EMC 2025

52nd European Muscle Conference

20-24th September 2025

Amsterdam

Programme Book

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Website: https://www.curibio.com/

At Optics11 Life we develop the Cuore, a non-destructive contractility platform that monitors 3D muscle-bundle dynamics, and a suite of mechanobiology tools, including high-precision, high-throughput instruments for cell-to-tissue nanoindentation. Together, these instruments quantify stiffness, viscoelasticity, and force generation under near-physiological conditions, giving researchers fast, actionable data for advanced disease modelling, drug screening and biomaterial quality control. Our products are used across diverse application areas, including cardiology, muscle diseases, fibrosis, oncology, and regenerative medicine.

Website: www.optics11life.com

IonOptix develops and manufactures cardiovascular data acquisition and analysis systems. Our new Cardiac Slice System allows simultaneous calcium and force measurements, mechanical work loop data, and characterization of tissue elastance/compliance. For higher throughput single cell studies, our MultiCell System features rapid stage movement and scanning speed, mark-and-find functionality for repeated measures of myocytes, and our CytoSolver batch analysis of high-content data. And our new CytoMotion algorithm expands our functional recordings to include high-speed, real-time iPSC-CM contractility data. Website: https://www.ionoptix.com/

IonOptix Myocyte Workshop

An in-person, free workshop will be held after the European Muscle Congress, offering live demonstrations on acquiring functional data from iPSC-derived, skeletal, and adult cardiac myocytes, as well as myocardial slices and (if available) myofibrils. Key topics include high-throughput myocyte measurements (IonOptix MultiCell), myocardial slice mechanics (Cardiac Slice System), and loaded myofibril recordings (MyoStretcher). The workshop will take place on Wednesday afternoon 24th September in the O|2 lab building (Amsterdam), hosted by the Department of Physiology, Amsterdam UMC. Spaces are limited and advance registration is required, via this link https://www.ionoptix.com/myocyte-workshop/ or info@ionoptix.com

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WELCOME

Dear friends and colleagues, dear muscle aficionados,

Welcome to the Royal Tropical Institute (KIT), welcome to Amsterdam!

We are happy and honored to welcome you to the 52nd European Muscle Conference. Thanks to the scientific programme committee and to your scientific contributions we were able to put together an exciting program, covering a broad range of muscle topics.

We are proud that, in the tradition of the EMC, this year's conference hosts many young enthusiastic researchers. To further expand their scientific network, before the official opening of the conference, the Early Career Association kicks off with the symposium for young researchers (O2 building, Amsterdam UMC). Next the conference will officially open at KIT with the Keynote Lecture by Professor Leslie Leinwand. The Jean Hanson Lecture will be given on September 23rd by Professor Jolanda van der Velden. The conference will end on September 24th with the award ceremony.

We thank the KIT institute for hosting us this EMC. The KIT is an independent knowledge institute – focusing on programmes improving global health, gender and sustainable economic development -, located in the Oosterpark, the heart of the vibrant Amsterdam-East area. We hope that this rich environment will stimulate creativity and exchange of ideas!

The social dinner will be held at the Vergulden Eenhoorn, a state monument dating back to 1702 and will feature DJ Fiësto, an Amsterdam legend. The canal boat tour will start in front of the KIT Institute and will reveal our beautiful city from the best perspective. This conference is also made possible by the sponsors (Ionoptix, Optics 11, Curi Bio, Aurora, Edgewise Therapeutics, Oroboros Instruments, MitoGlobal and Accelerated Muscle Biotechnologies) and by the contribution of NWO, the Dutch Research Council. We wish you all a great time in Amsterdam and look forward to discussing all new developments the muscle field has to offer!

Best,

EMC organizing committee:

Josine de Winter
Diederik Kuster
Rob Wüst
Coen Ottenheijm
Tyler Kirby

We thank the scientific committee and the Early Career Association for their valuable contributions in setting up the sessions.

Scientific committee:

Elisabetta Brunello, King's College London, United Kingdom
Kristina Djinovic-Carugo, EMBL Grenoble, France
Elisabeth Ehler, King's College London, United Kingdom
Neil Kad, University of Kent, United Kingdom
Alain Lacampagne, PhyMedExp Inserm, France
Wolfgang Linke, University of Münster, Germany
Michael Regnier, University of Washington, USA

Early Career Association:

Maicon Landim-Vieira; Illinois institute of Technology, USA
Christine Loescher, University of Muenster, Germany
Franziska Koser, University of Muenster, Germany
Cameron Hill, King's College London, United Kingdom
Vinicius Mariani, Duke University School of Medicine, USA
Emrulla Spahiu, Hannover Medical School, Germany
Katharina Voigt, Univeristy of Duesseldorf, Germany
Annika Klotz, Univeristy of Muenster, Germany
Maria Rosaria Pricolo, Spanish National Centre of Cardiovascular Research, Sp

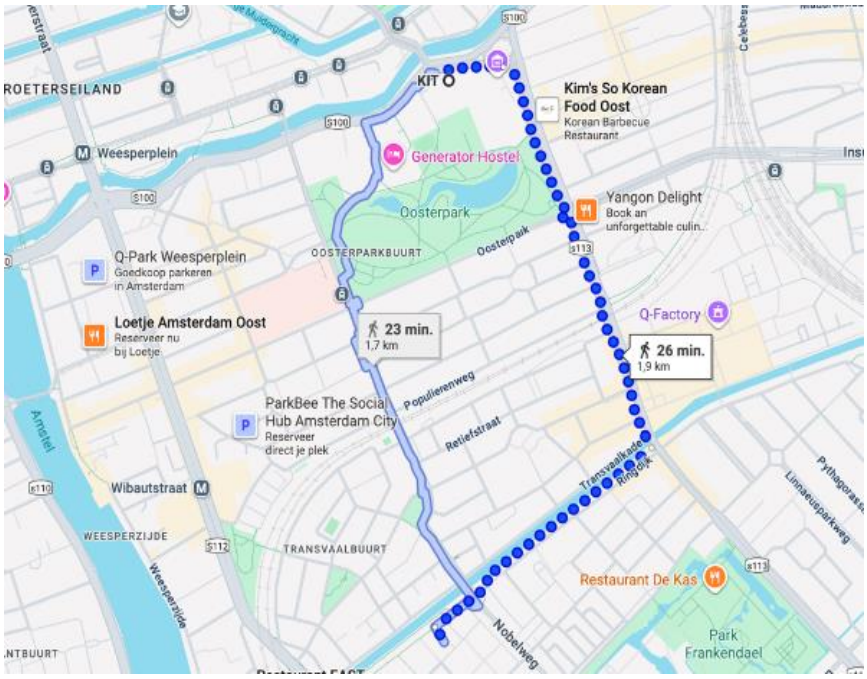
2025 ECA Sponsors

ADDRESSES

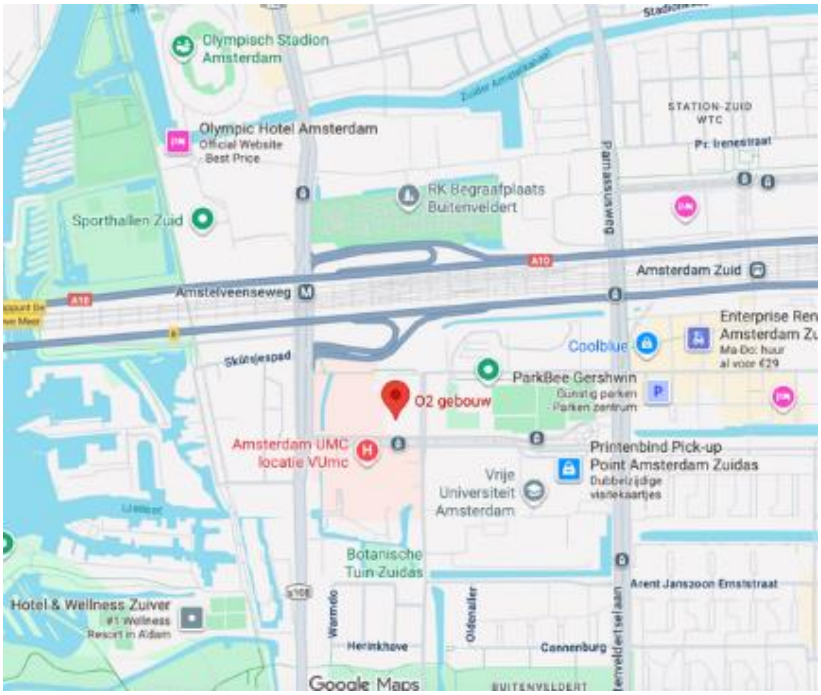
Conference venue
KIT Institute
Mauritskade 64
1092 AD Amsterdam

WIFI: Events
Password**:** welcometokit

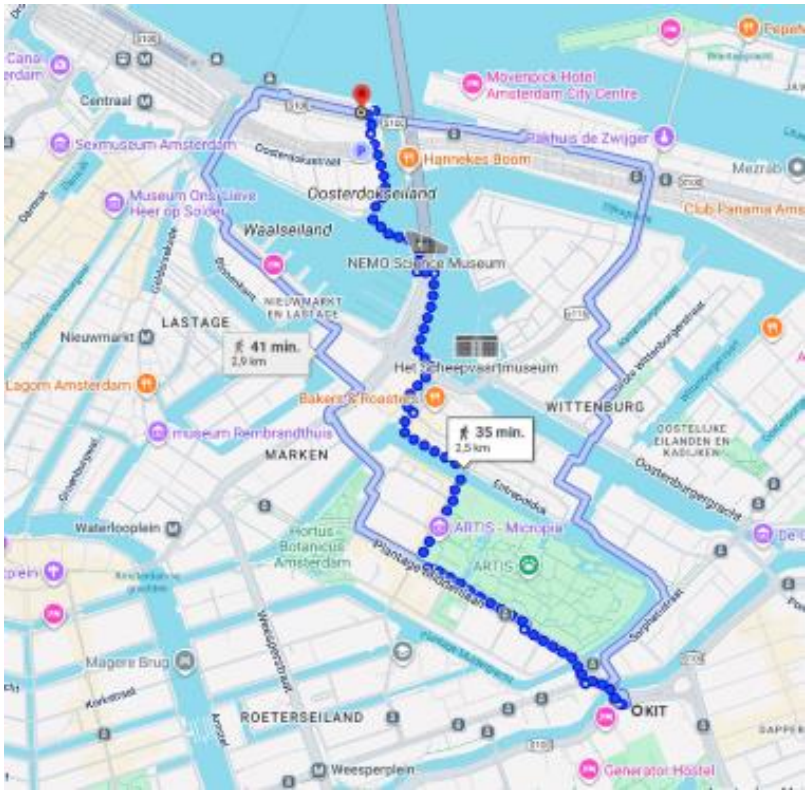
Conference boat tour
Mauritskade 59B (in front of KIT Institute, see map at top)



Conference dinner
De Vergulden Eenhoorn
Ringdijk 58
1097 BB Amsterdam



Early careers symposium venue
O2 building
De Boelelaan 1108
1081 HZ Amsterdam



Early careers social event
De Ruijterkade 151
1011 AC Amsterdam

BIOGRAPHIES KEYNOTE SPEAKERS

Leslie Leinwand (Universiteit van Colorado Boulder, VS)

Dr. Leinwand is a Distinguished Professor of Molecular, Cellular, and Developmental Biology (MCDB) and the Chief Scientific Officer of the BioFrontiers Institute at the University of Colorado Boulder. Her research laboratory focuses on the genetics and molecular physiology of inherited diseases of the heart and particularly how biological sex affects the heart in health and disease. She co-founded Myogen, Inc. (acquired by Gilead Pharmaceuticals), Hiberna, Inc., and MyoKardia, Inc. (acquired by Bristol Myers Sqibb), which develop therapeutics for inherited cardiomyopathies. She is a Fellow of the AAAS, a former MERIT Awardee of the NIH, an Established Investigator of the American Heart Association, and an elected member of the American Academy of Arts and Sciences and the National Academy of Inventors. She received her bachelor's degree from Cornell University, her PhD from Yale University, and did post-doctoral training at Rockefeller University.

JEAN HANSON LECTURE

Jolanda van der Velden (Amsterdam University Medical Center, The Netherlands)

Jolanda van der Velden, PhD, is Professor of Physiology, and chairs the Department for Physiology and the Department of Experimental Cardiology at the Amsterdam University Medical Center. The main research interest of the van der Velden group is to study the role of sarcomere proteins in cardiac performance. As mutations in sarcomere proteins are a frequent cause of heart disease, research on inherited cardiomyopathies is a central research line in Amsterdam, and experiments are performed from bench to the clinic. Expertise includes functional studies at single cardiac muscle cell and multicellular level, and mitochondrial studies in patient samples obtained during cardiac surgery and stem cell-derived heart models. Close collaboration with clinical departments allows translational research in which the functional studies in cardiac samples are combined with in vivo parameters of cardiac pump function and energetics. Moreover, methods have been developed to refine research in animal models, including high-throughput analyses of cardiomyocyte function. From June 2025, she will co-direct the Netherlands Heart Institute.

PROGRAMME

Saturday, September 20th - Early Careers Association Symposium

10.00 – 14.00	**Early Careers Association Symposium** (Note that this will take place at the O|2 building at the Vrije Universiteit Amsterdam!)	
10.00 – 11.30	Session A.	Chair: Annika Klotz and Franziska Koser
10.00 – 10.10	Welcome and opening remarks	
10.10 – 10.25	**Roberto Silva-Rojas (National Institute for Cardiovascular Research, Spain)** Sarcomere loss triggers partial reprogramming of adult myofibers	
10.25 – 10.40	**Kyrah Turner (Washington State University, USA)** Myosin light chain kinase and protein kinase A independently and cooperatively regulate contractile function via myofilament phosphorylation	
10.40 – 10.55	**Karina Ivaskevica (Hannover Medical School, Germany)** Functional impact of cell-to-cell variability in CMYBP-C expression in a human PSC model of hypertrofic cardiomyopathy	
10.55 – 11.10	**Arne Hofemeier (University Medical Center Göttingen, Germany)** Novel platform for bioengineering skeletal muscle for high resolution imaging and functional analysis to study Duchenne muscular dystrophy	
11.10 – 11.20	**Edgewise Therapeutics (Sponsor)**	
11.20 – 11.30	**AMB - Accelerated Muscle Biotechnologies (Sponsor)**	
11.30 – 12.30	**Lunch Break**	
12.30 – 13.45	Session B	Chair: Cameron Hill and Katharina Voigt
12.30 – 12.45	**Jose Medina (McGill University, Montreal, Canada)** Analyzing the effects of Drosophila Zasp mutants in myofibril assembly	
12.45 – 13.00	**Bilal Ahmad Mir (Lund University, Sweden)** Nature's own experiment: clarifying the role of MSS51 in human skeletal muscle metabolism and its contribution to T2D risk	

13.00 – 13.15 **Chao Su (University of Galway, Ireland)**
Decoding the role of microRNAs in cancer cachexia: a study using LLC tumor-bearing mice and in silico approaches

13.15 – 14.35 **Closing keynote talk – Sergej Pirkmajer (University of Ljubljana, Slovenia) & closing remarks**

Saturday, September 20th

15.00 – 16.00 Registration and check-in

16.00 – 17.00 Keynote Lecture – Prof. dr. Leslie Leinwand (University of Colorado Boulder)
"Translation python biology to mammals: novel therapeutic targets"

17.00 – 19.00 **Social: Networking and Drinks**

Sunday, September 21st

09.00 – 10.30 Session 1. Muscle structure
Chair: Kristina Djinovic

09.00 – 09.30 **Jorge Allegre Cebollada (National Institute for Cardiovascular Research, Spain)**
Tug-of-war gone wrong: unexpected tissue responses to titin inloading

09.30 – 09.45 **Chris Tiessen (University of Calgary, Canada)**
Can variation in contractile filaments in adjacent sarcomeres explain sarcomere length non-uniformities?

09.45 – 10.00 **Lina Herssi (Aix Marseille university, CNRS, IBDM, Marseille, France)**
Manipulating the mechanical integrity of Drosophila titin to investigate its role in sarcomere assembly and maintenance

10.00 – 10.30 **Anthony Hessel (University of Münster, Germany)**
The opportunities and pain points of using near-instant controllable phenotypes to study muscle structure function relationships

10:30 – 11:00 Coffee Break

11.00 – 12.30 Session 2. Molecular mechanisms of muscle plasticity
Chair: Tyler Kirby

11.00 – 11.30 **John McCarthy (University of Kentucky, USA)**
microRNA-1 Regulates Skeletal Muscle Metabolic Flexibility via Pyruvate Metabolism

11.30 – 11.45 **Alejandro Clemente – Manteca (Centro Nacional de Investigaciones Cardiovasculares (CNIC), Spain**
Short and sweet – crosslinking glycation stiffens diabetic titin

11.45 – 12.00 **Vanessa Todorow (Yale University, USA)**
Satellite Cell Fusion into Mature Myofibers Under Pathological Conditions

12.00 – 12.30 **Regula Furrer (Biozentrum, University of Basel, Switzerland)**
Molecular control of endurance training adaptation

12:30 – 14:00 Lunch and Posters (Sessions 1-4)

14.00 – 15.30 Session 3. Excitation-contraction coupling
Chair: Alain Lacampagne

14.00 – 14.30 **Isabelle Marty (INSERM, France)**
From pathophysiological mechanism to therapeutic development in RYR1-related disease

14.30 – 14.45 **Romane Idoux (CHEO Research Institute, Canada)**
Autosomal dominant rhabdomyolysis is associated with a missense variant in the Atp2a2 reducing SERCA2 calcium pump function in skeletal muscle.

14.45 – 15.00 **Ferdinand Conrad (Hannover Medical School, Germany)**
Influence of an HCM associated cMYBP-C truncation on contractile function of myofibrils within long-term cultured hiPSC-derived cardiomyocytes

15.00 – 15.15 **Edgar Nollet (University of Copenhagen, Denmark)**
Hyperacetylation disrupts myosin relaxation in diabetic patients with hypertrophic cardiomyopathy

15:15 – 15:30 Industry Presentation

15:30 – 16:00 ESMR Meeting

16.00 – 16.30 **Coffee Break**

16.30 – 18.00 Session 4. Data Science in Muscle: from -omics to Physiology
Chair: Rob Wüst

16.30 – 17.00 **Fabien Le Grand (NeuroMyoGène Institute, France)**
Skeletal muscle in development, health and disease at the single nuclei resolution

17.00 – 17.15 **Michaela Yuen (The Children's Hospital at Westmead and the Children's Medical Research Institute, Westmead, Australia)**
Characterizing a mouse model for pyroxd1 myopathy – pathway to therapy development

17.15 – 17.30 **Koen Zwetsloot (Vrije Universiteit Amsterdam, The Netherlands)**
The fibre makes the muscle: fibre type-specific distinction in proteasomal, sarcomeric, and metabolic pathways in gastrocnemius medialis fibres revealed by fibre type dependent proteomics

17.30 – 18.00 **Thibaux van der Stede (University of Gent, Belgium)**
A multicellular perspective on exercise-induced plasticity of human skeletal muscle

20.00 – 21.00 **ECA – Social Event (separate registration required)**

Monday, September 22nd

09.00 – 10.30 Session 5. Innovations in human disease models
Chair: Michael Regnier

09.00 – 09.30 **Theresia Kraft (Hannover Medical School, Germany)**
Patient-derived induced pluripotent stem cell cardiomyocytes and organoids as models for molecular mechanisms of early-stage Hypertrophic Cardiomyopathy

09.30 – 09.45 **Amélie Marais (Brock University, Canada)**
Partial Gsk3 knockdown improves muscle performance in mdx mice: investigating the cellular mechanisms

09.45 – 10.00 **Stuart Campbell (Yale University, USA)**
Enhancing myofilament isoform maturation in human engineered heart tissues

10.00 – 10.30 **Manuel Pioner (University of Florence, Italy)**
The Role of Cardiac Extracellular matrix - Cell interaction in the onset of dystrophic cardiomyopathies

10.30 – 11.00 Coffee Break

11.00 – 12.30 Session 6. Muscle energetics
Chair: Josine de Winter

11.00 – 11.30 **Lykke Sylow (University of Copenhagen, Denmark)**
Powering the aging muscle: Mitochondria, Metabolism and Molecular surprises

11.30 – 11.45 **Gretel Major (University of Canterbury, New Zealand)**
Disrupted glucose insulin signalling drives metabolic dysfunction in the mdx mouse model oF Duchenne muscular dystrophy

11.45 – 12.00 **Rianne Baelde (Amsterdam UMC, The Netherlands)**
Nicotinamide riboside supplementation prevents the onset of mitochondrial dysfunction in a mouse model for nemaline myopathy type 6

12.00 – 12.30 **Leonarda Nogara (University of Padova, Italy)**
Advantages and limitations of the mantATP turnover in skinned muscle fibers

12:30 – 14:00 Lunch and Posters (Sessions 5-8)

14.00 – 15.30 Session 7. Cytoskeleton
Chair: Elisabeth Ehler

14.00 – 14.23 ***Carol Gregorio (Mount Sinai, USA)***
Redefining actin dynamics of the pointed end complex in health and myopathy

14.24 – 14.47 **Thomas Iskratsch (Queen Mary university of London, United Kingdom)**
Pressure and stiffness sensing of vascular smooth muscle cells regulate phenotypic switching in arterial disease

14.47 – 14.57 **Sylvia Bogaards (Amsterdam UMC, The Netherlands)**
The replacement kinetics of the giant muscle protein nebulin are slow but much faster by a variant that disrupts an actin-binding motif

14.57 – 15.07 **Aishwarya Prakash (Queen Mary University of London, United Kingdom)**

Mechanotransduction at the Intercalated Disc via an LMCD1-Associated ProteinComplex

15.07 – 15.30 **Dieter Fürst (University of Bonn, Germany)**
From mechanosensation to myofibril repair: the regulation of filamin C by phosphorylation

15.30 – 16.00 Coffee Break

16.00 – 17.30 Session 8. Respiratory muscle mechanics in NMD
Chair: Coen Ottenheijm

16.00 – 16.30 **Jonne Doorduin (Radboud UMC, The Netherlands)**
Diaphragm Dysfunction in Critical Illness

16.30 – 16.45 **Stefan Matecki (University of Canterbury, New Zealand)**
Asynchronies related to mechanical ventilation exacerbates diaphragm dysfunction in mice model of Duchenne Muscular Dystrophy.

16.45 – 17.00 **Ricardo Galli (National Institutes of Health, USA)**
Spontaneous oscillatory muscle contractions are from sarcomeric origin in novel MYBPC1 variants associated with tremor

17.00 – 17.30 **Leo Ferreira (Duke University School of Medicine, USA)**
Diaphragm myopathy in heart failure with reduced ejection fraction

18:00 – 19:30 Canal Boat Tour (separate registration required)

Tuesday, September 23rd

09.00 – 10.30 Session 9. Regulation of contraction by thick and thin filaments
Chair: Elisabetta Brunello

09.00 – 09.30 **Vitold Galkin (Eastern Virginia Medical School, USA)**
The role of allosteric interactions in regulation of cardiac thin filament revealed by the troponin T pathogenic variant Ile79Asn

09.30 – 09.45 **Gabriel Watson (King's College London, UK)**
Calcium-dependence of the structural changes in the thick filament of demembranated myocardial slices from the rabbit heart

09.45 – 10.00 **Luca Fusi (King's College London, UK)**

Sub-sarcomeric regulation of thin and thick filaments in skeletal muscle myofibrils

10.00 – 10.30 **Marco Linari (University of Florence, Italy)**
Activation of cardiac myosin filament during systole with different afterloads

10.30 – 11.00 Coffee Break

11.00 – 12.30 Session 10. Targeting the nucleus
Chair: Diederik Kuster

11.00 – 11.30 **Claudia Crocini (Charité Berlin, Germany)**
Impact of Titin N2B Deletion on the nucleus of hiPSC-Derived Cardiomyocytes

11.30 – 11.45 **Ilhan Gokhan (Yale University, USA)**
Desmoplakin Loss Leads to PKC Dependent Insertion of Series Sarcomeres and Contractile Dysfunction in Cardiomyocytes

11.45 – 12.00 **Osman Esen (Amsterdam UMC, Netherlands)**
Altered perinuclear cytoskeleton and lamina in aged myonuclei: implications for impaired mechanoresponsiveness

12.00 – 12.30 **Matthew Stroud (King's College London, UK)**
The pleiotropic nuclear envelope and its role in striated muscle development, ageing, and disease

12:30 – 14:00 Lunch and Posters (Sessions 9-13)

14.00 – 15.30 Session 11. Cardiomyopathies
Chair: Wolfgang Linke

14.00 – 14.30 **Mathias Gautel (King's College London, UK)**
Thick-filament cardiomyopathies one mechanism or thousands?

14.30 – 14.45 **Laura Sen Martin (Centro Nacional de Investigaciones Cardiovasculares (CNIC), Spain)**
Broad therapeutic benefit of myosin inhibition in hypertrophic cardiomyopathy

14.45 – 15.00 **Poppy O. Smith (Queen Mary University of London, UK)**
Cardiomyocyte remodelling through mechanically tuned talin

15.00 – 15.30 **Natalie Weber (Hannover Medical School, Germany)**
Human induced pluripotent stem cell-derived cardiomyocytes integration and maturation on human living myocardial slices.

15.30 – 16.00 **Coffee Break**

16.00 – 17.00 **Jean Hanson Lecture: Jolanda van der Velden (Amsterdam University Medical Centers)**
Fueling the hypertrophied heart

18:30 – 00:00 **Conference Dinner and Party (separate registration required)**

Wednesday, September 24th

09.00 – 10.30 Session 12. Muscle modeling
Chair: Neil Kad

09.00 – 09.30 **Sam Walcott (Worcester Polytechnic Institute, USA)**
Successes and challenges In using mathematical modeling to bridge scales in muscle contraction

09.30 – 09.45 **Kenneth S. Campbell (University of Kentucky, USA)**
Multiscale simulations exhibit ventricular wall thickening when cMYBP-C does not suppress myosin cycling

09.45 – 10.00 **Momcilo Prodanovic (FilamenTech Inc., USA)**
In silico prediction of myosin modulator effects in DCM and HCM using multiscale simulations and translational matrices

10.00 – 10.30 **Joost Lumens (Maastricht University, Netherlands)**
Computational Cardiology: linking sarcomere function to cardiovascular system dynamics

10.30 – 11.00 **Coffee Break**

11.00 – 12.30 Session 13. Skeletal myopathies
Chair: Coen Ottenheijm

11.00 – 11.30 **Katia Kontrogianni-Konstantopoulos (University of Maryland, USA)**
Exploring the expanding Myotrem myopathy: what we know…much to learn

11.30 – 11.45 **Jochen Gohlke (University of Arizona, USA)**
Pathomechanisms of Monoallelic Variants in TTN Causing Skeletal Muscle Disease

11.45 – 12.00 **Sora Han (Research Institute of Women's Diseases, Sookmyung Women's University, South Korea)**

CTRP1 regulates skeletal muscle differentiation by quality control of mitochondrial dynamics and function

12.00 – 12.30 **Sandra de Haan (Leiden UMC, Netherlands)**
High-Resolution Spatial Transcriptomics Reveals Myofiber-Immune Cell Interactions in Inclusion Body Myositis

12:30 – 13:00 Awards ceremony and closing remarks

BIOGRAPHIES INVITED SPEAKERS

Session 1: Muscle structure

Jorge Allegre Cebollada (National Institute for Cardiovascular Research, Spain)
I earned my PhD in Biochemistry from Complutense University in 2008. Prompted by an interest in quantitative science, I trained in single-molecule biophysics and protein mechanics with Julio Fernandez at Columbia University. My independent research career started in 2014 at the National Institute for Cardiovascular Research (CNIC) in Madrid, Spain. At that time, I felt that I was in a privileged position to build on the biochemical and biophysical concepts I had learned to build a research program to examine protein mechanics in biological context, including connections to human disease. Some years later, I was ready for the next step: developing and exploiting novel tools to study protein mechanics in living cells and animals, an idea that raised funding from the ERC-consolidator program in 2020. My presentation will show some the insights we are getting with these new methods.

Anthony Hessel (University of Muenster, Germany)
Dr. Anthony Hessel is a muscle physiologist with a diverse background in muscle biomechanics, assistive devices like prostheses and exoskeletons, and biophysics. His current research program is focused on defining the role of the sarcomere proteins titin and myosin-binding protein C (MyBP-C) to cardiac and skeletal muscle performance. He recieved his PhD at Northern Arizona University (Major advisor Prof. Kiisa Nishikawa) and conducted Post Doc appointments at Ruhr university Bochum (Mentor Prof. Daniel Hahn) and University of Muenster (mentor Prof. Wolfgang Linke). He is currently operating under his own german research foundation grant.

Session 2: Molecular mechanisms of muscle plasticity

John McCarthy (University of Kentucky, USA)
Dr. McCarthy received a B.S. in Biology from the University of California at Irvine, a M.S. in Physical Education for California State University at Fullerton and a PhD in Exercise Physiology from the University of Oregon. He completed post-doctoral training at the University of Illinois at Urbana-Champaign and University of Missouri at Columbia. He is currently a Professor in the Department of Physiology at the University of Kentucky. The primary focus of his lab is to better understand the molecular and cellular mechanisms regulating skeletal muscle mass in response to exercise and with aging. Ongoing projects are investigating the role of satellite cells, the gut microbiome and muscle-specific microRNA miR-1 in hypertrophy, atrophy and aging. Dr. McCarthy's research is primarily supported by funding from NIH.

Regula Furrer (Biozentrum, University of Basel, Switzerland)
Regula Furrer completed her MSc and PhD in Human Movement Sciences at the Vrije Universiteit (VU) Amsterdam, The Netherlands. As a PhD candidate, she investigated the adaptive responses of muscle and bone to different types of training and the relationship between muscle function and bone quality on fracture risk in elderly people. Subsequently, she joined Prof. Handschin's group at the Biozentrum of the University of Basel in Switzerland as a postdoc where she now works as a senior scientist. She is investigating various aspects of exercise and muscle plasticity in health and disease, including the acute transcriptional response to exercise, multicellular cross-talk, effects of exercise on tumor-associated muscle mass loss and anemia and metabolic dysregulation in the muscular dystrophy dysferlinopathy.

Session 3: Excitation-contraction coupling

Isabelle Marty (INSERM, France)
Isabelle Marty is INSERM Research Director, leader of the team "Cellular Myology and Pathologies" at Grenoble Institute of Neurosciences (France). Working for more than 30 years in the field of muscle biochemistry, muscle cell biology, and calcium release for muscle contraction, she has published more than 80 papers on the calcium release complex and its alterations, both in skeletal and in cardiac muscles. Her research interests are centered on the ryanodine receptor and its associated protein triadin. Her lab, gathering basic researchers and geneticists, focuses on the identification of pathophysiological mechanisms resulting of mutation in a protein of the calcium release complex. She is working on the development of therapies for central core disease patients with mutations in the ryanodine receptor gene. Both pharmacological and gene therapy approaches are studied in her team. She has provided recently the first experimental demonstration of benefit of some gene therapies for RYR1 mutations.

Session 4: Data Science in Muscle: from -omics to Physiology

Fabien Le Grand (Claude Bernard University, Lyon, France)
Fabien Le Grand is a French researcher in the field of muscle cell biology. He is Director of Research at the CNRS and Principal Investigator at the Claude Bernard University of Lyon. Dr. Le Grand obtained his Ph.D. in Cell Biology from the University of Nantes in 2004. He then went on to complete a postdoctoral fellowship at Ottawa University, where he worked on the role of the Wnt signaling pathway, before starting his own lab in Paris. Dr. Le Grand's research focuses on the molecular mechanisms that regulate muscle development, regeneration, and disease. He made significant contributions to our understanding of the roles and functions of satellite cells, the adult muscle stem cells. He also identified new signaling pathways and transcription factors that control satellite cell fate. In recent years, the Le Grand lab delved into single cell/nuclei analyses to study the muscle tissue in all its states.

Thibaux van der Stede (VIB-UGent Center, Belgium)
Thibaux Van der Stede completed his joint PhD in 2024 at Ghent University and the University of Copenhagen, working in the labs of Dr. Wim Derave and Dr. Ylva Hellsten. His research focused on the molecular adaptations of human skeletal muscle to exercise, employing advanced omics technologies to explore muscle fiber heterogeneity and cellular diversity in muscle. Currently, he is employed as a postdoctoral researcher at the VIB-UGent Center for Inflammation Research, working on the molecular immunology and inflammation of musculoskeletal diseases. His work bridges fundamental musculoskeletal biology with translational research, aiming to contribute to a better understanding of muscle physiology and its implications for health and disease.

Session 5: Innovations in human disease models

Theresia Kraft (Hannover Medical School, Germany)
Theresia Kraft is Professor of Physiology at Hannover Medical School, Germany. During her PhD at Tübingen University with Professor Bernhard Brenner, she used skeletal muscle mechanics to study the role of weak-binding cross-bridge states during the force generating cycle. Her postdoc with Dr. Leepo Yu at NIH was focused on x-ray diffraction analysis of weak-binding acto-myosin complex conformation. In Hannover, she began to study the effects of missense mutations in the cardiac beta-myosin isoform in Hypertrophic Cardiomyopathy (HCM). Her present focus is on pathomechanisms of HCM. Biomechanical analysis of cardiomyocyte function, transcriptional activity, mRNA and protein expression in human adult and stem cell derived cardiomyocytes are employed to address this. She and her group have evidence that burst like, stochastic and independent transcription of mutated and wildtype alleles cause unequal fractions of the respective wildtype and mutated protein from cell-to-cell, which may lead to contractile imbalance among cardiomyocytes in HCM.

Manuel Pioner (University of Florence, Italy)
Josè Manuel Pioner (b. 26 January 1987, Bolzano-Bozen, Italy), PhD, is a tenure-track assistant professor in Physiology at the Department of Biology, University of Florence. He earned his PhD in Molecular Medicine from the University of Siena and trained at the University of Washington and the University of Cologne. His research focuses on cardiac muscle biophysics, with emphasis on human iPSC-derived cardiomyocytes and genetic models of hypertrophic and dystrophic cardiomyopathies. He has contributed to EU, Italian Ministry of Health, and Telethon-funded projects, coordinating advanced cell culture and precision medicine studies. Dr. Pioner has authored 28 peer-reviewed publications (Scopus ID: 57035436600) and received awards at international conferences. A member of the Biophysical Society, he collaborates with international institutions and mentors young researchers. He also lectures in general physiology and holds Italy’s National Scientific Habilitation for Associate Professorship.

Session 6: Muscle energetics

Lykke Sylow (University of Copenhagen, Denmark)
Lykke Sylow is an Associate Professor and group leader of the Molecular Metabolism in Cancer & Aging Group at the University of Copenhagen. With over 15 years of experience in muscle metabolism research, her focus is on understanding the molecular regulation of skeletal muscle adaptations in response to conditions of diseases such as cancer and diabetes, and the beneficial adaptations elicited by exercise. She aims to utilize her research on exercise adaptations to harness the positive effects of exercise on aging, diabetes, and cancer. She recently received the International Award by the Biochemical Society and the EFSD Novo Nordisk Foundation Future Leaders Award.

Leonardo Nogara (University of Padova, Italy)
Dr. Leonardo Nogara is an Assistant Professor in the Department of Biomedical Sciences at the University of Padua (Italy), working in the Muscle Contractility and Neuromuscular Plasticity unit. He has experience measuring muscle contractility at different scales, from in vivo murine models to ex vivo isolated muscle preparations, down to single muscle fibers mechanically isolated. His work centers on the Super Relaxed State (SRX) of myosin, the main skeletal muscle motor protein. Muscle tissue experiences about a 100-fold increase in metabolic load ranging from rest to active exercise; in this context, the SRX is a biochemical state evolved as an energy-saving mechanism. To explore the physiological and pathological effects of SRX modulation, he developed a single-fiber ATPase assay, which he applies to screen new compounds for the pharmacological modulation of basal metabolism and to biopsies of patients to evaluate myosin energy consumption in hypermetabolic or hypometabolic conditions.

