared to sed). Exercise apparently improved diaphragm histology and decreased creatine kinase.
Conclusion: Treadmill exercise alone or associated to corticoid/omega-3 may be beneficial to improve muscle function in aged mdx mouse.
Supported by: FAPESP, CNPq, CAPES, Faepex-Unicamp

P9-5

Contribution of muscle activity in leg muscles to metabolic rate during uphill slope running in middle-aged men

Kohji Hirakoba[1], Masato Tokui[2]

[1]Kyushu Institute of Technology, Japan; [2]Kyushu Kyoritsu University, Japan

It is acknowledged that metabolic rate increases with decreasing elastic energy during uphill slope exercise at a constant running velocity. The purpose of this study was to investigate contribution of muscle activity at each site of the exercising muscles to increased metabolic rate during a gradient-incremental running (GIR) test. Eight middle-aged men (60 ± 6.3 years) run on a treadmill the GIR test which was composed of increasing 2% gradient per 2 min from 0 to 10% at an individually chosen comfortable velocity. Oxygen uptake (VO_2) and electromyogram (EMG) signals on seven sites from superficial leg and buttock muscles were measured during the GIR test. VO_2 was found to linearly increase as a function of gradient. Standardized integrals of EMG data (%iEMG) of all the targeted muscles were remained a constant level until 4% gradient, and after that %iEMGs of major gluteus, vastus lateralis, biceps femoris and soleus muscles were started to increase with the elevation of gradient, while %iEMGs of the remaining three muscles did not change across over the examined gradients. The activity levels of the four muscles were considered to mainly contribute to the increased metabolic rate at higher ($\sim$ 6%) gradients during the GIR test. In addition, this finding suggested that activity levels of the unmeasured muscles would account partially for the increased metabolic rate at lower ($\sim$ 4%) gradients.

Poster Session 10: Contraction Regulation, EC Coupling

P10-1

Cardioprotective regimen of intermittent hypobaric hypoxia affects phosphorylated status of connexin 43 and expression of its upstream kinases in the rat heart

Kristyna Holzerová[1], Jan Neckář[1], Barbara Elsnicová[2], Ondřej Šebesta[2], Pavel Vebr[2], Barbara Szeiffova Bačová[4], Tamara Egan Beňová[4], Markéta Hlavačková[3], Olga Nováková[2], Daniela Horníková[2], Narcis Tribulová[4], František Kolář[1], Jitka M Žurmanová[2]

[1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic; [2]Department of Physiology, Faculty of Science, Charles University in Prague, Prague, Czech Republic; [3]Department of Development, Charles University in Prague, Prague, Czech Republic; [4]Institut for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic

Ventricular arrhythmias are the major cause of death in worldwide. Adaptation to intermittent hypobaric hypoxia (IHH) potentiates endogenous protective pathways reducing the incidence of ischemia/reperfusion (I/R) arrhythmias, however the molecular principle has not been fully elucidated.

We aimed to determine Cx43 expression, phosphorylated status (p-Cx43) and its upstream protein kinases PKA, PKG and casein kinase CK1 in normoxic (N) and IHH left ventircles.

Male Wistar rats were adapted to IHH (7000 m, 8-h/day, 25 daily exposures). Western blotting (WB), Mass spectrometry and quantitative immunofluorescence microscopy were used.

Results showed that IHH increased expression of Cx43 and p-Cx43(Ser368), as well as the ratio of transversal "end to end" and longitudinal "side to side" junctions. IHH also increased expression of PKA and PKG while level of CK1 has not changed. Beside that p-Cx43(Ser 364,365) were upregulated however p-Cx43(Ser278/289) and p-Cx43(Tyr265) decreased after IHH.

In conclusion, IHH afforded anti-arrhythmic effect is accompanied by changes in phosphorylated state of Cx43 which may influence turnover and assembly of gap junctions and thus conductivity.

P10-2

A A 3D diffusional model of the [Ca^{2+}] in cytosol, sarcoplasmic reticulum and mitochondria of murine skeletal muscle

Lorenzo Marcucci[1], Marta Canato[1], Feliciano Protasi[2], Ger J.M. Stienen[3], Carlo Reggiani[1]

[1]University of Padova, Italy; [2]University. G. d'Annunzio, Chieti, Italy; [3]VU University Medical Centre, Amsterdam Cardiovascular Sciences, The Netherlands

Variations of free [Ca^{2+}] control contraction in muscle cells. To fully understand the role of calcium redistribution upon contraction in skeletal muscle, the local [Ca^{2+}] in the cytosol, where myofibrils are embedded, the lumen of the sarcoplasmic reticulum (SR) and the mitochondrial matrix, need to be considered. Previously, models have been developed describing intracellular calcium handling in skeletal and cardiac muscle cells. However, a comprehensive model describing the kinetics of the changes in free [Ca^{2+}] in these three compartments is lacking.

We designed a new 3D compartmental model of the half sarcomere, which accounts for diffusion of Ca^{2+} into the three

compartments and simulates its dynamics at rest and at various rates of stimulation in mice skeletal muscle fibers.

This model satisfactorily reproduces the amplitude of the previously published variations of [Ca^{2+}]. To illustrate the applicability of the model, we investigated the effects of Calsequestrin (CSQ) ablation. CSQ knock-out mice muscles preserve a near-normal contractile behavior, but it is unclear whether this is caused by additional SR calcium buffering or a significant contribution of calcium entry from extracellular space, via stored-operated calcium entry (SOCE). The model enabled quantitative assessment of these two scenarios supporting the idea that SOCE has an important role in CSQ-KO contraction.

P10-3

Halothane-modulation of voltage-dependent Ca^{2+} release in malignant hyperthermia muscle fibres

Stefan Mall[1], Philipp Elischer[1], Alberto Zullo[2,3], Martin Textor[1], Andreas Alt[4], Werner Klingler[5,6], Werner Melzer[1]

[1]Institute of Applied Physiology, Ulm University, Ulm, Germany; [2]CEINGE–Biotecnologie Avanzate s.c. a r.l., Napoli, Italy; [3]Department of Sciences and Technologies, University of Sannio, Benevento, Italy; [4]Institute of Legal Medicine, Ulm University, Ulm, Germany; [5]Department of Neuroanaesthesiology, Ulm University, Günzburg, Germany; [6]Queensland University of Technology, Brisbane, Australia

Malignant hyperthermia (MH) is a fatal hypermetabolic state caused by anaesthetic-induced release of Ca^{2+} in skeletal muscle. In our experiments, Halothane, a volatile anaesthetic used in contracture testing for MH susceptibility, was applied to muscle fibres of knock-in mice heterozygous for the RyR1 MH mutation Y524S. The reaction to halothane and additional membrane polarization was investigated by intracellular Ca^{2+} recording. Fibres of the mutants showed a much stronger elevation of the baseline Ca^{2+} level than wildtype cells during application of the halothane-containing solution (0.5–3% at room temperature). To control the membrane potential, we used a two-electrode voltage clamp device. Rectangular voltage pulses from a holding potential of − 80 mV were repeatedly applied. The voltage of half-maximal activation by depolarization got shifted to more negative values. Hyperpolarizing pulses induced a rapid decrease in the steady state Ca^{2+} level. We conclude that halothane and voltage exhibit cross-influence on the Ca^{2+} release channels and a large fraction of the channels activated by halothane remains under the control of the transverse tubular voltage sensor.

P10-5

Elementary calcium release events and calcium waves in skeletal muscle fibers of the honey bee *Apis mellifera*

Claude Collet[1], Marianna Takacs[2], Laszlo Szabo[3], Laszlo Csernoch[4]

[1]Department Bees and Environment–Environmental toxicology, National Institute for Agricultural Research, Avignon, France; [2]Department of Animal Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary; [3]Department of Electrical Engineering, Sapientia Hungarian University of Transylvania, Tîrgu Mureş, Romania; [4]University of Debrecen, Faculty of Medicine, Department of Physiology, Debrecen, Hungary

Domestic and wild bees have a crucial role in vegetal biodiversity maintenance and food production, since they are amongst the most efficient pollinators. In recent years, beekeepers experienced a high level of colony mortality all around the world and this decline trend has recently been confirmed in other insect species. In this context, intense neuromuscular studies are needed to better characterize the deleterious effects of insecticides on pollinators and other useful insects. Most of approved substances are indeed targeting ion channels from nerves and muscles, e.g. those acting on the Ryanodine receptor, a channel which triggers cell contraction by releasing calcium in the cytoplasm. Electron microscopy and confocal imaging studies have shown that ultrastructural characteristics of bee skeletal muscle cells from the legs resemble those of mammals and that excitation-contraction coupling relies on calcium entry through voltage-gated calcium channels and a calcium-induced calcium release process (Collet, 2009). In the present work we characterized intracellular calcium signaling at the subcellular level.

Confocal 2D and line-scan images were taken in a physiological Tyrode's solution containing calcium, after loading of muscle fibers from 1 to 3 days old bees with the calcium indicator fluo-8 AM. Spontaneous calcium release events (CRE) were detected frequently of times, and occasionally, propagating calcium waves were observed. In fibers showing spontaneous activity, CRE's frequency was calculated to 2.20 ± 0.47 kHz/mm^2 (n = 15 fibers) from 2D image series taken at 10 Hz. Automatic images analysis program calculated the characteristic parameters of CRE. Their average spatial spread at half maximum was 3.71 ± 0.02 and 3.28 ± 0.02 μm (n = 5174 events) parallel with and perpendicular to the fiber axis, respectively. The mean amplitude of the events was 0.220 ± 0.001. They looked 'wider' and their frequency is much higher than events (sparks, embers) detected previously in cardiac myocytes, batrachian and mammalian skeletal muscle fibers. We presented first time subcellular calcium events monitored in isolated skeletal muscle cells from an arthropod. This new technique may help in understanding their role and regulation in muscles and the myotoxicity of insecticides.

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P10-6

Thick filament mechanosensing is a downstream mechanism in dual filament regulation of cardiac muscle performance

Marco Caremani[1], Francesca Pinzauti[1], Serena Governali[1], Joseph Powers[1], Massimo Reconditi[1], Theyencheri Narayanan[2], Ger Stienen[3], Marco Linari[1], Gabriella Piazzesi[1], Vincenzo Lombardi[1]

[1]Physiolab, University of Florence, Italy; [2]ESRF, The European Synchrotron, France; [3]VU University Medical Center, Amsterdam, The Netherlands

The X-ray reflections signalling the state of the thick filament in an electrically paced trabecula dissected from the ventricle of the rat heart (0.5 Hz, SL 2.15 μm, 27 °C), indicate that mechano-sensing in thick filament regulation operates also in cardiac muscle, as in skeletal muscle. During diastole most of the myosin motors lie on the surface of the thick filament packed into helical tracks in the OFF state in which they are unavailable for actin binding and ATP hydrolysis. During contraction, the myosin motors leave the OFF state in relation to the loading condition (Reconditi et al. PNAS 114, 3240–3245, 2017). Considering that in a heartbeat Ca^{2+} dependent thin filament activation is submaximal, we investigated the interdependency of the two regulatory mechanisms in electrically paced trabeculae, by recording the X-ray signals during two inotropic interventions that double the twitch force at SL 2.0 μm and external

[Ca^{2+}] 1 mM: either SL increase to 2.25 μm or addition to the solution of the β-adrenergic effector isoprenaline (10–7 M). In diastole none of the X-ray signals was affected by either intervention, indicating that thin-filament and thick-filament activation act independently and further supporting the idea of the thick-filament based regulation as an energetically well-suited downstream mechanism. Supported by Ente Cassa di Risparmio di Firenze 2016–2018.

P10-7

Early vertebrate origins and diversification of FXYDs and other small transmembrane regulators of ion transport

Sergej Pirkmajer[1], Henriette Kirchner[2], Leonidas Lundell[2], Pavel V. Zelenin[3], Juleen R. Zierath[2,4], Kira S. Makarova[5], Yuri I. Wolf[5], Alexander V. Chibalin[4]

[1]University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, SI-1000, Ljubljana, Slovenia; [2]Karolinska Institutet, Department of Physiology and Pharmacology, SE-171 77, Stockholm, Sweden; [3]Karolinska Institutet, Department of Neuroscience, SE-171 77, Stockholm, Sweden; [4]Karolinska Institutet, Department of Molecular Medicine and Surgery, SE-171 77, Stockholm, Sweden; [5]National Institutes of Health, National Center for Biotechnology Information, NLM, Bethesda, Maryland 20894, USA

Introduction: In vertebrates small transmembrane proteins, such as FXYDs (FXYD1-12), which regulate Na^{+}-K^{+}-ATPase, and phospholamban, sarcolipin, myoregulin and DWORF, which regulate sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), are fundamental to ion homeostasis in skeletal muscle and other tissues. Uncertain evolutionary history of FXYDs and regulators of SERCA led to inconsistencies in their classification across vertebrate species, thus hampering comparative studies of their functions.
Objective: To elucidate evolutionary origins and phylogenetic relationships of FXYDs and regulators of SERCA.
Methods and results: We discovered the first FXYD homologue in sea lamprey, a basal jawless vertebrate, suggesting that FXYDs predate the emergence of fishes and other jawed vertebrates. We also found that FXYDs diversified more extensively than SERCA regulators, indicating they operate under different evolutionary constraints. Furthermore, using a combination of sequence-based phylogenetic analysis and conservation of local chromosome context, we identified 13 gene subfamilies of FXYDs and propose a revised, phylogeny-based, FXYD classification that is consistent across vertebrate species.
Conclusions: Our findings provide an improved framework for investigating functions of small transmembrane regulators of ion transport in health and disease.

P10-8

Optical recordings of action potential initiation and propagation in mouse skeletal muscle fibers

Quinton Banks, Stephen Pratt, Shama Iyer, Richard Lovering, Erick O. Hernández-Ochoa, Martin F. Schneider

University of Maryland, Baltimore

Isolated skeletal muscle fibers are used to study many cellular functions. Action potential (AP) propagation has been used to assess the integrity and function of muscle. Here we use Di-8-ANEPPS, a potentiometric dye, and mag-fluo-4, a low-affinity intracellular calcium indicator, to non-invasively measure AP conduction velocity in muscle fibers. Extracellular bipolar electrodes were used to initiate an action potential at one end of the fiber. In enzymatically dissociated flexor digitorum brevis fibers, we show the strength and applicability of this method. Using high-speed line scans, we estimate the conduction velocity to be approximately 0.4 m/s. In addition, we measured the passive electrotonic potentials elicited by pulses by applying tetrodotoxin. In elevated extracellular potassium conditions, conduction velocity was significantly reduced compared to our control condition. Lastly, we made a circuit model of a muscle fiber to predict passive polarization of the fiber by the field stimuli. These predictions closely resemble our in vitro recordings. Our work shows that we can non-invasively examine how various pathologies affect AP propagation using Di-8-ANEPPS or mag-fluo-4.

Poster Session 11: Integrative Muscle Biology

P11-1

Muscle protein synthesis during early recovery from disuse atrophy: a role of stretch-activated channels in the activation of anabolic signalling

Timur Mirzoev, Sergey Tyganov, Boris Shenkman

Institute of Biomedical Problems of the Russian Academy of Sciences, Myology Lab

The study was aimed at evaluating a possible role of mechanosensors such as stretch-activated ion channels (SAC) in the anabolic response of the rat soleus muscle during early recovery from disuse atrophy. Wistar male rats were subjected to 14-day hindlimb unloading (HU) followed by 12-h reloading. In vivo blockade of SAC during recovery was performed by injection of gadolinium (Gd3+). The phosphorylation status of the key anabolic markers was assessed by WB. 12-h reloading resulted in a rapid increase in phosphorylation of GSK-3beta, p70S6K, rpS6 and 4E-BP1 ($p < 0.05$) accompanied by a trend towards enhanced PS ($p = 0.07$) vs. control. Gd3+ treatment during reloading returned p70s6k and 4E-BP1 phosphorylation to the control values and partly reduced the phosphorylation level of rpS6 and GSK-3beta. In 12-h reloading + Gd3 + group the rate of PS significantly decreased vs. 12-h reloading alone and did not differ from the baseline. We conclude that SAC may play an important role in the reloading-induced activation of muscle PS acting via mTORC1-dependent (p70S6K, 4E-BP1) and mTORC1-independent (GSK-3beta) signalling pathways. The study was supported by RFBR grant # 16-34-00530a.

P11-5

Physiological and molecular responses to high intensity interval training in flatwater kayak athletes

Apostolos Papandreou[1,2], Maria Maridaki[2], Tzanis Georgios[3], Athanasios Moustogiannis[1], Argyro Papadopetraki[1], Evangelos Zevolis[1], Michael Koutsilieris[1], Anastassios Philippou[1]

[1]Department of Physiology, Medical School, National and Kapodistrian University of Athens; [2]Faculty of Physical Education and Sport Science, National and Kapodistrian University of Athens; [3]Cardiopulmonary Exercise Testing and Rehabilitation Laboratory, Evaggelismos Hospital, Athens, Greece

Introduction: High-intensity interval training (HIIT) can improve physical condition and sports performance, while also stimulates systemic and local responses associated with skeletal muscle growth.
Purpose: This study investigated physiological, anaerobic and aerobic, systemic, and molecular adaptations of HIIT training in flatwater kayak athletes.

Method: Six male kayak paddlers followed an 8-week HIIT training program using a kayak ergometer. A VO_{2max} test and blood and deltoid muscle biopsy sampling were performed prior to and 48 hrs after the completion of the 8-week program. VO_{2max}, $PSVO_{2max}$, PSVT2, PE, HR_{peak}, and La + 2peak were measured. RT-qPCR was used to assess the mRNA expression of IGF-1Ea, IGF-1Eb, IGF-1Ec, TGF-β and VEGF-a. Plasma levels of IGF-1 and growth hormone (GH) were assayed by ELISA.
Results: HIIT protocol resulted in a significant improvement of PSVT2 ($p < 0.05$). VO_{2max}, $PSVO_{2max}$, PE, La + 2peak and HRpeak exhibited a trend of increase, however without reaching statistical significance ($p > 0.05$). Similarly, expression of IGF-1Ea, IGF-1Eb, IGF-1Ec, TGF-β and VEGF-a, and plasma levels of GH and IGF-1 showed a non-significant increase ($p > 0.05$), due to a large inter-individual variability.
Conclusion: Our findings suggest that HIIT training improves PSVT2 and probably other physiological and molecular factors related to aerobic and anaerobic capacity.