Session 7: Cytoskeleton

Carol Gregorio (Mount Sinai, New York City, USA)
Carol Gregorio, PhD started at Mount Sinai in April 2023 as the Irene and Dr. Arthur M. Fishberg Professor of Medicine, Senior Associate Dean for Basic Science, Founding Director for the Center for Cardiac Muscle Biology within the Cardiovascular Research Institute, and Vice-Chair for Strategic Innovation. Additionally, she is building a Duchenne Muscular Dystrophy Care Center at Mount Sinai. Dr. Gregorio is an active member of several Editorial and Philanthropic Boards and is a chair/grant reviewer at the National Institutes of Health. Dr. Gregorio is a Fellow of the American Association for the Advancement of Science, American Heart Association, and American Association for Anatomy. Dr. Gregorio runs a continuously-funded National Institutes of Health (NIH) research program broadly focused on deciphering the cellular mechanisms involved in development and regulation of contractile proteins in healthy hearts and cardiomyopathy. Her research program spans the fields of Cell Biology, Molecular Biology, Biophysics, Biochemistry, Bioengineering, and Genetics. She received her BS degree in Biological Sciences and a MA degree in Natural Sciences from the State University of New York at Buffalo, Subsequently, she received her Doctorate in Molecular

Immunology from Roswell Park Cancer Institute in Buffalo, NY and did her Postdoctoral Fellowship at the Scripps Research Institute in La Jolla, CA.

Dieter Fürst (University of Bonn, Germany)
Studied Biology at the University of Salzburg (Austria) from 1978 till 1986. PhD thesis at the Institute for Molecular Biology (Austrian Academy of Sciences) under the supervision of Prof. J.V. Small (1981-1986). From 1986 till1989 Postdoctoral fellow at the Max-Planck-Institute for Biophysical Chemistry, Department of Biochemistry and Cell Biology in Göttingen (Germany) with Alexander von Humboldt- and Max Planck Society fellowships, under the auspicies of Prof. Klaus Weber. Senior scientist at the Max-Planck-Institute for Biophysical Chemistry, Department of Biochemistry and Cell Biology in Göttingen (Germany; 1989-1995). Habilitation in Biochemistry and Cell Biology at the University of Salzburg (1993). Assistant Professor (C3) for "Cell Biology" at the Institute for Zoophysiology and Cell Biology, University of Potsdam (Germany; 1995-2004). Since 2004 Professor (C4) and Chair, Department of Molecular Cell Biology, Managing Director of the Institute for Cell Biology at the University of Bonn (Germany).

Session 8: Respiratory muscle mechanics in NMD

Jonne Doorduin (Radboud UMC, Netherlands)
Dr. Doorduin is a technical physician and assistant professor in Intensive Care at the Radboudumc in Nijmegen, the Netherlands. He is deeply passionate about the intricacies of breathing and an expert in respiratory (neuro)physiology, respiratory muscle ultrasound, medical technology and mechanical ventilation. In Nijmegen, he leads a research group on acute and chronic respiratory failure and mechanical ventilation, comprising multiple (international) PhD students and post-docs. Dr. Doorduin' approach to respiratory failure is unique: he focuses on the interaction between the brain, muscles and lungs. He has conducted numerous physiological and clinical studies in the field of respiratory failure, critical illness, neuromuscular disorders and mechanical ventilation (>80 peer-reviewed publications). Dr Doorduin' mission is to improve outcome for patients with respiratory failure. For his innovative ideas, he received the prestigious Academic Career award from the Royal Netherlands Academy of Arts and Sciences.

Leo Ferreira (Duke University, USA)
Leo Ferreira, PT, PhD, is an Associate Professor in Orthopaedic Surgery, Pathology, and the Cardiovascular Research Center at Duke University School of Medicine. Previously, he was Professor and Vice-Chair in Applied Physiology & Kinesiology and Director of the Center of Exercise Science at the University of Florida. He received a physiotherapy degree from Universidade Estadual de Londrina (Brazil), a PhD from Kansas State University, and completed postdoctoral training at the Center for Muscle Biology at the University of Kentucky Medical School. At Duke University School of Medicine, Leo Ferreira directs the Basic and Clinical Muscle Biology and Rehabilitation laboratory. The scientific mission of the laboratory is to examine mechanisms and advance therapies for striated muscle abnormalities in disease and aging performing experiments spanning from individual cells to non-invasive studies in humans.

Session 9: Regulation of contraction by thick and thin filaments

Vitold Galkin (Old Dominion University in Norfolk, USA)
Dr. Vitold E. Galkin obtained his doctorate at the Institute of Cytology, Russian Academy of Sciences, followed by postdoctoral training at the University of Virginia. In 2013, he joined the Department of Biomedical and Translational Sciences at Macon & Joan Brock Virginia Health Sciences at Old Dominion University in Norfolk, USA, where he currently serves in the rank of Associate Professor. Dr. Galkin uses high-resolution cryo-electron microscopy and advanced image analysis techniques to elucidate the structural dynamics of the native cardiac thin filament and its partners (myosin and myosin binding protein C). His current work demonstrates that the thin filament works as a molecular machine comprised of an array of allosterically coupled protein subunits suited to maintain adequate sarcomere response to physiological calcium levels during a heartbeat.

Marco Linari (University of Florence, Italy)
Marco Linari, PhD, studies the molecular mechanisms of skeletal and cardiac muscle contraction. These goals are pursued through biophysical techniques applied to skeletal and cardiac muscle at different levels in the hierarchical organization of the contractile machinery. The mechano-chemistry and structural dynamics of the muscle molecular motor myosin II has been determined in situ, combining sarcomere-level mechanics and X-ray diffraction from synchrotron light (at the ESRF, Grenoble, France and APS, Argonne, USA) in single intact and demembranated cells from the most suitable animal models.More recently the same approach has been applied to study thin and thick filament-based regulation of muscle. A new regulatory system based on myosin filament mechano-sensing has been defined firsts in the intact skeletal muscle fibre of the frog and then in intact trabeculae of the cardiac ventricle of the rat. The same techniques are currently applied to investigate the role of titin in muscle function and disease.

Session 10: Targeting the nucleus

Claudia Crocini (Charité Berlin)
Claudia Crocini obtained her PhD at the University of Florence, where she applied advanced imaging techniques to investigate excitation-contraction coupling in heart failure and hypertrophic cardiomyopathy. She completed her postdoctoral training in the Leinwand Lab at the University of Colorado Boulder, studying sex differences in cardiac physiology, mechanisms of physiological hypertrophy, fibrosis, and nuclear mechanotransduction. In 2023, she established her independent research group at the Charité Berlin, focusing on cardiac mechanobiology and sex differences.

Matthew Stroud (King's College London, UK)
Dr. Stroud's research centre of interest lies in elucidating the mechanisms underlying alterations to nuclear shape and the nuclear envelope, which impact ageing and lead to inherited forms of skeletal and cardiac myopathy. He completed his Wellcome Trust-funded PhD in Molecular Cell Biology at the University of Manchester with Professors Christoph Ballestrem and Richard Kammerer (2010). Subsequently, he relocated to California to pursue NIH and AHA-funded Postdoctoral Fellowships in Cardiology at UC San Diego, where he with Professor Ju Chen. During his postdoctoral fellowship, he uncovered the pathophysiological functions of the LINC complex proteins, which link the nucleoskeleton to the cytoskeleton, and the inner nuclear membrane protein TMEM43. In 2016, Dr. Stroud established his independent laboratory within the BHF Centre of Research Excellence at King's College London and was appointed as a Senior Lecturer (Associate Professor) in 2023. Throughout his academic career, Dr. Stroud has received numerous accolades, including the Chancellor's Award and the Schulman Prize during his tenure at UCSD (2015). In 2023, he was awarded the AHA's Paul Dudley White International Scholar Award. Notably, one of his PhD students, Ed Battey, was recognised with the Early Investigator Prize from the Physiological Society for his groundbreaking paper, which elucidated the effects of exercise and ageing on skeletal muscle nuclei (2024). Dr. Stroud serves as the academic lead for Early Career Researchers (ECRs) at the BHF Centre at King's. This role entails mentoring ECRs, organising ECR forums, and hosting an annual Fellows' day that are designed to enhance research culture, foster diversity, and promote inclusion at King's.

Session 11: Cardiomyopathies

Mathias Gautel (King's College London, UK)
Mathias Gautel received his MD from Heidelberg University in 1991, and moved to EMBL Heidelberg as post-doctoral fellow, where he worked on titin for his habilitation in Biochemistry until 1998. After a Heisenberg fellowship at the Max-Planck-Institute Dortmund, he joined King's College London in 2002. He is head of the School of Basic and Medical Biosciences and holds the British Heart Foundation Chair of Molecular Cardiology. He was awarded the International Society for Heart Research Outstanding Investigator Award in 2009 and is a Fellow of the Academy of Medical Sciences. His work is centred on sarcomeric structure and mechanosignalling using structural, biophysical and cellular approaches. His team identified and characterised many novel sarcomeric components and mechanisms like cardiac myosin-binding protein-C, the giant protein obscurin, the regulatory mechanism of sarcomeric alpha-actinin. Recent interests are the pathomechanisms of missense mutations and the role of proteostasis in titin-linked myopathies and cardiomyopathies.

Natalie Weber (Hannover Medical School, Germany)
Dr. Natalie Weber, MD, PhD, is a cardiovascular researcher at Hannover Medical School (MHH) at the Institute of Molecular and Translational Therapeutic Strategies, specializing in heart diseases such as hypertrophic cardiomyopathy (HCM), heart failure, and other cardiac conditions. She obtained her MD and PhD at MHH in the department of Molecular and Cellular Physiology. She utilizes patient-specific stem cell-derived cardiomyocytes and living myocardial slices to investigate early disease mechanisms and cardiac remodeling in HCM, heart failure, and injury. Her recent work includes studies on allelic and contractile imbalance in early-stage HCM and the development of novel cardiac injury and fibrosis models using living myocardial slices. Dr. Weber also explores novel drug effects, including myosin inhibitors and SGLT2 inhibitors, in multiple myocardial models. Her findings have been published in leading journals such as Journal of Molecular and Cellular Cardiology, Basic Research in Cardiology, and the European Heart Journal. Recognized with the Young Investigator Award at the European Molecular Cardiology (EMC) conference, Dr. Weber is committed to advancing therapies that improve outcomes in complex cardiovascular diseases.

Session 12: Muscle modeling

Sam Walcott (Worcester Polytechnic Institute, USA)
I received my undergraduate degree in Biology and my PhD in Theoretical and Applied Mechanics, both from Cornell University. I did my first postdoc with David Warshaw at the University of Vermont, performing measurements in the laser trap to understand smooth muscle regulation. I did my second postdoc with Sean Sun at Johns Hopkins, developing mathematical models for cell mechanosensation. I then became an independent investigator at UC Davis in the Department of Mathematics. I am currently at Worcester Polytechnic Institute in the Department of Mathematical Sciences. My primary research interest is using mathematical modeling to tie together experimental measurements of biological systems at the molecular, cellular, and larger scales.

Joost Lumens (Maastricht University, Netherlands)
Professor Joost Lumens is Full Professor of Computational Cardiology at the Cardiovascular Research Institute Maastricht (CARIM) of Maastricht University and chair of the CARIM Division Heart. His research bridges cardiovascular physiology and clinical cardiology through computational modeling and simulation technologies, developing multiscale approaches spanning subcellular mechanisms to electromechanical myocardial tissue function and whole-heart pump function. His team develops patient-specific cardiac Digital Twin solutions that reveal how cellular abnormalities manifest as clinical phenotypes in cardiovascular disease. This multiscale methodology enables mechanistic investigation from sarcomere-level perturbations to system-level cardiovascular function, facilitating experimental validation across biological scales. His laboratory developed and hosts the CircAdapt framework for cardiovascular simulation (www.circadapt.org), serving fundamental research and clinical decision support through computational modeling. Professor Lumens has established international recognition through leadership roles in cardiovascular societies (ESC) and editorial positions (European Heart Journal – Digital Health).

Session 13: Skeletal myopathies

Katia Kontrogianni-Konstantopoulos (U Maryland,USA)
Dr. Aikaterini Kontrogianni-Konstantopoulos received her PhD from Baylor College of Medicine. In 2007, she joined the Department of Biochemistry & Molecular Biology at the University of Maryland School of Medicine as Assistant Professor and was promoted to Professor in 2017. Using the muscle and epithelial cells, her laboratory has pioneered the molecular and functional characterization of major cytoskeletal proteins in health and disease, ranging from myopathies to breast cancer. Her research has been continually funded by the Muscular Dystrophy Association (MDA), the American Heart Association (AHA), and the National Institutes of Health (NIH). Dr. Kontrogianni-Konstantopoulos has received several awards, including the 2018 Dr. Patricia Sokolove Outstanding Mentor Award and the 2023 Teacher of the Year award. In addition to her roles as researcher and mentor, she serves as Director of the Interdisciplinary Training Program in Muscle Biology and the Biochemistry & Molecular Biology Graduate Program. Dr. Kontrogianni-Konstantopoulos has been a member of the AHA BCVS Council and has served or chaired AHA, MDA and NIH study sections.

Sandra de Haan (Leiden UMC, Netherlands)
Sandra de Haan is a postdoctoral researcher in Pietro Spitali's Neuromuscular Disease Biomarker Group at the Leiden University Medical Center (LUMC). She holds a PhD from the Karolinska Institute, Sweden, and the National Institutes of Health, USA, with a strong background in developmental biology. Her doctoral research focused on Notch signaling (de Haan et al., Development, 2024) and transcriptome-based lineage tracing (de Haan et al., Science, 2025). Currently, Sandra employs advanced spatial transcriptomics to unravel the complex disease mechanisms underlying muscle pathologies, with a particular focus on inclusion body myositis (IBM). Her work aims to deepen understanding of the immune and degenerative processes in IBM and to identify novel therapeutic targets to address the unmet clinical needs of IBM patients.

SELECTED ABSTRACTS FOR ORAL PRESENTATIONS

Session 1: Muscle structure

Can variation in contractile filaments in adjacent sarcomeres explain sarcomere length non-uniformities?

Chris Tiessen[1], Walter Herzog[1].

1. University of Calgary, Calgary, Canada

Introduction:
Sarcomere length non-uniformity (SLNU) is an intrinsic property in mammalian skeletal muscle [1], and can be observed at all levels of muscle hierarchical structure, including myofibrils. Since its discovery over 70 years ago, a definitive explanation remains elusive. We believe that the simplest explanation for SLNU is that the number of contractile proteins may vary between adjacent sarcomeres in the same myofibril. Previous work from our lab has shown that sarcomere length in a passively stretched myofibril correlates with sarcomere length upon activation; i.e. the longest sarcomeres after passive stretch remain the longest upon isometric activation in a myofibril [2]. Since titin is primarily responsible for passive force generation in myofibrils and exists in a predictible ratio with myosin and actin, this supports variable contractile protein expression may occur in a myofibril.

Methods:
We have developed a novel imaging process, whereby we can test mechanical properties of isolated myofibrils (rabbit psoas) using phase contrast microscopy, and then transfer *the exact same myofibril* for imaging using transmission electron-microscopy. Therefore, we can to track contractile protein numbers in adjacent sarcomeres using serial cross-sections down the length of the myofibril, and correlate sarcomere length with sarcomeric protein content in *an individual myofibril*.
We measure sarcomeric forces by attaching myofibrils to custom-built nano-levers with known spring constants and performing isometric activation.

Results:
Preliminary imaging tests were performed on skinned muscle fibres. We show that variation in contractile protein content between *different myofibrils* (n=6) within a fibre predicts similar SLNU to that seen in isolated myofibril preparations (Figure 1). We are now in the process of acquiring and analyzing isolated myofibril data to correlate variation in contractile protein content and SLNU in the *same myofibril*.

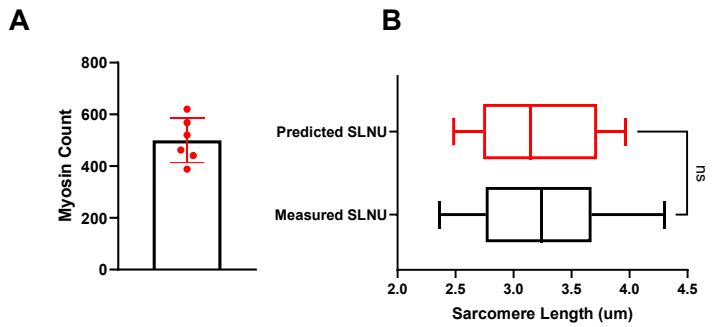


Figure 1: (A) Variation in number of myosin thick filaments in different myofibrils within the same fibre. (B) Comparison of predicted SLNU based on counts in A (n=6) to observed SLNU in myofibrils (n=72 sarcomeres) activated isometrically at an average sarcomere length of 3.2um.

Discussion:

Thus far, we have provided another piece of evidence back the variable contractile protein hypothesis for SLNU. By quantifying the variation in sarcomeric protein content in adjacent sarcomeres and correlating protein content with force measurements in the same myofibril, we may be able to finally definitively explain SLNU. This study also represents a significant technological advancement to mechanically test and then image the *exact same myofibril* using tEM.

References:

[1] Huxley A., & Peachey, L. (1961). *J. Physiology*, 156-165.
[2] Li, M., Leonard, T., Han, S., Moo, E., & Herzog W. (2024). *Frontiers in physiology*, 14.

MANIPULATING THE MECHANICAL INTEGRITY OF *DROSOPHILA* TITIN TO INVESTIGATE ITS ROLE IN SARCOMERE ASSEMBLY AND MAINTENANCE

Lina Herssi[1], Lucas Schröfl[1], Clara Sidor[1] and Frank Schnorrer[1]

1. Aix Marseille University, CNRS, IBDM, Turing Centre for Living Systems, Marseille, France

Abstract:
Titin is a central element in the sarcomere and a key mechanical link between Z-discs and thick filaments in mammalian sarcomeres. To better understand its mechanical role in *Drosophila* sarcomere structure and function, we manipulated the mechanical integrity of the *Drosophila* I-band titin Sallimus *in vivo* and analyzed the consequences from the molecular to the functional level in developing and mature *Drosophila* muscles. After generating a TEV cleavable *Drosophila* titin/Sallimus, we characterized this genetic tool by inducing TEV expression in developing muscles or in mature sarcomeres. This defined the timing and efficiency of titin cleavage. We then examined the consequences of cleaving titin in mature sarcomeres at the molecular and the functional level. We found that the intact TEV-cleavable Sallimus results in wild type muscles. However, once both copies of titin/Sallimus are cleaved in homozygous flies, both the flying and walking abilities of adults are lost within a few hours. The morphological severity of sarcomere phenotype after titin cleavage is more appreciated in muscles in which Sallimus is under high tension: in larval muscles, the sarcomeric structure falls apart within 6 hours after TEV expression. Finally, we investigated the role of intact Sallimus during sarcomere assembly. We found that cleaving both copies of titin during development was lethal at late embryonic stage 17, when wild type muscles begin to contract. Thus, intact titin is required to assemble larval sarcomeres. Cleaving Sallimus during sarcomere assembly in flight muscles results in sarcomeres with strong phenotypes, too, although these phenotypes are milder compared to the other muscle types in which Sallimus is under high tension. Together our data show that *Drosophila* titin is under high mechanical tension and physically links Z-discs to the thick filaments, both during sarcomere assembly as well as in mature sarcomeres.

Session 2: Molecular mechanisms of muscle plasticity

Satellite Cell Fusion into Mature Myofibers Under Pathological Conditions

Vanessa Todorow[1,3], Xaviere Lornage[2], Shinichiro Hayashi[3], Fiorella Grandi[2], Frederique Rau[2], Benedikt Schoser[1], Peter Meinke[1], Ichizo Nishino[3], Denis Furling[2]

Affiliations:

1. Ludwig-Maximilians-University, Friedrich-Baur-Institute, Munich, Germany
2. Institut de Myologie, Paris, France
3. National Center of Psychiatry and Neurology, Tokyo, Japan

Centrally located nuclei are a hallmark of many muscular dystrophies, including myotonic dystrophy type 1 (DM1), yet their cellular origin and functional identity remain unresolved. In DM1, which typically lacks overt fiber necrosis, classical regeneration through de novo fiber formation is unlikely. It has been speculated whether central nuclei arise from impaired nuclear migration or reflect the recent incorporation of progenitor-derived nuclei into mature fibers.

To investigate this, we generated a myofiber-specific Mbnl1/2 knockdown model using AAV-shmiR delivery in adult mouse muscle. Ten weeks after injection, knockdown muscles recapitulated key DM1 features, including myotonia, reduced specific force, MBNL-regulated mis-splicing, and increased centrally nucleated fibers. Single-nucleus RNA sequencing (snRNA-seq), combined with EdU labeling and Pax7-lineage tracing, revealed satellite cell activation and fusion into existing fibers, increasing myonuclear number and centralization. A prominent transcriptional feature was the emergence of Ankrd1-expressing myonuclei, absent in control muscle. Ankrd1, normally restricted to early postnatal stages, was reactivated in knockdown muscles. Comparison with developmental snRNA-seq data showed Ankrd1 expression peaks at postnatal day 21 and is lost in adult muscle, suggesting reversion to an immature myonuclear state.

To assess whether these features are conserved in humans, we performed snRNAseq on muscle biopsies from DM1 patients and controls. Ankrd1 was similarly upregulated across patient myonuclei. We also identified a unique myonuclear subset—Cluster X—present only in DM1. This cluster expressed genes typical of mononuclear progenitors, such as MEGF10, MAML2, DCLK1, and ANTXR1, suggesting recent fusion. CDKN1A, a marker of cell cycle exit, was also enriched and confirmed in situ by immunofluorescence.

These results support a model in which satellite cells—and potentially other progenitor populations—fuse into intact fibers under chronic stress, giving rise to transcriptionally distinct, centrally located nuclei. Rather than reflecting failed nuclear positioning, these nuclei may represent an adaptive mechanism to reinforce fiber function and preserve muscle integrity in the face of persistent molecular pathology.

***Short and sweet* – crosslinking glycation stiffens diabetic titin**

Alejandro Clemente-Manteca[1], Agata Bak[1], Diana Velázquez-Carreras[1], Inés Martínez-Martín[1,2], Enrique Calvo[1], Andrea Laguillo[1], Roberto Silva-Rojas[1], Elías Herrero-Galán[1], Jesús Vázquez[1], Miquel Adrover[2], Jorge Alegre-Cebollada[1].

Affiliations:
1. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.
2. Department of Physics, King's College London, London, UK.
3. Universitat de les Illes Balears, Palma de Mallorca, Spain.

Myocardial stiffening is an early hallmark of diabetes, leading to the development of diabetic cardiomyopathy and heart failure. Although the mechanism driving extracellular matrix stiffening is known, the molecular basis of cardiomyocyte stiffening remains poorly understood. To address this question, we investigate the mechanical alteration of cardiomyocytes produced by glycation, a highly prevalent posttranslational modification subsequent to high glucose levels. This modification generates non-crosslinking and crosslinking modifications in biomolecules. Our work focuses on the effect of glycation on the nanomechanical properties of titin, a protein that plays an essential role in cardiomyocyte's mechanical response.
To explore the extent of this modification in titin and in cardiomyocyte mechanics, we first demonstrate increased levels of non-crosslinking modifications in titin extracted from Ob/Ob mice (leptin KO obese mice) using mass spectrometry. Furthermore, we detect for the first time crosslinking glycations in native titin, by developing an *ad hoc* strategy based in titin domain's three-dimensional structure and mass spectrometry. We further support our mass spectrometry findings using single-molecule force spectroscopy magnetic tweezers. For this, we first demonstrate the efficacy of this technique to detect crosslinking glycations using recombinant titin domains. Then, using the features of the Halo mice (Halo-Tag titin KI mice), we measure an increase of crosslinking modifications in the native titin of Ob/Ob-Halo mice compared to their healthy counterparts. Finally, we test mutant recombinant titin using single molecule force spectroscopy AFM to reveal the spatial distribution of residues undergoing crosslinking modifications.
Our observations suggest that glycation reactions produce crosslinking modifications that stiffen titin and contribute to the altered mechanical landscape of the heart in diabetic patients.

Session 3: Excitation-contraction coupling

AUTOSOMAL DOMINANT RHABDOMYOLYSIS IS ASSOCIATED WITH A MISSENSE VARIANT IN THE ATP2A2 REDUCING SERCA2 CALCIUM PUMP FUNCTION IN SKELETAL MUSCLE.

Romane Idoux*[1,2], Sivasankar Malaichamy*[1,2], Kiran Polavarapu[1], Rachel Thompson[1], Sally Spendiff[1], Emily Freeman[1], Daniel O'Neil[1], Ricardo Carmona-Martinez[1], Andreas Roos[4], Ivo Barić[3], Hanns Lochmüller[1,2].

Affiliations:
[1]Children's Hospital of Eastern Ontario Research Institute, Ottawa, Canada
[2]Department of Cellular and Molecular Medecine, University of Ottawa, Ottawa, Canada
[3]Department of Pediatrics, University Hospital Center Zagreb, Zagreb, Croatia
[4]Department of Neuropediatrics, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

Rhabdomyolysis is a severe condition characterised by the breakdown of skeletal muscle, leading to the release of intracellular components into the bloodstream and causing acute myalgia and weakness. Its causes are diverse including metabolic and neuromuscular diseases (NMDs). In this study, we identified 14 affected individuals from three unrelated families carrying an identical heterozygous missense variant in the *ATP2A2* gene (c.1583G>A; p.R528Q). All patients experienced recurrent episodes of rhabdomyolysis, often triggered by viral infections, fever or intense physical activity. ATP2A2 encodes SERCA2, a calcium pump that is essential for maintaining intracellular Ca^{2+} homeostasis. In skeletal muscle, the SERCA2a isoform plays a critical role in reuptaking cytosolic Ca^{2+} into the sarcoplasmic reticulum (SR) during the relaxation phase of muscle contraction. We hypothesised that the *p.R528Q variant* impairs SERCA2a function by disrupting SR Ca^{2+} reuptake, leading to cytosolic Ca^{2+} overload, and, consequently, rhabdomyolysis. To investigate this, we conducted *in vitro* studies on patient-derived myotubes and *in vivo* analysis using atp2a2a *knockdown* and p.R528Q CRISPR/Cas9 zebrafish models. Zebrafish morphants exhibited gross morphological abnormalities and impaired muscle function, with reduced locomotor activity at 5 and 7 days post-fertilisation in both the light/dark transition and fatigue swimming tests. The p.R528Q CRISPR/Cas9 zebrafish model presented a milder phenotype, with no apparent morphological defects but a consistent reduction in swimming performance. Live calcium imaging in patient myotubes revealed slower SERCA-mediated Ca^{2+} reuptake. In slow muscle fibres of anaesthetised zebrafish larvae, calcium transients displayed reduced peak amplitudes, without alterations in reuptake kinetics, during trains elicited by electrical stimulation at 3 and 5 Hz. Our findings indicate that the p.R528Q variant alters SERCA2a function, leading to abnormal intracellular Ca^{2+} homeostasis in skeletal muscle, which can result in rhabdomyolysis. To date, heterozygous *ATP2A2* variants have only been associated with autosomal dominant skin disorders. Thefore, this study expands the phenotypic spectrum of *ATP2A2*-related disorders beyond autosomal dominant skin diseases and provides a diagnostic conclusion for the affected individuals in the three families studied.

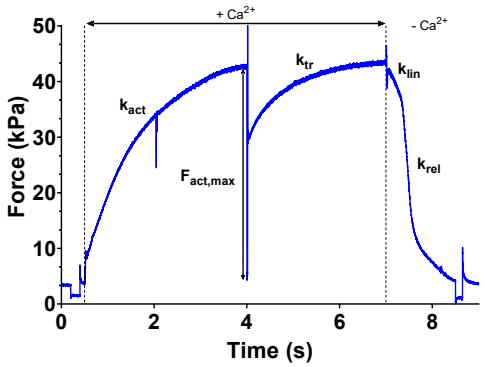
INFLUENCE OF AN HCM-ASSOCIATED CMYBP-C TRUNCATION ON CONTRACTILE FUNCTION OF MYOFIBRILS WITHIN LONG-TERM CULTURED HIPSC-DERIVED CARDIOMYOCYTES

Ferdinand Conrad[1], Karina Ivaskevica[1], Birgit Piep[1], Sarah Konze[1], Jana Teske[2], Robert Zweigerdt[2], Theresia Kraft[1], Bogdan Iorga[1]

Affiliations: 1 Institute of Molecular and Cell Physiology, Hannover Medical School (MHH), Hannover, Germany; **2** Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Clinic for Cardiac, Thoracic, Transplantation and Vascular Surgery, MHH, Hannover, Germany

Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disorder characterized by left ventricular wall thickening and primarily could affect contractile function of cardiomyocytes (CMs). Most HCM-causing mutations occur in genes encoding sarcomeric proteins, notably cardiac myosin binding protein C (*MYBPC3)* and myosin heavy chain (*MYH7)*. We consider that human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) carrying a heterozygous *MYBPC3* truncation can serve as a relevant cellular disease model for HCM. We analyzed force-related parameters of heterozygous *MYBPC3* c.927-2A>G, p.309fsX mutant (MUT) and isogenic (ISO, control) myofibrils (MFs) from demembranated hiPSC-CMs cultured on stiff substrates over two cultivation periods (d35+ and d56+), to investigate the subcellular effects of the mutation in cMyBP-C protein during CMs maturation.

For functional investigations, MFs within demembranated hiPSC-CMs were isometrically mounted in a temperature-controlled micromechanical setup, between a nN-sensitive force probe and a stiff needle. MFs were exposed at 15°C to activating solutions (containing either saturating or intermediate Ca^{2+} concentrations [Ca^{2+}]), or relaxing solution, to induce activation or relaxation, respectively. The resulting force traces were used to evaluate magnitudes of the isometric force (F_{act}) at different [Ca^{2+}] and rate constants of Ca^{2+}-induced force development (k_{act}), of force re-development (k_{tr}), following a short period of isotonic shortening, and kinetics of relaxation (k_{lin}, k_{rel}).



Representative force recording of a MF within hiPSC-CM

Our results indicated that, at 35+ days post-differentiation, MFs of MUT hiPSC-CMs exhibited increased isometric force levels at saturating [Ca^{2+}] ($F_{act,max}$) compared to isogenic controls. This functional change correlated with a reduction in full-length cMyBP-C levels, indicating haploisuffiency, as determined by protein analysis. It was proposed that the wild-type cMyBP-C might manifest an inhibitory role in cross-bridge formation. Therefore, its reduced expression may diminish this functional modulatory constraint, potentially explaining, at least in part, the enhanced force production observed in MFs of mutant cells at the early time point. Nevertheless, calcium sensitivity of myofibrillar force was not significantly altered between MUT and ISO hiPSC-CMs, suggesting minimal potential effect of the mutation on

Ca^{2+} regulation. k_{act} was higher in fully Ca^{2+}-activated MFs of mutant cells only at day 35+ compared to k_{act} corresponding to control MFs. However, no significant differences in k_{tr} at either time point were found. The mutation had no significant effect on k_{lin} and k_{rel}. From contraction (k_{tr}) and relaxation (k_{lin}) kinetic parameters the rate constants of forward (f_{app}) and backward (g_{app}) transitions between force-generating and non-force-generating cross-bridge states can be estimated. Our results suggest that the transition of cross-bridges toward force-generating states might be favored in MFs within hiPSC-CMs affected by haploinsufficiency due to the *MYBPC3* mutation. In conclusion, these findings suggest that the *MYBPC3* mutation may have an early functional effect on MFs within hiPSC-CMs.

Session 4: Data Science in Muscle: from -omics to Physiology

CHARACTERISING A MOUSE MODEL FOR PYROXD1 MYOPATHY – PATHWAY TO THERAPY DEVELOPMENT.

Gregory Dziaduch[1,2,3ł], **Michaela Yuen**[1,2,3ł], Joe Yasa[1,2,3ł], Leon Kiriaev[4], Peter Houweling[4], Emma Kettle[5], Ignatius Pang[6], Mark Graham[7], Frances Lemckert[1,2,3], Sandra T Cooper[1,2,3*] & Frances Evesson[1,2,3*] ł contributed equally to this work; *contributed equally to this work

Affiliations:

1. Kids Neuroscience Centre, Kids Research Institute, The Children's Hospital at Westmead, Westmead, Australia
2. Functional Neuromics, Children's Medical Research Institute, Westmead, Australia
3. Neuroscience, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, Australia
4. Murdoch Children's Research Institute, Department of Paediatrics, University of Melbourne, Melbourne, Australia
5. Westmead Research Hub Electron Microscope Core Facility, The Westmead Institute for Medical Research, Westmead, Australia
6. Bioinformatics Group, Children's Medical Research Institute, Westmead, Australia
7. Synapse Proteomics, Children's Medical Research Institute, Westmead, Australia

Recessive variants in *PYROXD1* cause PYROXD1 myopathy, an ultra-rare congenital or adult-onset disorder affecting skeletal muscle and connective tissue. Clinical features include generalised muscle weakness, respiratory and feeding difficulties, distal joint laxity, hypernasal speech, blue sclerae, and osteopenia. Fewer than 30 individuals have been reported worldwide.

PYROXD1 encodes pyridine nucleotide-disulphide oxidoreductase domain 1 (PYROXD1), a ubiquitously expressed oxidoreductase enzyme essential for cell viability; complete loss of PYROXD1 causes cell death in vitro and is embryonically lethal in mice. Although PYROXD1 is thought to contribute to mitochondrial respiration and regulation of the tRNA ligase complex, its precise biological function remains unclear. The pathomechanism underlying PYROXD1 myopathy is also poorly understood, and no targeted therapies are currently available.

To advance understanding and support therapy development, we generated a mouse model harbouring the *PYROXD1* variant p.N155S (*Pyroxd1*$_{N155S}$), found on at least one allele in 80% of reported cases. *Pyroxd1*$_{N155S}$ mice faithfully replicate the human myopathy, displaying marked skeletal muscle pathology—including fibre atrophy, internalised nuclei, disrupted sarcomeres, and abnormal mitochondrial distribution. We also identified a significant bone phenotype, with reduced cortical thickness and bone mineral density. Functional assessments revealed that *Pyroxd1*$_{N155S}$ mice exhibit decreased skeletal muscle strength, particularly in fast-twitch fibres, as shown by *in vivo* grip strength and *ex vivo* contractile testing. However, muscle performance during force-frequency stimulation, fatigue, and eccentric contraction protocols was comparable to wild-type controls. Proteomic analysis of mutant muscle further supports PYROXD1's role in essential cellular processes, including mitochondrial function and protein synthesis.

In summary, we have established a robust and well-characterised mouse model of *PYROXD1* myopathy that recapitulates the human phenotype. This model is now being used to evaluate therapeutic strategies including PYROXD1 replacement and voluntary exercise.

THE FIBRE MAKES THE MUSCLE: FIBRE TYPE-SPECIFIC DISTINCTION IN PROTEASOMAL, SARCOMERIC, AND METABOLIC PATHWAYS IN GASTROCNEMIUS MEDIALIS FIBRES REVEALED BY FIBRE TYPE-DEPENDENT PROTEOMICS

Koen A.E. Zwetsloot[1], Moritz Eggelbusch[1,2], Wendy Noort[1], Emma Kraal[1], Remco Klaassen[3], Ryan Awadhpersad[1], Richie P. Goulding[1], August B. Smit[3], Rob C.I. Wüst[1]

Affiliations:
1. Department of Human Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands
2. Professorship of Exercise Biology, Department Health and Sport Sciences, TUM School of Medicine and Health, Technical University of Munich, Munich, Germany
3. Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Aim & Methods: Fibre type-dependent differences are often studied by comparing muscles dominated by specific fibre types. However, such fibre type specificity may be confounded by overall muscle activation profiles. Direct intramuscular comparisons are rare but essential to understand intrinsic differences in the proteome. To study this, C57BL/6N mice (N=6) were terminated, and the gastrocnemius medialis muscle (mixed fibre type composition) was dissected, sectioned and stained for succinate dehydrogenase to distinguish between fibre types. Laser microdissection was used to isolate low and high-oxidative fibres for LC-MS/MS proteomics, and subsequent gene set enrichment analysis. Functional confirmation of some proteomics results was performed using high-resolution mitochondrial respirometry.
Results: The myosin thick filament proteins and the Na/K-ATPase protein confirmed that the distinguishment based on oxidative capacity was similar to the distinguishment between fast, and slow-twitch fibres. Interestingly, proteins of the M-band, actin thin filament, and Z-disc proteins titin, and PDLIM7 were more abundant in the low oxidative fibres, while Z-disc proteins myotilin, and titin-cap were more abundant in high oxidative fibres.
Proteins of the ubiquitin-proteasomal system were more abundant in the low oxidative fibres, particularly the ATP-independent regulator (lid) 19s unit (PSMD1/2/8/11/13) the ATP-dependent cataclysmic 20s unit (PSMB5, chymotrypsin-activity), subunits of the 26s complex (PSMC2), and ubiquitin (RPS27A).
Despite the strong distinguishment of fibres based on mitochondrial capacity, three mitochondrial proteins were more abundant in the low-oxidative fibres: GPD2, SQOR, and SFXN3. These proteins are implicated in the glycerol-3-phosphate shuttle, sulphate-, and serine metabolism, respectively. GPD2 likely functions in the glycerol-phosphate shuttle as a reactive oxygen species (ROS) protective mechanism in low oxidative fibres and a by-pass from the glycolysis directly to the oxidative phosphorylation system. High-resolution respirometry confirmed the functional contribution of the glycerol-3-phosphate shuttle to mitochondrial respiration, with an increased respiration during ROS exposure (with pyrogallol producing H_2O_2) in the EDL (+410%, $p<0.05$) compared to the soleus (+17,7%, non-significant).