P11-6

Signaling pathways involved in the slow-to-fast myosin transition during unloading

B.S. Shenkman, N.A. Vilchinskaya, I.I. Paramonova, K.A. Sharlo

SSC RF Institute of Biomedical Problems, RAS, Myology Laboratory, Moscow, Russia

The skeletal muscle inactivity is usually accompanied with the increase of the fast myosin isoforms (fast MyHC) expression and the decrease of the slow myosin isoform (slow MyHC) expression. We have suggested that at the early stage of hindlimb unloading the AMP-activated protein kinase (AMPK) dephosphorylation through histone deacetylases (HDAC) myonuclear import facilitates the slow MyHC expression decline in rat soleus. We demonstrated that after 24 h of unloading in rat soleus muscle AMPK activator AICAR administration prevented AMPK dephosphorylation, the HDAC4 myonuclear accumulation and decrease of both slow MyHC expression. Thus the early AMPK dephosphorylation determines the decrease of slow MyHC expression through HDAC4 myonuclear import. After 7 day unloading we found that decline of slow MyHC expression is accompanied with the decreased GSK3β phosphorylation (in NO-dependent manner) after 7 day unloading. In the studies with NO content modulation and GSK3β inhibition it was observed that increased activity of dephosphorylated GSK3β during unloading mediated the decrease of slow MyHCIβ expression. The study was funded by Russian Science Foundation grant #18-15-00107.

P11-7

Role of microRNAs in endurance-exercise-induced skeletal muscle adaptation

Satoshi Oikawa[1], Minjung Lee[2], Seiji Maeda[1]

[1]University of Tsukuba; [2]The University of Tokyo

The contractile and metabolic properties of adult skeletal muscle change in response to endurance exercise. The mechanisms of transcriptional regulation in exercise-induced skeletal muscle adaptation have been investigated intensively, whereas the role of microRNA (miRNA)-mediated posttranscriptional gene regulation is less well understood. We used tamoxifen-inducible Dicer1 knockout (iDicer KO) mice to reduce the global expression of miRNAs in adult skeletal muscle, and subjected these mice to 2 weeks of voluntary wheel running. Dicer mRNA expression was completely depleted after tamoxifen injection. However, several muscle-enriched miRNAs were reduced by only 30–50% in skeletal muscle. Furthermore, an endurance-exercise-induced fast-to-slow fiber-type switch occurred normally in the fast muscle of the iDicer KO mice. Consistent with these data, protein expression of myosin heavy chain IIa was also increased in the iDicer KO mice after 2 weeks of voluntary running. These data indicate that muscle-enriched miRNAs were detectable even after 4 weeks of tamoxifen treatment and there was no apparent specific endurance-exercise-induced muscle phenotype in the iDicer KO mice.

P11-8

Endocrine responses after a single bout of moderate aerobic exercise in healthy adult humans

Maria Dourida[1], MarinelaTzanela[2], Athina Asimakopoulou[2], Efi Botoula[2], Michael Koutsilieris[1] and Anastassios Philippou[1]

[1]National and Kapodistrian University of Athens, Department of Experimental Physiology, Medical School, Athens, Greece; [2]Department of Endocrinology- Diabetes centre, “Evaggelismos” Hospital, Athens, Greece

Introduction: Exercise affects the homeostatic mechanisms of the human body, depending on the type, duration, intensity and frequency of exercise. Nevertheless, it is a stress stimulus for the human organism.
Purpose: The aim of this study was to determine the effects of an acute bout of moderate aerobic exercise on the hypothalamo-pituitary-adrenal (HPA) axis hormonal responses in healthy adult humans.
Methods: Twelve healthy male and female volunteers (age: 30.6 ± 4.4 years, body mass: 77.3 ± 12.3 kg, height: 1.77 ± 0.07 m) performed a single bout of a 30 min aerobic exercise at 70% VO_{2max} on a treadmill, following standard diet. Blood samples were collected before (t0), at the end of the exercise bout (t30), and 30 min later (t60) and serum adrenocorticotropic hormone (ACTH), cortisol (COR), aldosterone (ALDO) and renin (REN) were measured.
Results: ACTH and COR decreased immediately after exercise reaching a significant decrease 30 min after the completion of exercise compared to pre-exercise levels ($p < 0.01$). ALDO increased at the end of exercise and 30 min after its completion, however it failed to reach significance. Renin significantly increased immediately after exercise ($p < 0.05$) and remained elevated 30 min after the end of exercise.
Conclusion: This exercise regimen had beneficial effects on the stress axis thus can be recommended and prescribed for healthy and diseased populations.

Poster Session 12: Muscle Development, Regeneration and Disease

P12-3

Identification of a new alpha-sarcoglycan degradation inhibitor using high content screening to treat LGMD2D

Lucile Hoch[1], Celine Bruge[1], Sara Henriques[2], Manon Benabides[1], Isabelle Richard[2], Xavier Nissan[1]

[1]CECS, INSERM U861, I-STEM, AFM, Institute for Stem Cell Therapy and Exploration of Monogenic Diseases, 28 Rue Henri Desbruères, 91100, Corbeil-Essonnes, France; [2]INSERM, U951,

INTEGRARE research Unit, Evry, F-91002, France; Généthon, Evry, F-91002, France

Limb-girdle muscular dystrophy type 2D (LGMD2D) is a rare genetic disease characterized by a progressive proximal muscle weakness. LGMD2D is due to mutations in the gene encoding alphasarcoglycan (alpha-SG), a dystrophin-associated glycoprotein, leading to the proteasomal degradation of alpha-SG proteins through the endoplasmic reticulum quality control process (ERQC). Recent findings described the positive impact of ERQC inhibitors on alpha-SG localization to the plasma membrane opening the development of new therapeutical perspectives. Here, we report the first in vitro high-throughput screening assay monitoring the proper localization of the most frequent mutant form of alpha-SG (R77C substitution). Using this pharmacological assay, a library of 2560 FDA-approved drugs and bio-active compounds was tested, identifying a new drug candidate potentially repurposable to LGMD2D.

P12-4

Exercise prevents formation of Tubular Aggregates in ageing skeletal muscle fibers.

Claudia Pecorai, Antonio Michelucci, Laura Pietrangelo, Feliciano Protasi, and Simona Boncompagni

CeSI-MeT, Center for Research on Ageing and Translational Medicine, Univ. G. d'Annunzio, I-66100 Chieti

Background: Tubular aggregates (TAs) are clusters of ordered SR tubes found in various muscle disorders including TA myopathy (TAM), a disease linked to STIM1 or Orai1 mutations, two main players in store-operated Ca^{2+} entry (SOCE). TAs are also found in EDL fibers of aging mice where they appear positive for both STIM1 and Orai1.
Objective: Verify the functional impairment of muscles containing TAs and determine if exercise prevent their formation.
Method; Using electron microscopy and a fatiguing protocol (30 × 1 s 60 Hz pulses every 5 seconds) we studied EDL muscles from adult (4 m old) and aged mice, either control (24 m old) or exercised in wheel cages for 15 m (9–24 m of age).
Results: (i) 24 m old muscles exhibit a faster decay of contractile force than 4 m old; (ii) this force-decay is likely caused by impaired Ca^{2+} entry (suggested by experiments in Ca^{2+}-free extracellular solution); (iii) exercise reduce formation of TAs (found in 7 vs. 50% of fibers in trained vs. untrained), and rescued fatigue resistance during repetitive stimulation.
Conclusion: Exercise reduces formation of TAs and improves muscle function during repetitive stimulation, possibly improving SOCE function.

P12-5

Gα12/13 signaling plays a critical role in satellite cell quiescent maintenance and skeletal muscle regeneration

Yundong Peng[1], Shuichi Watanabe[1], Nina Wettschureck[2], Stefan Offermanns[2], Andre Schneider[1], Thomas Braun[1]

[1]Department of Cardiac Development and Remodelling, Max Planck Institute for Heart and Lung Research; [2]Department of Pharmacology, Max-Planck-Institute for Heart and Lung Research

Background: It's still unclear of the specific functions of Gα12/13 signaling in quiescent satellite cell and how it's worked in muscle regeneration process.
Objective: This study focuses on deciphering the specific functions and mechanism of Gα12/13 signaling in skeletal muscle regeneration process especially the potential roles of Gα12/13 in satellite cell quiescent maintenance.
Methods: We use the G12/13-Pax7CreErt2 mouse strain to conditional inactivate Gα12/13 in satellite cells by tamoxifen injections. The muscle injury was induced by IM injection of 50 µl of cardiotoxin(CTX).
Results: Gα12/13 cKO mice show severe dysregeneration phenotype in CTX induced muscle regeneration model. After 10 times tamoxifen injection, many of the nuclei central localized myofibers were observed in the TA muscle of Gα12/13 cKO mice and further immunofluorescence staining results showing that compared with wild-type, the skeletal muscle of the Gα12/13 cKO mice have dramatically increased number of the Pax7, Myod or Pax7, Ki67 double positive cells.
Conclusion: Gα12/13 is crucial to maintaining quiescent state of satellite cells and also very important to skeletal muscle regeneration process.