Conclusion: Muscle fibre type distinction based on oxidative capacity proved effective and revealed unexpected fibre type-specific expression of sarcomeric proteins. Low oxidative fibres showed higher abundance of proteasomal components. The glycerol-3-phosphate shuttle appears crucial in these fibres for both energy metabolism and ROS protection.

Further study on the potential role for hydrogen sulphide metabolism is needed. These findings highlight the value of intramuscular fibre type proteome analyses in uncovering biologically relevant differences that may be overlooked by bulk tissue approaches.

Session 5: Innovations in human disease models

PARTIAL GSK3 KNOCKDOWN IMPROVES MUSCLE PERFORMANCE IN *MDX* MICE: INVESTIGATING THE CELLULAR MECHANISMS

Amélie A.T. Marais[1], Bianca M. Marcella[1], Briana L. Hockey[1], Ryan W. Baranowski[1], Rene Vandenboom[1], Paul J. LeBlanc[1], Rebecca E.K. MacPherson[1], Val A. Fajardo[1]
Affiliations: 1. Brock University, St. Catharines, Canada

INTRODUCTION: Duchenne muscular dystrophy (DMD) is a severe muscle-wasting disease caused by an X-linked mutation to the dystrophin gene that primarily affects boys. The absence of functional dystrophin protein leads to muscle wasting and weakness that progressively worsens over time with affected boys typically being wheelchair-bound by their teenage years and living a shortened lifespan of 30-40 years. Current treatment plans aim to enhance quality of life and preserve muscle strength, primarily using glucocorticoids. Although these are effective in delaying loss of ambulation, side effects often result in discontinuation. Our lab has recently shown that knocking down the enzyme glycogen synthase kinase 3 (GSK3) improved muscle strength and fatigue resistance in a preclinical model for DMD, the *mdx* mouse. However, the exact cellular mechanisms leading to these benefits remain unknown, and uncovering these mechanisms is the purpose of the proposed study.

METHODS: Male muscle-specific GSK3 knockdown mice (C57BL/6J background) were bred with female homozygous D2 *mdx* mice to produce 1) *mdx* mice with partial (30-40%) muscle-specific GSK3 knockdown (*mdx*/GSK3^{KD}) and 2) *mdx* mice with GSK3 intact (*mdx* flox control, herein referred to as *mdx*). To determine how GSK3 knockdown improves muscle strength and endurance, muscle glycogen content, fibre type composition, % necrosis, and oxidative stress were assessed in extensor digitorum longus muscle at 4-6 weeks and 10-14 weeks of age. These age groups were chosen based on the progression of pathology seen in *mdx* mice, with the first bout of myonecrosis occurring at 4-6 weeks, and muscle regeneration cycles happening at 10-14 weeks.

RESULTS: Force-frequency curve and % force remaining analysis suggest that GSK3^{KD} mice had greater force production and fatigue resistance when compared to their *mdx* counterparts at both age groups. We observed a significant increase in muscle glycogen content, along with an increase in oxidative fibre type composition and select mitochondrial markers as potential pathways for fatigue resistance. The *mdx*/GSK3^{KD} group also exhibited decreased oxidative stress as observed by significantly lower % necrosis and central nuclei, suggesting muscle quality improvements due to GSK3 knockdown contributing to both greater strength and fatigue resistance. We saw no significant difference to muscle cross-sectional area when comparing *mdx* to *mdx*/GSK3^{KD} groups.

CONCLUSION: Gaining a better and deeper understanding of the mechanisms leading to the improvement in muscle health in *mdx*/GSK3 knockdown mice provides further evidence in support of targeting GSK3 for DMD. Our findings suggest that GSK3 knockdown increases oxidative fibre type composition and decreases % necrosis and oxidative stress, resulting in improved muscle quality and function. Future efforts from our laboratory will include RNASeq experiments to determine how GSK3 knockdown influences the *mdx* transcriptome.

ENHANCING MYOFILAMENT ISOFORM MATURATION IN HUMAN ENGINEERED HEART TISSUES

Stuart G. Campbell[1,2], Xia Li[1], Xianmu Li[1], Jack Sendek[1]

Affiliations:
1. Department of Biomedical Engineering, Yale University, New Haven, Connecticut, USA
2. Department of Cellular & Molecular Physiology, Yale University, New Haven, Connecticut, USA

Introduction: Engineered heart tissues (EHTs) derived from human induced pluripotent stem cells (iPSC) have achieved broad use in the field for several applications, including muscle physiology, cardiomyopathy modeling, and therapy development. While the advantages of human-derived EHTs are numerous, a persistent challenge is the incomplete maturation of cardiomyocytes when derived in vitro. Among the several potential facets of maturation that could be considered, our work on sarcomere mutations has led us to prioritize improvement of correct myofilament protein isoforms in EHTs.

Methods: EHTs were formed by seeding iPSC-derived cardiomyocytes into thin, decellularized porcine myocardial scaffolds suspended between plastic clips. During subsequent culture, EHTs were exposed to one of several possible conditioning paradigms designed to alter protein isoform expression. Contractile testing was performed on EHTs after long-term culture, at which time they were also homogenized and assayed for protein isoform content.

Results: Two culture conditions were tested with respect to their effect on isoform content, both inspired by events in normal cardiac development. First, we examined the effects of thyroid hormone (T3). Following birth, there is a transient surge in T3. We sought to mimic this hormonal phase by supplementing tissue media with 15 nM T3 until Day 16 after their formation. Thereafter, only the small amount of T3 present in B27 supplement remained. At the time of T3 withdrawal, EHTs were found to express ~20% cardiac troponin I (cTnI), compared with <1% in the untreated controls. At the same time, β-myosin heavy chain (β-MHC) content had dropped to 40% of total myosin (owing to the well-known effect of T3 on enhancing α-MHC expression). However, by 12 days post-T3 withdrawal, β-MHC expression had rebounded to ~80% of total, while cTnI levels remained at 20%. In a second set of experiments, we used a dynamic culture bioreactor to simulate normal chamber growth that occurs after birth, in which cardiomyocytes are gradually stretched by increasing blood volume until they reach adult size. We administered a 40% total stretch relative to starting EHT length over the course of five days in culture. At the conclusion of progressive stretch conditioning, EHTs were found to express ~8% cTnI as a fraction of total, in comparison with <1% in unstretched, control EHTs.

Discussion: Other investigators have reported the ability of T3 to enhance expression of cTnI, but we were initially reluctant to utilize this method of maturation owing to the counterproductive changes in MHC isoform. We hypothesized that a developmentally inspired ‘T3 pulse’ would trigger transition to cTnI expression while allowing β-MHC expression to recover. This hypothesis was supported by the experimental data. We also

note that progressive stretch of EHTs on its own, without T3 pulse, was sufficient to trigger a meaningful increase in cTnI. We anticipate that a simultaneous administration of a T3 pulse, progressive stretch, and ramped electrical pacing, all of which mimic events in development, will yield high cTnI and β-MHC expression in an accelerated 21-day protocol.

Session 6: Muscle energetics

Disrupted glucose-insulin signalling drives metabolic dysfunction in the *mdx* mouse model of Duchenne muscular dystrophy

Gretel Major[1], Ekaterina Salimova[2], Joel Eliades[3], Gang Zheng[2], Rong Xu[2], Christoph E. Hagemeyer[2], Karen Caeyenberghs[3], Michael de Veer[2], Jiayi Chen[1], Charlie Najt[4] and Angus Lindsay[1,5]

Affiliations:
1. University of Canterbury, Christchurch, New Zealand
2. Monash University, Clayton, Australia
3. Deakin University, Geelong, Australia
4. Virginia Tech University, Virginia, USA
5. University of Otago, Christchurch, New Zealand

Skeletal muscle, as the largest insulin-sensitive tissue, plays a key role in systemic metabolism through its metabolic plasticity and insulin-dependent glucose uptake. Duchenne muscular dystrophy (DMD), a severe muscle wasting disease, is associated with systemic metabolic dysfunction, including adiposity, hyperinsulinemia, and glucose intolerance; as well as neurobehavioral comorbidities, such as stress hypersensitivity. These metabolic and neurobehavioral phenotypes coincide with altered energy metabolism in both skeletal muscle and the brain and are replicated in the *mdx* mouse model of DMD. In the current study, we leverage the stress hypersensitivity phenotype as a tool to amplify metabolic dysfunction in the *mdx* mouse model of DMD and determine the consequences for metabolic function systemically, as well as in the brain and skeletal muscle, the body's primary consumers of glucose. Tube-restraint stress induced sustained hyperglycaemia and worsened glucose intolerance in *mdx* mice. Using positron emission tomography, *mdx* mice showed altered tissue metabolic responses to stress, with no change in brain and reduced striated muscle ^{181}F-fluorodeoxyglucose uptake, in contrast to wild-type (WT) mice, which exhibited decreased brain and slightly increased skeletal muscle uptake—indicating disrupted glucose-insulin signalling in *mdx* mice. *Mdx* mice had lower serum C-peptide levels, a byproduct of insulin synthesis, under basal and stress conditions relative to WT mice. Furthermore, glucose-stimulated-insulin-secretion assays performed on isolated pancreatic islets supported these findings whereby islets from *mdx* mice secreted less insulin in response to glucose insults relative to islets from WT mice – highlighting decreased endogenous insulin production in a basal state. When administered exogenous insulin, stress-induced hyperglycaemia was prevented in *mdx* mice, suggesting that *mdx* mice are not insulin insensitive and that the glucose intolerance is likely due to impaired insulin synthesis. *Mdx* mice also demonstrated elevated corticosterone levels during and following the stress exacerbation, which likely further suppressed insulin secretion. Taken together, this work 1) presents a mechanistic understanding of how DMD may affect glucose-insulin signalling and metabolic dysregulation in DMD, and 2) demonstrates that exogenous insulin administration can mitigate glucose intolerance in this model. These findings not only fill a gap in our understanding of metabolic dysregulation in DMD but also suggest that a targeted therapeutic strategy could improve systemic metabolic function and possibly quality of life for patients with the disease.

NICOTINAMIDE RIBOSIDE SUPPLEMENTATION PREVENTS THE ONSET OF MITOCHONDRIAL DYSFUNCTION IN A MOUSE MODEL FOR NEMALINE MYOPATHY TYPE 6

Rianne Baelde[1], Alexcia Fortes Monteiro[1], Edgar Nollet[1], Ricardo Galli[1], Sultan Bastu[3], Edoardo Malfatti[3], Bauke Schomakers [4], Kasper Vinten [4], Riekelt Houtkooper [4], Jolanda van der Velden[1], Coen Ottenheijm[1,2] and Josine de Winter[1]

1 Dept. of Physiology, Amsterdam UMC, location VUmc, The Netherlands
2 Dept. of Cellular and Molecular Medicine, University of Arizona, Tucson, USA
3 AP-HP, Neuromuscular Pathology Reference Centre, Henri Mondor Hospital, Créteil, France
4 Dept. of Genetic Metabolic Diseases, Amsterdam UMC, location AMC, The Netherlands

Nemaline Myopathy type 6 (NEM6) is caused by variants in Kelch-repeat-and-BTB-(POZ)-Domain-Containing-13 (*KBTBD13*). The majority of the NEM6 patients harbors the Dutch founder mutation *KBTBD13*^{R408C} (c.1222C>T, p.Arg408Cys), resulting in a hypercontractile phenotype caused by sarcomere-based impaired muscle relaxation. Histological characterization of NEM6 patient biopsies by NADH staining show the presence of cores, indicating the absence of complex I (NADH) activity and mitochondrial dysfunction.

In this study, we aimed to perform a natural history study in homozygous *Kbtbd13*^{R408C}-knockin mice (NEM6 mouse model) to investigate whether hypercontractility contributes to mitochondrial dysfunction in NEM6. First, enzymatic NADH staining showed absence of cores at 1 month, onset at 3 and progression of cores at 9 months. Therefore we can conclude that the NEM6 mouse model phenocopies core formation found in NEM6 patients. Second, mitochondrial respiration was investigated by *in vitro* high-resolution respirometry. In line with the presence of NADH cores at 3 and 9 months old, soleus muscle of NEM6 mice showed significant decreased total OXPHOS and NADH-linked respiration. To study the functional consequences *in vivo*, metabolic treadmill experiments were performed. These experiments showed significant impaired running performance, decreased VO_2max and increased respiratory exchange ratio (RER) in NEM6 mice. Next, we studied the pathways underlying mitochondrial dysfunction in NEM6 muscle. Metabolomics and proteomics were performed on soleus muscle, and joint pathway analysis revealed that TCA cycle related pathways are most affected in NEM6 mice. Of special interest, NAD^+ levels in 3 and 9 months old NEM6 mice were significantly decreased. We attempted to prevent the onset of the mitochondrial phenotype with supplementation of nicotinamide ribose (NR), a NAD^+ precursor that is generally considered safe and is currently tested in clinical trials. One month old WT and NEM6 mice received nicotinamide riboside (NR) supplementation for 8 weeks. Our data showed that chronic NR supplementation prevented the onset of both total OXPHOS and NADH-linked respiration in NEM6 mice. To conclude, this study provided insights into the natural history of mitochondrial dysfunction in NEM6 and provides proof-of-concept for the ability of NR to revert the mitochondrial phenotype in NEM6.

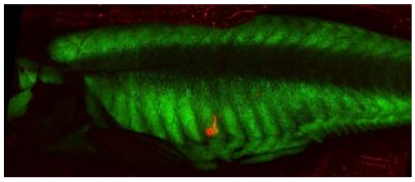
Session 7: Cytoskeleton

THE REPLACEMENT KINETICS OF THE GIANT MUSCLE PROTEIN NEBULIN ARE SLOW BUT MUCH FASTER BY A VARIANT THAT DISRUPTS AN ACTIN-BINDING MOTIF

Sylvia Bogaards[1], Yeszamin Onderwater[1], Lacramioara Fabian[3], Jim Dowling[3] and Coen Ottenheijm[1,2]

Affiliations: 1. Department of Physiology, Amsterdam UMC, Amsterdam, the Netherlands; 2. Cellular and Molecular Medicine, University of Arizona, Tucson, USA; 3. Departments of Paediatrics and Molecular Genetics, Hospital for Sick Children, University of Toronto, Canada

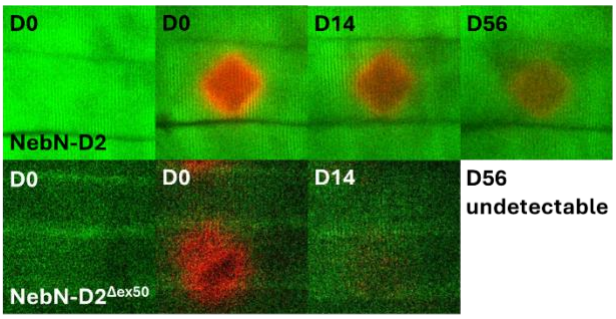
1. Intubated fish on the microscope



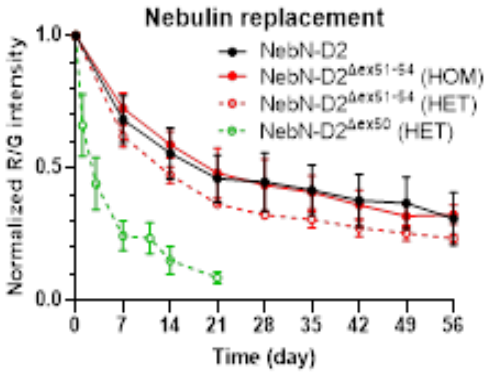
2. Example of green fluorescent fish

To date, it is unknown how giant muscle proteins are integrated and replaced in continuously contracting muscles. A prime example is the sarcomeric, thin filament-associated protein nebulin (~800 kDa), variants in which cause nemaline myopathy. To establish the replacement kinetics of nebulin in sarcomeres, we designed a zebrafish model with the photoconvertible fluorescent protein dendra2 inserted in nebulin's N-terminus (NebN-D2), and used *in vivo* imaging in adult, full grown zebrafish. For imaging, fish were sedated and intubated (fig 1) for ~15 minutes to maintain proper sedation and oxygenation. Fish were followed for 2 months. In between imaging the fish was free to swim around freely. Sarcomeric striation was observed when a fish was imaged for green fluorescence (fig 2&3). In at least 6 ROIs (20x20 µm) per fish, green dendra2 was converted to the red fluorescent state and fluorescence was followed over time (fig 3). After conversion, green fluorescence can only increase via replacement of red fluorescent nebulin with new (green fluorescent) nebulin. Data are displayed as red/green intensity (fig 4), therefore, a value for normalized R/G intensity of 0.25 represents 50% replacement. Data show that nebulin's replacement is very slow, with <50% of converted (red) dendra2 being replaced after 56 days (n=15 fish). Zebrafish with a variant in nebulin leading to a deletion of one of the 22 super repeats (NebN-D2$^{\Delta ex51\text{-}54}$), without disrupting an actin binding motif, showed similar replacement kinetics as NebN-D2 (n=3 per genotype, pilot data). Zebrafish with a heterozygous variant in nebulin, which disrupts an actin binding motif (NebN-D2$^{\Delta ex50}$), show a less bright striation pattern, indicating less of the mutated protein is present and incorporated. Data of these fish show a much faster replacement of nebulin (n=13 fish; ~8x faster).

Summarizing, our data indicate that the replacement kinetics of the giant protein nebulin in adult zebrafish are very slow. In NebN-D2$^{\Delta ex50}$ fish, replacement is much faster, suggesting that the binding of nebulin to actin plays an important role in the replacement kinetics of nebulin.



3. Example of converted ROI right before and after conversion (day 0) and at day 14 or 56 in NebN-D2 or NebN-D2$^{\Delta ex50}$ fish



4. Replacement kinetics of nebulin in zebrafish

Mechanotransduction at the Intercalated Disc via an LMCD1-Associated Protein Complex

Aishwarya Prakash[1], Emilie Marhuenda[1,2], Andrew Tinker[3], Cesare Terraciano[4], Thomas Iskratsch[1]

Affiliations:
1. School of Engineering and Materials Science, Queen Mary University of London, UK
2. Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, F-33600 Pessac, France
3 William Harvey Heart Centre, Queen Mary University of London, UK.
4 National Heart & Lung Institute, Imperial College London, UK.

Introduction:
Dilated cardiomyopathy (DCM) is a major contributor to heart failure, marked by ventricular dilatation, reduced cardiac output, and increased myocardial stiffness. While these macroscopic changes are well characterized, the cellular mechanisms—particularly at the level of cardiomyocyte connectivity—remain poorly understood. Previous work suggested that structural remodelling of the intercalated disc (ICD), the specialized junction between cardiomyocytes, may be a hallmark characteristic in DCM. LIM domain proteins, known for their roles in mechanotransduction, have been implicated in this process. This study aimed to identify and characterize a mechanosensitive protein complex involving LIM domain proteins at the ICD and to explore its role in cardiomyocyte mechanosignaling.
Methods:
Neonatal rat cardiomyocytes (NRCs) were cultured on polydimethylsiloxane (PDMS) substrates with microgrooved patterns (to facilitate cell-cell contacts) and tunable stiffness (6 kPa to mimic neonatal heart tissue and 66 kPa to mimic fibrotic myocardium). Immunostaining, co-immunoprecipitation, and proteomic analyses were employed to investigate protein localization and interactions. An N-Cadherin tension sensor was used to assess mechanical tension at the ICD under varying stiffness conditions.
Results:
Ten LIM domain proteins previously associated with heart failure were screened for stiffness-dependent localization to the ICD. Dyxin/LMCD1 emerged as a key candidate, showing consistent ICD localization and structural impact under high-stiffness conditions. Overexpression of LMCD1 led to ICD abnormalities, including increased ICD width, reminiscent of DCM pathology. Loss of LMCD1 increased tension at the ICD, as confirmed by the N-Cadherin tension sensor. Proteomic analysis identified a novel LMCD1-associated complex comprising the atypical cadherin FAT1, Tec kinase, WAVE3, and β-actin, all of which co-localized with β-catenin at the ICD. Several of these proteins are linked to cardiac disease, underscoring the relevance of this complex.
Discussion:
This study identifies a novel mechanosensitive complex at the ICD that includes LMCD1 and several proteins implicated in cardiac remodelling. The stiffness-dependent behaviour of this complex suggests a critical role in mechanotransduction and pathological signalling in cardiomyocytes. These findings provide new insights into how mechanical stress is sensed at the cellular level and open avenues for targeting mechanosensitive pathways in heart failure therapy.

Session 8: Respiratory muscle mechanics in NMD

Asynchronies related to mechanical ventilation exacerbates Diaphragm Dysfunction in mice model of Duchenne Muscular Dystrophy.

Mohamad Yehya[1#], Haikel Dridi[2#], Eric Estève[1], Johan Moreau[1,3], Steven Reiken[2], Andrew R Marks[2], Alain Lacampagne[1], Stefan Matecki[1,3].

1: PhyMedExp, Montpellier University, INSERM, CNRS, CHRU Montpellier, 34295-Montpellier, France
2: Department of Physiology and Cellular Biophysics, Clyde and Helen Wu Center for Molecular Cardiology Columbia University College of Physicians and Surgeons New York USA
3: Physiological Department, Montpellier University and Montpellier University Health Care Center, 34295-Montpellier, France
#: Authors contributed equally to this work.

Mechanical ventilation (MV) with non-invasive positive pressure (NIVPP) is recommended for patients with Duchenne Muscular Dystrophy (DMD) experiencing daytime hypercapnia or symptoms of alveolar hypoventilation. Nevertheless, the use of mechanical ventilation which unload the diaphragm is associated with a detrimental condition known as ventilator-induced diaphragmatic dysfunction (VIDD) in non-dystrophic patients, which may accelerate the progression of respiratory muscle disease and increase dependence on ventilatory support in DMD patients.
Specifically, the asynchrony between diaphragm contraction in response to the patient's air demand and the air delivery by the ventilator can lead to a mechanical stress resembling eccentric contraction, which is particularly harmful in the context of dystrophic deficiency diaphragm.
In healthy mouse models, MV induces early remodeling of the Ca2+ release channel (RyR1) and disrupts intracellular Ca2+ homeostasis, resulting in VIDD. We hypothesized that VIDD is exacerbated in dystrophic patients due to increased fragility of the diaphragmatic membrane caused by the absence of dystrophin, amplifying mechanical strain and worsening intracellular Ca2+ dyshomeostasis and dysfunction.
To model asynchrony, we imposed eccentric stress on a mechanically ventilated mdx diaphragm. We observed that MV impairs RyR1 function, increases susceptibility to eccentric contraction, and causes injuries and weakness in the diaphragm. This effect can be mitigated by specific RyR1 stabilizers, such as S107, showing comparable efficacy to ebselen treatment, a specific inhibitor of NOX2. Our findings highlight the importance of minimizing asynchrony during NIVPP in DMD patients and pave the way for future preventive treatments targeting RyR1 functional deficits.

Session 9: Regulation of contraction by thick and thin filaments

CALCIUM-DEPENDENCE OF THE STRUCTURAL CHANGES IN THE THICK FILAMENT OF DEMEMBRANATED MYOCARDIAL SLICES FROM THE RABBIT HEART

Watson G.[1,2], Arcidiacono A.[1,2], Kalakoutis M.[1,2], Hill C.[1,2], Shattock MJ.[2,3], Clark JE.[2,3], Theyencheri N.[4], Irving M.[1,2], Fusi L.[5], Brunello E.[1,2]

Affiliations:
[1] Randall Centre for Cell and Molecular Biophysics, School of Basic and Medical Biosciences, King's College London, London, United Kingdom;[2]British Heart Foundation Centre of Research Excellence, King's College London, London, United Kingdom; [3]School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, United Kingdom; [4]ESRF – The European Synchrotron Radiation Facility, Grenoble, France; [5]Centre for Human and Applied Physiological Sciences, School of Basic and Medical Biosciences, King's College London, London, United Kingdom.

Contraction of the myocardium is regulated at the level of the myofilaments. Calcium-dependent structural changes in the thin filaments control access to the myosin binding sites on actin, while thick filament-based regulation dynamically controls the number of myosin motors available to interact with actin. Myosin motors are folded and helically ordered on the surface of the thick filament backbone in diastolic or relaxed cardiac muscle, and become more perpendicular to the thick filament surface during maximal calcium activation. This dual-filament model of regulation of cardiac contractility was developed from recent X-ray diffraction work performed in both electrically-paced and demembranated rat cardiac trabeculae (Reconditi et al., 2017 PMID:28265101; Brunello et al. 2020 PMID:32220962; Wang et al., 2024 PMID:39552044), which express the fast alpha isoform of the cardiac myosin heavy chain. However, the ventricles of larger mammals, including humans, express the slow beta myosin isoform, which recent studies have suggested may have different mechanisms of thick filament-based regulation (Mohran et al., 2024 PMID:39302315).
Here we used Small Angle X-ray Diffraction (SAXD) at beamline ID02 (ESRF, France) to measure the calcium-dependence of the structural changes in the thick filament in freshly demembranated myocardial slices obtained from the left ventricular wall of cardioplegically arrested rabbit hearts, which predominantly express the beta-myosin isoform. SAXD patterns were collected either at 31m or 3.2m sample-to-detector distance to measure changes in sarcomere length, or to collect the SAXD signals associated with changes in myofilament structure, respectively. Myocardial slices were activated with a temperature jump protocol to 37°C at steady calcium concentrations ranging from pCa 9 to pCa 4.7, in the presence of 3% Dextran T500 and at 2.25 μm sarcomere length. Active force had a sigmoidal dependence on calcium concentration and was 50±10 kPa (mean±SD, N=5 slices) at saturating calcium. Increased calcium concentration was accompanied by an increase in the equatorial intensity ratio ($I_{1,1}/I_{1,0}$) associated with the movement of the myosin motors from the thick filament surface towards the thin filaments. Furthermore, thick filament activation at increasing calcium concentrations was signaled by the increase in the spacing of the M6 reflection, associated with the axial periodicity of the thick filament, and by a progressive decrease in the intensity of the first myosin layer line, associated with the helical order of the myosin motors in relaxed muscle. The spacing and intensity of the M3 reflection had biphasic behavior, signaling first the loss of the relaxed folded motors at intermediate calcium concentrations and then the

increase in the population of perpendicular force generating motors at high calcium concentrations. Inhibition of myosin by 25 μM Mavacamten suppressed active force and the structural changes in the thick filament associated with increasing calcium concentration. These data are consistent with comparable experiments performed in demembranated rat cardiac trabeculae (Kalakoutis et al 2025, JMCC), suggesting that the alpha and beta myosin isoforms have similar mechanisms of thick filament-based regulation.
The present results elucidate the calcium-dependence of the regulatory structural changes in the thick filament in cardiac muscle expressing the beta isoform of the cardiac myosin heavy chain, at physiological temperature and lattice spacing, and demonstrate the suitability of myocardial slices for SAXD studies of thick filament regulation in the heart of large mammals.
Supported by British Heart Foundation (UK).

SUB-SARCOMERIC REGULATION OF THIN AND THICK FILAMENTS IN SKELETAL MUSCLE MYOFIBRILS

Kristina Sliogeryte[1] and **Luca Fusi[1,2]**

Affiliations:
1. Randall Centre for Cell and Molecular Biophysics, School of Basic and Medical Biosciences, King's College London, London, United Kingdom.
2. Centre for Human and Applied Physiological Sciences, School of Basic and Medical Biosciences, King's College London, London, United Kingdom.

The contractility of skeletal muscle is regulated by a dual-filament mechanism in which thin filament-based regulation controls the number of actin binding sites available to myosin motors, while thick filament-based regulation modulates the number of myosin motors available for contraction (Brunello et al., 2023; PMID:37216507). Calcium binding to troponin induces movement of tropomyosin along the thin filament, partially exposing myosin binding sites on actin. Myosin motors bound to actin further enhance thin filament activation; however, it remains unclear whether these myosin-dependent structural changes in the thin filament occur both in the filament overlap region (A-band) and in the non-overlap region (I-band) of the sarcomere. Thick filaments are structurally divided into P, C, and D zones, defined by the localisation of Myosin-Binding Protein-C (MyBP-C) in the C-zone and distinct titin domains in the C- and D-zones. However, the regulation of OFF/ON states of myosin motors within these filament domains is not fully understood.
We developed a novel approach using Fluorescence Polarisation Microscopy (FPM) on single myofibrils from rabbit psoas muscle to map the activation states of troponin and myosin motors within distinct filament zones at the single sarcomere level. Myofibrils were exchanged with bifunctional rhodamine probes on either troponin C (TnC) or the regulatory light chain (RLC) of myosin. FPM was used to spatially resolve TnC and RLC probe orientations with ~400 nm resolution along thin and thick filaments, respectively, in rigor (pCa 9 and 4.7), relaxed (pCa 9), and calcium-activated (pCa 6.6 and 4.7) myofibrils. In rigor in the absence of calcium (pCa 9) actin-attached myosin motors induced large conformational changes in troponin within the A-band, whereas the orientation changes of the TnC probes in the I-band were smaller. The addition of calcium (pCa 4.7) enhanced the activation of the thin filament in the A-band but not in the I-band. These results align with the "Closed" and "Open" thin filament conformations, in the I- and A-bands respectively, revealed by cryo-electron tomography of rigor skeletal myofibrils (Wang et al., 2021; PMID:33765442). In relaxed myofibrils at near-physiological temperature (30 °C) and lattice spacing (in the presence of Dextran T-500), troponin probe orientation was uniform along the thin filament and largely unaffected by changes in sarcomere length between 2.4–3.3 μm. In contrast, RLC orientation in the C-zone was more parallel to the filament axis than in the P and D zones, suggesting that MyBP-C stabilises the folded-OFF state of myosin in this filament domain. Upon stretching relaxed myofibrils to a sarcomere length of 3.3 μm, C- and D-zone motors became partially activated, while P-zone motors remained folded. Activation of the myofibril at both low (pCa 6.6) and high (pCa 4.7) calcium concentrations caused uniform structural changes in troponin along the thin filament, in both A- and I-bands. At high calcium, myosin motors were fully activated along the thick filament, whereas at low calcium, ON motors were concentrated in the D-zone, and OFF motors in the P and C zones.

All together, our results reveal the zonal regulation of the OFF/ON states of thin and thick filaments by calcium and filament force at single sarcomere level under near-physiological conditions.
(Supported by the Wellcome Trust and The Royal Society, UK).

Session 10: Targeting the nucleus

DESMOPLAKIN LOSS LEADS TO PKC-DEPENDENT INSERTION OF SERIES SARCOMERES AND CONTRACTILE DYSFUNCTION IN CARDIOMYOCYTES

Ilhan Gokhan[1], Xia Li[1], Jack M. Sendek[1], Alex J. Mora Pagan[2], Fadi G. Akar[1,3], Stuart G. Campbell[1,2,4]

Affiliations:
1. Dept. of Biomedical Engineering, Yale University, New Haven, CT, USA
2. Dept. of Cellular and Molecular Physiology, Yale University, New Haven, CT, USA
3. Division of Cardiology, Department of Internal Medicine, Yale University, New Haven, CT, USA

Background
Mutations in *DSP*, which encodes the protein desmoplakin, lead to cardiomyopathy with unusually high penetrance. Clinical features include ventricular tachyarrhythmias, fibro-fatty infiltration of both ventricles, and ultimately dilated cardiomyopathy. While some data have been gathered to explain the mechanism of desmoplakin cardiomyopathy, a comprehensive mechanism linking *DSP* mutations to ventricular dilation and heart failure remains elusive.

Methods
We use iPSC-derived engineered heart tissue (EHT) bearing a functional desmoplakin haploinsufficiency to model the heart failure phenotype that occurs in desmoplakin cardiomyopathy. Desmoplakin depletion is secondary to a missense mutation, R451G, that results in rapid proteolytic degradation of protein. We complement functional data obtained in tissue-engineered constructs with cell biology assays in 2D cardiomyocytes to elucidate the mechanism of DSP cardiomyopathy.

Results
Engineered heart tissues harboring a desmoplakin insufficiency recapitulate a patient phenotype notable for hypocontractility without alterations in calcium handling (Fig. A). Interestingly, DSP-mutant tissues exhibited enhanced length-dependent activation. Sarcomere length in both 2D and 3D contexts was shortened as a result of DSP depletion, potentially accounting for these contrasting mechanical phenotypes (Fig. B). Shortened sarcomere lengths in DSP-mutant cells and tissues could be rescued by broad-spectrum inhibition of protein kinase C (PKC), and PKC activation was accordingly increased in DSP tissues. Finally, mechanical loading on adherens junctions, as measured by immunofluorescence of extended-conformation α-catenin, was enhanced in DSP-mutant vs. WT cells, suggesting a redistribution of force from desmosomes to adherens junctions. Therefore, desmosomal instability, which results in excessive loading of adherens junctions, may act as a stimulus for PKC-induced insertion of series sarcomeres. This shortens the length per sarcomere and may result in a contractile deficit consistent with ventricular dilation.

Conclusions
Our study uncovers a novel mechanism underlying systolic dysfunction in desmoplakin cardiomyopathy that, to our knowledge, is the first to implicate shortened sarcomere length

and PKC activation. We not only recapitulate the disease phenotype, but we identify sarcomere length regulation through altered force transmission at the intercalated disc as a previously-unrecognized mechanism, which may be a potential therapeutic target.

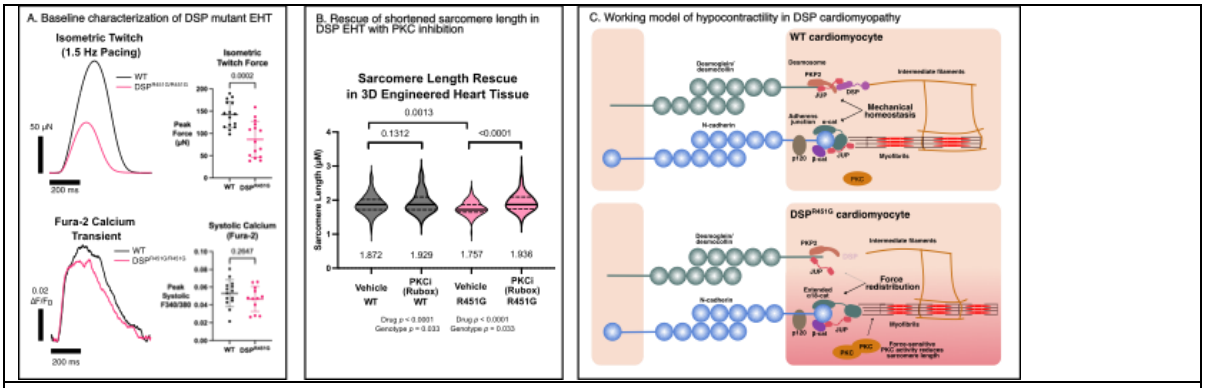


Fig: A. Baseline mechanical characterization; B. Shortened sarcomere length in DSP-mutant tissues, rescuable by PKC inhibition; C. Working model of force redistribution at the intercalated disc resulting in avid series sarcomere addition with shortened length-per-sarcomere.

ALTERED PERINUCLEAR CYTOSKELETON AND LAMINA IN AGED MYONUCLEI: IMPLICATIONS FOR IMPAIRED MECHANORESPONSIVENESS

Osman Esen[1], Leander A. Vonk[1], and Tyler J. Kirby[1]

Affiliations:
1. Department of Pyhsiology, Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

Skeletal muscle cells (i.e. myofibers) generate large forces through actin-myosin contraction, exposing their organelles to constant mechanical stimuli. Mechanical forces are transmitted to myonuclei through a physical connection between the cytoskeleton (i.e., microtubules) and the nucleus via the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. The LINC complex then interacts with the nuclear lamina, a dense, intermediate filament meshwork that tethers chromatin at the nuclear periphery. Thus, this mechanical coupling can deform the nucleus and influence chromatin organization and gene expression.
Age-related alterations in myonuclear morphology have been reported, but their underlying causes and functional implications for nuclear mechanoresponsiveness remain unclear.