P12-6

Adipose tissue derived mesenchymal stem cells in regeneration of large damages of skeletal muscles

Iwona Grabowska, Karolina Maciejewska, Aleksandra Wojcik, Karolina Kielczyk, Maria A. Ciemerych

Department of Cytology, Faculty of Biology, University of Warsaw

In case of large injuries of skeletal muscles the pool of endogenous stem cells, i.e. satellite cells, might be not sufficient to secure proper regeneration. Such failure in reconstruction is often associated with loss of muscle mass and excessive formation of connective tissue. Therapies aiming to improve skeletal muscle regeneration and prevent fibrosis often rely on the transplantation of different types of stem cell. Among such cells are adipose derived mesenchymal stem cells (ADSCs) which are relatively easy to isolate, culture, and differentiate. Our study aimed to verify applicability of ADSCs in the therapies of severely injured skeletal muscles. We tested whether 3D structures obtained from Matrigel populated with ADSCs and transplanted to regenerating mouse Gastrocnemius muscles could improve the regeneration. In addition, ADSCs used in this study were pre-treated with various cytokines and other factors modifying their ability to differentiate and migrate. Analyses performed one week after injury allowed us to show the impact of 3D cultured control and pre-treated ADSCs at muscle mass and structure, as well as fibrosis development.

P12-7

Cardiomyoblast (H9c2) molecular responses during differentiation

Evangelos Zevolis[1], Athanasios Moustogiannis[1], Antonis Chatzigeorgiou[1,2], Michael Koutsilieris[1], Anastassios Philippou[1]

[1]Department of Experimental Physiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece; [2]Department of Clinical Pathobiochemistry and Institute for Clinical Chemistry and Laboratory Medicine, University Clinic Dresden, TUD, Germany

Introduction: H9c2 myoblasts possess the ability to differentiate into cardiomyocytes, however the molecular signature of these cells has not been fully characterized.

Objective: This study aimed at revealing molecular responses of H9c2 myoblasts during their differentiation towards a cardiomyocyte phenotype.
Methods: H9c2 cells were switched to differentiation and harvested at their day 0, 3, and 5 of differentiation. RT-qPCR was used to assess the mRNA expression of Cyclin D1, cardiac troponin T (cTnT) and of myogenic regulatory factors (MRFs; MyoD, Myogenin, MRF4), growth (IGF-1 isoforms: IGF-1Ea, IGF-1Eb), pro-apoptotic (Foxo, Fuca) and atrophy (Atrogin 1, Myostatin) factors. Connexin 43 (Cx43), cardiac actin (cACT), phospho(P)-ERK1/2 and P-Akt were also evaluated by western blot.
Results: Compared to day 0, The expression of MRFs, IGF-1 isoforms, pro-apoptotic factors, myostatin and cTnT increased in differentiating H9c2 and a down-regulation of Cyclin D1 and Atrogin 1 was observed ($p < 0.01$). P-ERK1/2 decreased while P-Akt increased ($p < 0.05$). As expected, Cx43 initially increased and subsequently decreased, and cACT was progressively detected only in differentiated cells ($p < 0.01$).
Conclusion: Our findings indicate that during the differentiation of mononucleated H9c2 cells into cardiomyotubes multiple myogenic, growth, atrophy and survival factors are modulated.

P12-9

Eccentric exercise training improves soleus muscle morphology and function of mdx mice

Paulo S. Pedrazzani[1], Tatiana O. P. Araújo[1], Emilly Sigoli[1], Isabela R. Silva[1], Dilson E. Rassier[2], Anabelle S. Cornachione[1]

[1]Department of Physiological Sciences, Federal University of Sao Carlos, Sao Paulo, Brazil; [2]Department of Kinesiology and Physical Education, Physics and Physiology, McGill University, Montreal, Canada

There are not clear guidelines regarding the benefits of exercise applied to Duchenne muscular dystrophy (DMD) patients. The aim of this study was to investigate the effects of eccentric training on muscle from mdx mice that mirrors human DMD. Male mice four weeks of age (C57Bl/10-control and-mdx) were assigned in trained and sedentary group. Single permeabilized fibers from soleus muscles were dissected and set-up in an experimental chamber for contractile measurements and maximum active force (pCa^{2+} 4.5) was measured. Histological and immunofluorescence techniques were applied to analyze the morphological alterations and changes in fiber type. Single fibers from mdx mice showed a reduction of isometric force when compared to the control group. Training increased the force, but this effect was not dependent on the group of mice. Qualitative analysis showed signals of cell degeneration in dystrophic muscles. After training, signals of regeneration were observed in the mdx mice, including nuclear centralization and reduction of connective tissue. Our results suggest that exercise is a therapeutic strategy to improve functional and morphological characteristics of dystrophic muscles.

P12-11

Isolation of specific titin RNA-binding proteins using the streptomycin-binding RNA aptamer

Sandra Swist[1], Marcus Krüger[2], Wolfgang A. Linke[3]

[1]Dept. of Systems Physiology, Ruhr University Bochum; [2]Department of Cardiac Development and Remodeling, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim; [3]Institute of Physiology II, University of Münster, Germany

Background: Alternative splicing contributes to the tissue-specific expression of the giant muscle protein titin (TTN), but there is only minor knowledge of how this tissue-specific splicing is regulated. Our goal is to identify factors involved in the splicing of Ttn exon49 solely expressed in cardiac but not in skeletal muscle cells. We hypothesize that differentially expressed RNA binding proteins regulate the tissue-specific splicing of Ttn.
Methods: The Ttn exon49 comprising pre-mRNA was attached to a streptomycin-binding aptamer, immobilized on a streptomycin affinity matrix, and incubated with either cardiac or skeletal muscle cell lysates. RNA-trapped proteins were identified by mass spectrometry and gene ontology analyses were performed.
Results and conclusions: The titin pre-mRNA captured 134 proteins specifically from cardiac and 84 proteins specifically from skeletal muscle lysates. We show that RNA binding proteins are overrepresented among these captured proteins and comprise several well-known splicing factors. Thus, our data provide a resource of candidates involved in the regulation of alternative tissue-specific Ttn splicing.

P12-12

The role of non-muscle myosin II in the angiogenesis and neuronal regeneration after stroke

Mate Penzes[1], Gabriella Pal[1], Demeter Turos[1], Csilla Homonnay[2], Gabor Szegvari[1], Arpad Dobolyi[1,2], Daniel S. Veres[3], Ildiko Horvath[3], Nikolett Hegedus[3], Domokos Mathé[4], Krisztián Szigeti[3], Miklos Z. Kellermayer[3], Mihaly Kovacs[1], Mate Gyimesi[1], András Málnási-Csizmadia[1,5]

[1]Eotvos Lorand University; [2]Hungarian Academy of Sciences; [3]Semmelweis University; [4]CROmed Translational Research Centers; [5]Optopharma Ltd.

Given the significant functions of non-muscle myosin-2 (NM2), NM2 is becoming an important therapeutic target in diseases including stroke. Stroke is a leading cause of disability and mortality. Currently no effective therapy is available. Our laboratory developed a biologically safe myosin-2 inhibitor, para-aminoblebbistatin (pAmBleb), to repair the damaged tissues through the formation of vascular and neuronal networks. For modelling stroke, transient middle cerebral artery occlusion (MCAO) was used in rats which was followed by the injection of pAmBleb to the damaged area. To study the effects of pAmBleb SPECT/CT combined with MRI was used up to 21 days after MCAO. To test whether pAmBleb contributes to brain recovery and provide an accurate evaluation of neurological function, general and focal deficits were monitored. In treated animals cerebral blood flow evaluation and MRI images revealed increased blood flow and decreased cerebral oedema within the lesion site as well as substantial behavioural improvement was observed. Our results suggest that the direct myosin-2 inhibition by the newly developed pAmBleb can contribute to the repair of brain following ischemic stroke.

P12-15

Effects of insulin on statin-induced myopathy and insulin resistance in c2c12 myotubes

Gerda M. Sanvee, Jamal Bouitbir, Stephan Krähenbühl

Clinical Pharmacology and Toxicology, University Hospital Basel

Background: Statins are effective and widely used lipid-lowering drugs. They exert several beneficial effects but are associated with myopathy.

Objectives: Goals were to characterize processes leading to simvastatin-induced myotoxicity in skeletal muscle cells and to investigate effects of insulin on the induced myopathy, on the Akt pathway and on the induction of insulin resistance.
Methods: Mouse C2C12 myotubes were treated separately or in co-treatment with simvastatin (10 μM) and insulin (10–100 ng/mL). Cytotoxicity assays, Western blots, PCRs and glucose uptake assays were performed after 24 h exposure.
Results: Simvastatin induced toxicity at 10 microM. Insulin exposure prevented and rescued the cytotoxicity, reducing it by 50% with the highest insulin concentration. Simvastatin suppressed the phosphorylation of the insulin receptor, Akt and S6rp, while upregulating atrophy genes up to 2-fold and inducing ER stress. Furthermore, simvastatin induced insulin resistance by decreasing the glucose transport rate into myotubes by half. Co-treatment with insulin was able to prevent these adverse effects.
Conclusion: This study shows the importance of IR/Akt signaling in skeletal muscle and demonstrates the positive role of insulin in the prevention of myopathy and insulin resistance associated with simvastatin.

P12-16

Absence of properly dimerizing FLNC leads to Z-disc destabilization and lesion formation in skeletal muscle fibers.

Julia Schuld[1], Zacharias Orfanos[1], Frédéric Chevessier[2], Christoph Clemen[3,4], Kathrin Markus[5], Andreas Unger[6,7], Gregor Kirfel[1], Peter F. M. van der Ven[1], Rolf Schröder[2], Dieter O. Fürst[1]

[1]Institute for Cell Biology, Department of Molecular Cell Biology, University of Bonn, Bonn, Germany; [2]Institute of Neuropathology, University Hospital Erlangen, Erlangen, Germany; [3]Institute of Biochemistry I, Medical Faculty, University of Cologne, Cologne, Germany; [4]Department of Neurology, Heimer Institute for Muscle Research, University Hospital Bergmannsheil, Ruhr-University Bochum, Bochum, Germany; [5]Medizinisches Proteom-Center, Medical Faculty Ruhr-University Bochum, Bochum, Germany; [6]Department of Cardiovascular Physiology, Ruhr University Bochum, Bochum, Germany; [7]Institute for Genetics of Heart Diseases, University Hospital Münster, Münster, Germany

Introduction: Filamin C (FLNC) is expressed in striated muscles and localizes to Z-discs, myotendinous junctions (MTJ) and intercalated discs. Mutations in FLNC cause skeletal muscle and heart disease. The mutation p.W2710X deletes the carboxyterminal 16 amino acids from FLNC, leading to its inability to dimerize properly. Humans, heterozygous for this mutation develop myofibrillar myopathy (MFM) characterized by Z disc disintegration, myofibrillar lesions and sarcoplasmic protein aggregates. Muscle fibers of patient-mimicking knock-in mice (heterozygous p.W2711X mice) show increased myofibrillar instability.
Objective: To analyze skeletal muscle function and structure in mice homozygous for this mutation.
Results: Mice homozygous for the p.W2711X mutation are viable, fertile, and show no gross morphological abnormalities. Mutant FLNC RNA and protein is expressed at lower levels, when compared to wild-type mice. Mutant FLNC is localized in MTJ with only small quantities associated with Z-discs. Instead it is located in myofibrillar lesions that were also seen, albeit less frequently, in MFM patients and heterozygous mice. Ultrastructural studies confirmed that lesions are filamentous and structured, and not amorphous aggregates.
Conclusion: In sedentary, homozygous p.W2711X mice skeletal muscles are functional. Muscle fiber assembly and function do not depend on FLNC dimerization. The presence of multiple lesions, however, indicates a role for FLNC in Z-disc stabilization.

P12-17

Molecular mechanisms of muscle dysfunction resulting from the myopathy-causing E41K mutation in the TPM2 gene

Stanislava V. Avrova[1], Yurii S. Borovikov[1], Olga E. Karpicheva[1], Armen O. Simonyan[1,2], Vladimir V. Sirenko[1], Nikita A. Rysev[1], Charles S. Redwood[3]

[1]Institute of Cytology RAS, St Petersburg, Russia; [2]Saint Petersburg State University, St Petersburg, Russia; [3]Radcliffe Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

The E41K mutation in TPM2 gene encoding muscle regulatory protein β-tropomyosin (Tpm) causes nemaline and cap myopathy. The presence of the E41K-Tpm in muscle fibres results in reduced myofilament Ca^{2+}-sensitivity and muscle weakness. To understand the structural basis of these changes, we labeled Tpm, actin and myosin subfragment-1 by fluorescent probes and incorporated the proteins into ghost muscle fibre. The multistep changes in spatial arrangement of the proteins were studied at various stages of the ATPase cycle in reconstituted ghost fibres using the polarized fluorescence microscopy. The E41K mutation inhibits troponin's ability to shift Tpm to the closed position at high Ca^{2+}, thus restraining the transition of the thin filaments from Off to On state and contributing to the low Ca^{2+}-sensitivity. The E41K mutation inhibits the ability of S1 to shift Tpm towards the open position. As a result, the amount of the myosin heads bound strongly to actin decreases at high Ca^{2+} and increases at low Ca^{2+}, which may contribute to muscle weakness. As the mutation has no effect on troponin's ability to switch actin monomers on at high Ca^{2+} and inhibits their switching off at low Ca^{2+}, the use of reagents that increase the Ca^{2+}-sensitivity of troponin may not be appropriate to restore muscle function in patients with the E41K mutation. The work is supported by Russian Science Foundation (No 17-14-01224).

P12-18

Destabilization of blocked functional state of thin filaments by cap myopathy-causing mutation Glu150Ala in TPM3 gene

Olga E. Karpicheva[1], Armen O. Simonyan[1,2], Elena A. Rogozovets[1], Charles S. Redwood[3], Yurii S. Borovikov[1]

[1]Institute of Cytology RAS, St Petersburg, Russia; [2]Saint Petersburg State University, St. Petersburg, Russia; [3]Radcliffe Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

The elucidation of how the mutations in contractile proteins associated with various congenital myopathies affect the mechanisms of muscle contraction is necessary for early diagnosis of diseases and the development of therapeutic approaches to their treatment. The purpose of this work was to study the effect of the Glu150Ala substitution in tropomyosin (Tpm3.12) identified in cap myopathy on the molecular mechanisms of regulation of actin-myosin interaction by troponin and Tpm in the ATP hydrolysis cycle. Using polarized fluorescence microscopy, we showed that the mutant Tpm is located much closer to the centre of the thin filaments at low Ca^{2+} than the wild-type Tpm, and induces switching-on of actin monomers. Such localization of Tpm partially opens the sites on actin for interaction with the myosin heads and stimulates the formation of force-generating cross-bridges in blocked functional state of thin filaments. This

is indicative of the high Ca^{2+}-sensitivity of the thin filaments in the presence of the mutant Tpm. An abnormal rise in the proportion of the strong-binding myosin heads in the ATP hydrolysis cycle, along with an increase in the Ca^{2+}-sensitivity of thin filaments, may be one of the reasons for contractures and muscle weakness observed in muscle fibres containing the Glu150Ala-mutant Tpm. This work is supported by the Russian Science Foundation (grant no 17-14-01224).

P12-19

Improper thin filament activation by γ-tropomyosin with the Arg90Pro mutation associated with congenital fibre type disproportion

Elena A. Rogozovets[1], Armen O. Simonyan[1,2], Vladimir V. Sirenko[1], Charles S. Redwood[3], Yurii S. Borovikov[1], Olga E. Karpicheva[1]

[1]Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia; [2]Saint Petersburg State University, St. Petersburg, Russia; [3]Radcliffe Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

Over 50 mutations in skeletal muscle tropomyosin (Tpm) were identified to be associated with congenital myopathies. The primary effects of these mutations can be conditionally divided into loss- and gain-of-function, but the result in both cases is a disturbance in the mechanisms of regulation of muscle contraction by troponin-Tpm and muscle weakness. Type 1 hypotrophy of more than 12% of the muscle fibres in the absence of any structural anomalies is a diagnostic feature for congenital fibre type disproportion (CFTD). However, the molecular mechanisms of CFTD vary with different mutations in Tpm. The aim of this work is to study the effect of the Arg90Pro mutation in Tpm3.12 identified in CFTD on the spatial organisation of actin, myosin and Tpm in ghost muscle fibre using fluorescent probes. Although the Arg90-Arg91 residues are involved in the interaction with actin, the mutant Tpm retains the normal ability to incorporate into the thin filaments of muscle fibre. The mutant Tpm does not occupy an open position, at low Ca^{2+} markedly inhibits the switching actin monomers off and increases the number of myosin heads strongly bound with actin, and induces a high Ca^{2+}-sensitivity of thin filaments. It is concluded that the cause of muscle weakness with the Arg90Pro mutation in Tpm may be an incorrect activation of the thin filament. The work was supported by the Russian Science Foundation (no 17-14-01224).

P12-20

Quantitative analysis of sialyltransferase expressions in mouse skeletal muscle by real time RT-PCR

Katerina Gencheva, Svetlozara Petkova, Ani Georgieva, Ivan Iliev, Russy Russev, Dimitar Ivanov, Valeria Dilcheva, Ivelin Vladov

Institute of Experimental Morphology, Pathology and Anthropology with Museum—BAS

A great part of the congenital muscular diseases are due to mutations of glycosyltransferases and several recent studies on myopathies brought to a conclusion that skeletal muscles are sensitive to losses of sialic acids. We still have very little information about the substrates of the mutated glycosyltransferases, because of the limited knowledge about the glycoproteome of the skeletal muscles, especially about the expressions of sialylated glycoproteins.

With this work we investigated the expressions of mRNAs of eleven sialyltransferases from different families in mouse skeletal muscles.