We hypothesize that age-related changes in cytoskeletal organization at the nuclear exterior and interior alter myonuclear morphology and subsequent mechanoresponsiveness. To test this, we analysed myofibers from aged (18 months) and young (3-5 months) mice. We used immunofluorescence to examine the organization and abundance of microtubules, nesprin-1 (a key LINC component), and lamin A/C, along with live-cell imaging to assess nuclear deformation during contraction. To gain mechanistic insights into the role of nucleo-cytoskeletal coupling on nuclear deformation, we either disrupted the entire LINC complex by AAV-mediated expression of a dominant-negative KASH construct or only the microtubules using nocodazole treatment.

Aged myofibers demonstrated nuclear elongation and disrupted perinuclear microtubule network, particularly microtubules located at the long axis of the nucleus. While the spatial distribution of nesprin-1 was unchanged, its overall expression was reduced. The nuclear lamina in aged myonuclei showed altered conformation, as assessed by immunodetection of mechanosensitive cryptic epitopes, whereas total lamin A/C levels were not affected by age. Interestingly, both microtubule disruption and LINC complex interference in young myofiber induced nuclear elongation. However, the nuclear lamina showed no alteration in conformation, suggesting that nuclear lamin conformational occur slowly over time. Intriguingly, despite the above-mentioned changes, the contraction-induced percentage of nuclear deformation in aged myonuclei retained normal. However, they exhibit increased absolute nuclear deformability and shorter creep-relaxation times, indicative of reduced nuclear viscosity.

Together, these findings reveal that aging alters myonuclear mechanical properties, morphology, and cytoskeletal organization. Such adaptations may serve to preserve nuclear deformation under contractile stress, thereby contributing to the regulation of nuclear mechanosensitivity in aged skeletal muscle.

Session 11: Cardiomyopathies

BROAD THERAPEUTIC BENEFIT OF MYOSIN INHIBITION IN HYPERTROPHIC CARDIOMYOPATHY

Laura Sen-Martín[1], Ángel Fernández-Trasancos[1], Miguel Á. López-Unzu[1], Agata Bak[1], Divya Pathak[2], Alessia Ferrarini[1], Verónica Labrador-Cantarero[1], David Sánchez-Ortiz[1,3], María Rosaria Pricolo[1], Natalia Vicente[1], Diana Velázquez-Carreras[1], Lucía Sánchez-García[1], Jose Ángel Nicolás-Ávila[1], María Sánchez-Díaz[1], Saskia Schlossarek[4,5], Lorena Cussó[1,6,7], Manuel Desco[1,6,7,8], María Villalba-Orero[1,9], Gabriela Guzmán-Martínez[10,11], Enrique Calvo[1], Roberto Barriales-Villa[12,13,14], Jesús Vázquez[1,14], Fátima Sánchez-Cabo[1], Andrés Hidalgo[1], Lucie Carrier[4,5], James A. Spudich[2], Kathleen M. Ruppel[2], Olivier Cazorla[15], Annika Klotz[16], Michel N. Kuehn[16,17], Khoi D. Nguyen[16], Anthony L. Hessel[16,17], Jorge Alegre-Cebollada[1]

Affiliations:
1. Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; 2. Department of Biochemistry and Stanford Cardiovascular Institute, Stanford University School of Medicine; 3. Servicio de Cardiología, Hospital Puerta de Hierro de Majadahonda, Madrid, Spain; 4. Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 5. 2DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Hamburg, Germany; 6. Laboratorio de imagen para pequeño animal de experimentación, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain; 7. CIBER de salud mental, Instituto de salud Carlos III, Madrid, Spain; 8. Departamento de Bioingeniería, Universidad Carlos III de Madrid, Madrid, Spain; 9. Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain; 10. Department of Cardiology, La Paz University Hospital, IdiPaz, Madrid, Spain; 11. Department of Medicine, Faculty of Biomedical and Health Sciences, Universidad Europea de Madrid, Spain; 12. Unidad de Cardiopatías Familiares, Servicio de Cardiología, Complexo Hospitalario Universitario de A Coruña, Servizo Galego de Saúde (SERGAS); 13. Instituto de Investigación Biomédica de A Coruña (INIBIC), Universidade da Coruña, A Coruña, Spain ; 14. CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain; 15. PhyMedExp, University of Montpellier, INSERM, CNRS, Montpellier, France; 16. Accelerated Muscle Biotechnologies Consultants, Boston, MA, USA; 17. Institute of Physiology II, University of Muenster, Muenster, Germany

Many myocardial pathologies are caused by inherited genetic mutations that result in anatomical alterations and compromised cardiac function. In humans, the missense variant R502W in cardiac myosin-binding protein C (cMyBP-C) is the most frequent mutation leading to hypertrophic cardiomyopathy (HCM). However, the molecular mechanisms sustaining the pathogenicity of the R502W variant remain unknown, since both cMyBP-C's mRNA and protein structure have been proposed not to be perturbed by the mutation. Using CRISPR/Cas9-based genetic engineering, we have generated a knock-in mouse model that harbors the R502W mutation in murine cMyBP-C and characterized the resulting cardiac phenotype. R502W mice exhibit progressive myocardial remodeling without alterations in cMyBP-C levels or localization. In addition, while we observe changes in myosin structural states (ON/OFF) in accordance with increased calcium sensitivity, passive force, and slightly decreased binding affinity between mutant cMyBP-C and myosin. Despite these changes, the myosin conformational states (super-relaxed (SRX) and disordered-relaxed (DRX)),

which are defined by the rate at which myosin heads consume ATP, remain unaltered. Although the knockout (KO) and R502W cMyBP-C animals present distinct pathomechanisms, with no affected SRX/DRX ratio in the R502W model, mavacamten effectively attenuates HCM myocardial remodeling in both. Furthermore, R502W mice exhibited enhanced exercise tolerance following treatment. We observed that mavacamten increases the proportion of myosin heads in the OFF state, which could explain the mechanism of action underlying the beneficial effects of myosin inhibition in our R502W model.

CARDIOMYOCYTE REMODELLING THROUGH MECHANICALLY TUNED TALIN

Poppy O. Smith[1], Emilie Marhuenda[1,2], Aishwarya Prakash[1], Till Kallem[3], Benjamin T. Goult[3], Thomas Iskratsch[1]

1. Queen Mary University of London, London, UK; 2. University of Bordeaux, Bordeaux, France. 3. University of Liverpool, Liverpool, UK.

Introduction

Heart failure, the leading cause of death worldwide, lacks effective treatments. Disease progression is characterized by changes in the cardiac extracellular matrix (ECM) stiffness and composition. Cardiomyocytes, the contractile cells of the heart, sense these environmental changes, resulting in phenotype remodeling. The mechanosignaling driving this phenotypic change remains unclear.
The protein talin is an essential cardiomyocyte mechanosensing hub, connecting integrins, which sense ECM changes, to the cell actin cytoskeleton. The talin rod domains unfold and refold under different ECM stiffnesses, exposing cryptic binding sites for other proteins. This worked aimed to explore the stiffness-dependent regulation of the talin interactome in cardiomyocytes.

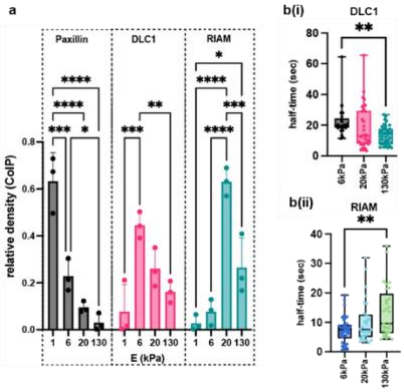


Fig. 1. *Co-IP (a) and FRAP (b) of talin with binding proteins paxillin, DLC1 (bi) and RIAM (bii) in NRCs at different stiffnesses.*

Methods

Neonatal rat cardiomyocytes (NRCs) were cultured on PDMS substrates mimicking healthy and diseased cardiac stiffnesses. Protein interactions with talin were investigated using co-immunoprecipitation (Co-IP), fluorescence recovery after photobleaching (FRAP), fluorescence resonance energy transfer (FRET), and LOVTRAP optogenetics. *In situ* proximity ligand was performed on wildtype and MLP knockout mouse hearts. Fluorescence polarization binding assays were performed with purified synthetic talin domains and talin-interacting proteins. Dynamic stiffness environments were created using PEG-anthracene hydrogels (UV controlled) and magnetic PDMS.

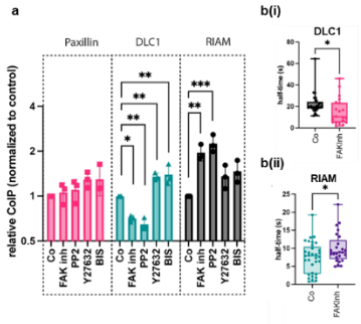


Fig. 2. *Co-IP (a) and FRAP (b) of talin with binding proteins DLC1 (bi) and RIAM (bii) in NRCs treated with kinase inhibitors.*

Results

We identified three talin-bindings proteins, DLC1, RIAM and paxillin, which preferentially bound to talin at specific ECM stiffnesses in NRCs: paxillin preferred 1 kPa, DLC1 6 kPa and RIAM 20-130 kPa (Fig. 1). This was confirmed subsequently *in situ* in healthy and diseased mouse hearts. The interaction of DLC1 and RIAM with talin was regulated through direct competition for binding at the cellular and molecular level, yielding Kd values of 4.9 μM and 9.2 μM for DLC1 and RIAM, respectively. These

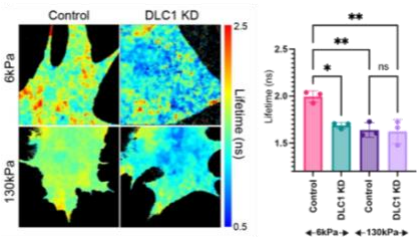


Fig. 3. *FRET with RhoA biosensor in NRCs on 6 kPa or 130 kPa PDMS substrates, control versus DLC1 knockdown (KD).*

interactions persisted when talin was in a tension-free state, suggesting the competitive interactions might be imprinted by other mechanisms, such as phosphorylation. Focal adhesion kinase (FAK) activity regulated the interaction between talin and DLC1 or RIAM: inhibition of FAK altered the talin interactions, promoting the talin-RIAM interaction over the talin-DLC1 at 6 kPa (Fig. 2). DLC1 knockdown at 6 kPa, but not 130 kPa, resulted in altered RhoA activity, a G-protein with a crucial role in heart failure progression, alongside cytoskeletal disruptions (Fig. 3). This highlighted a stiffness-dependent regulatory role of DLC1. These findings were further explored in NRCs seeded on substrates capable of real-time stiffness changes, elucidating the dynamics of talin binding partners and exploring cardiomyocyte mechanical memory.

Conclusions

We showed that mechanical sensing via activation of integrin-related kinase pathways altered the talin interactome. This began to uncover how mechanical information could be imprinted through talin, regulating the cardiomyocyte phenotype, advancing our understanding of heart failure pathophysiology.

Session 12: Muscle modeling

MUTLISCALE SIMULATIONS EXHIBIT VENTRICULAR WALL THICKENING WHEN cMYBP-C DOES NOT SUPPRESS MYOSIN CYCLING

[1] Kerry S. McDonald, [2] Julian E. Stelzer, [3] **Kenneth S. Campbell**

Affiliations:
1. University of Missouri, Columbia MO, USA
2. Case Western Reserve University, Cleveland OH, USA
3. University of Kentucky, Lexington KY, USA

Multiscale models of the cardiovascular system can provide insights into both physiological and pathophysiological processes. MyoVent is a computer model that bridges from molecular to organ-level function and that simulates a left ventricle pumping blood around a systemic circulation (PMID 36107358). In prior work, we adapted MyoVent to allow the ventricle to grow concentrically (adding / removing myofibrils in parallel) and eccentrically (adding / removing sarcomeres in series). Simulations in which concentric growth was regulated by the intracellular ATP concentration and eccentric growth was regulated by the passive stress in titin molecules mimicked ventricular growth patterns associated with hypertension, aortic valve insufficiency, and mitochondrial dysfunction.

In this work, we enhanced the multiscale MyoVent framework by replacing the original contractile module (which predicted contraction using Huxley-type cross-bridge distributions) with the spatially-explicit FiberSim framework for half-sarcomeres (PMID 34932957). This allowed us to predict how modifying the function of cardiac myosin binding protein-C (cMyBP-C) molecules influenced growth.

In the new model termed FiberVent, cMyBP-C molecules were localized to the C-zone of half-sarcomeres and assumed to transition between: (1) a null state (no effect), (2) an actin-attached state (which increased cooperative activation of the thin filament and exerted a drag force during interfilamentary movement), and (3) a myosin-suppressing state (which biased nearby myosin heads towards a super-relaxed / interacting-heads motif-like state where they could not interact with actin).

When the cMyBP-C molecules were prevented from entering the myosin-suppressing state, half-sarcomere contractility and the ventricular ATPase both increased. This initiated concentric growth and subsequent wall thickening. These simulations mimic aspects of organ-level growth seen in patients who develop hypertrophic cardiomyopathy linked to variants in cMyBP-C.

These results demonstrate how computer modeling can help bridge between molecular and organ-level scales. Ongoing computing work is investigating non-cMyBP-C mechanisms which can be perturbed to reverse abnormal growth.

IN SILICO PREDICTION OF MYOSIN MODULATOR EFFECTS IN DCM AND HCM USING MULTISCALE SIMULATIONS AND TRANSLATIONAL MATRICES

Momcilo Prodanovic[1,2], Vanja Cvetkovic[1,3], Kristi Kooiker[4], Farid Moussavi-Harami[4], Michael Regnier[5], Srboljub M. Mijailovich[1]

Affiliations:
1. FilamenTech Inc., Newton, MA, USA
2. Institute for Information Technologies Kragujevac, Kragujevac, Serbia
3. Faculty of Physics, University of Belgrade, Belgrade, Serbia
4. Division of Cardiology, University of Washington, Seattle, WA, USA
5. Department of Bioengineering, University of Washington, Seattle, WA, USA

Dilated (DCM) and hypertrophic (HCM) cardiomyopathies are major causes of heart failure, often resulting from mutations that impair sarcomeric protein function. Small-molecule modulators such as Danicamtiv and Aficamten, acting as a myosin activator and inhibitor respectively, offer promising therapeutic strategies by modulating myocardial contractility. However, assessing their efficacy in DCM and HCM patients from experimental data across species and animal disease models is challenging. Rodent models, while genetically tractable, differ substantially from humans in cardiac physiology, particularly in myosin isoform expression and heart rates. Porcine models provide better physiological relevance but lack robust disease models, limiting drug testing primarily to healthy tissue, while myocardium tissue from cardiomyopathic patients is rarely available. To bridge this gap, we used observed rodent responses to drugs to develop species-specific translational matrices within the MUSICO simulation platform, enabling prediction of drug effects in hypothetical diseased porcine and human hearts. Observed twitch contractions from trabeculae of wild-type (WT) and transgenic I61Q cTnC mice were used to calibrate MUSICO simulations under baseline conditions and following treatment with 1 μM and 3 μM Danicamtiv. Starting from previously published simulations, we adjusted intracellular calcium transients and key temperature-dependent kinetic rates. Consistent with experimental observations, simulations under Danicamtiv treatment were adjusted by decreasing the ADP release rate and increasing the number of active myosin heads. While these changes reproduced twitch behavior in WT, simulating I61Q responses required an additional increase in the dissociation rate of cTnI from actin, suggesting a mutation-specific enhancement of thin filament activation by Danicamtiv. From calibrated twitch simulations, we simulated demembranated force–pCa relationships and validated them against experimental data. Using translation matrices method, we then reversed the process to predict intact twitches in healthy and DCM porcine myocardium in presence or absence of Danicamtiv, starting from demembranated force–pCa porcine data. These results were extended to human simulations, showing restored tension and relaxation in DCM with Danicamtiv, closely resembling healthy contractile function. The same methodology was applied to investigate the effects of Aficamten in HCM, starting from observed data in WT mouse and porcine trabeculae. Simulations predicted reduced contractility, consistent with the observed effect of Aficamten in attenuating hypercontractility in HCM patients. This approach demonstrates how in silico multiscale frameworks, tightly coupled with experiments, can support the development of personalized treatment strategies for cardiomyopathy patients.

Session 13: Skeletal myopathies

Pathomechanisms of Monoallelic Variants in TTN Causing Skeletal Muscle Disease

Jochen Gohlke[1], Johan Lindqvist[1], Zaynab Hourani[1], Sarah Heintzman[2], Paola Tonino[3], Bakri Elsheikh[2], Ana Morales[4], Matteo Vatta[4], Arthur Burghes[5], Jennifer Roggenbuck[2], Henk Granzier[1]
[1]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ
[2]Department of Neurology, The Ohio State University Wexner Medical Center, Columbus, OH, USA
[3]Research, Innovation and Impact Core Facilities Department, University of Arizona, Tucson, AZ, USA
[4]Invitae Corporation, San Francisco, CA, USA
[5]Department of Biological Chemistry and Pharmacology, The Ohio State University Wexner Medical Center, Columbus, OH, USA

Titin (TTN), the largest known human protein, is essential for sarcomere function, contributing to passive stiffness in striated muscle via its function as a molecular spring. The TTN gene contains 364 exons (363 in the meta-transcript) and generates diverse cardiac and skeletal isoforms through extensive alternative splicing of its spring region. Pathogenic TTN variants are associated with a growing spectrum of cardiac and musculoskeletal disorders with varying onset throughout life, with skeletal myopathy mostly attributed to biallelic variants.
We identified monoallelic truncating variants (TTNtv), splice site or internal deletions in TTN in probands with mild, progressive axial and proximal weakness, with dilated cardiomyopathy frequently developing with age. These variants segregated in an autosomal dominant pattern in 7 out of 8 studied families, adding to recent examples of dominant titinopathies. We investigated the impact of these variants on mRNA, protein levels, and skeletal muscle structure and function.
Results reveal that nonsense-mediated decay likely prevents accumulation of harmful truncated protein in skeletal muscle in patients with TTNtvs (reduced amount of TTN transcript along with a shift in allelic balance, Figure A). In contrast to recent studies in cardiac tissue, we did not observe truncated protein in TTNtvs. For an internal large heterozygous deletion of titin with particularly severe early-onset phenotypes (*TTN*$^{\text{del 346-362}}$, Figure B), allelic balance analysis indicated that titin deletion transcript did not get degraded. Titin protein with an internal deletion of ~3800 AA and an intact C-terminus accumulated to a significant degree (Figure C) and electron microscopy revealed irregular Z-disks and M-lines (Figure D), which might be a result of integration of shortened and regular-sized titin into the sarcomere.

RNA-seq demonstrated that splice variants and an out-of-frame deletion induce aberrant exon skipping, while the *TTN*$^{\text{del 346-362}}$ in-frame deletion produces shortened titin with intact N- and C-termini. All variant types were associated with genome-wide changes in splicing patterns (for example in Ankyrin-2, Figure E), which represent a hallmark of disease progression. Lastly, gene expression analysis revealed that GDF11, a member of the TGF-β superfamily, is upregulated in diseased tissue, indicating that it might be useful as a biomarker and/or therapeutic target in skeletal muscle titinopathies.

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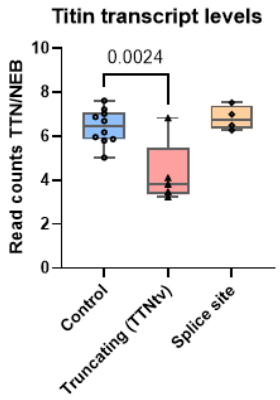

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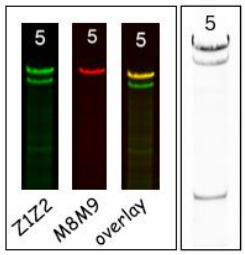

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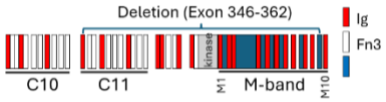

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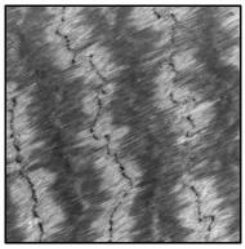

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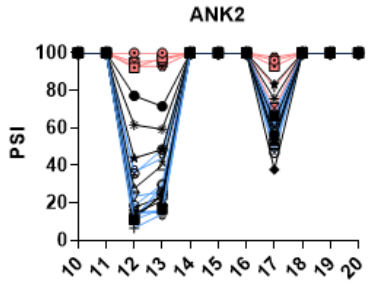


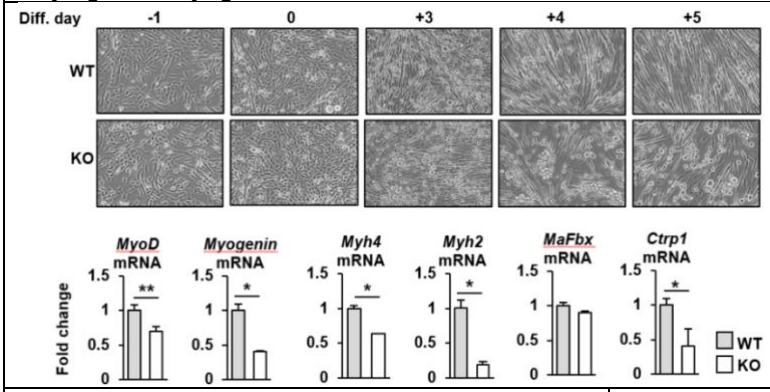
CTRP1 REGULATES SKELETAL MUSCLE DIFFERENTIATION BY QUALITY CONTROL OF MITOCHONDRIAL DYNAMICS AND FUNCTION

Sora Han, Young Yang

Affiliations:
Research Institute of Women's Diseases, Sookmyung Women's University, Seoul, Korea

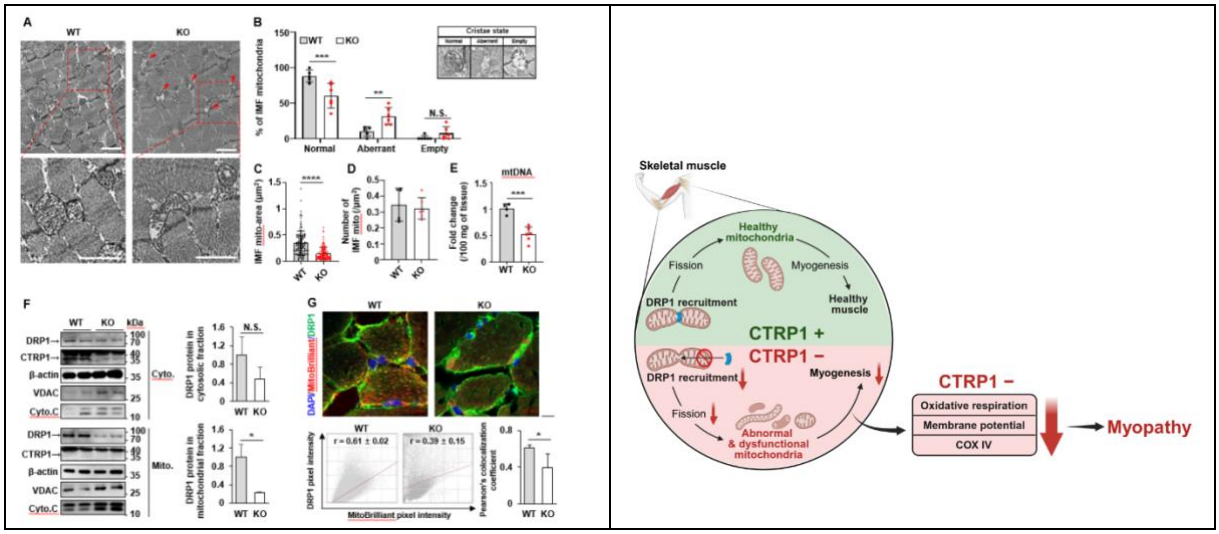
Mitochondrial dysfunction is hallmark of myopathies and impaired skeletal muscle differentiation. Here, we demonstrate that C1q/TNF-related protein 1 (CTRP1) is essential for maintaining mitochondrial dynamics and supporting myogenic differentiation. Loss of CTRP1 in myoblasts and skeletal muscle-specific knockout (KO) mice led to impaired myotube formation, reduced muscle fiber cross-sectional area, and decreased muscle strength. CTRP1 deficiency also shifted a muscle fiber composition from oxidative type IIA to glycolytic type IIB fibers, indicating compromised mitochondrial capacity. At the cellular level, CTRP1 loss resulted in elongated, disorganized mitochondria with diminished cristae density, membrane potential, and oxidative respiration. These mitochondrial abnormalities were associated with defective recruitment of dynamin-related protein 1 (DRP1), a central mediator of mitochondrial fission. Restoring CTRP1 expression or performing mitochondrial transplantation in CTRP1 KO myoblasts rescued mitochondrial function and reestablished differentiation capacity. Furthermore, CTRP1 expression was progressively decreased in accordance with disease severity in skeletal muscle biopsies from patients with polymyositis, dermatomyositis, and Duchenne muscular dystrophy, supporting its potential relevance to human myopathies. Together, these findings identify CTRP1 as a novel regulator of mitochondrial quality and myogenic differentiation and highlight its potential as a therapeutic target in mitochondrial myopathies.

KeyFigure1. Myogenic differentiation defects in skeletal muscle-specific CTRP1 knockout mice (KO)



KeyFigure2. CTRP1 deficiency leads to DRP1-engaged mitochondrial defect.

KeyFigure3. A graphical summary of this study.



All unpublished. Manuscript prepared.

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#10	Katharina Voigt	Lipid droplet accumulation in isolated cardiomyocytes affects titin-based cardiomyocyte properties
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#13	Rostyslav Bubnov	SHEAR WAVE ELASTOGRAPHY FOR ASSESSING ACHILLES TENDON OVERLOAD IN STANDING POSTURE AND ITS NORMALIZATION VIA ULTRASOUND-GUIDED DRY NEEDLING

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#24	Roberto Silva-Rojas	Sarcomere loss triggers partial reprogramming of adult myofibers
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#26	Sergej Pirkmajer	CROSSTALK BETWEEN INTERLEUKIN-6 AND LEUKEMIA INHIBITORY FACTOR IN CULTURED HUMAN MYOBLASTS
#27	Stefano Cagnin	SATELLITE CELLS COMMUNICATION: miR-26a-5p AND -431-5p ARE SECRETED FROM SATELLITE CELLS IMPACTING ON THEIR DIFFERENTIATION AND MOTOR NEURON DEVELOPMENT.
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#29	Xuedan Zhao	IGF-1 and TGF-β1 additively stimulate collagen formation in C2C12 myoblasts and myotubes
#30	Claude COLLET	DIAMIDE INSECTICIDES AND THEIR MODE OF ACTION ON HONEY BEE CALCIUM CHANNELS
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#37	Maira Rossi	DECIPHERING TRANSCRIPTIONAL ADAPTATIONS IN A HUMAN MODEL OF PHYSICAL INACTIVITY.
#38	Oriol Gracia I Carmona	TITINdb2—expanding annotation and structural information for protein variants in the giant sarcomeric protein titin
#39	Petr Zouhar	COLD-INDUCED CHANGES IN MUSCLE LIPIDOME
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#45	Matteo Marcello	THERAPEUTIC SCREENING OF A DUAL AAV-SPLIT INTEIN MIDI DYSTROPHIN BY FUNCTIONAL AND STRUCTURAL ANALYSIS OF EX VIVO MICE SKELETAL MUSCLE AND HUMAN MYORGANOIDS
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Posters – Monday, September 22nd

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#5	Chiara Tesi	Im pact of the reduction of mitochondrial calcium uptake on the contractile properties of fast and slow mouse skeletal muscle with constitutive deletion of MCU (skmcu-/-)
#6	Chuqi He	Protective effects of metformin against statin-induced myotoxicity: through ampk-mediated protective metabolic inhibition
#7	Cosimo De Napoli	Reduced ATP turnover during hibernation in relaxed skeletal muscle
#8	Jan Kopecky	Muscle non-shivering thermogenesis increases energy cost of physical activity
#9	Jelle Huijts	Exercise-induced glut4 translocation toward sites of high glucose delivery and utilization (2/2)
#10	Leonardo Nogara	A combined experimental and computational analysis of mantATP turnover in skinned muscle fibers
#11	Lev Kalika	Biomechanical consequences of gigantomastia: shoulder dyskinesia, neurovascular compression, and functional recovery with targeted interventions

#12	Lucrezia Puccini	Deletion of a muscle-specific promoter in the 3' region of the ank1 gene affects glucose homeostasis and the expression of genes involved in various metabolic pathways.
#13	Marta Hanczar	Muscle-in-a-dish: a novel model to study the microenvironmental contribution to muscle glucose metabolism and contraction
#14	Mauro Montesel	Enlighten the decay: mantatp chasing assay optimization and new insight
#15	Rianne Baelde	Nicotinamide riboside supplementation prevents the onset of mitochondrial dysfunction in a mouse model for nemaline myopathy type 6
#16	Daniela Rossi	Loss of CCDC78 impairs muscle mass and force in a knockout mouse model
#17	Eliza Guti	Effect of simulated microgravity and proton irradiation on PIEZO1 mechanosensitive ion channel and cytoskeletal Septin 7 in C2C12 myogenic cell line
#18	Dieter O. Fürst	From mechanosensation to myofibril repair: the regulation of filamin c by phosphorylation
#19	Katja Gehmlich	Hspb7 regulates filamin c dimerisation
#20	Maria Rosaria Pricolo	Titin cleavage in living cardiomyocytes induces sarcomere disassembly but does not trigger cell proliferation
#21	Miklos Kellermayer	Actin assembly-modulating effect of titin's disordered pevk domain
#22	Mónika Gönczi	Genomic and proteomic analysis of septin7 knockdown myogenic cell line and mammalian skeletal muscle samples
#23	Seong-won Han	Glycerol storage increases passive stiffness of muscle fibers through effects on titin extensibility

#24	Ayesha Sarfraz	Investigating the effects of Mavacamten in diaphragm muscle myosin
#25	Stefan Matecki	Asynchronies related to mechanical ventilation exacerbates Diaphragm Dysfunction in mice model of Duchenne Muscular Dystrophy.
#26	Alice Arcidiacono	Molecular basis of lenght-dependent activation (LDA) in cardiac muscle
#27	Anthony Hessel	Defining the structural function of slow and fast myosin-binding protein c isoforms in mouse extensor digitorum longus
#28	Cameron Hill	Structural changes in thick filaments and myosin motors produced by active stretch of mouse edl muscle
#29	Charles Chung	Differences in twitch and Mechanical Control of Relaxation under increasing Mavacamten and Aficamten doses
#30	David Heeley	Phosphate groups covalently bonded to serines-283 of striated muscle tropomyosin 1.1 (alpha) alter thin filament structure and function. A biochemical study.
#31	Doug Swank	The Myosin Essential Light Chain is not Essential for Muscle Function
#32	Felix Osten	Replating stem cell-derived cardiomyocytes alters contractile function and associated myosin isoform expression
#33	Kyrah Turner	Myosin light chain kinase and protein kinase a independently and cooperatively regulate contractile function via myofilament phosphorylation
#34	Marco Linari	Activation of cardiac myosin filament during systole with different afterloads
#35	Michaeljohn Kalakoutis	Orientation changes of a c-terminal region of troponin i during contraction of cardiac muscle
#36	Samantha P. Harris	The m-domain of cardiac myosin binding protein-c is necessary to damp spontaneous oscillatory contractions (spoc) in mouse cardiomyocytes
#37	Thomas Kampourakis	Spatial control of myosin regulatory light chain phosphorylation modulates cardiac thick filament mechano-sensing

#38	Maral Azodi	Mechanosensing dysfunction following n2b deletion in human ipsc-derived cardiomyocyte
#39	P. Bryant Chase	Possible roles of troponin, tropomyosin and ca2+ in transcriptional regulation in striated muscle myocyte nuclei: could they modulate rna polymerase ii activity in conjuction with nuclear actin?
#40	Hamed Ghazizadeh	Benchmarking functional maturation and pharmacological responsiveness in hipsc-derived engineered heart tissues for preclinical applications
#41	Po Lin (Brian) Chen	Skeletal muscle metabolite alterations in long covid patients revealed by an optimized untargeted metabolomics pipeline
#42	Jasmine Wollenhaupt	Disorganization of micortubule contributes to pathological phenotyoe of DCM in LMNA-MUTATED hipscs
#43	Laura Lechner	Investigation of z-disc and cytoskeletal remodeling in Hypertrophic Cardiomyopathy using hipsc-derived cardiomyocytes
#44	Linda Micali	Nuclear abnormalities and dna damage in hypertrophic cardiomyopathy
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#47	Maria Tishkova	Disruption to the twisting and untwisting of actin and tropomyosin filaments during muscle contraction can lead to muscle dysfunction
#48	Venus Joumaa	Functional and structural properties of cardiac muscle bundles in response to a high fat and sucrose diet in female rats
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Posters - Tuesday, September 23rd

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#3	Christine Delligatti	Methylglyoxal Glycation Competes With Ubiquitination, Disrupting Sarcomere Function
#4	Cristian Revnic	THE BEHAVIOUR OF PHYSIOLOGICAL PARAMETERS OF ISOLATED PERFUSED RAT HEART IN EXPERIMENTALY INDUCED CARDIAC HYPERTROPHY WITH ISOPROTERENOL
#5	Franziska Koser	CARDIAC PROTEOMIC AND PHOSPHOPROTEOMIC SCREENING OF HUMAN HEART FAILURE WITH PRESERVED VERSUS REDUCED EJECTION FRACTION
#6	Ilse Altenburg	CONTRACTILITY MEASUREMENTS COMBINED WITH SINGLE CELL PERFUSION REVEAL KINETICS AND VARIABILITY OF ISOPRENALINE RESPONSE IN MOUSE CARDIOMYOCYTES
#7	Johanna Freundt	SPECIFIC CLEAVAGE OF CARDIAC TITIN IN VIVO DEMONSTRATES THE IMPORTANCE OF TITIN IN THE GENERATION OF RESTORING FORCES DURING DIASTOLE.
#8	Karina Ivaskevica	FUNCTIONAL IMPACT OF CELL-TO-CELL VARIABILITY IN CMYBP-C EXPRESSION IN A HUMAN IPSC MODEL OF HYPERTROPHIC CARDIOMYOPATHY
#9	Larissa Hartmann	Investigating VCP-related Cardiomyopathies: From Zebrafish Model to ipsc-derived Cardiomyocytes
#10	Maya Noureddine	HYPERTROPHIC CARDIOMYOPATHY-LINKED MISSENSE VARIANT IN ALPHA-ACTININ-2: STRUCTURAL AND FUNCTIONAL CHARACTERISATION
#11	Natalia Suyana Rojas Galvan	PRECISE GENOME EDITING OF INHERITED TITINOPATHIES IN HIPSCS-DERIVED CARDIOMYOCYTES
#12	Nicoletta Piroddi	TIME RESOLVED MODULATION OF FAST SKELETAL AND SLOW CARDIAC CONTRACTILE PROPERTIES BY AFICAMTEN IN THE 12-25 °C TEMPERATURE RANGE

#13	Tom P. Mitchell	INVESTIGATING THE PROTEASOMAL REGULATION AND SARCOMERIC PROTEIN COMPOSITION OF MYOPATHY-ASSOCIATED AGGREGATES
#14	Vittoria Di Mauro	A SKELETAL MUSCLE–SYMPATHETIC NEURON AXIS CONTROLS CARDIAC FUNCTION
#15	Yaniv Hinits	THE STRUCTURE AND FUNCTION OF ZEBRAFISH NEBULETTE
#16	Zsigmond Kovács	THE SLOW DELAYED RECTIFIER POTASSIUM CURRENT (IKS) IS DIFFERENTLY REGULATED UNDER BASELINE CONDITIONS AND FOLLOWING β-ADRENERGIC STIMULATION IN CANINE VENTRICULAR CARDIOMYOCYTES
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#18	Arne Hofemeier	NOVEL PLATFORM FOR BIOENGINEERING SKELETAL MUSCLE FOR HIGH RESOLUTION IMAGING AND FUNCTIONAL ANALYSIS TO STUDY DUCHENNE MUSCULAR DYSTROPHY
#19	Frans van Kaam	FROM MOLECULAR PUSH TO FILAMENT PULL IN ZERO LENGTH THEORY (L0T)
#20	Jaakko Sarparanta	C2C12-CTE – A VERSATILE MYOTUBE MODEL FOR C-TERMINAL TITIN STUDIES
#21	Kenneth S. Campbell	MUTLISCALE SIMULATIONS EXHIBIT VENTRICULAR WALL THICKENING WHEN cMYBP-C DOES NOT SUPPRESS MYOSIN CYCLING
#22	Lea Rems	Computational analysis of the effect of electroporation on action potentials in isolated adult rat cardiomyocytes
#23	Malte Tiburcy	SCALABLE GENERATION OF MUSCLE SPHEROIDS FOR TISSUE ENGINEERING OF HUMAN MUSCLE

#24	Angus Lindsay	DYSTROPHIN-DEFICIENCY STIFFENS SKELETAL MUSCLE AND IMPAIRS ELASTICITY – AN IN VIVO RHEOLOGICAL EXAMINATION
#25	Anja Srpčič	INTERLEUKIN-6 SIGNALLING AND ANTI-IL-6 AUTOANTIBODIES IN IDIOPATHIC INFLAMMATORY MYOPATHIES
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#27	Fanny Rostedt	Novel insights into molecular mechanisms leading to congenital fibre-type disproportion (CFTD)
#28	Jelle Huijts	LIPOFUSCIN AS A MARKER FOR SKELETAL MUSCLE BIOLOGICAL AGEING (1/2)
#29	Jenni Laitila	DEFINING PATHOGENETIC MECHANISMS IN CASQ1 DISORDERS THROUGH SINGLE-FIBRE STUDIES
#30	Jochen Gohlke	Characterization of NEB pathogenic variants in patients reveals novel nemaline myopathy disease mechanisms and omecamtiv mecarbil force effects
#31	Kerstin Filippi	Exploring BAG3P209L Myofibrillar Myopathy: In Vitro Models for Targeted Therapy
#32	Leander Vonk	A NOVEL KBTBD13 G67R KNOCK-IN MOUSE MODEL OF NEMALINE MYOPATHY TYPE 6 SUGGESTS A MILD CONTRACTILE PHENOTYPE
#33	Malte Rinn	ADVANCED HUMAN TISSUE MODELS TO INVESTIGATE COLLAGEN-VI RELATED MUSCULAR DYSTROPHY
#34	Martin Rees	DISTINGUISHING PATHOGENIC FROM BENIGN MISSENSE VARIANTS IN TITIN DOMAIN M10
#35	Martina Esposito	FBXO30/MUSA1 IS A NOVEL CRITICAL REGULATORY ELEMENT FOR Z-LINE HOMEOSTASIS AND SKELETAL MUSCLE FUNCTION
#36	Michaela Yuen	CHARACTERISING A MOUSE MODEL FOR PYROXD1 MYOPATHY – PATHWAY TO THERAPY DEVELOPMENT.
#37	Nienke ten Cate	Mechanically-induced nuclear damage leads to global transcriptional heterogeneity across Lmna KO myonuclei

#38	Recep Küçükdoğru	Impact of TPM2 Myopathy-Linked Mutations on Thin Filament Length Regulation
#439	Stefan Conijn	VARIANT IN MYOSIN BINDING PROTEIN C1 (MYBPC1) CAUSES SPONTANEOUS OSCILLATORY CONTRACTIONS IN MICE
#40	Tom Kerkhoff	The effects of myositis-specific autoantibodies in Immune-Mediated Necrotizing Myopathy on muscle fiber contractility
#41	Xueying Huang	CAPILLARY RAREFACTION AND ASSOCIATED ALTERATIONS IN VEGF/VEGFR IN HUMAN DUCHENNE MUSCULAR DYSTROPHY
#42	Yongyong Yan	Static skeletal muscle exosomes inhibit osteogenic differentiation of BMSCs via the miRNA6363/S100A8 axis
#43	Laura Sen Martin	BROAD THERAPEUTIC BENEFIT OF MYOSIN INHIBITION IN HYPERTROPHIC CARDIOMYOPATHY

POSTER ABSTRACTS

#1 Sunday Poster Session

Making muscles big: Expansion Microscopy of the skeletal myofibril.