After isolation of RNA, cDNA was synthesized and real time PCR was performed. We found that the enzymes ST6GalNAc1 and ST6Gal2 are not expressed in healthy mouse skeletal tissue. The enzymes ST3Gal1, -2, -3, -4, -6, ST6Gal1 and ST6GalNAc2, -3 and -4 are expressed in this tissue.

The investigated enzymes have high specificity towards their oligosaccharide substrates. The obtained results will allow us to predict the carbohydrate compositions of the glycoproteome in healthy skeletal muscles and can help molecular studies in skeletal muscle pathology.

P12-21

The muscle phase of trichinellosis is associated with up-regulation of the enzyme ST6GalNAc1

Rositsa Milcheva, Svetlozara Petkova, Ani Georgieva, Ivan Iliev, Russy Russev, Dimitar Ivanov, Valeria Dilcheva, Ivelin Vladov

Institute of Experimental Morphology, Pathology and Anthropology with Museum—BAS

We previously showed that the de-differentiation of the occupied portion of muscle fibers toward Nurse cell after invasion by Trichinella spiralis is associated with increased intracellular accumulation of α-2,6-sialylated glycoproteins. The aim of this work was to analyze α-2,6-sialyltransferase expressions in mouse skeletal muscles invaded by T. spiralis. Muscle samples were collected at days 0, 10, 14, 18, 2 and 35 after invasion. Expressions of mRNA of α-2,6-sialyltransferases were analyzed by real time PCR. Immunohistochemistry was performed using rabbit polyclonal antibody against ST6GalNAc1 sialyltransferase. We found strong up-regulation of the enzyme ST6GalNac1 at day 14 after invasion that faded within the transformation of the occupied area into a Nurse cell. The enzyme ST6GalNAc1 is not synthesized in healthy mouse muscle tissue and is rarely expressed in normal tissues. It is responsible for the formation of the cancer-associated sialyl-Tn antigen in variety of carcinomas, blocking regular carbohydrate chain elongation. The functional role of this enzyme for the Nurse cell formation of T. spiralis in muscles remain to be elucidated.

P12-23

Drosophila model of myosin myopathy rescued by overexpression of a TRIM-protein family member

Martin Dahl-Halvarsson[1], Montse Olive[2], Malgorzata Pokrzywa[1], Ruth Palmer[3], Katarina Ejeskär[4], Anne Uv[3], Homa Tajsharghi[4]

[1]Department of Pathology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden; [2]Institute of Neuropathology, Department of Pathology and Neuromuscular Unit, Department of Neurology, IDIBELL-Hospital de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain; [3]Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden; [4]Translational Medicine, School of Health and Education, University of Skövde, Skövde, Sweden

Myosin is a molecular motor indispensable for body movement and heart contractility. Apart from pure cardiomyopathy, mutations in MYH7 encoding slow/ß-cardiac myosin heavy chain can also cause skeletal muscle disease with or without cardiac involvement. Mutations mainly residing within α-helical rod domain of MYH7 are associated with Laing distal myopathy. We developed a Drosophila

melanogaster model of Laing distal myopathy in order to investigate the pathobiological mechanisms of the recurrent causative mutation (K1729del MYH7). Homozygous MhcK1728del animals die during larval or pupal stages and both homozygous and heterozygous larvae display reduced muscle function. Heterozygous MhcK1728del adult flies and flies with exclusive expression of MhcK1728del in indirect flight and jump muscles were flightless and exhibited reduced jump abilities and a declined lifespan. The sarcomere of mutant indirect flight muscles and larval body wall muscles was disrupted with disorganised muscle filaments. Larvae homozygous, but not heterozygous, for MhcK1728del also demonstrated structural and functional impairments of heart muscle, indicating a dosage effect of the mutated allele. The phenotypes associated with MhcK1728del, including reduced jump ability, lack of flight ability and the myopathy of indirect flight and leg muscles, were fully suppressed by overexpression of Abba/Thin, which has an essential role in maintaining sarcomere integrity. The data shows the first Laing distal myopathy model in Drosophila, recapitulating certain morphological phenotypic features seen in Laing distal myopathy patients with the recurrent Lys1729del mutation, which was rescued by Abba/Thin overexpression. The findings may warrant immediate clinical and molecular genetic investigation for diagnosis and possible therapeutic intervention.

P12-24

Engineering evolution: tetraploidization of human cardiac stem cells to enhance functional activity

K. M. Broughton[1], D. Yaareb[1], C. Esquer[1], R. Adamson[2], W. Dembitsky[2], G. Pompilio[3], M. A. Sussman[1]

[1]San Diego State University Heart Institute and the Integrated Regenerative Research Institute, San Diego, CA, USA; [2]Sharp Hospital, Division of Cardiology, San Diego, CA, USA; [3]University of Milan, Monzino IRCCS Cardiological Center, Milan, Italy

Reparative and regenerative capacity is consistently observed in lower vertebrates, but clinical implementation fails to yield comparable results. Rodent cardiac stem cells (CSC) possess 4n DNA capacity, but comparable human 4n CSCs are not present. Since polyploidization correlates with enhanced regenerative capacity, ploidy raise questions regarding translational applicability of myocardial regeneration. We hypothesize mononuclear chromatin duplication in human stem cells improves functional capabilities by inhibiting senescence and increasing stress resistance. Tetraploidy was induced in human CSCs, EPCs and MSCs from multiple patient samples. Mononuclear 4n content was consistently induced and stable in hCSCs and hEPCs, unlike hMSCs which underwent apoptosis. Tetraploid hCPCs escape senescence-related cell cycle arrest unlike 2n hCPCs, although doubling time was similar. H2O2 stress induction demonstrate 4n hCSCs respond better than 2n hCSCs. Double strand DNA breaks in 4n hCSCs were reduced by half compared to 2n hCSCs, measured by gamma H2AX per total DNA. Ongoing studies focus upon potential of 4n hCSCs to enhance capability to mediate myocardial repair and regeneration.

P12-26

A pilot physiological study on a novel muscle myopathy in broiler pectoralis major muscle

Jiao Liu[1,2], Eero Puolanne[3], Anders Arner[1,2]

[1]Lund University, Dept Thoracic Surgery, Lund, Sweden; [2]Karolinska Institutet, Dept Physiology and Pharmacology, Stockholm, Sweden; [3]University of Helsinki, Dept of Food and Nutrition Sciences, Helsinki, Finland

Poultry meat from broilers is increasingly consumed today. In order to produce the meat at low costs, broilers have been bred to grow muscle, especially breast muscle pectoralis major, in shorter time with heavier weight. However, a newly described condition, the “woody breast syndrome”, has been identified within the rapidly growing broiler lines all around the world with an incidence rate around 20%. Intact affected muscle exhibits abnormal hardness by palpation after being excised from slaughtered animal carcass. Previous work has reported degeneration of muscle fibres, edema and fibrosis in the breast muscle and changes in biochemical profile of “woody breast”. In the present study we analyzed the woody breast condition from a muscle physiology perspective. The aim was to examine the mechanical function of affected muscles and the structure of contractile system. Woody breast (WB) and normal breast (NB) were identified in the slaughter house 30 min post mortem. Histology of WB revealed a pathological myopathy with rounded swollen fibres, fibrosis, centralized nuclei and degenerated fibre profiles. Single skinned muscle fibers were analyzed. Length force curves showed no differences in maximum active stress and in optimal sarcomere length. However, WB fibres had less steep descending arm of the length active force relationship compared to NB and were slightly more compliant at large stretches. The results showed muscle hypertrophy (increased fibre diameters) in WB with a contribution of swollen sarcomeres (determined by small angle x-ray diffraction). In summary, the rapid growth of the particular poultry strain is associated with significant structural changes in the pectoralis muscle, with an increase in cell and sarcomere unit cell diameter.

P12-27

LMNA G232E and R482Q mutations prevent progression from fetal developmental program to adult skeletal muscle differentiation in vitro

Natalia Khromova, Ksenia Perepelina, Anna Kostareva

National Almazov Medical Research Centre, Institute of Molecular Biology and Genetics. Saint-Petersburg, Russia

Background: Lamin A/C provides mechanical steadiness to the nucleus and regulates genetic machinery. Mutations in the LMNA gene cause of laminopathies, which include tissue-specific dystrophies affecting different types of tissues. Different localizations of LMNA mutations result in different pathological phenotype. R482Q mutation in the LMNA gene lead to Familial partial lipodystrophy, Dunnigan variety (FPLD). The clinical features of this disorder are muscle hypertrophy, severe myalgia of the lower extremities on exercise and at rest, generalized lipodystrophy sparing the face and neck; G232E mutation in the LMNA gene associated with the development of Emery–Dreyfus muscular dystrophy. Molecular mechanisms involved in development of laminopathies remain unclear.

Purpose: The aim of this work was to uncover molecular markers, and regulators of skeletal muscle degeneration in laminopathy associated with progressive muscle wasting.

Methods and study design: We used in vitro differentiation model based on C2C12 mouse myoblasts. C2C12 cells were transduced with lentiviruses bearing either LMNA WT encoding wild type lamin A/C, lentivirus bearing LMNA G232E mutation or lentivirus bearing LMNA R482L. mutation. Confluent C2C12 cells were induced to differentiate with two types of media: pro-myogenic stimulation and mixed stimulation (combined pro-myogenic and pro-adipogenic stimulation). mRNA samples were collected at day 0, day 2 and 7 after stimulation of differentiation for expression analysis. The

expression of genes that control muscle metabolism and development was analyzed: MYH3, MYF6, FABP4, ATGL, Desmin, Myomarker was analyzed. Efficiency of transduction controlled by immunostaining with antibody specific for hLMNA, lipids accumulation was detected by OilRed staining, muscle differentiation was visualized by MHC immunostaining.
Results: Morphology of myotubes formed by C2C12 cells bearing G232E or R482Q mutations or WT or LMNA and treated with promyogenic and mixed differentiation media differ significantly in their morphology: C2C12 cells bearing LMNA R482Q mutation differentiated into hypertrophied myotubes; C2C12 cells bearing both LMNA G232E and R482Q mutations, demonstrated alterations in regulation of intracellular lipid droplets development and metabolism. Furthermore, both types of mutations promoted expression of embryonic (MYH3) and neonatal (MYH8) myosins expression, but not adult myosins (MYH1, MYH4, MYH7).
Conclusions: We created an experimental model to study molecular mechanisms of R482Q mutation in LMNA gene—related disorders in lipid droplets metabolism, and muscle hypertrophy. We have also demonstrated that muscle wasting and degeneration associated with LMNA mutation G232E can be related to defects in formation, maintenance and metabolism of lipid droplets in adipose tissue and ectopic lipid depots in muscle. Both mutations prevented progression from fetal muscle development program to adult skeletal muscle differentiation in vitro.

P12-28

Effect of cardiomyoblast secretome, with and without mechanical preconditioning, on hypoxia reoxygenation injury

Argyro Papadopetraki[1], Evangelos Zevolis[1], Athanasios Moustogiannis[1], Antonios Chatzigeorgiou[1,2], Michael Koutsilieris[1], Anastassios Philippou[1]

[1]National and Kapodistrian University of Athens, Medical School, Department of Physiology; [2]Technische Universität Dresden, Faculty of Medicine, Institute for Clinical Chemistry and Laboratory Medicine, Department of Clinical Pathobiochemistry

Introduction: Reperfusion after myocardial infarction (MI) can worsen cardiac tissue damage and in vitro models of hypoxia/reoxygenation (H/R) have been developed to simulate the in vivo ischemia/reperfusion injury.
Purpose: The present study investigated the cardiomyoblast paracrine effects in H/R, by treating them with the conditioned media of mechanically loaded or unloaded cells, in vitro.
Method: H9C2 cardiomyoblasts underwent a cyclic stretching (Flexcell tension system) and then their conditioned media (secretome) was collected (stretch media, SM). Conditioned media of unstretched H9C2 was also collected (non-stretch media, NSM). H9C2 were subjected to 6 h hypoxia followed by 8 h reoxygenation (H/R) while during R, they were treated either with SM or NSM. Cell apoptosis was subsequently assessed by flow cytometry (Annexin V/PI).
Results: The percentage of early apoptotic cells decreased significantly in the SM- and NSM-treated cells compared with the non-treated cells ($p < 0.01$). No significant differences ($p > 0.05$) were found between the three groups in the total number of early and late apoptotic cells. Live cells exhibited a trend of increase in the SM and NSM groups ($p > 0.05$).
Conclusion: Our findings suggest that H9C2 secretome is able to delay but not inhibit their apoptosis after H/R injury, independently of their mechanical load “preconditioning”.

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The effects of muscle cell aging on myogenesis

Athanasios Moustogiannis[1], Orjona Taso[1,2], Evangelos Zevolis[1], Antonios Chatzigeorgiou[1,3], Michael Koutsilieris[1], Anastasios Philippou[1]

[1]National and Kapodistrian University of Athens, Medical School, Department of Physiology; [2]University College London, Department of Neuroscience Physiology and Pharmacology, Division of Biosciences, London, United Kingdom; [3]Technische Universität Dresden, Faculty of Medicine, Institute for Clinical Chemistry and Laboratory Medicine, Department of Clinical Pathobiochemistry

Introduction: The process of myogenesis gradually deteriorates as skeletal muscle ages. However, less is known about the molecular responses of aged muscle cells during myogenesis.
Objective: This study investigated the effect of cell senescence on myoblast differentiation, using an in vitro model of myoblast aging.
Methods: C2C12 myoblasts were continuously cultured for 50 days, reaching passage 36 (aged myoblasts) while myoblasts passage 14 were used as controls. SA-β-gal activity was used as marker of cell ageing. Cells were switched into differentiation medium and harvested at their day 0, 2, 6 and 10 of differentiation. qRT-PCR was utilized to measure changes in expression levels of the myogenic regulatory factors (MRFs; MyoD, Myogenin, MRF4), growth (IGF-1 isoforms: IGF-1Ea, IGF-1Eb), apoptotic (Foxo, Fuca), atrophy (Murf1, Atrogin, Myostatin) and inflammatory (IL-6) factors.
Results: Compared to controls, aged differentiating myoblasts exhibited increased activity of SA-β-gal and reduced expression of MRFs and IGF-1 isoforms ($p < 0.01$), along with increased expression of the apoptotic, atrophy and inflammatory factors ($p < 0.05$–0.01).
Conclusion: A diminished differentiation capacity characterizes the aged myoblasts, which in combination with the induction of apoptotic and atrophy factors, indicates a disrupted myogenic process in senescent muscle cells.

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Molecular mechanisms underlying maintenance of the quiescent state of muscle satellite cell

So-ichiro Fukada

Project for Muscle Stem Cell Biology, Graduate School of Pharmaceutical Sciences, Osaka University

Muscle stem cells named satellite cells are indispensable for skeletal muscle growth and regeneration. In a steady condition, muscle satellite cells are sustained in a mitotically quiescent state with low energy production and RNA contents. For maintaining this dormant state, some signaling pathways actively function to inhibit the activation and differentiation in muscle satellite cells. We have focused on two pathways, canonical Notch and Calcitonin receptor, in the quiescent muscle satellite cells. Canonical Notch pathway maintains MuSCs in the undifferentiated state, and Calcitonin receptor pathway maintains MuSCs in the quiescent state. However, these downstream molecules are still unclear. Here, I would like to present the potential downstream molecules of Notch or Calcitonin for maintaining undifferentiated or quiescent state in muscle satellite cells.

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Skeletal muscle contractile properties in experimental autoimmune encephalomyelitis mice

Jan Spaas, Pieter Van Noten, Charly Keytsman, Bert O. Eijnde

Hasselt University (REVAL Rehabilitation Research Center, BIOMED Biomedical Research Institute)

Background: Neurological disorders such as multiple sclerosis (MS) can affect skeletal muscle properties.
Purpose: To investigate muscle mass and in vitro contractile properties of fast and slow hindlimb skeletal muscles in experimental autoimmune encephalomyelitis (EAE), an animal MS model.
Method: Intact m. soleus (SOL) and m. extensor digitorum longus (EDL) from 10 EAE mice (18 d after immunization) and 10 healthy mice underwent electrical stimulation to assess twitch and tetanic force, force-frequency relation, and muscle fatigue (10 min) and recovery.
Results: EAE mice were classified as mild (n = 6) and severe (n = 4). Severe EAE reduced EDL muscle weight (− 30%, $p < 0.01$) and cross sectional area (CSA, − 26%, $p < 0.05$), SOL tetanic force (− 34%, $p < 0.01$), and EDL twitch force (− 30%, $p < 0.05$), tetanic force (− 45%, $p < 0.001$) and force/CSA (− 30%, $p < 0.05$). Submaximal force (< 25 to 50 Hz) was preserved and the force-frequency curve shifted leftwards in severe EAE. No differences were found during fatigue, whereas force recovery (normalized to initial force) was faster in severe EAE.
Conclusion: Severe, but not mild, EAE affects skeletal muscle properties, which is more pronounced in fast (EDL) muscle tissue.

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An optimized protocol for the differentiation and analysis of fiber types from murine muscles by laser microdissection and mass spectrometry

Britta Eggers[1], Katalin Barkovits[1], Malte Sielaff[2], Julian Uszkoreit[1], Stefan Tenzer[2], Rolf Schröder[3], Christoph Clemen[4], Katrin Marcus[1]

[1]Ruhr-Universität Bochum, Medizinisches Proteom-Center; [2]Universitätszmedizin Mainz, Institut für Immunologie; [3]Universitätsklinikum Erlangen, Institut für Neuropathologie; [4]Universitätsklinikum Bergmannsheil, Neurologie

Background: Muscle tissue is comprised of different fiber types, type I, IIa, IIB and IIx fibers. The fiber type composition is highly variable between different muscle groups and can change due to aging or training. These shifts can also be observed in several neuromuscular diseases and could play a role in disease progression. Utilization of laser microdissection (LMD) combined with mass spectrometry (MS) enables an accurate subclass specific analysis.
Methods: LMD samples from murine muscles were either used to establish an optimized workflow for proteomic analysis including: (1) sample lysis; (2) tryptic digestion (3) peptide separation, or fiber type differentiation by fluorescent staining. The optimized protocols were further used for the analysis of different fiber types (type I and type II) in male and female mice.
Results: All fiber types could be clearly distinguished by specific antibody staining. For LMD generated samples lysis with formic acid followed by an in solution digest was the most effective method identifying more than 4500 peptides. The analysis of different fiber types of male and female mice, identified several differential expressed proteins between the two sexes.
Conclusions: We established a fast, simple and cost effective workflow for the differentiation of fiber types and their label free quantitative analysis by LMD-MS and could by that identify gender specific differences in type I and II fibers.