Alicia Cuber Caballero [1], *Callum Muirhead* [1], *Dr Pauline Bennett* [1], *Dr Richard Marsh* [1], *Prof Mathias Gautel* [1], *Dr Siân Culley* [1]

1. *The Randall Centre for Cell and Molecular Biophysics, King's College London, London, United Kingdom*

Striated muscle comprises skeletal and cardiac muscle, and its structure, organisation and biophysical mechanisms are essential for the function of these organs, in both health and disease. The development of super-resolution fluorescence microscopy techniques such as STED and SMLM has brought the molecular specificity of immunofluorescence beyond the diffraction limit and up to 20nm. More recently, Expansion Microscopy (ExM) has democratised the super-resolution realm, and opened new avenues to achieve higher resolutions, when combined with more conventional SR approaches.

ExM is a novel imaging approach that works by embedding the sample in a hydrogel and, after a sample disruption step, physically expanding it by submerging it in deionised H2O. When making quantitative measurements from structures such as the sarcomere, it is critical that expansion is homogeneous. However, myofibrils have a set of unique, intrinsic mechanical properties that pose a challenge to the most widely used ExM approaches. While limited examples of expansion of muscle tissue and cardiomyocytes can be found in the current published literature, there is no precedent of assessment of the isotropy of these, or of the expansion of individual myofibrils.

In this research, we have considered different ExM approaches like ultrastructure expansion microscopy (U-ExM)—which uses heat denaturation for the homogenisation process—and protein retention expansion microscopy (Pro-ExM)—which uses enzymatic digestion. We see that enzymatic digestion is necessary for muscle and have therefore optimised Pro-ExM for skeletal myofibrils and used pan-labelling methods to contextualise and assess the expansion quality. By imaging the same myofibrils pre- and post-expansion, the isotropy of the expansion can be benchmarked and the reliability of the data assessed. We also offer a hypothesis to explain the anisotropies that can still be observed after extensive enzymatic digestion, and data to support the uses of this technique despite cases of anisotropic expansion.

#2 Sunday Poster Session

Mapping nanoscale muscle sturcture with X-ray diffraction in human DMD samples

Hendrik Bruns[1], Titus Czajka[1], Andras Major[1], Riccardo Scodellaro[2], Jiliang Liu[3], Jana Zschüntzsch[4], Frauke Alves[2], Tim Salditt[1]

Affiliations:
1. Institute for X-ray Physics, University of Georg August University, Göttingen, Germany
2. Research Group Translational Molecular Imaging, Max Planck Institute for Multidisciplinary Sciences and UMG, Göttingen, Germany
3. European Synchrotron Radiation Facility (ESRF), Grenoble, France
4. Department of Neuromuscular Diseases, University Medical Center (UMG), Göttingen, Germany

We use scanning X-ray diffraction (sXRD) in order to map the nanometer structures of muscle tissue over a large field of view up to the centimeter scale. The muscle diffraction patterns reveal the two dimensional organization of specific molecular structure parameters such as actomyosin spacing ($d_{1,0}$, $d_{1,1}$) myosin motor spacing (M3) and actin spacing (ALL6, ALL7). We have acquired scanning diffraction maps of human skeletal muscle tissue, in healthy states and pathological states associated with Duchenne muscular dystrophy (DMD). DMD is a progressive disease that, among other symtoms, impairs the transduction of force from the cytoskeleton to the extracellular matrix (ECM) and causes muscle replacement by connective tissue. We show that, in addition to the muscular tissue components sXRD can reveal the distribution of collagen, lipid droplets associated with adipocytes and myelin on neurons inside the tissue. In the case of the sarcomere structure we correlate structural parameters from $5x10^5$ diffraction patterns in a single muscle slice. In this contribution we both explain the fundamentals, experimental methods, analysis, constraints and potential of the field.

#3 Sunday Poster Session

TITIN'S P-ZONE DOMAINS A164-A167 ARE ESSENTIAL FOR THICK FILAMENT ORGANIZATION AND FUNCTION

Catherine Hoover, John Smith, Justin Kolb, Paola Tonino, Jochen Gholke, Seong-won Han, Gerrie Farman, Robbert van der Pijl, Henk Granzier

Affiliations:
1. University of Arizona, Tucson, AZ, USA

The sarcomeric protein titin plays a central role in thick filament structure and mechanosensitive signaling through its modular A-band domains, including the under-studied P-zone region (domains A164-A170). Titin's P-zone links its M-band and C-zone segments and contains unique structural features. We probed the contribution of titin's P-zone domains A164-A167 to A-band regulatory function by deleting these four domains in a novel mouse model ($Ttn^{\Delta A164\text{-}167}$). The mouse model exhibits muscle type-specific phenotypes: the fast-twitch EDL muscle has a prominent contractile deficit, while the soleus is unaffected and the heart exhibits only mild diastolic dysfunction. Single EDL fibers additionally showed increased passive tension. Cardiomyocyte mechanical analysis indicated normal cardiomyocyte function. RNA-sequencing analysis of EDL and left ventricular muscle revealed altered expression of metabolic and mitochondrial genes. Immuno-electron microscopy and super-resolution microscopy revealed that titin A-band epitopes and MyBP-C are shifted, on average, ~43 nm toward the sarcomere center, that titin's alpha and beta conformations are disrupted, and that the thick filament is shorter. Taken together, these results suggest that a myosin helical repeat may be missing from each half of the thick filament. These findings establish a key structural role for titin's P-zone domains A164–A167 in templating thick filament protein arrangement, specifically for establishing titin's alpha and beta conformations.

#4 Sunday Poster Session

HIGH-THROUGHPUT MUSCLE X-RAY DIFFRACTION EXPERIMENTS AT NSLS-II and PETRA-III SYNCHROTRONS

Christine E. Delligatti[1,2], Michel N. Kuehn[1,3], Anthony L. Hessel[1,3], and Khoi D. Nguyen[1]

Affiliations:
1. Accelerated Muscle Biotechnologies, Boston, USA
2. Loyola University, Chicago, USA
3. University of Muenster, Muenster, Germany

The molecular motor complex of cardiac and skeletal muscle tissues is central to their function and contractility. Currently, the only way to directly measure muscle motor proteins in living tissues under near-physiological conditions is an imaging technology based on principles of diffraction known as muscle small-angle X-ray diffraction (MyoSAXD). This technology applies high-powered X-ray light sources available only at synchrotron radiation facilities, of which there are only a few worldwide, to resolve nanometer-scaled details regarding thick and thin filament structural order, muscle motor protein dynamics, and downstream effects of regulatory proteins. Here, we present recent successes at two synchrotron labs, the LiX beamline at the NSLS-II synchrotron (Brookhaven National Laboratory, USA) and P03 beamline PETRA-III synchrotron (DESY, Germany), to support high-throughput muscle X-ray diffraction experiments. We designed both hardware and software from the ground up to optimize multiple muscle experiments in parallel. The hardware comprises of mobile modular rigs that can be configured with length controllers, force transducers, electrodes, solution exchangers, and temperature control as needed based on experimental design. Rigs can be rapidly mounted/dismounted from the beamline, allowing for muscle incubation or loading and muscle sample interchange. This interchanging on-and-off beamline is crucial because each sample typically requires only a few minutes for X-ray diffraction analysis, a minor fraction of time when compared to other steps such as sample loading, calibration, and treatment incubation times, which can be done away from the beamline. This approach minimizes deadtime and makes the main limitation to throughput only the skilled scientists available to run the experiments. At full operational capacity, we typically run 30-90 samples per 24-hours of beamtime, depending on the experimental complexity. These rigs are now available at LiX and P03 and both beamlines actively encourage new users interested in incorporating muscle X-ray diffraction analysis into their research programs.

#5 Sunday Poster Session

IMPACT OF LIPID DROPLET ACCUMULATION ON CONTRACTILE FUNCTION IN CARDIAC SLICES AND CALCIUM HANDLING IN CARDIOMYOCYTES

Constanze Zieschang[1], Katharina Voigt[1], Joachim Schmitt[2], Martina Krüger[1]

1. Heinrich Heine University Düsseldorf, Institute of Cardiovascular Physiology, Medical Faculty, Düsseldorf, Germany

2. Heinrich Heine University Düsseldorf, Institute of Pharmacology, Medical Faculty, Düsseldorf, Germany

Introduction: In patients with type 2 diabetes mellitus, the accumulation of non-esterified fatty acids (NEFAs, e.g. oleat, palmitate) as lipid droplets (LDs) in the myocardium is associated with impaired cardiomyocyte function and the development of heart failure. Here, we established an ex-vivo protocol to induce lipid droplet accumulation in cardiac tissue slices and isolated cardiomyocytes from mouse hearts within 10-90 min, and analyzed the effects of LD formation on contractile function and cardiomyocyte calcium cycling.

Methods: Cardiac slices (300 µm thick) from adult C57BL/6N wildtype mice (N = 3-6, n = 3-9) were mounted in a MyoDYNAMIC Muscle Strip System (DMT) using custom adapters to fix the slices between the integrated force transducer and length adjuster. Slices were continuously electrically stimulated at 0.5 Hz and incubated for 10-90 minutes with either 4 mM oleate and 200 µM palmitate (OP) or vehicle-treated controls. Cardiac slices were snap-frozen in liquid 2-methylbutane, transversely cryosectioned, and stained with LipidTOX™ to detect lipid accumulation. Calcium handling was studied using Fura-2 imaging using the calcium measurement setup from IonOptix. Isolated cardiomyocytes (N = 13) from adult C57BL/6N wildtype mice were incubated for 2 to 4 hours in high glucose medium with either 200 µM oleate and 200 µM palmitate (OP) or vehicle.

Results: Active force development was determined in 10-minute intervals and a significant decrease from minute 20 onwards was observed in the OP-treated group compared to controls (measured as relative force pre-treatment set to 100%; at 90 minutes: 41.87% ± SEM 7.86% vs 84.2% ± SEM 9.27%). No significant differences in contraction kinetics, as determined by time-to-peak (TTP) and time-to-baseline (TTBL), were observed between groups. Histological analysis demonstrated that all OP-treated slices showed significantly increased LipidTOX™ staining intensity within the outer 40 µm of the tissue compared to controls. LipidTOX™ staining was significantly stronger in the 90-minute OP-treated group in comparison to the 10-minute treatment group and the control group, with differences within the outer 130 µm being detectable. OP-treatment of isolated adult cardiomyocytes did not significantly affect the 340/380 nm excitation ratio for Fura-2 or the time to peak and time to baseline, compared to controls.

Discussion: In cardiac tissue sections, exposure to elevated oleate/palmitate concentrations lead to marked LD accumulation in the cardiomyocytes and a significant decrease in maximal contractile force development within 20 min.
Calcium imaging of isolated cardiomyocytes did not detect effects of NEFA-treatment on calcium transient height.
We conclude that acute LD accumulation induces a rapid decline in contractile force in cardiac tissue slices, which cannot be explained by alterations of calcium homeostasis.

#6 Sunday Poster Session

A MULTI-TARGETED DIETARY SUPPLELEMT ATTENUATES THE INCREASE IN THE INTRAMUSCULAR TOTAL AMINO ACID AND AMMONIA POOLS FOLLOWING EXPOSURE TO IONIZING RADIATION IN A MOUSE MODEL

D.A MacLean[1], T. Hawke[2], M.V. McCue[3], D. Boreham[1] and R. Vemula[2,4]

[1]Division of Medical Sciences, Northern Ontario School of Medicine University, [2]Department of Pathology & Molecular Medicine, McMaster University [3]Biomolecular Sciences, Laurentian University, [4]Medical Sciences, McMaster University.

Exposure to ionizing radiation (IR) increases the risk of cancer and musculoskeletal degradation. In an effort to combat the deleterious effects of IR a Multi-Targeted Dietary Supplement (MTDS) has been specially formulated to protect against radiation-induced molecular damage. The purpose of the present study was to investigate the impact that IR and a MTDS have on intramuscular amino acid and ammonia balance. Seven groups of ten-week-old male BALB/c mice underwent the following treatments (n=8/group). The true control group received no treatment, the MTDS control group were administered the MTDS for a total of 56 days. The radiation (Rad) group received IR at doses of 0.8 Gy at day 0 and 14 for a total of 1.6 Gy. The MTDS+Rad group underwent a 28 day MTDS pre-feeding period followed by IR administered as outlined above. All tissue was harvested at day 21 and day 28 post IR. Tibialis Anterior (TA) samples were collected and analyzed for amino acids via HPLC. IR resulted in an increase ($p<0.05$) in the total amino acid (TAA) pool from true control (90±2 mmol/kg dw) to 96±2 mmol/kg dw at day 21 and 95±2 mmol/kg dw at day 28. In contrast, when the MTDS was administered the TAA pool remained unchanged in the MTDS+Rad group at day 21 (89±3 mmol/kg dw) and day 28 (85±4 mmol/kg dw) as compared to the MTDS group at day 21 (89±2 mmol/kg dw) and day 28 (86±5 mmol/kg dw) as well as true control. Intramuscular ammonia levels were increased ($p<0.05$) from true control (26±1 mmol/kg dw) to 34±2 mmol/kg dw) at day 21 but had returned to baseline by day 28 (28±2 mmol/kg dw) following IR exposure. However, MTDS administration maintained ammonia levels similar to those of true control throughout the study at both time points. These data demonstrate that exposure to 1.6 Gy of IR resulted in an increase in the intramuscular TAA pool suggesting an alteration in protein turnover favouring protein degradation. This is further supported by the observation that intramuscular ammonia levels were also elevated suggesting that the amino acids freed up as a result of net protein degradation were used for cellular metabolism. In contrast, the administration of the MTDS appears played a protective role as the TAA and ammonia pools were unaltered following exposure to IR. These findings suggest that a MTDS may be beneficial in protecting skeletal muscle protein balance when exposed to IR and may be an option in the future development of countermeasures to protect those exposed (e.g. astronauts and cancer patients) to the deleterious effects of radiation.

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#7 Sunday Poster Session

ANALYZING THE EFFECTS OF *DROSOPHILA* ZASP MUTANTS IN MYOFIBRIL ASSEMBLY.

José Luis Medina Quintana.

Affiliations:
1. McGill University, Montreal, Canada.

The sarcomere is the basic contractile unit of muscle fibers bordered by Z-discs. In *Drosophila*, Z-disc structure and maintenance is mediated by actinin and the PDZ and LIM domain protein Zasp52, a structural component of Z-discs and a member of the Alp/Enigma protein family. Actinin is regarded as a central organizer of Z-discs because it cross-links actin filaments at the Z-disc; however, actinin null mutants do not completely disrupt the Z-disc structure in *Drosophila*. Previous reports have shown that Zasp52 can bind both actin and actinin, as well as itself, and Zasp52 may complement the function of actinin at the Z-disc. With this knowledge, the present works propose that the full scope of Zasp functions is likely hidden because of functional redundancy. To shed light on this, we have generated a CRISPR null mutant in Zasp52 for the first time, completely deleting the 50 kb genomic locus and presenting its phenotype in adult flies' indirect flight muscle. We also generated a double null mutant of Zasp52 and Zasp67, an Alp/Enigma family member exclusively expressed in indirect flight muscles. This work characterizes null mutants and shows how the Z-disc structure gets severely disrupted. Another reason the Zasp protein family has gained attention in the muscle field is its significance in muscle diseases when the protein members are mutated. We have studied and characterized the human disease variant “Zasp-608P>L” by expressing the exact ortholog of this disease variant “Zasp52-PR-607P>L” in the *Drosophila* indirect flight muscles. Our data suggest that molecular functions underlying human muscle disorders can be analyzed in *Drosophila*.

#8 Sunday Poster Session

AN INTEGRATIVE APPROACH TO STRUCTURALLY AND BIOPHYSICALLY CHARACTERISE THE HUMAN α-ACTININ 2: ZASP SARCOMERIC COMPLEX

Jules van der Walt[1], Kristina Djinović-Carugo[1,2]
Affiliations:
1. EMBL Grenoble, Grenoble, France
2. Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Austria

The sarcomere is the smallest repeating unit of muscle fibers. Its structure is maintained by the Z-disk, an ultrastructure of 60+ proteins which are also involved in signaling processes such as mechanotransduction and autophagy turnover in muscles. The primary component of the Z-disk is alpha-actinin 2, responsible for crosslinking actin and titin filaments. One of its binding partners is ZASP, a protein which is essential for the structural integrity of the sarcomere. One interaction site between the ZASP PDZ and ACTN2 EF-34 is known, but there is evidence to indicate that ZASP motif (ZM), downstream of the PDZ domain, might interact with alpha-actinin's spectrin repeats (SRs). However, as the ZM motif is contained within an intrinsically disordered region (ZASP IDR), the structure and the interaction of this region with alpha-actinin have so far remained elusive.

This study aims to characterize the ZASP:alpha-actinin 2 interaction, biophysically and structurally, using an integrative approach.

Complexes were formed between ZASP C-terminal truncation (ZASP ΔC) and both the ACTN2 full-length (ACTN2 FL) and ACTN2 half-dimer (ACTN2 HD), a monomer of ACTN2 which binds onto itself, forming a half-dimer. These were validated using Mass Photometry (MP).

The ZASP:ACTN2 HD complex was imaged using single particle Cryo Electron microscopy (CryoEM) to get high resolution structural information of the binding sites. In order to characterise the binding site between the ZASP IDR and the ACTN2 rod, High Speed Atomic Force Microscopy (HS-AFM), Small Angle X-ray Scattering (SAXS) and Crosslinking Mass Spectrometry (XL-MS) were performed. To accurately model the flexibility of the complex in the SAXS data, Ensemble Optimization Method (EOM) was performed with the ACTN2 neck, ZASP HisTag and ZASP IDR set as disordered regions, and the rest of the domains set as structured.

The XL-MS data indicated 7 different cross-links of the ZASP IDR (including the ZM motif) along the ACTN2 rod, mostly on SR2-3, confirming the interaction between the ZASP ZM motif and the ACTN2 rods. Analysis of SAXS further validated the assembly of the ZASP:ACTN2 FL complex, with D_{max}=39.6 nm indicating a complex of a molecular weight of 252 kDa, validating the 2:2 stoichiometry of the complex. EOM analysis was performed on an Alphafold 3 model of the ZASP:ACTN2 FL complex in 2:2 stoichiometry, yielding a chi-square of 1.88. This indicates that the Alphafold 3 model was able to explain the data to a large degree.

Integration of the XL-MS data of the second binding site (ZM motif with the rod) is expected to further improve fitting of SAXS data. Using HS-AFM, the complex dynamics could be followed over time. Protrusions were observed stemming from rod and disappearing, suggesting that the disordered region of ZASP was binding and unbinding the ACTN2 rod. In Cryo-EM, high

resolution 2D classes of the ZASP:ACTN2 HD complex were generated, as well as a 9 Å resolution 3D reconstruction. Issues with preferential orientation and aggregation prevented high-resolution 3D reconstruction.

In conclusion, XL-MS has confirmed a binding site between the rod of ACTN2 and the ZASP IDR, with SAXS and HS-AFM suggesting that this is a transient binding site. Further modelling of SAXS data using EOM analysis, as well as HS-AFM, is needed to understand the dynamics of the interaction.
Further optimization of single particle analysis of CryoEM is planned to elucidate the structure of this complex at high resolution.

#9 Sunday Poster Session

Z-DISK THICKNESS REGULATION IS INDEPENDENT OF TITIN AND NEBULIN Z-REPEATS

Justin Kolb, Xavier Aguilar, Jochen Gulke, Zaynab Hourani, Robbert van der Pijl, John Smith, Henk Granzier

Affiliations:
1. University of Arizona, Tucson, USA

The Z-disk of the sarcomere plays a crucial role in transmitting both active and passive forces between sarcomeres by anchoring thin and titin filaments. Its thickness varies across muscle types—from ~ 50 nm in fast skeletal muscles like the extensor digitorum longus (EDL) to ~100 nm in cardiac and slow skeletal muscles like the soleus. Although the mechanism regulating Z-disk thickness is unknown, it has been hypothesized that the myofilament proteins titin and nebulin, which span the Z-disk, may act as regulators. This idea is based on the presence of variable numbers of repeat motifs in each protein: titin contains up to seven Z-repeats (Zr), with Zr1-3 and Zr7 found in all striated muscles, and Zr4-6 expressed only in cardiac and slow skeletal muscles—where Z-disks are thickest. Similarly, nebulin includes 18 Z-repeats, with more expressed in muscles that have thicker Z-disks. To test whether titin and nebulin determine Z-disk thickness, we generated two mouse models: one lacking all titin Z-repeats ($Ttn^{\Delta 8\text{-}14}$), and another lacking nebulin's differentially expressed Z-repeats ($Neb^{\Delta 152\text{-}160}$). Both models survived to adulthood and appear normal. Unexpectedly, electron microscopy revealed that Z-disk thickness in the EDL, soleus, and cardiac muscle of $Ttn^{\Delta 8\text{-}14}$ mice was unchanged compared to wild-type. Immunofluorescence confirmed that titin's T12 epitope, located near the Z-disk, remained properly positioned despite the deletion. In the $Neb^{\Delta 152\text{-}160}$ model, the number of nebulin Z-repeats in the soleus matched that of the EDL, yet Z-disk thickness remained soleus-like—again contradicting expectations. To explore whether titin and nebulin might act together to regulate Z-disk thickness, we created a double mutant. Surprisingly, instead of a thinner Z-disk, we observed a slight increase (~10 nm) in Z-disk width and greater variability. In conclusion, using genetically engineered mouse models, we tested the long-standing hypothesis that titin and nebulin regulate Z-disk thickness. The findings demonstrate that neither protein is a primary determinant of Z-disk thickness.

#10 Sunday Poster Session

Lipid droplet accumulation in isolated cardiomyocytes affects titin-based cardiomyocyte properties

Katharina Voigt, Maurice Brucker, Martina Krüger
Affiliations: Heinrich-Heine University, Düsseldorf, Germany

Introduction Cardiac lipid droplets (LD) in type 2 diabetes mellitus patients are associated with heart failure and impaired cardiomyocyte function. In our current study, we investigated how LD accumulation affects sarcomere structure and contractile properties of isolated cardiomyocytes.

Material and Methods We established an in vitro model to induce LDs in isolated cardiomyocytes from adult C57BL/6N mice, by incubating cells with non-esterified fatty acids (NEFA) or DMSO/BSA vehicle. For microscopic analysis, cells were treated for 4h, fixed and stained with LipidTOX and anti-alpha-actinin antibodies and counting in 3x100 µm^2 regions of interest (ROI), (n=5-6). For confocal microscopy cells were treated for 4 h and fluorescently labeled with either LipidTOX and anti-alpha-actinin antibodies or LipidTOX and Phalloidin to stain actin filaments. Furthermore, Perilipin expression (n=5-7), and relative phosphorylation of titin PEVK domain (n=2-6) were determined after 4 h treatment by Western blot analysis. Intact cardiomyocytes were electrically paced at 0.5 Hz after 2-4 h treatment to assess fractional shortening and contraction kinetics with the MyoTronic System Myoscope-Cell and analyzed with imageJ (n=6-8).

Results 4 h NEFA treatment increased LD number to 37.0±8.3 LDs in NEFA-treated cells compared to 4.9±2.5 LDs per ROI in controls. LD size was increased in NEFA-treated cells with diameters of 0.87±0.16 µm, compared to 0.67±0.24 µm in controls, but the area to length ratio of the cardiomyocytes remained unchanged. NEFA-treatment reduced sarcomere length to 1.66±0.14 µm, compared to 1.86±0.11 µm in controls. Confocal microscopy revealed that LDs are embedded between actin filaments and remain spatially separated, without overlapping. Further image analyses indicated that LDs may also accumulate between myofibrillar bundles, impairing the cohesion of aligned myofibrils into contractile units. In line with increased LD formation, Western blot analysis showed increased expression of Perilipin 2 and 5 in NEFA-treated cells. Relative titin PEVK phosphorylation increased at S12022 by around 20 % compared to controls, but relative phosphorylation of S11878 was unchanged. PKC phosphorylation at T497 and S660 were unchanged. Cardiomyocyte fractional shortening during contraction was reduced in NEFA-treated cells with 6.48±0.89 % of slack length compared to 8.26±0.77 % in controls. Relaxation time was increased to 0.35±0.09 s in NEFA-treated cells compared to 0.30±0.07 s in controls. Contraction time was not significantly changed with 0.13±0.01 s in NEFA-treated cells and 0.12±0.01 s in controls.

Conclusions Our study demonstrates that LD accumulation in mouse cardiomyocytes is inducible by short-term NEFA-treatment. LD accumulation was associated with increased titin

PEVK phosphorylation, which is expected to increase passive stiffness of the titin filament. Stiffer titin could explain the increased relaxation times observed in NEFA-treated cells and may also lead to decreased fractional cell shortening. We conclude from our findings that LD accumulation in cardiomyoycytes affects the contractile structures and may thus acutely impair contractile function.

#11 Sunday Poster Session

NON-ESTERIFIED FATTY ACID INDUCED CHANGES IN ACTIVE FORCE AND CONTRACTILE PROPERTIES IN PAPILLARY MUSCLES OF RAT LEFT VENTRICLE

Lukas Bungard[1], Katharina Voigt[1], Sabine Bongardt[1], Martina Krüger[1]

Affiliations:
1. Cardiovascular Physiology, University Hospital of Düsseldorf, Germany

Introduction: Accumulation of non-esterified fatty acids (NEFAs) as lipid droplets (LDs) in the myocardium can impair cardiomyocyte function and is associated with heart failure. We established a protocol for rapid lipid droplet accumulation in rat papillary muscle to test for changes in contractile function.

Materials and Methods: Left ventricle papillary muscles of wild-type Wistar rats aged between 8-10 weeks were rapidly dissected and pinned to a length adjuster and a force transducer in a MyoDYNAMIC Muscle Strip System (DMT). Papillary muscles were electrically stimulated at 0.5 Hz, equilibrated for 30 minutes and manually pre-stretched until a force of about 1 mN was achieved. After a second 30 minute equilibration phase the muscles were incubated with either low (2 mM) or high (18 mM) concentrations of oleate and palmitate (200 µM) for 90 minutes under continuous electrical stimulation (N=6-11 per group). After 90 minutes of stimulation, 1 µM Isoprenaline was applied to assess tissue responsiveness to beta-adrenergic stimulation. Papillary muscles were frozen in liquid 2-methylbutane and cryosections were prepared and stained with LipidTox™ Green to detect lipid accumulation.

Results: Relative active force was determined in 10-minute intervals and dropped significantly in muscles incubated with the high-oleate solution compared to controls. Muscles incubated with low-oleate solution showed a significant drop in active force development within the first 10 minutes, but not after longer incubation. Contraction kinetics, determined as time-to-peak (TTP) and time-to-baseline (TTBL), remained unchanged. All muscles showed an increase in force shortly after being treated with Isoprenaline, which was concentration-dependent. Relative increase in isoprenaline-induced force production was lower in the high-oleate group. Histological analysis showed a significantly increased LipidTox™ fluorescence in the outer 60 µm of the muscles in the high-oleate group compared to control after 90 minutes, indicating significant fatty acid uptake. No significant increase in LipidTox™ staining intensity was detected in papillary muscles after only 10 minutes of incubation. Evaluations were carried out using LabChart, ImageJ and GraphPad Prism.

Discussion: We conclude that NEFA uptake rapidly impacts active force development in a concentration-dependent manner, while it impairs, but not abolishes, the papillary muscles' ability to respond to beta-adrenergic stimulation. Despite the unchanged force kinetics we cannot rule out disturbances of cellular calcium cycling, but rather we suspect that LDs directly impair contractile function and lead to reduced force production.

#12 Sunday Poster Session

Mavacamten facilitates myosin head ON-to-OFF transitions and shortens thin filament length in relaxed skeletal muscle

Michel Nicolas Kuehn[1], Nichlas M. Engels[2], Devin Nissen[3], Weikang Ma[3], Thomas Irving[3], Wolfgang A. Linke[1] & Anthony L. Hessel[1]

Affiliations:
[1]Institute of Physiology II, University of Muenster, Muenster, Germany
[2]Department of Physiology, University of Arizona, Tucson, AZ USA
[3]BioCAT, Department of Biology, Illinois Institute of Technology, Chicago, IL USA

The first-in-its-class cardiac drug mavacamten shifts myosin heads towards a structural inactive position where they lay along the helical tracks of the thick filament. However, mavacamten is not completely specific to cardiac myosin and can also affect skeletal muscle myosin, an important consideration since mavacamten is administered orally and so will also be present in skeletal tissue. Indeed, emerging clinical reports indicate mavacamten-induced generalized skeletal myopathy in elderly patients. These findings raise important safety considerations for vulnerable populations, while also highlighting the drug's potential as a novel basic research tool to probe thick filament regulation and myosin head availability in skeletal muscle mechanics experiments. Using small-angle X-ray diffraction (MyoXRD), we tracked these structural changes in the thick filaments of relaxed muscle before and after mavacamten incubation and found that mavacamten treatment reduced the proportion of ON myosin heads but did not eliminate length-dependent structural changes in passive muscle that are linked to changes in contraction performance upon activation, demonstrating similar effects to those observed in cardiac muscle. These findings provide valuable insights for the potential use of mavacamten as a tool to study skeletal muscle contraction across striated muscle.

#13 Sunday Poster Session

Shear Wave Elastography for Assessing Achilles Tendon Overload in Standing Posture and Its Normalization via Ultrasound-Guided Dry Needling

Rostyslav Bubnov[1], Lev Kalika[2]

Affiliations

1 Clinical hospital 'Pheophania' of State Affairs Department, Ukraine

2 New York Dynamic Neuromuscular Rehabilitation & Physical Therapy https://nydnrehab.com/, New York, USA

Background: Tendinopathy is often the result of chronic overload, frequently driven by altered posture and compensatory muscle activity. In patients with low back pain (LBP) and leg pain, evaluating postural influence on distal tendons can aid in targeted treatment. Shear Wave Elastography (SWE) offers a real-time, non-invasive method to quantify tendon stiffness and monitor therapeutic outcomes.

Methods: Twenty subjects were studied: 10 healthy volunteers and 10 patients with chronic LBP and leg pain. SWE was performed on the Achilles tendon in relaxed (supine) and standing positions, simulating mid-stance gait. Both linear and convex ultrasound probes were used; moderate-frequency convex probes provided superior penetration and visualization for deeper structures. Active myofascial trigger points and sacroiliac joint (SIJ) dysfunction were identified. Ultrasound-guided dry needling (US-DN) targeted gluteal and lumbar (L-level) multifidus trigger points.

Results

- **Relaxed (supine)** condition: Achilles tendon SWE values ranged from 0.8–1.4 m/s.
- **Standing**: Values increased to 2.2–3.0 m/s in healthy volunteers and 2.5–4.0 m/s in patients, indicating overload.
- After US-DN, SWE values returned to baseline (1–2.0 m/s), correlating with reduced overload and clinical improvement.
- Post-intervention, posture improved significantly, particularly in pelvic alignment and lumbar multifidus activation, suggesting partial correction of SIJ and lumbar segmental dysfunction.

Conclusion: Achilles tendon overload in standing posture is more pronounced in patients with LBP, likely due to proximal instability and compensatory mechanics. SWE is a sensitive tool for detecting tendon stress and evaluating treatment effects. Ultrasound-guided dry needling effectively reduces stiffness and facilitates postural correction, especially at the SIJ and lumbar multifidus level. Convex probes proved advantageous for deeper muscle and joint visualization.

Keywords:Shear Wave Elastography, Achilles Tendon, Tendinopathy, Posture, Low Back Pain, Sacroiliac Joint, Multifidus, Trigger Points, Dry Needling, Ultrasound-Guided Therapy

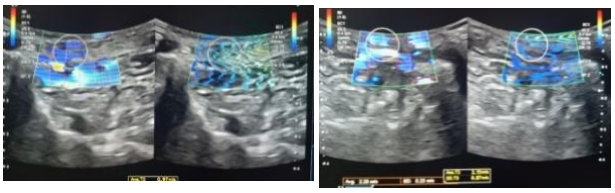

Figure 1. Achilles tendon SWE: Relaxed (supine) condition (left); standing position (right)

#14 Sunday Poster Session

Cytoplasmic YAP Promotes Sarcomere Assembly and Cell Growth Independently of YAP-TEAD Transcriptional Activity in Human Cardiomyocytes In Vitro.

Vladimir Vinarsky[1], Cristina Mazzotti[2], Bacel Aldabash[1], Stefania Pagliari[1,2], and Giancarlo Forte[1,2]

Affiliations:
1. St Anne's University Hospital, Brno, Czech Republic
2. King's College London, London, United Kingdom

Introduction

The ability of YAP-TEAD transcriptional activity to promote re-entry of cardiomyocytes (CMs) into cell cycle has been convincingly established (Von Gise A, 2012). However, other studies suggest that beneficial effects are present only when YAP activity is tightly regulated. Overt YAP-TEAD activity in response to pressure overload (PO) leads to disruption of sarcomere structure (Ikeda S, 2019), that is not normally observed in hypertrophic response to PO (Byun J, 2019), rising questions about other YAP-TEAD independent function(s). To isolate contributions of TEAD dependent and independent effects on sarcomere assembly, and CM growth we re-expressed different variants in YAP deficient CMs.