P12-34

Extraocular muscles in desmin knockout mice

Maria Angels Rodriguez[1], Kimmo Parkkonen[2], Jing-Xia Liu[2], Zhenlin Li[3], Fatima Pedrosa Domellöf[1,2]

[1]Dept. of Clinical Sciences, Ophthalmology, Umeå University, Umeå, Sweden; [2]Dept. of Integrative Medical Biology, Anatomy, Umeå University, Umeå, Sweden; [3]Sorbonne Université-CNRS UMR 8256-ISERM U1164, Paris, France

Extraocular muscles (EOMs) are defined as a separate muscle class that shows a unique gene expression profile and different properties at structural, cellular and molecular level. One of most remarkable characteristics is their response to disease. EOMs are selectively spared in several muscular dystrophies that severely affects other muscle types, however data on the EOM in desminopathies are lacking.

We investigated the effect of the lack of desmin on the morphology and mitochondria of the EOMs using histochemical methods. We also investigated the distribution of important cytoskeletal desmin-binding proteins (synemin, syncoilin, plectin and nestin) in EOMs lacking desmin.

The structure of the EOMs in desmin−/− mice was remarkably unaffected with no signs of muscular pathology in contrast to limb muscles. Staining for mitochondrial enzyme succinate dehydrogenase demonstrated abnormal subsarcolemmal accumulation of mitochondria.

The distribution and staining intensity of synemin, syncoilin and plectin in EOM of desmin−/− mice were comparable with that observed in controls and no nestin labeling was found in control or desmin−/− EOMs

In summary, in spite of mitochondrial alterations on EOMs lacking desmin, no alterations in synemin, syncoilin, plectin and nestin labeling patterns were detected and, in contrast to other skeletal muscles, the EOMs appear well preserved in the absence of desmin.

P12-35

Connexin-based hemichannels mediate the skeletal muscle damage induced by dysferlin deficiency

Gabriela Fernández[1], Jorge A. Bevilacqua[2], Juan C. Sáez[3], Pablo Caviedes[4], Luis A. Cea[1]

[1]Instituto de Ciencias Biomédicas, Facultad de Ciencias de la salud, Universidad Autónoma de Chile; [2]Programa de Anatomía y Biología del desarrollo, Facultad de Medicina, Universidad de Chile; [3]Departamento ed Fisiología, Pontificia Universidad Católica de Chile; [4]Programa de Farmacología Molecular y Clínica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile

Dysferlinopathy, is a genetic disease caused by mutations in the gene encode to dysferlin, a sarcolemma protein that participates in membrane repairing. The onset is around third decade of life, as a progressive lower-limb weakness. In dysferlin-deficient (DD) mice (model of dysferlinopathies) the recovery of membrane resealing function by expression of a mini-dysferlin does not arrest progressive muscular damage, suggesting the presence of dysferlin-dependent pathogenic mechanisms unknown. In this regard, we demonstrated a persistent expression of functional connexin-based hemichannels (Cx HCs) in pathological conditions that affect skeletal muscles. Such membrane channels are permeable to Ca^{2+} and critically contribute to muscular damage. DD myofibers also showed positive immunostaining for Cxs 39, 43 and 45 and elevated Cx HCs activity concomitant with elevated resting intracellular free Ca^{2+} level compared with wt myofibers. Also, it was detected a lower performance of

DD mice in rotarod type exercise compared to wt mice. All these changes were prevented in DD mice deficient in Cx43 and 45, suggesting that Cxs HCs are responsible of the pathogenic mechanism. In addition, the presence of Cxs 40.1, 43 and 45 was detected in human muscle biopsies from Chilean dysferlinopathy patients. Therefore, Cx HCs could be good candidate as a new therapeutic target.

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MARP1 is a negative regulator of passive stretch-induced hypertrophy

Robbert van der Pijl[1,2], Shenyi Shen[1], Stefan Conijn[2], Johan Lindqvist[1], Joshua Strom[1], Siegfried Labeit[3], Henk Granzier[1], and Coen Ottenheijm[1,2]

[1]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, United States; [2]Dept of Physiology, VU University Medical Center, Amsterdam, The Netherlands; [3]Department of Integrative Pathophysiology, Medical Faculty Mannheim, Mannheim, Germany

Background: MARP1 is a stress-induced protein involved in trophic signaling. Though extensively studied in the heart, MARP1's function in skeletal muscle has remained elusive. Here, we studied the role of MARP1 in skeletal muscle hypertrophy.
Methodology and results: We used unilateral diaphragm denervation (UDD), a model associated with stretch-induced hypertrophy of the denervated hemidiaphragm, and a 320-fold increase of MARP1 protein, that primarily localizes to the titin N2A-segment, without nuclear localization.
To study the role of MARP1 in stretch-induced hypertrophy, we used a knock-out mouse model for the Ankrd1 gene (MARP1 KO). Applying UDD to MARP1 KO mice, we found that that hypertrophy is increased in the absence of MARP1 (6 d after UDD: 56 ± 4% tissue mass increase vs. 43 ± 3% in WT mice).

To determine how MARP1 affects hypertrophy, we first performed pull-down assays with MARP1. Subsequent mass spectrometry on the pulled-down proteins revealed that MARP1 interacts with various members of the Hsp70-family, tropomyosin (Tpm1 and -3) and Casq1.

Working hypothesis: MARP1 is a negative regulator of passive stretch-induced hypertrophy by one of two (or both) mechanisms:

1. Altering localization/activation of trophic signaling proteins through competitive binding to titin's N2A segment;
2. Altering the compliance of titin, thereby affecting its mechanosensory activity.

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Pathological changes in muscle signaling mechanisms in muscle contractures of children with cerebral palsy

Jessica Pingel

[1]Department of Neuroscience, University of Copenhagen, Copenhagen N, Denmark; [2]Section of Forensic Genetics, Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark; [3]Department of Orthopedic Surgery, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark

Background: Cerebral palsy (CP) is a non-progressive motor disorder that affects the posture and gait of the patients. Aim: The aim of the present study was to elaborate whether specific signaling pathways in the muscle are correlated to increased passive stiffness and reflex activity in CP.
Methods: Next Generation Sequencing of 92 candidate targets was performed in muscle biopsies from the m. Gastrochnemius muscle. In addition, stretch reflexes and passive stiffness was measured in the lower legs.
Results: Passive stiffness was significantly correlated to mRNA expressions of HSPG2 ($p = 0.02$), PRELP ($p = 0.002$), RYR3 ($p = 0.04$), COL5A3 ($p = 0.0007$), ASPH ($p = 0.002$) and COL4A6 ($p = 0.03$). In addition the reflex activity was significantly correlated to mRNA expressions of HSPG2 ($p = 0.02$), LAMc1 ($p = 0.04$) and COL4A6 ($p = 0.05$). Subsequently children with CP showed more pathogenic variants in the mitochondria especially in COII, COIII and ATPase6.
Conclusion: The present study shows that some of the investigated targets potentially might be associated to the severity of passive stiffness and reflex activity in cerebral palsy.

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IGF-I modifies microRNA expression and release in rat skeletal myoblasts during differentiation

T. Domoradzki, A. Majewska, M. Milewska, M. Błaszczyk, A. Chojak, K. Grzelkowska-Kowalczyk

Warsaw University of Life Sciences (SGGW), Faculty of Veterinary Medicine, Department of Physiological Sciences, Warsaw, Poland

Background: MicroRNAs modulate gene expression post-transcriptionally and, when released from cells, provide humoral signals for tissue homeostasis.
The purpose of the study was to examine effects of IGF-I on miRNA expression and secretion in rat skeletal primary myoblasts.
Method: Microarray and qPCR analyses were used to assess and validate miRNA profiles in myoblasts, subjected to 11-day differentiation with IGF-I (25 nmol/l). Student t-test was used for comparison the results vs control.
Results: Microarray analysis revealed increased expression of 45 miRNAs and decreased expression of 20 miRNAs in myoblasts treated with IGF-I. Ten secreted miRNAs were increased and 3 miRNAs were decreased by presence of IGF-I. PCR analysis confirmed increased expression and secretion of miR-100 (fold change, FC, values: 1.78 and 1.21, respectively), miR-107 (FCs: 5.14 and 3.59), and decreased expression of miR-21 (FC: 0.6). Expression of miR-322 was increased (FC: 1.65), but its secretion was decreased (FC: 0.41) by IGF-I.
Conclusion: IGF-I, an anabolic factor for skeletal muscle, can modulate micro-environment of differentiating myoblasts through the effects on miRNA expression and secretion.

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