Methods

WT and YAP deficient H9 hESCs were differentiated into cardiomyocytes using modified GiWi protocol. Lentiviral vectors were used to re-express normal, and YAP-TEAD inactive, cytoplasmic YAP variants in spontaneously contracting cardiomyocytes. Gene expression and super resolution microscopy were used to characterize YAP-TEAD activity, cardiomyocyte maturation, and sarcomere assembly. Proximity Ligation Assay (PLA) was used to assess protein-protein interaction between YAP and structural proteins of sarcomere.

Results

Both WT and YAP deficient hESCs differentiated into spontaneously beating cardiomyocytes. However, growth, sarcomere assembly, and functional maturation of YAP deficient cardiomyocytes was compromised (Vinarsky V, 2024). Re-expression of full-length YAP variant restored YAP-TEAD transcriptional activity, increased sarcomere maturation, and modestly enlarged projected cell area. Surprisingly, the YAP-TEAD inactive YAP variant not only induced comparable sarcomere maturation, but significantly increased cardiomyocyte projected area and improved myofibril content while lacking the apparent YAP-TEAD transcriptional activity. PLA showed abundant interaction of YAP-TEAD inactive variant with TnnT2. Interestingly, YAP-TnnT2 interaction was limited in cardiomyocytes re-expressing full-length YAP, and absent in WT CMs.

Discussion

YAP-TEAD transcriptional activity using constitutively active nuclear variants of YAP is being investigated as a therapeutic modality of post proliferative myocardium. These studies show YAP-TEAD to disrupt sarcomere structure (Ikeda S, 2019). Our preliminary results suggest

important previously unobserved YAP-TEAD independent cytoplasmic function of YAP in cardiomyocytes. Our preliminary observations lead us to hypothesize that correct sarcomere assembly, maturation, and growth may require cytoplasmic activity of YAP.

#15 Sunday Poster Session

DYNAMIC REMODELING OF THE CYTOSKELETON IN REMOTE CARDIOMYOCYTES OF MICE AFTER ISCHEMIA/REPERFUSION

Ana-Nicole Sirbu, Peter F.M. van der Ven, Dieter O. Fürst, Joachim P. Schmitt, Martina Krüger

Affiliations:
Heinrich-Heine-University, Düsseldorf, Germany
University of Bonn, Institute of Cell Biology, Bonn, Germany

Introduction: Myocardial infarction is a leading cause of death worldwide. Heart failure with left ventricular dysfunction following the changes in the nonischemic remote myocardium is responsible for a high mortality. Mechanical overload induces cytoskeletal and contractile adaptations, particularly via higher myofiament stiffness. Proteins such as filamin C (FLNC), microtubules and intercellular junction components like connexin 43 (CX43) are suspected to play a key role in these early adaptive processes. Previous data show, that phosphorylation of FLNC increases its stability and is observed in cells with acute mechanical stress. In ischemic cardiomyocytes an increased presence of detyrosinated tubulin was reported and associated with enhanced celluar stiffness and a reduction in contractile force. Our present study investigates structural and molecular changes of these proteins in remote myocardium 24 and 72 hours after I/R in a murine model. We used mice who underwent transverse aortic constriction (TAC) to mimic direct mechanical overload as comparison.
Methods: C57BL/6 mice were subjected to 60 minutes of cardiac ischemia by binding of the left anterior descending artery followed by reperfusion in a closed chest model. Another group of C57BL/6 mice was subjected to TAC using a 27 gauge blunt needle. Sham-operated mice served as controls. At 24h and 72h post intervention the hearts were extracted for biochemical and histological analysis. Western blotting and immunofluorescence staining were employed to analyse the expression, post translational modification and distribution of FLNC, CX43 and tubulin.
Results: Western blot analysis revealed dynamic changes in FLNC phosphorylation and total expression following I/R and TAC. FLNC phosphorylation was significantly increased at 24h post I/R compared to 72h. An opposite trend was observed in TAC hearts, with an increase of phosphorylation from 24h to 72h.There were no significant modifications of phosphorylation within the time points (24h, 72h) when compared to sham-operated mice. Total FLNC expression significantly increased from 24h to 72h post I/R. A comparable trend was seen at 72h post I/R compared to the sham controls. At 24h no significant changes in overall FLNC expression were detected. In TAC mice there were no differences visible across time points. Immunofluorescence staining revealed significant alterations in intercalated disc (ID) morphology measured as FLNC mediated ID width in response to I/R and TAC. At 24h both groups showed significantly wider ID compared to the control, while after 72h no more differeneces could be detected. This effect was most pronounced in the TAC 24h group. Control hearts showed stable ID widths over time.

CX43 expression did not show significant modifications in western blot analysis. However, immunofluorescence staining showed a broader distribution of CX43 at the ID region in TAC mice after 24h, compared to the control. This was no longer visible at 72h. A similar trend was observed with the I/R mice with a non-significant width increase after 24h.
Total α-tubulin expression remained unchanged at 24h post-intervention but significantly increased at 72h post I/R compared to the control. No significant changes in tubulin levels were observed in the TAC mice over all time points. Post translational modification analysis revealed a significant increase in detyrosinated tubulin at 72h post I/R compared to control. This effect was not observed at 24h or in TAC mice at any time point. Tyrosinated tubulin levels remained stable across all groups and time points.
Discussion: The data show distinct, time-dependent responses to both TAC and I/R in the remote myocardium. The increased FLNC phosphorylation at 24h post I/R, suggests an acute response to ischemia-related mechanical stress, increased protein stability and a contribution to cytoskeletal resilience. In contrast, the delayed increase upon TAC, indicates that regulation of FLNC involves more than just acute mechanical load. The increased total FLNC expression only after I/R at 72h, supports a specific ischemia-driven adaptation process. Widening of ID was observed in both I/R and TAC at 24h but normalized by 72h, suggesting an early transient remodeling rather than long term pathological alterations. CX43 protein levels remained unchanged but immunofluorescence revealed broader ID distribution after TAC at 24h, further indicating a temporary junctional remodeling. The significant increase in detyrosinated tubulin at 72h only after I/R, points to delayed, ischemia-specific cytoskeletal stiffening.

#16 Sunday Poster Session

AN INABILITY TO RECOVER: IMPAIRED REGENERATIVE CAPACITY OF PATIENTS WITH ME/CFS AND LONG COVID

Braeden T. Charlton[1], Anouk Slaghekke[1], Jelle Y. Huijts[1], Brent Appelman[2], Po Lin Chen[1], Richie P. Goulding[1], Frank Bloemers[3], Jelle J Posthuma[4], Paul van Amstel[3], Wendy Noort[1], Bauke Schomakers[5], Michel van Weeghel[5], Richard T. Jaspers[1] Michèle van Vugt[5], Rob CI Wüst[1]
[1]Department of Human Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam, NL
[2]Centre of Experimental and Molecular Medicine, Amsterdam University Medical Centre, Amsterdam, NL
[3]Department of Trauma Surgery, Amsterdam University Medical Centre, Amsterdam, NL
[4]Department of Trauma Surgery, Flevoziekenhuis Almere, Almere, NL
[5] Core Facility Metabolomics, Amsterdam UMC Location University of Amsterdam, NL
[6]Amsterdam Institute of Infectious Disease, Amsterdam University Medical Centre, Amsterdam, NL

Aim: Patients with long COVID and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) often experience worsening of symptoms with physical or mental exertion, known as post-exertional malaise (PEM), and frequently exhibit signs of muscular damage. Appropriate muscle regeneration is dependent on satellite cell presence and function, however it is unknown whether local inflammation associated with long COVID or ME/CFS impacts skeletal muscle regenerative capacity. This study aimed to assess markers of regeneration and metabolic function of skeletal muscle in ME/CFS and long COVID patients.

Methods and Results: Biopsies from the vastus lateralis of 26 ME/CFS patients, 25 long COVID patients, and 30 healthy age- and sex-matched controls were obtained, and sections stained with Pax7 to visualize satellite cells, which were normalized to myonuclear count, and eMHC to assess regenerating myofibres. Mitochondrial respiration was assessed using high resolution respirometry. Liquid chromatography mass spectrometry was used to assess metabolite concentrations in skeletal muscle.

Both long COVID and ME/CFS patients displayed less satellite cells than controls following exercise (P=0.008 and P=0.014 respectively). Satellite cell abundance was proportional to oxidative phosphorylation capacity across all groups (r=0.55, P=0.033), suggesting a relationship between regenerative capacity and mitochondrial function. ME/CFS patients displayed higher proportions of eMHC-positive fibres prior to exercise.

Metabolomic analysis revealed that citric acid and FAD were reduced in both long COVID and ME/CFS patients, further corroborating the impaired oxidative capacity in patients. Both patient groups also displayed markedly lower glutathione-CoA and taurine, indicating impaired antioxidant function. Additionally, patients exhibited reductions of glutamate, which is necessary for several metabolic pathways including antioxidation, energy metabolism, and consequently regenerative ability.

Conclusion: The reduction in satellite cell abundance in both patient groups indicates a likely decreased ability to repair muscle following damage, while the increased expression of eMHC suggests ongoing damage and regeneration events in patients. The reduction in mitochondrial function and metabolites related to energy production may also contribute to the impairment of muscle repair in patients with long COVID and ME/CFS.

#17 Sunday Poster Session

FIBRO-ADIPOGENIC PROGENITORS MAINTAIN MUSCLE MASS IN HOMEOSTATIC MUSCLE THROUGH PREVENTION OF IMMUNE CELL INVASION

Elisabeth R. Barton, Yangyi E. Luo, Young il Lee, Zoe Abe-Teh, Lan Wei-LaPierre

Affiliations:
University of Florida, Gainesville, USA

Fibro-adipogenic progenitors (FAPs) are essential for maintaining muscle mass. When FAPs are depleted, rapid muscle atrophy ensues. Previous studies have implicated the loss of neuromuscular junction (NMJ) integrity as a potential cause of atrophy in the absence of FAPs. However, additional mechanisms may also contribute to muscle mass loss. To address those questions, we generated an inducible FAP knockout (FAP-KO) model by crossing *Pdgfra*$^{CRE-ERT2}$ and *Rosa26*^{DTA} mice. Following tamoxifen (TAM) administration, we confirmed ablation of FAPs and atrophy that mirrored previous findings. Isolated muscle functional testing of the extensor digitorum longus (EDL) and soleus (SOL) muscles revealed proportional force and mass deficits, leading to a preservation of specific force when muscles were stimulated directly. NMJ integrity was evaluated in both muscles using whole-mount immunofluorescence and confocal microscopy. The NMJ structure remained intact, with no signs of denervation, muscle fiber fragmentation, or Schwann cell pathology, suggesting NMJ dysfunction was not responsible for the observed atrophy. Instead, we observed a robust immune activation in FAP-KO muscles, including increased neutrophil and macrophage infiltration and a 10- to 50-fold upregulation of pro-inflammatory cytokines, chemokines, and interleukins. The inflammatory response in muscles of FAP KO mice was accompanied by a ~10-fold increase in atrogene expression. These data suggest that FAPs serve as gate keepers to immune cell invasion, and that the loss of FAPs enables an immune response which later drives atrophy. Together, these findings reveal a previously unrecognized protective mechanism by FAPs for preservation of skeletal muscle mass under homeostatic conditions.

#18 Sunday Poster Session

EFFECTS OF PHYSICAL AND NUTRITIONAL COUNTERMEASURES BEFORE AND DURING DECONDITIONING ON SINGLE MUSCLE FIBER PROPERTIES

Margot Issertine[2], Valérie Montel[1], Julian Theuil[2], Thomas Brioche[2], Angelina Caiazzo[1], Jules Petit[1], Angèle Chopard[2] and **Laurence Stevens[1]**

1. APMS (Activite Physique, Muscle et Sante), ULR 7369, University of Lille, France
2. DMEM (Dynamique du Muscle et Metabolisme), UMR INRA, University of Montpellier, France

The main purpose of this study was to evaluate the combined effects of a physical and nutritional countermeasure before and after a long term exposure to partial gravity in view of future lunar space missions. 70 female wistar rats were divided into different groups. They were submitted either to CONT conditions or 3 weeks of preconditionning conditions (PREC group) exercices en résistance et endurance, normal gravity), followed by a 7-day period of simulated microgravity (HU7 group ; harness unloaing) followed by a 15d-period of partial simulated weightbearing (Dr Marie Mortreux, Harvard Medical School, NASA validation), comprising resistance and endurance exercises. A nutritional countermeasure (HMB, Ergothioneine, Nacre powder) was administrated to all groups all along the entire protocol. Quadriceps intermedius was taken off and single muscle fibers were isolated from all the groups (20 fibers per group i.e. a total of fibers). After contractile experiments (pCa-tension relationships) and diameter measurements, SDS-PAGE electrophoresis was realized in order to determine their myosin heavy chain isoform profile i.e. slow, fast or hybrid types. After 7 days of HU as well as after PWB15 conditions (without preconditionning), there was a significant atrophy of ~15% in fiber diameter from both slow and fast fiber types. When preconditioning conditions were applied, the atrophy observed after HU7 was less important that the one observed in cont conditions. This was not the case for the PREC PWB15 where the atrophy was maintained. The fiber type distribution after HU7 or PWB15 conditions was modified since a large pool of hybrid fibers appeared, coexpressing a large pannel of slow and fast MHC isoforms. Thus, preconditionning conditions permitted to reduce muscle atrophy related to microgravity. For lunar gravity, our results could be due to the duration of the experiment, to the double exposure to HU and PWB conditions, or simply to the fact that the gravity of 0.2g induces a smaller effect than 0G.

#19 Sunday Poster Session

CAPILLARIZATION AND AEROBIC FITNESS: DO TORTUOUS CAPILLARIES MATTER?

Leopold G.D. Kalemba[1], Jelle Y Huijts[1], Veerle Post[1], Alec Kleiman[1], Moritz Eggelbusch[1,2], Wendy Noort[1], Richie P Goulding[1], Rob C.I. Wüst[1]
1. Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands
2. Professorship of Exercise Biology, Department Health and Sport Sciences, TUM School of Medicine and Health, Technical University of Munich, Munich, Germany

Aim: Capillary tortuosity—the degree to which capillaries deviate from a straight path—is a key structural feature of the microvasculature in skeletal muscle that facilitates efficient oxygen exchange. By increasing the effective length and surface area of capillaries within a given volume of tissue, tortuosity enhances the diffusive interface between capillaries and myofibres, particularly under conditions of high metabolic demand. This structural adaptation seems especially prominent in oxidative muscle fibres and might correlate with aerobic fitness levels. Whether (a combination of) capillary-to-fibre (C:F) ratio, capillary density, capillary tortuosity or the capillary-to-fibre-perimeter ratio (CFPR) best relates to maximal oxygen uptake (VO_2max) is currently unknown. In this study, we determined the correlation between maximal oxygen uptake (VO_2max), and various markers for capillarity, including capillary tortuosity in a large heterogeneous human population. Interventions such as a 60-day bed rest and a 4-week aerobic exercise training were used to study the effects of altered physical activity on capillary tortuosity.

Methods & Results: In total, 31 participants were included in this analysis. All underwent cardiopulmonary exercise testing on an electronically braked cycle ergometer to assess aerobic exercise capacity. Muscle biopsies were obtained from the vastus lateralis. Capillarization was assessed by staining 10 µm thick sections for Ulex Europaeus Agglutinin 1 lectin. Analysis was performed using custom-made macros in Fiji. Capillary tortuosity was defined as 1) the ellipticity of the individual capillaries, with higher values representing a higher tortuosity, and as the C:F relative to the fibre perimeter (CFPR, assuming a hexagonal fibre shape).
Capillary-to-fibre ratio related to VO_2max, and ellipticity weakly correlated to VO_2max (R^2=0.065, P=0.06). VO_2max correlated to CFPR (R^2=0.0186, P=0.001). Long-term bed rest (n=23) reduced the CFPR (P=0.024), but the ellipticity did not change (P=0.53). On the other hand, a 4-week aerobic exercise training (n=8) significantly increased CFPR (P=0.045) and ellipticity (P=0.033). Data analysis and interpretation are still ongoing.

Conclusions: While a significant correlation was found between capillary-to-fiber ratio and VO_2max, higher tortuosity was only weakly associated with higher VO_2max. Likely, the relationship between tortuosity and muscle aerobic capacity is most likely affected by local structural factors including fibre size, oxidative phosphorylation capacity, and neighbouring cells with different metabolic profiles.

#20 Sunday Poster Session

Unique pattern of satellite cells in the Blind mole rat Spalax, a long-lived and hypoxia-tolerant mammal, is involved in maintaining skeletal muscle homeostasis and function throughout life

[1]**Mark Mazin**, [2]Yulia Pollak, [1,2]Imad Shams and [2]Irena Manov

[1]Department of Evolutionary and Environmental Biology, University of Haifa
[2]Institute of Evolution, University of Haifa, Haifa, Israel.

In most mammals, aging is associated with a depletion of satellite cells and a decline in their functional activity. Prolonged quiescence may lead to cellular senescence, impairing regenerative capacity and contributing to sarcopenia - a progressive loss of muscle mass and function.

Spalax, a hypoxia-tolerant, long-lived subterranean rodent of the Middle East, shows remarkable physiological resilience, including resistance to age-related decline in muscle function, structure, and fiber type composition. We previously reported that *Spalax* retains balanced proportions of fast- and slow-twitch muscle fibers with age. In our previous physiological assessments, Spalax skeletal muscles also demonstrated remarkable stability in contractile performance across age groups. Unlike rats, which exhibited age-related reductions in maximum force, slower relaxation times, and increased fatigue following repeated stimulations, *Spalax* muscles maintained consistent contractile force and timing throughout life. These findings suggest that Spalax possesses not only molecular but also functional adaptations that contribute to muscle integrity and fatigue resistance with age. Here, we extend these findings by analyzing expression patterns of key myogenic regulatory factors - PAX7, MyoD1, and MYF5 - in young and old individuals. Tibialis anterior, Extensor digitorum longus and Trapezius muscles were isolated from young (2–4 years) and old (10 years and older) and subjected to western blot analysis and qPCR.

Our results demonstrate a distinct age-associated shift in the expression profile of satellite cell markers. While PAX7 expression levels are elevated in young muscles, older *Spalax* exhibit relatively lower expression of PAX7 and higher levels of MyoD1 and MYF5. This altered ratio may indicate a transition from a quiescent, stem-like phenotype toward an activated or differentiating state in aging muscle. Unlike in typical mammalian sarcopenia-sensitive models (rats and mice), where aging leads to satellite cell depletion or dysfunction, *Spalax* may preserve muscle homeostasis through alternative, possibly compensatory, regulation of the myogenic program.

Its adaptation to chronic hypoxia may further contribute to the maintenance of skeletal muscle integrity during aging, making *Spalax* a valuable model for uncovering mechanisms of sarcopenia resistance and long-term muscle health.

#21 Sunday Poster Session

3D IMAGING OF INDIVIDUAL HUMAN MUSCLE FIBERS SHOWS CLEAR REGIONAL MYH-PHENOTYPES IN HYBRID FIBERS

Ullrich M[1], Van der Stede, T[1], Pende, M[2], Turner, DC[3] Derave, W[1]
[1]Ghent University, Ghent, Belgium
[2]MDI Biological Laboratory, Maine, USA
[3]University of Pavia, Pavia, Italy

Introduction: Skeletal muscle fibers are centimeters long multinucleated syncytia, comprised of thousands of myonuclei that collectively maintain homeostasis. Whether gene expression is coordinated between myonuclei within the same muscle fiber or if myonuclei specialize in response to region-specific homeostatic perturbations is still poorly understood. We therefore developed a method for in-situ volumetric quantification of gene expression within myonuclear domains along the length of single human skeletal muscle fibers. Using the three main myosin heavy chain-isoforms, classically used for cross-sectional identification of type I (MYH7), IIa (MYH2) and IIx (MYH1) fibers, we targeted hybrid fibers co-expressing more than one MYH-isoform to better understand the elusive IIa/IIx hybrid fiber.

Methods: Single muscle fibers, > 3mm in length, were manually dissected from healthy human vastus lateralis biopsies and suspended in a hydrogel to maintain 3D morphology. Fibers were subsequently hybridization with *MYH7*, *MYH2* and *MYH1* mRNA probes together with nuclei (DAPI) and cell membranes (WGA). High resolution 5-channel confocal image stacks covering the full length of the fiber were obtained, and gene expression quantified within myonuclear domains by fitting a 3D mesh around all mRNA annotated to each myonuclei (Fig 1G).

Results: By comparing myonuclear domains within the same single fiber, we revealed a striking spatial heterogeneity in MYH transcript distribution along the fiber length (fig 1A). The labelling of *MYH7*, *MYH2* and *MYH1* does not correspond to pure MYH-isoform expression in any fiber. Although the dominant MYH-isoform comprised more than 95% of all three MYH-mRNAs in “pure” fibers, there was always co-expression of the other two isoforms. In IIa/IIx hybrid fibers, this co-expression was highly variable, where one fiber expressed ~27-fold higher MYH1 mRNA in myonuclear domains at one end compared to the opposite end of a 200 μm fiber segment (Fig 1B-E). Within multiple IIa/IIx hybrid fibers, we observed segmental *MYH*-isoform expression switching in ~100-500 μm long segments dominated by either *MYH2* or *MYH1* along the same fiber.

Conclusion: We successfully developed a scalable in situ 3D volumetric quantification of gene expression in the exact same fiber at high sub-cellular resolution. Here we show the first evidence for myonuclear domain-level MYH-isoform specialization in regions of hybrid fibers. Future directions could easily expand beyond MYH expression to explore myonuclear domain-specific expression of other myofiber functions and exercise responses.

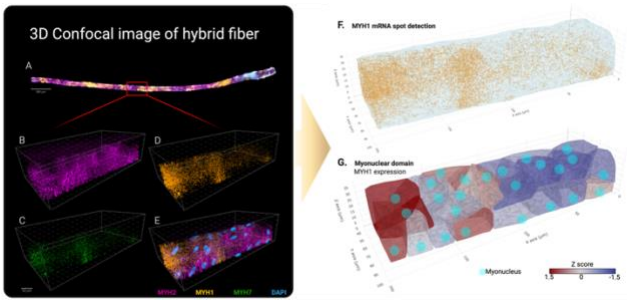

Figure 1. Heterogeneity of MYH-isoform mRNA expression along the length of single human myofiber. **A.** 5-channel fluorescent image of whole-length manually isolated hybrid muscle fiber on Zeiss Axioscan 7. **B-E.** High resolution (40x oil immersed objective) with 4-channel airyscan Zeiss LSM980 confocal 3D image of myofiber segment portrayed in A, in-situ hybridized with RNAscope probes for *MYH2*, *MYH1* and *MYH7* mRNA & DAPI with a shared scalebar. **B-D.** Individual channels, **E.** Combined all four channels. **F.** Quantification of MYH1 signal and myofiber shape projection using the R package alphashape3d (1.3.2) and plotly (4.10.4). **G.** Myonuclear domain calculated by annotating all mRNA to the closest myonucleus and fitting a 3D mesh around all spots annotated to each individual myonucleus (cyan). Z-score shows how many standard deviations above/below the mean MYH1 expression level is for each myonuclear domain ((norm counts-mean norm counts)/sd norm counts).

#22 Sunday Poster Session

EFFECT OF ELECTRICAL PULSE STIMULATION ON C2C12 MYOTUBE EXTRACELLULAR VESICLES' miR CARGO AND EV EFFECTS ON EPS NAÏVE MYOBLAST MIGRATION AND DIFFERENTIATION

Kathryn H Myburgh, John Hingle, Rhys McColl.

Stellenbosch University, Stellenbosch, South Africa.

Skeletal muscle releases extracellular vesicles (EVs) during exercise. These EVs may have autocrine or paracrine effects mediated by the cargo contained in the EVs. MicroRNA (miR) constitutes a significant EV cargo and some miRs may be preferentially released by specific cell types. This study aimed to determine if skeletal muscle miRs, myomiRs, are preferentially released by myotubes in response to two electrical pulse stimulation (EPS) protocols. A further aim was to determine if EVs harvested from myotube media following EPS would have functional effects on EPS naïve myoblasts.

Methods: C2C12 cells were differentiated for 5 days in media containing 2% horse serum. Myoblast culture for EV collection was done with EV depleted fetal bovine serum (no-stimulation control group; 2 EPS stimulation groups). EPS was applied using C-Pace (IonOptix). The low Hz group received pulses of 2 ms, 13 V at a frequency of 2 Hz bringing about rhythmic twitch contractions. The high Hz group received pulses of 2 ms, 13 V with a frequency 30 Hz for 5 s followed by 5 s rest, 5 Hz for 5 s and then 5 s rest. This protocol brought about intermittent tetanic contractions. Media was collected after 10 hours of EPS. Media was replaced and harvested again after 10 hours of rest. Media was harvested from 24 x 6-well plates per experimental group. EVs were isolated using differential ultracentrifugation and characterised using standard methods. EV pellets were resuspended in phosphate buffered saline and combined for a single pooled sample per experimental group. MiRs were isolated from 200 uL of resuspended EVs (Qiagen kit) and sequenced (Illumina platform, Macrogen). Reads per million (rpm) were generated (miRDeep2). The remainder of the pooled EVs were used to test functional effects of EV treatment (2.19×10^8 EVs per mL) including migration (scratch closure; images taken every 20 min for 24 hr) and differentiation (myotube area and length).

Results: The best culture plate coating for myotube formation was Geltrex ($p < 0.0001$ compared to uncoated; $p < 0.05$ compared to ECL coated). Both low and high Hz EPS protocols brought about myotube contractions, although not in the majority of myotubes in the video field of view. EVs collected were enriched in small EVs: mode sizes were 169.5 nm ± 8.86 nm for control, 162.3 nm ± 10.96 nm for low Hz and 148.7 nm ± 14.70 nm for high Hz groups. MiRNA sequencing revealed over 300 known miRs to be packaged inside C2C12 myotube released EVs. All myomiRs were present with miR-206 being the most packaged miRNA (6-fold higher than the next most prominent). There were positive fold differences for various pro-differentiation miRs in EVs from stimulated myotubes compared to rested controls.

The majority of miRs were unchanged or had negative fold-change. A number of miRNAs were present in myotube EVs that have not been reported before. Some of these increased in stimulated myotube EVs, possibly indicating new mechanisms by which muscle responds to exercise. Myotube-derived EV treatment increased myoblast differentiation compared to non-treated myoblasts with similarly high significance in low Hz and high Hz groups ($p < 0.001$). Myoblasts grown in depleted media with no EV treatment had the slowest scratch closure. At 15 h myoblasts receiving treatment with EVs derived from the EV-depleted media no-stimulation group had better % scratch closure ($p<0.05$), whilst treatment with EVs derived from the low Hz group improved myoblast scratch closure significantly more ($p<0.01$).

Discussion: The EPS protocols utilised *in vitro* mimicked electrical induction of muscle contraction. Of the known myomiRs, miR-206 and miR-133a/b were upregulated in EVs from contracting myotubes with miR-1a and miR-486a/b being downregulated. Therefore, regardless of the EPS stimulation protocol it was confirmed that myotubes released some expected miRNAs, but that EPS can have either a positive or a negative effect on the number released. Functional effects on myoblasts were seen as a result of EV depletion from FBS. Generally, function could be restored by treatment using EVs from EPS stimulated myotubes. In conclusion, the autocrine effect of skeletal muscle contraction on satellite cell function may be, at least in part, due to the cargo contained in the EVs released.

#23 Sunday Poster Session

TITIN'S N2A-ELEMENT FUNCTIONS AS A STRESS RESPONSE ELEMENT TO STABILIZE MARP PROTEINS IN MUSCLE

Robbert van der Pijl[1], Stefan Conijn[1,2], Eva Peters[1], Johan Lindqvist[1], Ricardo Galli[2], Mei Methawasin[1], Coen Ottenheijm[1,2], Henk Granzier[1]

Affiliations:
1. University of Arizona, Tucson, United States of America
2. Amsterdam UMC, location Vumc, Amsterdam, Netherlands

Titin plays a critical role in regulating passive tension in muscle. Traditionally, titin-based passive tension is modulated through alternative splicing of its I-band spring region and post-translational modifications. Recent findings from our group and others (Zhou et al.) revealed an additional regulatory mechanism involving Muscle Ankyrin Repeat Protein 1 (MARP1), which tethers titin's N2A-element to the actin thin filament (TF). This TF-MARP1-N2A interaction sequesters part of the I-band spring, resulting in increased passive tension. In this study, we aimed to establish a functional role for this tethering mechanism. We generated a mouse model with a conditional deletion of the MARP1-binding site on titin (domains I80–I81; exons 103–106), referred to as cTtn$^{\Delta I80\text{-}I81}$. This model was bred with an MCK-Cre transgenic mouse to induce striated muscle-specific deletion, or Myh6-Cre transgenic mouse to induce cardiac specific deletion. A global deletion model (Ttn$^{\Delta N2A}$) was also created using EIIa-Cre. Mice were born at Mendelian ratios and exhibited no overt phenotype. Functional assessments via *ex vivo* contractility testing in EDL and soleus muscles showed no differences between genotypes. Echocardiography of the heart indicated modest diastolic dysfunction, with modest atrophy of the ventricles. Passive tension measured in chemically permeabilized skeletal muscle bundles (soleus) and isolated myofibrils (tibialis) also revealed no significant differences. However, when recombinant MARP1 was applied to myofibrils, only wild-type myofibrils exhibited a ~70% increase in passive tension (sarcomere length range 2.3–2.8 μm), whereas cTtn$^{\Delta I80\text{-}I81}$ samples remained unchanged. To test in vivo relevance, we used AAV-mediated MARP1 overexpression. AAV-MARP1 was only detectible in EDL muscle of wildtype mice, indicating the N2A-element is required for stabilizing MARP1 in skeletal muscle. These findings demonstrate that TF-MARP1-N2A tethering is a stress-responsive mechanism that enhances passive tension.

#24 Sunday Poster Session

SARCOMERE LOSS TRIGGERS PARTIAL REPROGRAMMING OF ADULT MYOFIBERS

Manuel Gavilán-Herrera[1], Consolación Marín-Vicente[1,2], Alba Pobes-Lagartos[1], Natalia Vicente[1], Jesús Vázquez[1,2], Jorge Alegre-Cebollada[1], **Roberto Silva-Rojas[1]**

Affiliations:
1. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Calle de Melchor Fernández Almagro 3, Madrid, Spain
2. CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain

Cell reprogramming has been traditionally achieved through the combined expression of Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc; OSKM). The response and effectiveness of these factors are highly tissue-dependent, with skeletal muscle fibers demonstrating notable resistance to reprogramming. Their multinucleated architecture and terminally differentiated state present significant structural barriers to reverse their differentiated identity using OSKM. In this study, we employ an engineered system based on the expression of Tobacco Etch Virus protease (TEVp) to cease force transmission across titin *in vivo*. Sarcomere disassembly following titin cleavage interferes directly in the functional identity of skeletal muscle fibers, driving them into a fundamentally reprogrammed state. This state is characterized by the downregulation of terminal differentiation markers and the reactivation of core developmental and myogenic programs. Additionally, we identified a shared transcriptional pattern with muscle denervation, pointing to a sarcomere-to-neuron crosstalk that we are currently characterizing. Further steps will include the identification of the transcriptional factors driving this partial reprogramming and to what extent they modulate muscle physiology in health and disease.

#25 Sunday Poster Session

THE EFFECT OF EXERCISE-INDUCED MECHANICAL LOADINGS ON MACROPHAGE ACTIVATION AND THEIR ROLE IN MUSCLE REGENERATION

Rui Yang [1], Jianfeng Jin[2], Jan Van de Bossche[3], Astrid D. Bakker[2], Richard T. Jaspers[1]

[1] Laboratory for Myology, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, Netherlands;
[2] Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, Netherlands;
[3] Department of Molecular Cell Biology and Immunology, Amsterdam Institute for Infection and Immunity, Amsterdam UMC, Amsterdam, Netherlands.

Introduction: Early mobilization or exercise has been recommended and proven effective in promoting muscle regeneration. Exercise may also stimulate the activation of macrophages characterized by cytokine secretion associated with inflammation and cell-induced growth. Upon myofiber stretch-shortening cycles, muscle stem cells are subjected to tensile and shear deformation (*Haroon et al. 2021*). It is conceivable that macrophages are also subjected to tensile and shear forces, considering their close vicinity to satellite cells. However, it is still uncertain whether macrophage activation can be triggered independently by exercise-related mechanical loadings, other than damage signaling. Moreover, how the paracrine effect of mechanically activated macrophages affects muscle stem cells or the muscle regeneration post-exercise remains to be clarified. **Purpose:** To explore the effect of exercise on macrophage activation and cytokine expression patterns. To investigate whether activated macrophages stimulate muscle stem cells' proliferation and differentiation. **Methods:** The dataset (GSE214544) of a single-cell sequence was collected from the GEO database, in which 3 human participants took 3x30s bouts of exercise and analyzed with R. DEGs were separated, followed by a GO and GSVA enrichment analysis. A Cellchat package was used to evaluate the cell-cell interactions. For the in vitro validation, RAW 264.7 cells were subjected to pulsating fluid shear stress(1h), and growth factor gene expression was quantified by RT-qPCR. The proliferation ratio of C2C12 was detected by an EdU assay. The expression of *MyoG*, *MyoD*, *Ccnd1*, and *Mki67* in C2C12 was detected by RT-qPCR. **Results:** M1 macrophages increased, and immune/inflammatory pathways were mostly enriched after the acute exercise. Interaction number and strength between cells increased, and the interaction between M1 and SC was enriched mostly on the EGF-EGFR pathway after exercise. The proliferation ratio of C2C12 increased in the epiregulin group (1ng/mL). *Ereg* (6h) expression increased in macrophages after PFSS. *MyoD* (1h, 24h), *Ccnd1* (24h), *Mki67* (24h) expression increased while *MyoG* (1h) decreased in C2C12 of the epiregulin group (1ng/mL). **Conclusions:** Acute exercise activates the immune and phagocytosis processes, and promotes the polarization of the M1 population with high pro-inflammatory and growth factor-related gene expression in human participants. The EREG/EGFR signaling pathway exhibits the highest interaction probabilities between M1 and SCs, influencing muscle regeneration after exercise. Ereg expression in macrophages is upregulated in response to fluid shear stress, and epiregulin promotes the proliferation of C2C12 *in vitro*. These findings suggest that exercise after injury promotes muscle regeneration by mechanical loading-induced activation of macrophages and expression of epiregulin in macrophages.

#26 Sunday Poster Session

CROSSTALK BETWEEN INTERLEUKIN-6 AND LEUKEMIA INHIBITORY FACTOR IN CULTURED HUMAN MYOBLASTS

Anja Srpčič[1, 2,*], Barbara Žvar Baškovič Gantar[1,*], Katarina Miš[1], Katja Lakota[2], Katja Perdan Pirkmajer[1,2], **Sergej Pirkmajer**[1]

Affiliations:
1. Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
2. University Medical Centre Ljubljana, Ljubljana, Slovenia

*These authors contributed equally to this work.

Interleukin-6 (IL-6) and leukaemia inhibitory factor (LIF), myokines that play key roles in myogenesis and muscle regeneration, bind to distinct ligand-binding receptors (IL-6Rα or LIFR), but bring about activation of the same signal-transducing receptor IL6ST (aka gp130) and the STAT1/STAT3 pathway. Skeletal muscle cells, such as myoblasts, can simultaneously serve as a source and target of IL-6 and LIF, which led us to hypothesise that IL-6 and LIF may modulate each other's signalling via IL6ST in regenerating skeletal muscle.

After having knocked down (using siRNA) the expression of receptors for IL-6 (*IL6R*) or LIF (*LIFR*) or the expression of endogenous *IL6*, we treated primary human myoblasts with recombinant human IL-6 (rhIL-6) or LIF (rhLIF) and tested their downstream effects by measuring the phosphorylation of STAT1 (Tyr701) and STAT3 (Tyr705). *IL6R* knockdown, which diminished the response to rhIL-6, enhanced the response to rhLIF. These responses were paralleled by *LIFR* knockdown, which decreased the response to rhLIF, but increased the response to rhIL-6. A similar effect was achieved by knocking down endogenous *IL6*, which decreased the action of rhLIF, but increased the action of rhIL-6. The mRNA and protein levels of IL6ST remained similar across all experimental groups, suggesting that enhanced signalling responses in *IL6R*, *LIFR*, or *IL6* knockdown myoblasts were not secondary to upregulation of IL6ST.

In conclusion, we show that suppression of *LIFR* enhances IL-6 action, while suppression of *IL6R* enhances LIF action in myoblasts. Moreover, suppression of endogenous *IL6* in myoblasts promotes their response to rhIL-6, while reducing their response to rhLIF. Our findings suggest that IL6R and LIFR signalling are mutually suppressive, which may have important functional consequences for IL-6 and LIF actions in skeletal muscle.

#27 Sunday Poster Session

SATELLITE CELLS COMMUNICATION: miR-26a-5p AND -431-5p ARE SECRETED FROM SATELLITE CELLS IMPACTING ON THEIR DIFFERENTIATION AND MOTOR NEURON DEVELOPMENT.

Caterina Peggion [1], Raphael Severino Bonadio [1], Roberto Stella [2], Silvia Scalabrin [1], Laura Pasetto [3], Caterina Millino [1], Laura Camporeale [3], Beniamina Pacchioni [1], Valentina Bonetto [3], Alessandro Bertoli [4 5 6], **Stefano Cagnin** [1 7], Maria Lina Massimino [6]

Affiliations:
[1] Department of Biology, University of Padova, Italy
[2] Department of Chemistry, Istituto Zooprofilattico Sperimentale delle Venezie, Italy
[3] Research Center for ALS, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy
[4] Department of Biomedical Sciences, University of Padova, Italy
[5] Padova Neuroscience Center, University of Padova, Italy
[6] Neuroscience Institute, Section of Padova, National Research Council (CNR), Italy
[7] CIR-Myo Myology Center, University of Padova, Italy

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset paralytic disorder, characterized primarily by a progressive loss of motor neurons in whose the involvement of skeletal muscle has been demonstrated. Given the established relationship between motor neurons and skeletal muscle in this pathology, we employed ALS animal models to assess the communication between these tissues. Differently from central nervous system (CNS), skeletal muscle is a plastic tissue that responds to insults through proliferation and differentiation of satellite cells to permit its regeneration. Skeletal muscle degeneration and regeneration are finely regulated by signals that regulate satellite cell proliferation and differentiation. It is known that satellite cell differentiation is impaired in ALS, but little is known about the involvement of miRNAs and their role in intercellular communication and influence on motor neuron maintenance. We demonstrated that satellite cells from different ALS mice have an impaired differentiation related to the impairment of myogenic p38MAPK and PKA/pCREB signaling pathways that can be regulated by miR-882 and -134-5p. These miRNAs participate in autocrine signaling in association with miR-26a-5p, that, secreted from WT and captured by ALS myoblasts, enhances ALS-related myoblast differentiation by repressing Smad4-related signals. Notably, miR-26a-5p and miR-431-5p exert a paracrine effect, influencing the differentiation of motor neurons. These findings underscore the imperative to elucidate the intricacies of intercellular communication and its pivotal role in the pathogenesis and progression of muscle disorders. Furthermore, they propose that miRNAs may serve as potential therapeutic targets or agents for the regeneration of myofibers and peripheral motor neurons.

#28 Sunday Poster Session

EARLY DYNAMIC REGULATION OF THE NON-NEURONAL CHOLINERGIC SYSTEM IN THE HEART AFTER ISCHEMIA/REPERFUSION AND PRESSURE OVERLOAD

Steffen Bönner[1], Michael Thaddäus Singer[1], Joachim Schmitt[2], Martina Krüger[1]

Affiliations:
1. Heinrich Heine University, Düsseldorf, Institute of Cardiovascular Physiology, Medical Faculty, Düsseldorf, Germany
2. Heinrich Heine University, Düsseldorf, Institute of Pharmacology, Medical Faculty, Düsseldorf, Germany

Introduction: The non-neuronal cholinergic system (nNCS) is increasingly recognized as a modulator of cardiac function, influencing processes such as contractility, inflammatory responses, and cell survival. While the physiological relevance of the nNCS has been well studied in several organs, its acute molecular adaptations in the heart under pathological conditions remain largely unexplored. In particular, little is known about the regulation of key nNCS components in the heart following acute pressure overload (transverse aortic constriction, TAC) or ischemia/reperfusion (I/R). The aim of this study was therefore to investigate early changes (24 and 72 hours after intervention) in major nNCS proteins in mouse cardiomyocytes in order to provide new insights into initial cardiac adaptation processes.

Material and Methods: In this proof-of-principle study, male mice (N=4 per group) were randomly assigned to three experimental groups: sham operation, transverse aortic constriction (TAC), and ischemia/reperfusion (I/R). The I/R model was induced by temporary ligation of the left anterior descending coronary artery for 60 minutes, followed by reperfusion. Pressure overload was achieved via transverse aortic constriction (TAC) by surgical narrowing of the aortic lumen between the brachiocephalic artery and the left common carotid artery using a ligature placed around the aorta against a 27-gauge blunt needle. In the control group, animals underwent the same surgical procedures without ligation of the artery or aorta (sham). Mice were killed by cervical dislocation 24 and 72 hours after I/R or TAC induction, and myocardial samples for protein and RNA analyses were obtained from the non-ischemic left ventricular free wall. Relative changes in protein expression of the nNCS components vesicular acetylcholine transporter (VAChT), choline acetyltransferase (ChAT), high-affinity choline transporter (CHT), and acetylcholinesterase (AChE) were analyzed by Western blot, normalized to alpha-actinin. Additionally, quantitative PCR (qPCR) was performed to assess mRNA expression of these proteins (n = 3–4).

Results: Following I/R, early protein levels of all key nNCS components showed mild, yet non-significant increases at 24 hours, with CHT and AChE remaining elevated at 72 hours. At the mRNA level, I/R induced a significant downregulation of AChE at 24 hours, followed by a significant decrease in VAChT and CHT at 72 hours.
After TAC, CHT expression was significantly increased at 24 hours, but not at 72 hours. Other nNCS proteins remained unchanged. At the mRNA level, TAC induced a significant downregulation of AChE at 24 hours. By 72 hours, mRNA levels of all nNCS components were reduced, with VAChT and CHT mRNA showing statistically significant decreases.

Conclusions:

Our findings demonstrate that the non-neuronal cholinergic system in cardiomyocytes undergoes rapid and distinct molecular adaptations in response to acute mechanical stress. Both I/R and pressure overload trigger early, stimulus-specific alterations in key cholinergic proteins, including dynamic changes in nNCS components involved in acetylcholine synthesis, uptake, and storage. In both models, we observed early increases in key proteins involved in acetylcholine synthesis and storage, particularly following I/R, suggesting a transient upregulation of the cholinergic system as an initial adaptive response to cardiac injury. Notably, AChE mRNA was reduced at early time points in both models, which may represent a compensatory feedback mechanism to limit acetylcholine degradation and support cardioprotective cholinergic signaling during acute stress.

#29 Sunday Poster Session

IGF-1 and TGF-β1 additively stimulate collagen formation in C2C12 myoblasts and myotubes

X. Zhao[1,2], A Shi[1], E van de Berg[2], C Offringa[2], M Steinz[2], J Pathak[1] and R T Jaspers[1,2]
Affiliations:

1. Guangzhou Medical University, Guangzhou, China.
2. Laboratory for Myology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands.

Introduction: *Maintaining adequate and appropriate collagen formation is essential for muscle health and function. However, after injury dysregulated immune activity and/or myogenic stimuli can lead to excessive collagen deposition and muscle fibrosis and impaired myogenesis. Transforming Growth Factor β1(TGF-β1) and Insulin-like Growth Factor 1 (IGF-1) play crucial roles in skeletal muscle regeneration and adaptation. TGF-β1 is strongly expressed by macrophages and promotes collagen synthesis by regulating collagen mRNA transcription, while IGF-1 expressed by several cells including macrophages, fibroblasts, muscle stem cells and myofibers may promotes collagen synthesis via enhancement of the rate its mRNA translation. As yet single and combined effects of these growth factors on collagen formation in muscle cells are unknown. This study aimed to explore the independent and synergistic effects of both growth factors myoblasts and myotubes.*

Methods: *C2C12 myoblasts and myotubes were cultured in growth or differentiation with or without recombinant human IGF-1 (100 ng/mL), TGF-β1 (10 ng/mL), or their combination*. *Myoblast proliferation was assessed by EDU labelling. Myotubes size and fusion index were determined by immunofluorescence. Collagen deposition was assessed by Sirius Red staining. Gene expression related to fibrosis and* endoplasmic reticulum (ER) *stress was analysed by qPCR. Phosphorylation of AKT and Smad protein levels were examined by Western blotting*.

Results: IGF-1 and TGF-β1 did not affect myoblast proliferation in the first 24 h. At 48 hours IGF-1 treated myoblasts showed a higher fraction of EDU (1.5 fold) positive cells than untreated cells, indicating that IGF-1 promoted myoblast proliferation. IGF-1 treatment for 24h showed 40% larger diameter of myotubes and 51% higher fusion index. TGF-β1 blunted IGF-1 induced promotion of myoblast fusion and increase in diameter. After 6 days of treatment by IGF-1 or TGF-β1 collagen content in differentiating myoblasts cultured in growth medium was almost doubled compared to that of untreated cells ($p<0.01$), while culture of myoblasts treated by both IGF-1 and TGF- β1 showed a 3-fold higher collagen production ($p<0.001$). TGF-β1 upregulated expression levels of fibrosis-related genes (Fgf2, Ccn2 and Col1a1, Col1a2). Interestingly, IGF-1 and TGF-β1 increased Col1a1 and Col1a2 mRNA expression in myotubes at 48 hours. Moreover, we observed that TGF-β1 upregulated expression levels of ER stress genes Perk and Atf4 in myoblasts and Atf4 in myotubes, suggesting an inhibitory role for TGF-β1 in protein translation. IGF-1 and TGF-β1 supported protein synthesis in myotubes via

upregulation of gene expression of metabolic enzymes Phgdh and Pkm2, which are both involved in anabolic signalling as well as serine and nucleotide synthesis. IGF-1 stimulated AKT phosphorylation, whereas TGF-β1 activated Smad2.

Conclusion: Although IGF-1 and TGF-β1 play different roles in the proliferation and differentiation of C2C12 cells, both growth factors additively promote collagen synthesis via enhancement of the rate of collagen mRNA expression and its mRNA translation, respectively. Their interplay may influence muscle regeneration dynamics, making it essential to understand the transient and temporal expression of these factors and how their expression is modulated by systemic factors as well as by exercise, mechanical loading, nutrition and other factors to regulate fibrosis and preserve muscle health.

Keywords: Muscle regeneration, Collagen, Fibrosis, TGF-β1, IGF-1, Connective tissue, ER stress

#30 Sunday Poster Session

DIAMIDE INSECTICIDES AND THEIR MODE OF ACTION ON HONEY BEE CALCIUM CHANNELS

Mercedes CHARRETON[1], Jerome MUTTERER[2], Michel PELISSIER[1], Aklesso KADALA[1], Pierre CHARNET[3], Laszlo CSERNOCH[4], Peter SZENTESI[4] & **Claude COLLET**[1]

1. INRAE, UR 406, Abeilles & Environnement, Avignon, France
2. CNRS, UPR 2357, Institut de biologie moléculaire des plantes, 67084, Strasbourg, France
3. IBMM, UMR 5247 CNRS, Université de Montpellier, Montpellier, France
4. Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

In vitro, anthranilic and phthalic diamides disrupt intracellular calcium homeostasis in peripheral neurons, skeletal muscle cells and cardiomyocytes of the honey bee *Apis mellifera*. We have also shown that calcium conductances are decreased at the plasma membrane of central neurons and muscle cells. At the tissular level, these insecticides compromise cardiac rhythmicity and contraction kinetics as well as force production from leg muscles. At high doses, compatible with field recommendations, they are lethal to bees and our results demonstrated differential toxicity levels depending on exposed body parts. At lower doses, a single contact exposure modifies behaviour in a persistent manner, which may create a durable vulnerability in the field. These long-lasting behavioural deficits may be ascribed partially to the multiple toxic effects observed in the heart, muscles and peripheral and central nervous systems, as well as other tissues where calcium is involved in the modulation of vital functions. Synthetic insecticides have been clearly identified as a major driver of a worrying worldwide insect diversity decline, and the global market sales and field usage of this recent neurotoxic insecticide family increased substantially in Europe. This has put the wisdom of their overuse under review. We will not only give an overview of the knowledge collected these late years on the global toxicity of diamides to bees but will also emphasize the benefit of using these molecules as pharmacological tools for the study of calcium homeostasis in insects.

#31 Sunday Poster Session

PHYSIOLOGICAL MUSCLE FUNCTION IS CONTROLLED BY THE SKELETAL ENDOCANNABINOID SYSTEM IN MURINE SKELETAL MUSCLES

Nyamkhuu Ganbat[1,#], Zoltán Singlár[1,#], Péter Szentesi[1,2], Elena Lilliu[3], Zoltán Márton Kohler[4], László Juhász[5], Anikó Keller-Pintér[4], Xaver Koenig[3], László Csernoch[1,2], **Mónika Sztretye[1,2]**

Affiliations:
1. Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
2. HUN-REN Cell Physiology Research Group of University of Debrecen, Debrecen, Hungary
3. Department of Neurophysiology, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria
4. Department of Biochemistry, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary
5. Institute of Surgical Research, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary
# These authors contributed equally

The endocannabinoid system (ECS) regulates and controls many of our most critical bodily functions including healthy muscles however, its potential role(s) in physiological skeletal muscle function and its disorders are unclear. Earlier using the Cre/LoxP system we generated and characterized a tamoxifen-inducible skeletal muscle-specific CB_1R knockdown (skmCB_1-KD) mouse model. Here, we sought to elucidate the mechanisms underlying reduced muscle force generation. To address this, we measured calcium movements following electrical stimulation-induced muscle fatigue, evaluated store-operated calcium entry (SOCE), and conducted functional analysis of respiring mitochondria. Based on our measurements, it is likely that the reduced muscle performance recorded *in vivo* may occur due to complementary changes in ATP production by mitochondria. Furthermore, in skmCB_1-KD mice, we observed a significant decrease in one component of the respiratory chain and a delayed mitochondrial membrane potential dissipation upon the application of an un-coupler.
We propose a putative link between ECS (more precisely CB_1R) and physiological muscle force generation along with a role of ECS in maintaining physiological cellular and mitochondrial homeostasis and function.
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#32 Sunday Poster Session

SEQUENTIAL OPENING OF RYR_1 DURING CALCIUM SPARKS IN FROG SKELETAL MUSCLE

Péter Szentesi[1,4], Henrietta Cserné-Szappanos[2], László Z. Szabó[3], Martin F. Schneider[2], László Csernoch[1,4]

[1]Department of Physiology, University of Debrecen, Debrecen, Hungary
[2]Department of Biological Chemistry, University of Maryland, Baltimore, MD, USA,
[3]Department of Electrical Engineering, Sapientia Hungarian University of Transylvania, Târgu Mureş, Romania
[4]HUNREN-UD Cell Physiology Research Group, Debrecen, Hungary

Calcium (Ca^{2+}) sparks can arise as both voltage-dependent and voltage-independent ligand-activated release events in amphibian skeletal muscle. Calcium sparks were recorded from intact frog skeletal muscle fibers using high temporal resolution confocal microscopy (linescan: 15 and 50 µs/line). Sparks were triggered by 1 mmol/l caffeine to open ryanodine receptors (RyR) or by subthreshold depolarization to +65 mV membrane potential to activate dihydropyridine receptors (DHPR). Images were analyzed using a custom MATLAB-based computer program. The properties of Ca^{2+} sparks observed under control conditions were compared to those measured in depolarized cells and those following caffeine treatment. Both treatments increased the frequency of sparks and altered their morphology. The amplitude (in $\Delta F/F_0$; 0.227±0.001 *vs*. 0.193±0.001; n = 19196 and 16316, respectively; mean±SE, $p<0.001$), the full width at half maximum (FWHM, in µm; 2.22±0.01 *vs*. 1.79±0.01, $p<0.001$), the rise time (in ms; 7.29±0.04 *vs*. 4.13±0.02, $p<0.001$), and the duration (in ms; 17.35±0.07 *vs*. 10.81±0.04, $p<0.001$) of sparks were significantly greater after caffeine treatment than in depolarized cells. The signal mass of sparks (i.e., the amount of calcium released) resembled the amplitude in shape. Additionally, the calcium release flux followed a linear function during the activation of sparks.

The detailed analysis of the sparks' time profile revealed that the activation and deactivation of the events occurred in a stepwise manner. The average step size (in $\Delta F/F_0$; 0.071±0.003) remained independent of the scanning speed. Furthermore, the steps during the rising and falling phases of the sparks were identical. The number of steps in the rising phases followed a linear function based on the spark's amplitude.

Our results suggest that the activation of neighboring release units may occur sequentially, and the amplitude of the sparks depends linearly on the number of activated RyR groups.

#33 Sunday Poster Session

STRAIN-DEPENDENT MUSCLE CONTRACTILITY IN RESPONSE TO COLD EXPOSURE AND HIGH-FAT DIET IN MICE WITH DISTINCT THERMOGENIC STRATEGIES

Sara Stanic[1], Petra Janovska[1], Kristina Bardova[1], Petr Zouhar[1], Jan Kopecky[1]

Affiliations:
1. Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Thermogenesis plays a key role in whole-body energy homeostasis, yet the contribution of muscle-based non-shivering thermogenesis (NST) to muscle metabolism and function remains poorly understood. In this study, we examined muscle function in two mouse strains that rely on distinct thermogenic strategies and differ in their susceptibility to diet-induced obesity: C57BL/6 (B6) mice, which predominantly utilize brown adipose tissue (BAT), and A/J mice, which appear to rely more heavily on muscle-based NST during cold exposure (CE). To determine how CE affects muscle function, we housed mice either at thermoneutrality or at 8°C for 7 days and subsequently assessed muscle force production.
Preliminary data show that CE results in reduced muscle mass across strains, yet absolute muscle force is preserved. As a result, force normalized to muscle mass is elevated in CE groups. Moreover, we observed distinct changes in muscle contractility between strains. In B6 mice, CE prolongs twitch duration, whereas in A/J mice, twitch force becomes faster. A/J mice also display inherently slower contractile kinetics at all stimulation frequencies and reach maximal force at lower frequencies compared to B6 mice. Interestingly, CE shifts the force-frequency response in A/J mice toward a more typical profile, suggesting an adaptation in their muscle excitation-contraction coupling.
To assess how diet influences contractile properties, we also evaluated the effects of high-fat diet (HFD). HFD feeding reduced muscle force production in B6 mice at both thermoneutrality and cold, while this effect was absent in A/J mice, indicating a potential strain-specific resilience to HFD-induced muscle dysfunction. We plan to further investigate the underlying mechanisms focusing on the role of muscle lipid content and substrate utilization

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#34 Sunday Poster Session

EFFECTS OF ELECTROPORATION ON PROLONGED PACING ABILITY OF ISOLATED CARDIOMYOCYTES AND THEIR RELEVANCE FOR PULSED FIELD ABLATION TREATMENT OF CARDIAC ARRHYTMIAS

Vid Jan 1, Tina Turk 1, Jernej Jurič 1, Marko Stručić 1, Matej Reberšek 1, Martina Perše 2, Damijan Miklavčič 1, Lea Rems 1

Affiliations:
1. University of Ljubljana, Faculty of Electrical Engineering, Ljubljana, Slovenia
2. University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

Background: Pulsed Field Ablation (PFA) uses high-voltage pulses that cause electroporation of cell membranes to treat cardiac arrhythmias. Following PFA, intracardiac electrograms (iEGMs) show immediate attenuation but may reappear minutes later. We investigated how different high-voltage waveforms affect cardiomyocyte excitability and recovery dynamics.

Methods: Primary adult rat cardiomyocytes were exposed to three different waveforms: monophasic 100 µs, biphasic 2 µs, and monophasic 200 ns pulses. Cells were initially paced at 2 Hz, exposed to high-voltage pulses, and then paced again to assess recovery. For prolonged experiments, cells were continuously paced for 20 minutes post-treatment, while intracellular Ca^{2+}, and sarcomere shortenings were recorded simultaneously at 5-minute intervals. In short term experiments transmembrane voltage was also measured. Computational modelling was used to simulate electroporation effects on action potentials and Ca^{2+} transients, and machine learning analysis was applied to identify predictive features from experimental signals that indicate electroporation occurrence.

Results: Six distinct response types were observed across all waveforms at different electric field thresholds (50-350 V/cm for monophasic 100 µs, 300-650 V/cm for biphasic 2 µs, and 1250-3500 V/cm for monophasic 200 ns pulses). Cell orientation affected sensitivity differently - parallel cells were more sensitive at lower field strengths when using longer pulses, perpendicular cells when using shorter pulses. Computational modelling demonstrated that increased membrane conductivity due to electroporation could reproduce experimental responses by varying membrane pore number and resealing time. In prolonged pacing experiments, 80.0-91.7% of excitable cells maintained excitability throughout 20 minutes, while 14.8-35.7% of initially unexcitable cells recovered within 10-20 minutes. Machine learning identified calcium dynamics features as the most predictive biomarkers of electroporation..

Conclusions: Our findings shed light on the cellular basis for transient iEGM attenuation and recovery observed clinically following PFA. The consistency of response patterns across different waveforms, despite varying electric field thresholds, suggests a common underlying mechanism of action for diverse PFA protocols.

#35 Sunday Poster Session

Evaluating the role of mitochondrial CB1 receptors in murine skeletal muscles

Zoltán Singlár[1], Nyamkhuu Ganbat[1], Péter Szentesi[1,2], Mónika Gönczi[1,2], Anikó Keller-Pintér[3], Attila Oláh[1], László Csernoch[1,2], Mónika Sztretye[1,2]

1. Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
2. HUN-REN Cell Physiology Research Group of University of Debrecen, Debrecen, Hungary
3. Department of Biochemistry, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

The endocannabinoid system (ECS) includes the cannabinoid receptors (CB1R and CB2R), endocannabinoids, metabolic enzymes, and transport proteins. CB1R is highly expressed in both the central nervous system and peripheral tissues, including skeletal muscle, where it is localized not only to the plasma membrane but also to mitochondria (mtCB1R). While previous work has suggested a role for the ECS in muscle function and certain pathologies, the physiological function of mtCB1R in skeletal muscle remains poorly understood. To explore this, we used a genetically engineered mouse model (DN22-CB1) in which the mitochondrial localization of CB1R is globally ablated (mtCB1-KO). We performed a comprehensive analysis on young adult mtCB1-KO mice using a combination of in vivo and in vitro assays to evaluate muscle function and mitochondrial performance. In vivo, mtCB1-KO mice displayed reduced performance in the hang test, while no significant differences were observed in the grip strength or rota-rod tests. Additionally, spatial learning and memory assessed by the Barnes maze, remained unaffected by the absence of mtCB1. In vitro, STED microscopy confirmed the specific subcellular distribution of CB1Rs. Isolated EDL and soleus muscles exhibited significantly reduced isometric force generation. Analysis of mitochondria respiration on gastrocnemius muscles revealed metabolic changes in samples from mtCB1-KO mice, whereas mitochondrial membrane potential depolarization dynamics were evaluated following TMRE fluorescence decay following FCCP exposure. Altogether, our findings point to a previously unrecognized role of mitochondrial CB1R in maintaining skeletal muscle bioenergetics and contractile function, particularly through its impact on mitochondrial bioenergetics. These data provide new insight into the intracellular mechanisms by which the ECS, specifically CB1Rs contribute to skeletal muscle physiology and function.

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#36 Sunday Poster Session

Decoding the role of microRNAs in cancer cachexia: a study using LLC tumor-bearing mice and in silico approaches

Chao Su[1] **,** Katarzyna Goljanek-Whysall[1]
1. Physiology, School of Medicine and REMEDI, University of Galway, Galway, Ireland.

Introduction
Cancer cachexia is a multifactorial syndrome marked by severe weight loss and skeletal muscle atrophy, which contributes substantially to morbidity and mortality in cancer patients. MicroRNAs (miRNAs) have emerged as critical regulators of diverse pathological processes and are increasingly recognized as both biomarkers and therapeutic targets. In the context of cancer cachexia, specific miRNAs modulate signaling cascades that drive muscle wasting. Here, we investigated the role of miRNAs in lung cancer–associated cachexia, aiming to elucidate their mechanistic contribution to muscle degradation and to identify candidate molecular markers.

Methods
To this end, we used the Lewis lung carcinoma (LLC) mouse model, which recapitulates systemic inflammation and progressive muscle wasting characteristic of cancer cachexia. Gastrocnemius muscle samples were analyzed by next-generation RNA sequencing to generate a comprehensive profile of miRNA expression changes. Differentially expressed miRNAs were identified by comparing cachectic muscles to those from non-tumor controls. We then predicted miRNA target genes by intersecting results from miRDB, miRTarBase, miRWalk, and TargetScan. Subsequent bioinformatic analyses—including Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment and protein–protein interaction (PPI) network reconstruction—were performed to pinpoint key miRNAs and their downstream effectors involved in cachexia-associated muscle wasting.

Results
Our analysis revealed five miRNAs with significant dysregulation in cachectic skeletal muscle: miR-9-3p, miR-1291, miR-124-3p, and miR-5126 were upregulated, whereas miR-5122 was downregulated. Predicted target sets comprised 53, 70, 835, and 3 genes for miR-9-3p, miR-1291, miR-124-3p, and miR-5126, respectively, while miR-5122 had only two predicted targets. Network analysis of these candidates identified a central cluster of interacting proteins, with the top ten hub proteins enriched in pathways critically linked to muscle wasting, including NF-κB signaling and the MAPK cascade. Among the dysregulated miRNAs, miR-124-3p emerged as a particularly compelling candidate: its expression was significantly elevated in the blood of cachectic patients, in cachectic muscle tissues, and in a C2C12 myotube model of cachexia.
Functional assays in vitro confirmed that miR-124-3p impairs myogenesis: transfection of C2C12 myoblasts with a miR-124-3p mimic led to a marked increase in miR-124-3p levels (as verified by RT-qPCR) and resulted in the formation of thinner myotubes (quantified by MF20

immunostaining) compared with scrambled controls. Integration of miR-124-3p target prediction with RNA-sequencing data from cachectic mice identified 30 candidate target genes. PPI network analysis of these 30 genes (constructed via the STRING database) revealed 11 high-confidence interactions, suggesting coordinated regulation of muscle homeostasis. Validation by RT-qPCR demonstrated that ARRB1, TLN1, ANXA5, and PDE4A were consistently downregulated in both gastrocnemius muscle from cachectic mice and in the C2C12 cachexia model, indicating that they are likely direct targets of miR-124-3p.

Conclusion

Collectively, our findings implicate miR-9-3p, miR-1291, miR-124-3p, miR-5122, and miR-5126 in the regulation of muscle wasting in LLC tumor–bearing mice, predominantly through modulation of NF-κB and MAPK signaling pathways. In particular, miR-124-3p and its validated targets represent promising biomarkers and mechanistic mediators of cachexia-induced muscle atrophy. These results lay the groundwork for future therapeutic strategies aimed at mitigating muscle loss in cancer cachexia.

#37 Sunday Poster Session

DECIPHERING TRANSCRIPTIONAL ADAPTATIONS IN A HUMAN MODEL OF PHYSICAL INACTIVITY.

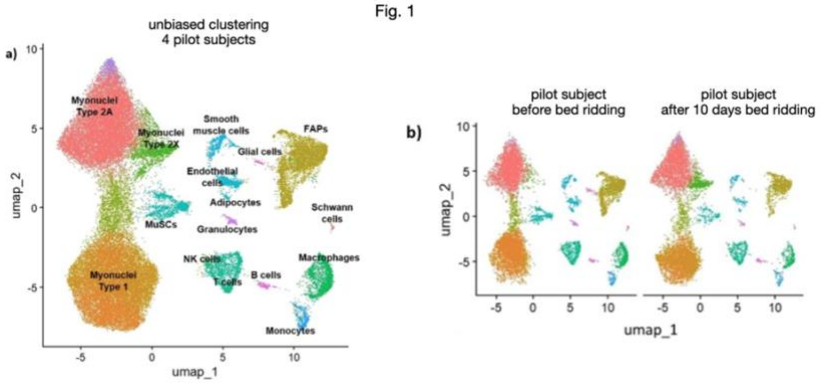
Maira Rossi[1,2], Monica Nicolau[2], Jimmy Massenet[2], Cristiana Sazzi[1], Lorenza Brocca[1], Maria Antonietta Pellegrino[1], Pier Lorenzo Puri[2], Roberto Bottinelli[1]
1. Department of Molecular Medicine, University of Pavia, Italy
2. Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA, USA

Physical inactivity (PI) is a major risk factor for chronic diseases and mortality, reaching pandemic levels. PI decreases exercise tolerance and elevates disease risks, impacting muscle mass and strength, nervous system function, and metabolism. Despite its clinical relevance, the molecular mechanisms underlying disuse-induced muscle atrophy remain unclear.

We investigated the cellular and transcriptional mechanisms driving skeletal muscle adaptations to disuse and subsequent recovery. We hypothesized that critical drivers of these changes include mononuclear muscle-resident cells, particularly fibro-adipogenic progenitors (FAPs), and the integrity of neuromuscular junctions (NMJs). Single-nucleus RNA sequencing (snRNA-Seq) of *Vastus Lateralis* biopsies from healthy young men (n=5) undergoing controlled bed rest (BR) – a model of whole-body deconditioning – was performed. Biopsies were collected at baseline, after 10 and 21 days of BR, and after 21 days of recovery.

Preliminary findings demonstrate successful yield in terms of number and quality of nuclei collected. Unbiased clustering (Fig. 1a) identified all major skeletal muscle cell populations. BR induced rapid alterations in myofiber type distribution and intrinsic metabolic profiles, shifting towards type 2X fibres (Fig. 1b). Changes in mitochondrial-related gene expression across different fiber type clusters were induced. FAPs exhibited significant modulation to BR, with dynamic subcluster transitions indicating disuse-induced plasticity. Cell-cell communication analysis revealed notable signalling interactions between FAPs and myonuclei. These findings highlight metabolic remodelling in myonuclei and dynamic changes in FAP subpopulations during muscle disuse and can contribute to a deeper understanding of PI-related pathophysiology. Ultimately, these findings will highlight potential targets for therapeutic strategies aimed at counteracting inactivity-induced muscle loss and preserving function across the lifespan.

Fig. 1



#38 Sunday Poster Session

TITINdb2—expanding annotation and structural information for protein variants in the giant sarcomeric protein titin

Oriol Gracia Carmona[1,2,3], Timir Weston[2], Joseph Ng[2,3], Mathias Gautel[1], Franca Fraternali[2,3]

Affiliations:
1. Randall Centre for Cell & Molecular Biophysics, School of Basic & Medical Biosciences, King's College London, London SE1 1UL, United Kingdom
2. Institute of Structural and Molecular Biology, University College London, London WC1E 6BT, United Kingdom
3. Department of Biological Sciences, Birkbeck, University of London, London WC1E 7HX, United Kingdom

Titin, the largest protein in humans, plays a central structural and regulatory role in muscle sarcomeres. Mutations in the titin gene have been linked to several skeletal and cardio myopathies. Due to its extremely large size and numerous isoforms, interpreting titin missense variants remains a significant challenge in both clinical and research settings. Here we present TITINdb2, a major update to our previously published TITINdb platform, designed to support the comprehensive analysis of titin variants across its full isoform complexity.

TITINdb2 integrates updated population data, literature-curated pathogenic variants, and harmonized annotations across chromosomal, transcript, and protein coordinates. The web-sever now offers 20 computational variant effect predictors, providing a consensus-driven view of potential pathogenicity. We have expanded the structural coverage of titin through domain-level AlphaFold2 models and introduced domain-domain interface annotations, supporting more nuanced mechanistic interpretations of variant effects. A new saturation mutagenesis module enables users to explore variant tolerance across any titin variant, supported by the seven top performing impact predictors. Additionally, a variant conversion tool facilitates the translation of genomic, transcript, and protein variant formats, simplifying different annotation methods to stream-line variant interpretation.

By combining structural annotation, extensive predictive analytics, and a user-friendly interface, TITINdb2 servers as a critical resource for researchers and clinicians investigating the role of titin in cardiac and skeletal muscle disorders. Its application to variant prioritization in skeletal and cardiac myopathies will help bridge the gap between large-scale sequencing data and functional understanding.

The web-sever is freely available at https://titindb.kcl.ac.uk/ and the accompanying manuscript at https://doi.org/10.1093/bioadv/vbaf062

#39 Sunday Poster Session

COLD-INDUCED CHANGES IN MUSCLE LIPIDOME

Petr Zouhar[1], Petra Janovska[1], Tatyana Kobets[1], Lenka Steiner Mrazova[1,2], Viktor Stranecky[1], Tomas Cajka[1], Kristina Bardova[1], Jan Kopecky[1]

1. Institute of Physiology of the Czech Academy of Sciences, Prague, Czechia
2. Department of Pediatrics and Inherited Metabolic Disorders, 1st Faculty of Medicine of Charles University, Prague, Czechia

Cardiolipin (**CL**) is a unique phospholipid located almost exclusively in the inner mitochondrial membrane, which plays a critical role in mitochondrial functions, including the organization of respiratory chain complexes and the regulation of mitochondrial energy metabolism. In skeletal muscle, CL is essential for maintaining mitochondrial quality, supporting muscle regeneration, and ensuring efficient energy production. CL synthesis in skeletal muscle can be modified by factors such as age and diet, suggesting that environmental stimuli like cold could influence its dynamics. We have published (PMID: 36720306) that C57BL/6J (**B6**) mice, which are predisposed to obesity, activate non-shivering thermogenesis (**NST**) predominantly in BAT, in contrast to A/J mice, in which impaired NST activation in BAT was associated with increased NST capacity in skeletal muscle and resistance to obesity. However, a potential role of mitochondrial CL in muscle NST is not known.

In this study, A/J and B6 mice were maintained at 30°C for three weeks and then exposed to mild cold (22°C) or cold (8°C) for two weeks. As a controls, mice maintained at thermoneutrality (30°C) were used. Lipidomic and transcriptomic analyses were performed in gastrocnemius muscle.

At the thermoneutral condition, there was no difference in total CL content between the genotypes. Cold acclimation significantly increased total CL content only in the muscle of B6 mice. CL composition in the muscle of B6 mice differed from that of A/J mice in CL 68:6, CL 70:6, and CL 72:6, regardless of acclimation temperature, and cold exposure deepened the difference. It resulted in increased amount of CL-containing (i) 16:0, 16:1, 18:1, 20:3, or 22:6 fatty acids in B6 mice; and (ii) 16:0, 18:1, or 20:3 fatty acid in A/J mice. Although we found no difference in the expression of key protein genes of CL synthesis and remodeling (*Crls1*, *Taz*) the effect of genotype and temperature was observed for genes associated with cardiolipin metabolism. Higher expression of *Ptpmt1*, a gene for a protein involved in phosphatidylglycerol phosphate dephosphorylation, and *Serac1*, a gene for a protein involved in phosphatidylglycerol remodelling, was found in the muscle of B6 mice compared to AJ mice. Both of these genes are involved in synthesis of precursors CL. *Pla2G6*, gene for protein participating directly in the remodeling and repair of CL, especially after oxidative damage, was increased in A/J mice compared with B6 mice and the expression was decreased by cold.

In conclusion, our findings suggest that cold affects skeletal muscle CL content and composition in mice, depending on the muscle capacity for NST.

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#40 Sunday Poster Session

Single-Nucleus RNA Sequencing Reveals Transcriptomic and Cell–Cell Signaling Alterations in Type I Myonuclei of Long COVID Skeletal Muscle

Po Lin (Brian) Chen[1], Braeden T Charlton[1], Wendy Noort[1], Sabrina Jagot[2], Richard T. Jaspers[1], Fabien Le Grand[2], Rob C.I. Wüst[1]

Affiliations:
1 Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, The Netherlands
2 Institut NeuroMyoGène, Université de Lyon - Université Claude Bernard Lyon, Lyon, France.

Introduction Long COVID poses an increasing burden on healthcare systems. The skeletal muscle of long COVID patients undergoes several pathological changes, including a shift toward glycolytic metabolism, a higher proportion of glycolytic fibers, muscle necrosis, atrophy, and reduced capillary-to-fiber ratios, all of which may contribute to symptoms such as muscle pain, diminished exercise capacity, and fiber-type switching. However, the transcriptomic signatures underlying these changes remain unknown, posing a major barrier to developing effective therapeutic strategies and diagnostic methods for long COVID-related muscle abnormalities.
Objective This study aims to utilize single-nucleus RNA sequencing (snRNA-seq) to investigate the transcriptomic signature and signaling pathways for type I myonuclei in the skeletal muscle of long COVID patients.
Method Vastus lateralis muscle biopsies from 6 healthy controls and 6 long COVID patients were analyzed using snRNA-seq to evaluate metabolite profiles and cell-type-specific gene expression.
Results Our results from snRNA-seq showed that type I myonuclei exhibited decreased expression of genes related to mitochondrial complexes and protein synthesis, along with an altered transcriptomic profile indicative of fiber-type switching toward a fast-twitch phenotype. Additionally, these myonuclei exhibited altered ligand–receptor interactions with other cell populations, particularly showing exclusive collagen-related and anabolic signaling.
Conclusions The results of this study suggest that type I fibers in long COVID patients with muscle abnormalities may undergo fiber-type switching, mitochondrial dysfunction, impaired protein synthesis, and excessive collagen deposition. These findings highlight potential biomarkers and signaling pathways that may serve as valuable targets for developing effective diagnostic methods and therapeutic strategies to counteract long COVID-associated muscle abnormalities.

#41 Sunday Poster Session

Targeting lysosomal damage is a new therapeutic perspective for Duchenne Muscular Dystrophy

Abbass JABER[1,2], Laura Palmieri[1,2], Ai Vu Hong[1,2], Rania Bakour[1,2], Nathalie Bourg[1,2], Elise Lachiver[1,2], Jérôme Poupiot[1,2], Sonia Albini[1,2], Daniel Stockholm[1,3], Laetitia Van Wittenberghe[1], Adeline Miranda[1], Nathalie Daniele[1], Inés Barthélèmy[4], Stephan Blot[4], Mai Thao Bui[5], Teresinha Evangelista[5], Isabelle Richard[1,2] & and David Israeli[1,2,]

Affiliations:

[1] Genethon, 91000 Evry, France

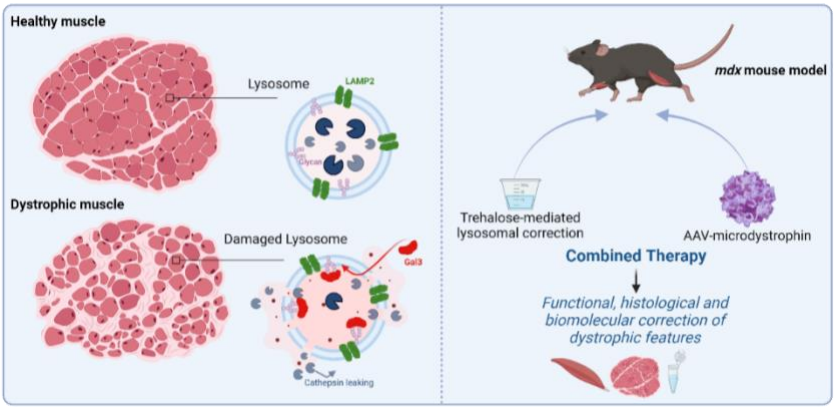
[2] Université Paris-Saclay, Univ Evry, Inserm, Généthon, Integrare research unit UMR_S951, 91000, Evry-Courcouronnes, France.

[3] Ecole Pratique des Hautes Etudes, PSL Research University, Sorbonne Université, INSERM, CRSA, Paris, 75012, France.

[4] Univ Paris-Est Créteil, INSERM U955 IMRB, Ecole Nationale Vétérinaire d'Alfort, EFS, IMRB, Team BNMS, Maisons-Alfort, France

[5] Muscle Pathology Unit, Institut de Myologie and Neuropathology department, Sorbonne Université, Pitié-Salpêtrière Hospital, APHP, Paris, France

Duchenne Muscular Dystrophy (DMD), a muscle degenerative disease affecting young boys, arises from the loss of dystrophin. Current gene therapy strategies focus on delivering a truncated but functional form of dystrophin (microdystrophin) via Adeno-Associated Virus (AAV). While recent clinical trials show promise, therapeutic efficacy remains incomplete, necessitating improved approaches. In this study, we identified lysosomal perturbations in the myofibers of DMD patients and animal models, an overlooked mechanism of cellular damage in muscular dystrophies. These were notably marked by the upregulation and recruitment of Galectin-3, a known biomarker of lysosomal membrane permeabilization, to damaged lysosomes, alongside alterations in lysosome structure, number and and activation of endolysosomal damage response. Importantly, microdystrophin gene therapy in *Dmd*^{mdx} mice fails to fully correct these damages. However, combining it with trehalose, a lysosome-protective disaccharide, significantly improves outcome, enhancing muscle function, histology and overall transcriptome. These findings highlight lysosomal damage as a novel mechanism in DMD pathogenesis and suggest that combining trehalose with gene therapy could enhance therapeutic efficacy.



#42 Sunday Poster Session

NATURE'S OWN EXPERIMENT: CLARIFYING THE ROLE OF MSS51 IN HUMAN SKELETAL MUSCLE METABOLISM AND ITS CONTRIBUTION TO T2D RISK

Bilal Ahmad Mir [1], Mikko Lehtovirta [1,2], Kristoffer Ström[1], Ola Ekström[1], Om Prakash Dwivedi[2], Sebastian Kalamajski[1], Olga Göransson[1], Maria Lindahl[1], Karin Stenkula[1], Tiinamaija Tuomi[1,2], Ola Hansson[1,2]

Affiliations:
1. Lund University, Lund/Malmö, Sweden
2. University of Helsinki, Helsinki, Finland

Type 2 diabetes (T2D), driven primarily by insulin resistance (IR), is a growing global health concern, with over 590 million people affected. Skeletal muscle plays an essential role in glucose clearance and whole-body glucose homeostasis yet remains an underexplored target for insulin-sensitizing therapies. Mitochondrial dysfunction is implicated in muscle IR, but unifying mechanisms are lacking.

MSS51 (also known as ZMYND17), a mitochondrial translational activator, is highly expressed in skeletal muscle. In literature, two Mss51 knockout mouse models have been published with clear, detailed and distinct, BUT opposite phenotypes, i.e. one diabetes prone and the other protected from diabetes. To clarify MSS51's role in humans, 139 individuals with muscle-specific loss-of-function (LoF) variants in MSS51 were identified in the Botnia Family Study. Compared to ~7,000 controls, these individuals exhibited elevated glucose, insulin, and triglyceride levels, indicative of reduced insulin sensitivity and dyslipidemia.

To explore underlying mechanisms, siRNA-mediated MSS51 knockdown was performed in primary human myotubes. Knockdown led to reduced MYH2 expression but did not affect GLUT4. Although basal glucose uptake increased, insulin-stimulated uptake did not improve. Phosphorylation of AS160 increased, and Seahorse analysis revealed a shift towards glycolysis, without changes in mitochondrial respiration. Interestingly, these effects resembled the beneficial metabolic profile seen in one mouse model, but not the human LoF phenotype.

Under lipid-induced stress (palmitic acid), MSS51 knockdown caused lipid accumulation, impaired insulin-stimulated glucose uptake, reduced PGC1α and PDK4 expression, and decreased mitochondrial membrane potential, mimicking the metabolic disturbances observed in humans with MSS51 LoF. These findings suggest that MSS51 plays a context-dependent role in regulating skeletal muscle insulin sensitivity and mitochondrial function. The outcomes may advance the identification of MSS51 as a potential therapeutic target, offering a novel approach to tackling muscle IR and counteracting T2D progression.

#43 Sunday Poster Session

An Anti-sense Oligonucleotide Targeting MYPT1 Exon24 Alters LZ+/LZ- MYPT1 Expression in Vascular Smooth Muscle

Young Soo Han & **Frank V Brozovich**

Mayo Clinic, Department of Cardiovascular Disease, Rochester MN USA

The vascular response to NO is a fundamental property of the vasculature and NO mediated vasodilatation is dependent on PKG mediated activation of MLC phosphatase, a trimeric enzyme, consisting of a catalytic, 20kDa and myosin targeting (MYPT1) subunits. COOH-terminal leucine zipper (LZ+/LZ-) MYPT1 isoforms are produced by alternative mRNA splicing of exon 24 (E24) of the MYPT1 transcript; E24 exclusion produces a LZ+ MYPT1, while E24 inclusion produces a LZ- MYPT1. The importance of LZ+/LZ-MYPT1 expression for the NO-cGMP-PKG signaling pathway in regulating vascular tone and blood pressure is well established; a hypertensive mouse is produced by mutation of the N-terminal domain of PKG, which is important for the interaction of PKG with LZ+ MYPT1, while transgenic mice with increased LZ+ MYPT1 expression are hypotensive. We used an octo-guanidine conjugated antisense oligonucleotide targeting the 5' splice site of MYPT1 E24 (ASO-E24) to alter LZ+/LZ-MYPT1 expression. Our data demonstrate that IP injection of this anti-sense oligonucleotide (12.5mg/kg, qodx3) in mice completely suppresses E24 splicing of the MYPT1 transcript, resulting in exclusive expression of the NO responsive, LZ+ MYPT1 isoform in vascular smooth muscle. The increase in LZ+ MYPT1 expression should increase in the sensitivity of the vasculature to NO mediated vasodilatation. These results demonstrate that antisense technology can alter vascular reactivity, Further, the 50bp sequences flanking MYPT1 E24 are highly conserved in mammals, and thus, these results suggest that antisense technology targeting E24 of the MYPT1 transcript represents a novel technology to alter vascular reactivity in health and disease.

#44 Sunday Poster Session

A 3D HUMAN ENGINEERED MUSCLE TISSUE PLATFORM FOR LONGITUDINAL MUSCLE ATROPHY, DIFFERENTIAL INSULIN SENSITIVITY, AND METABOLISM

Daniel Lih[1], **Hamed Ghazizadeh**[1], Ellie Sullivan[1], Shawn Luttrell[1], Kelly Kernan[1], Neda Kabi[1], Katharina Gees[1], Thomas Leahy[1], Christal Worthen[1], Greg Luerman[1], and Nicholas A. Geisse[1]

Affiliations:

1. Curi Bio; Seattle, Washington, USA

Introduction: Insulin resistance in skeletal muscle impairs systemic glucose regulation and is a hallmark of metabolic disorders such as obesity and type 2 diabetes. While GLP-1-based therapeutics have demonstrated clinical efficacy in managing these conditions, emerging evidence of treatment-associated muscle loss (i.e., sarcopenia) underscores the need for physiologically relevant models to study human muscle metabolism and atrophy. Traditional 2D culture systems lack the structural and functional complexity of native tissue, and rodent models pose translational challenges due to species-specific differences in metabolic enzyme expression, adipokine signaling pathways, and lipid handling. To address these limitations, we employed a 3D human engineered muscle tissue (EMT) platform to pursue three objectives: (1) develop a functional in vitro model of skeletal muscle atrophy, (2) establish an experimental framework for differential insulin sensitivity, and (3) assess the effects of glucose deprivation, insulin stimulation, and exercise on glycogen and lactate metabolism.

Methods: Human primary skeletal myoblasts were cast into contractile 3D EMTs using commercially available instruments, consumables, and media products from Curi Bio. To address the first objective, we evaluated the morphological and functional response of EMTs to high-dose dexamethasone or activin A treatment, which we hypothesized would induce an atrophic response. To address the second objective, EMTs were cultured in either standard or insulin-sensitizing media. After 20 days in culture, both groups were starved of glucose for 2 hours, stimulated with 10 nM insulin for 15 minutes, and analyzed for insulin-responsive markers (GLUT4 and phosphorylated AKT) by western blot. To address the third objective, EMTs were maintained in standard media for 40 days and subjected to glucose starvation, insulin stimulation, and electrical stimulation-induced exercise. Metabolic responses were measured by quantifying glycogen in EMTs and lactate content in the culture media.

Results: For the first objective, exposure to both high-dose dexamethasone and activin A led to significant reductions in muscle size and contractile force in EMTs (**Fig 1A**; activin A data not shown), validating our *in vitro* model of muscle atrophy. For the second objective, EMTs cultured in standard media exhibited low GLUT4 expression and no detectable AKT phosphorylation following insulin stimulation (**Fig 1B**), consistent with an insulin-resistant phenotype. In contrast, EMTs cultured in insulin-sensitizing media displayed elevated GLUT4 expression and a robust AKT phosphorylation response to insulin stimulation, indicating restoration of insulin sensitivity. For the third objective, exercise induced via electrical stimulation following glucose starvation decreased glycogen content and elevated lactate production (**Fig 1C-D**), reflecting metabolic activation. Glucose starvation, with or without insulin, had no effect on glycogen levels but increased lactate concentrations. Finally, glucose reintroduction and insulin stimulation led to significant increases in both glycogen and lactate levels.

Discussion: This study establishes EMTs as a sophisticated and physiologically relevant *in vitro* model for investigating human skeletal muscle atrophy, insulin sensitivity, and

metabolism. Moreover, these experiments illustrate our platform's ability to perform label-free, non-destructive, longitudinal measurements of muscle size and contractile function alongside parallel biochemical analyses on the same tissues. Taken together, these capabilities offer a powerful framework for metabolic drug discovery and preclinical screening of candidate therapeutics by enabling simultaneous assessment of therapeutic efficacy and the potential risk of treatment-induced sarcopenia.

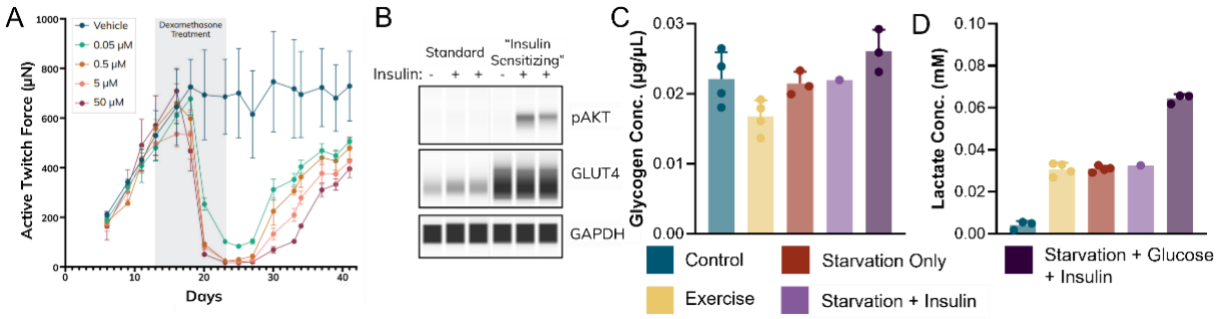


Figure 1. A) Dexamethasone generates an atrophic response in contractile force. B) "Insulin-sensitizing" media promotes GLUT4 expression and pAKT response to insulin stimulation. C) Glycogen and D) lactate media concentrations are modulated in response to glucose starvation and reintroduction, exercise, and insulin stimuli.

#45 Sunday Poster Session

THERAPEUTIC SCREENING OF A DUAL AAV-SPLIT INTEIN MIDI DYSTROPHIN BY FUNCTIONAL AND STRUCTURAL ANALYSIS OF EX VIVO MICE SKELETAL MUSCLE AND HUMAN MYORGANOIDS

M. Marcello, L. Palmieri, M. Ferrand, I. Richard and S. Albini

Affiliations:
1. Genethon, 91000 Evry, France
2. Université Paris-Saclay, Univ Evry, Inserm, Généthon, Integrare research unit UMR_S951, 91000, Evry-Courcouronnes, France.

Duchenne muscular dystrophy (DMD) is a rare genetic disorder caused by mutations in the gene encoding dystrophin. The absence of this structural protein compromises the integrity of muscle fibers, leading to progressive degeneration and loss of muscle function. The most promising treatment currently involves gene replacement using Adeno-Associated Viruses (AAVs) to deliver a shortened yet functional version of dystrophin, known as micro-dystrophin (µDys), which fits within the limited packaging capacity of AAV vectors. While microdystrophin has shown promise in clinical trials, its effectiveness has been incomplete, likely because it lacks key structural domains of the full-length protein. Consequently, expressing larger, more complete versions of dystrophin may be necessary to achieve a more effective correction of the disease phenotype.

We have recently generated two larger versions of dystrophin (MIDI dystrophin) (MIDI-Dys1 and MIDI-Dys2-EP23306256.1) using a dual AAV system with a trans-splicing intein approach, showing high protein reconstitution efficacy and therapeutic benefits in a severe DMD mouse model (D2-mdx). Our constructs differ between each other in key structural domain combinations, favoring dystrophin anchoring to the sarcolemma and the cytoskeleton. To assess the functional impact of these MIDI-dystrophins, we employed specific assays to evaluate the contractile properties of the extensor digitorum longus (EDL), a fast-twitch muscle that is particularly affected by DMD pathology. This approach enabled us to thoroughly assess the therapeutic effects on ex vivo EDL performance during both isometric and isotonic contractions. Our results show that MIDI-Dys1 confers superior recovery of contractile properties compared to both MIDI-Dys2 and micro-dystrophin. These results correlate well with the membrane restoration of dystrophin-associated proteins, including neuronal nitric oxide synthase (nNOS) and beta-dystroglycan.

To address the translational gap existing between animal studies and patient outcomes, we also established a human disease model that recapitulates critical hallmarks of DMD severity, providing a more predictive framework for therapeutic investigation. By employing a muscle engineering approach, we generated MYOrganoids from iPSC-derived muscle cells co-cultured with fibroblasts, which enhance the structural and functional maturation of myotubes. Testing our therapeutic candidates in MYOrganoids demonstrated that the dual vector approach, and particularly MIDI-Dys1, is the most effective in restoring a phenotype more closely resembling the WT condition in DMD MYOrganoids.

Overall, these findings emphasize the importance of further exploring the changes in muscle mechanical properties following gene replacement, which could lead to improvements in current therapeutic strategies. They also highlight the potential of functional assays — which better mimic real-world muscle movements — as valuable tools for therapeutic screening in DMD muscle models.

#46 Sunday Poster Session

EXPERIMENTAL AND COMPUTATIONAL STUDIES OF THE MYH7 VARIANT G256E ASSOCIATED WITH HYPERTROPHIC CARDIOMYOPATHY

Kerry Kao[1], Matthew Childers[1,2], **Michael Regnier[1,2,3,4]**

Affiliations:
1. Department of Bioengineering, University of Washington, WA, USA
2. Center for Translational Muscle Research, University of Washington, WA, USA
3. Institute for Stem Cell and Regenerative Medicine, University of Washington, WA, USA
4. Center for Cardiovascular Biology, University of Washington, WA, USA

Familial forms of Hypertrophic and Dilated Cardiomyopathy are diseases of the sarcomere. Advances in stem cell biology and gene editing technologies have made it possible to study a myriad of variants in sarcomeres that are associated with these diseases. The value of these stem cell models are the isogenic background, making them more directly comparable than stem cells derived from patients with different background histories. We have developed stem cell lines for more than a dozen of these variants in the MYH7 gene, coding for β-myosin. For each of these MYH7 variant lines we are performing multi-scale structural and chemo-mechanical and contractile kinetics analysis. Molecular Dynamics (MD) and Brownian Dynamics (BD) simulations are used to provide protein level structure based mechanistic insight to functional abnormalities caused by the MYH7 variants. For the G256E variant, measures of myofibril contraction and relaxation showed greatly enhanced force and slower relaxation compared with myofibrils from WT cells. In particular, the rate of crossbridge detachment, determined from the slope of the early, linear phase of relaxation (kREL,slow) was slowed suggesting the rate limiting process in loaded crossbridge cyling, ADP release, may be impaired. Further measures in the presence of elevated ADP supported this idea, as the relaxation kinetics of G256E myofibrils were less affected. Stopped-flow kinetics analysis demonstrated an approximate 25% higher ADP binding affinity for purified G256 vs WT myosin, and a somewhat slower rate of ATP binding to G256E myosin. MD of post-rigor, M.ATP, demonstrated that the G256E variant causes changes in local contacts that results in destabilization of hydrogen bonds in the first three beta strands of the transducer region. Steered MD simulations of the A.M.ADP state demonstrated that G256E myosin requires on average 1.5x as much work to displace ADP compared to WT due to aterations in the molecular release pathway. In a previous report, we showed that the G256E mutation results in a reduction in low ATPase heads, sometimes called the super relaxed state (SRX) of myosin. Taken together, these studies suggest the beta myosin variant G256E results in activation of myosin and slowing of the crossbridge cycle, via a retardation of ADP release from the nucleotide binding pocket, resulting in increased myofibrillar force and slowed relaxation. Our studies also demonstrate the value of multi-scale, multi-discipline research to provide a thourogh understanding of the molecular mechanisms of functional pathology.

#47 Sunday Poster Session

CHRONIC MYOTROPE TREATMENT INDUCES CONTRACTILE AND MYOFIBRILLAR REMODELING IN HIPSC-CMS

Miguel Sanchez-Lozano[1], Gabriela Villalpando Torres[1], Janae Gayle[2], Anela Bautista[2], Samuel D. Feinstein[1,2], Prof. Beth L. Pruitt[1,2]
Affiliations:
1. Department of Mechanical Engineering, University of California, Santa Barbara, USA
2. Department of Bioengineering, University of California, Santa Barbara, USA

Hypertrophic and dilated cardiomyopathies (HCM and DCM) are genetically inherited diseases that impair cardiac contractility. At the cellular level, hallmarks of HCM and DCM include changes in contractility and alterations in myofibril structure. These conditions are increasingly treated with cardiac myotropes such as Mavacamten and Omecamtiv Mecarbil (OM). Mavacamten reduces contractility and has been FDA-approved for patients with HCM. In contrast, OM enhances contractility and is a promising treatment for patients with DCM, but has not yet received regulatory approval. We previously found that 1-hour treatment of 50 nM Mavacamten reduced human-induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) contractile force measured by traction force microscopy (TFM) (Pardon, Nature Communications, 2024). While 100 nM of OM disrupted hiPSC-CM's myofibril structures immediately (Ribeiro, Circulation Research, 2017). Other research (Solis, FEBS PLOS, 2023) have treated cardiomyocytes with myotropes in vitro for 12 hours and there is no data in PUBMED of longer treatments studies, which normally use 1 hour treatment (Toepfer, Circulation Research, 2019). However, the chronic effects of these therapies on contractile force and cardiomyocyte's cytoskeleton remain poorly understood. To investigate how altering the contractile force influences cytoskeletal remodeling, hiPSC-CMs endogenously labeled with α-actinin 2 were cultured on single-cell micropatterned (1500 μm2, aspect ratio 7:1) polyacrylamide gels with a 10-kPa stiffness and treated with Mavacamten, OM, or DMSO control for 5 days. After 5-day treatment, contractile force was measured via TFM for OM (100 nM and 1 μM). Following TFM, we fixed cells and characterized myofibrils morphology by Z-line width and number of Z-lines by segmentation with Labkit, plugin in Fiji. Preliminary results suggest a trend toward increased contractile force with 1 μM of OM has an increased of 300 nN more in the mean value respect to the DMSO control; while the 0.1 does not show a significant difference with respect to DMSO control. And myofibril morphology illustrates that hiPSC-CMs treated with 1 μM of OM increased in Z-line in 34%, without a significative variance for 0.1 μM; and there is no significant change in the number of Z-lines in DMSO, 0.1 μM, and 1 μM of OM after 5-day treatment. While, we are still processing the results with Mavacamten chronic treatment. It is expected to show a sustained reduction in force and we see changes in myofibril structure (width and z-lines). These findings provide insight into cardiomyocytes' cytoskeletal and functional responses to myotropic modulation. The platform establishes a robust foundation for extended in vitro studies aimed at uncovering long-term effects of pharmacologic therapies on cardiomyocyte remodeling.

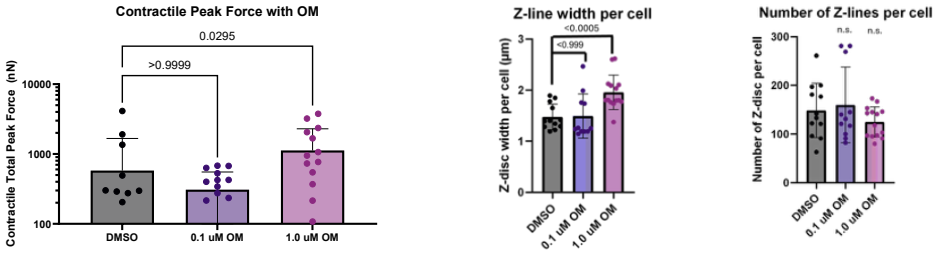


Figure 1. Contractile peak force measured By TFM on the right; in the center is the Z-line width per cell; and on the left Z-line number for DMSO (control), 0.1 and 1 µM of OM after 5-day treatment in each plot.

#48 Sunday Poster Session

INVESTIGATING THE EFFECT OF PRE- AND POST-MENOPAUSAL IGF-1 CONCENTRATIONON SKELETAL MUSCLE MASS IN VITRO

Zhenjia Zhong[1], Esmée van der berg[1], Carla Offringa[1], Richard T Jaspers[1], Maarten Michiel Steinz[1,2]

Affiliations:
1. Lab. for Myology, Dpt. Of Human Movement Sciences, Fac. of Behavioral & Movement Sciences, Vrije University Amsterdam, Amsterdam Movement Science
2. Department of Rheumatology, Amsterdam University Medical Center Amsterdam, The Netherlands

Background Menopause marks the permanent end of menstrual cycles due to the cessation of ovarian follicular activity. It induces a decline of systemic circulating concentrations of growth factors including insulin-growth factor (IGF-1). Menopause is also associated with an increased risk to develop sarcopenia: a progressive and age-related decline in skeletal muscle mass which is leading to decreased muscle strength, increased risk of falls and disabilities. The complexity of menopause fluctuations in IGF-1 and the pathophysiological effects on skeletal muscle mass are not yet fully understood. Moreover, in-vitro models that mimic pre- and postmenopausal conditions remain poorly characterized.

Objectives The main objective of this study was to investigate the effect of IGF-1 (100 ng/ml) on muscle mass and gene expression of genes related to cell-proliferation, protein synthesis and protein degradation in three different in-vitro skeletal muscle models that are potentially suitable for menopause *in-vitro* research.

Method Human induced Pluripotent Stem Cells (iPSCs)-derived myoblasts, primary human myoblasts, or immortalized mouse myoblast (C2C12) were cultured in DMEM with 10% fetal bovine serum (FBS), and differentiated into muscle-fibers (myotubes) using 2% horse serum. Myotubes were incubated with or without IGF-1 (100 ng/ml) to mimic pre- and post-menopausal IGF-1 conditions (N= 6/group). Bright-field images were taken to measure myotube diameter, as outcome measure for muscle mass. Gene expression was assessed for muscle cell-proliferation (CCND1, PKM2), protein synthesis (PHFDH) and protein degradation (TRIM63, FBXO32 and MYC) with qPCR (N= 6/group).

Results IGF-1 had no effect on the diameter or gene expression of iPSC-derived myotubes compared to control conditions. In primary human myotubes, IGF-1 increased diameter by 13.9% and upregulated CCND1 (*$p<0.05$), with a trend toward decreased FBXO32 ($p=0.08$) compared to control. In C2C12 myotubes, IGF-1 significantly altered gene expression: FBXO32, MYC, and TRIM63 were downregulated, while PHGDH, PKM2, and CCND1 were upregulated (**$p<0.01$). The gene-expression differences in C2C12 compared to control were greater than those observed in primary human myotubes.

Conclusion This study demonstrated *in-vitro* muscle model-specific differences in IGF-1 sensitivity between iPSC, human primary and C2C12 myotubes for *in vitro* skeletal muscle research in the context of menopause. These findings emphasize the need for further characterization of the *in-vitro* skeletal muscle models to better replicate pre- and post-menopausal conditions. It can ultimately improve the knowledge of how IGF-1 and other menopause-related hormones play a role in menopause-associated loss of muscle mass. Future experiments will be conducted in 3D tissue engineered muscle cells.

#49 Sunday Poster Session

Stretch Activation Combats Decreased Muscle Force Production from Fatigue In Mouse Type II Fibers

Philip C. Woods[1], Mark S. Miller[1] and **Douglas M. Swank**[2]
1. University of Massachusetts, Amherst, USA; 2. Rensselaer Polytechnic Institute, Troy, USA

Stretch activation (SA) is a delayed increase in muscle force following a rapid stretch and improves muscle performance during repetitive cyclical contractions in insect flight and cardiac muscles. Although historically considered too low to be physiologically relevant in skeletal muscle, our recent work showed that higher phosphate concentrations ([Pi]) increased SA 4-fold in mouse soleus muscles fibers (Straight, et al., 2019). These results suggested SA may have a role combating muscle fatigue, which increases [Pi], lowers pH and reduces activating calcium concentration ($[Ca^{2+}]$). To test this new idea, we measured SA during Control (pCa 4.5, pH 7.0, 5 mM Pi), High $[Ca^{2+}]$ Fatigue (pCa 4.5, pH 6.2, 30 mM Pi) and Low $[Ca^{2+}]$ Fatigue (pCa 5.1, pH 6.2, 30 mM Pi) conditions by inducing the SA response in myosin heavy chain (MHC) type I and IIA fibers from mouse soleus muscles and IIX and IIB fibers from EDL muscles. **SA force (F_{SA}) in myosin heavy chain type IIA fibers from mouse soleus muscles increased 46% under High $[Ca^{2+}]$ Fatigue conditions** and was unchanged under Low $[Ca^{2+}]$ Fatigue conditions compared to Control conditions. F_{SA} of Type IIX and IIB fibers from EDL were unchanged under both fatigue conditions compared to Control conditions. We found that calcium-activated isometric force (F_0) decreased going from Active to High $[Ca^{2+}]$ Fatigue and further decreased from High $[Ca^{2+}]$ Fatigue to Low $[Ca^{2+}]$ Fatigue in all type II fibers. Combined with our F_{SA} measurements, this means that stretch activation's percent contribution to total muscle force production ($F_{SA}/(F_0 + F_{SA})$) is much greater under fatigue than control conditions. **F_{SA}'s contribution to total force production under fatiguing conditions ranged from a 58% increase to a 114% increase depending on the MHC II isoform and fatigue conditions.** Interestingly, F_{SA} from soleus type I fibers was 80% lower than solus type II fibers under control conditions and the SA peak (phase 3 peak) was not visibly apparent under either fatigue condition. Comparing the timing of the stretch activation response of Type II fibers with *in vivo* mouse soleus and EDL muscle length change and activation patterns suggests the delayed SA force increase would occur during *in vivo* shortening thus increasing work and power. **These results suggest SA improves force production under fatiguing conditions in MHC type II fibers. This could play an important role in increasing muscle endurance for muscles that are lengthened prior to shortening by supplementing calcium-activated force production.**

Further analysis of fibers from our previous stretch activation experiments with mouse soleus fibers' F_{SA} values at different [Pi]s (Straight, et al., 2019) revealed that only MHC type IIA fibers' F_{SA} increased with Pi while type I F_{SA} was unchanged. Type IIB EDL fiber F_{SA} values decreased with increasing [Pi]. To explain the [Pi] and the fatigue experimental results, and our previous *Drosophila* myosin versus [Pi] findings (Zhao, et al., 2013), we propose two myosin-based mechanisms. The main difference between these two mechanisms is that **for some myosin isoforms and conditions, stretch causes a reversal of the myosin power-stroke** allowing for a subsequent "second" power-stroke, while in other cases, stretch forcibly detaches myosin from actin in a post-power stroke state.

Straight, C.R., K.M. Bell, J.N. Slosberg, M.S. Miller and D.M. Swank (2019) *Am. J. Physiol. Cell Physiol.* 317:C1143-C1152.; Zhao, C., and D.M. Swank **(**2013). *Biophys. J.* 104:2662-2670.

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#1 Monday Poster Session

Study of the muscular secretome in a prematurely aged model

Giulia Ferrarese[1,2], G. Masiero[1,2], M. Scalabrin[1,2], G.A. Dumitras[1,2], B. Blaauw[1,2], C. Tezze[1,2]* and M. Sandri[1,2,3]*
[a] *Dulbecco Telethon Institute at Venetian Institute of Molecular Medicine, Padova, Italy;*
[b] *Department of Biomedical Science, University of Padova, Padova, Italy*
[c] *Department of Medicine, McGill University, Montreal, Quebec, Canada*

Mitochondrial dysfunction is a recognized contributor to the pathogenesis of sarcopenia and aging. Tezze et al. (2017) showed that in adult mice acute muscle-specific blocks of fusion, throw the deletion of mitochondrial protein Optic Atrophy 1 (OPA1), induce a profound muscle loss, systemic inflammatory response, and precocious epithelial senescence that culminates with animal death. Mitochondrial dysfunction, caused by Opa1 ablation, triggers a dramatic increase in muscle fibroblast growth factor 21 (FGF21) expression and secretion from the skeletal muscle tissue. Impressively, the double muscle-specific ablation of Opa1/Fgf21 reverted completely the aging phenotype and prevented precocious death in mice. Importantly, the block of fission (Drp1 ko) or the concomitant block of fusion and fission (Drp1/Opa1 ko) showed higher FGF21 levels but not the precocious aging phenotype and the precocious death. Interestingly, sterile inflammation is visible in the iOpa1 mKO mice but not in the iDrp1 mKO and iOpa1/Drp1 DKO mice, suggesting that these pathways may contribute to the early aging phenotype observed in the iOpa1 mKO mice. This latter exhibited an elevated level of pro-inflammatory cytokines, particularly IL-6, specifically produced in skeletal muscle, which was mitigated by the double deletion in iFgf21-Opa1 m2KO mice. These observations support the concept that FGF21 needs the presence of other factors to elicit the precocious senescence phenotype of OPA1 KO mice. Investigating whether skeletal muscle contribute to inflammatory processes in OPA1 KO mice, we generated double Tamoxifen-inducible muscle-specific Opa1/IL-6 knockout mice (iOpa1-il-6 mDKO). Interestingly, we observed that muscle-specific deletion of IL-6 leads to increased survival in transgenic animals, attenuates systemic inflammation, and reduces cellular senescence. At the same time, however, IL-6 ablation negatively affects muscle function, mass, and calcium homeostasis, suggesting a potential role for this cytokine in the adaptive response to changes occurring during sarcopenia. To identify other factors that, synergistically with Fgf21, induce premature senescence in Opa1 KO mice, we generated a novel mouse model by crossing inducible muscle-specific Opa1 knockout mice with floxed MetRS* knock-in mice. The latter expresses a mutant methionyl-tRNA synthetase that allows the incorporation of azidonorleucine (ANL), a non-canonical amino acid, in place of methionine in muscle proteins. This amino acid can be conjugated to different affinity tags and the labeled proteins can be identified by tandem mass spectrometry (MS/MS) both in the circulation and in the target tissue. To streamline the in vivo model, we opted to collect proteins tagged with ANL secreted by muscles into the extracellular fluids (EFs) for MS/MS analysis. Numerous studies have shown that while most serum-detectable proteins can be identified in EFs, proteins present in tissues are often difficult to detect in serum. This

highlights the potential of EFs as a highly effective and efficient approach for identifying novel secreted factors.

#2 Monday Poster Session

ANALYSIS OF MOTOR PERFORMANCE IN *Stbd1* KO MICE

B. Bastide[1], MH. Canu[1], V. Montel[1], C. Cieniewski-Bernard[1,2], A. Demetriadou[3] and P.P Petrou[3].

[1]Univ. Lille, Univ Artois, Univ Littoral Côte d'Opale, URePSSS - Unité de Recherche Pluridisciplinaire Sport Santé Société, ULR7369, Lille, F-59000, France.

[2.] Université de Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, F-59000, Lille, France

[3]Biochemical Genetics Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.

Stbd1 is a glycogen-binding protein that localizes to the ER membrane and ER-mitochondria contact sites, with a poorly characterized role in glycogen metabolism. A recent report demonstrated that Stbd1 promotes glycogen clustering during endoplasmic reticulum (ER) stress in mouse myoblasts, a process that supports cell survival against apoptotic cell death (Lytridou A et al., J Cell Sci, 2020). Mice with targeted inactivation of *Stbd1* display insulin resistance and significantly lower levels of glycogen in the liver, suggesting a role for this protein in controlling glucose homeostasis. As glucose and glycogen metabolism are related to muscle contractile capacity, we aimed at determining whether the deletion of *Stbd1* in mice could impact motor function. The *in situ* isometric contractile properties were determined for the fast EDL in response to contractions induced by stimulating the sciatic nerve. Our findings revealed a significant decrease in twitch peak tension and peak tetanic tension obtained at 16, 80, 100 and 120 Hz in *Stbd1* KO mice, while the fatigue index remained unchanged. These results are corroborated by *in vivo* data which revealed a significant decrease in muscle strength, as evaluated by the grip test, with no alteration in fatigue, as assessed by the hanging wire test. Moreover, the free running wheel test showed that the *Stbd1* KO mice are less active, as compared to wild-type control mice (lower running distance and lower activity duration). Footprint analysis using the Catwalk system revealed changes in locomotor parameters. However, the motor coordination evaluated using the beam walk test was not altered in *Stbd1* KO mice.

Our findings demonstrate that *Stbd1* ablation in mice results in a loss of muscle force and activity. Further experiments are underway aiming to shed light on the metabolic and molecular basis of skeletal muscle contractile and motor function defects observed in *Stbd1* KO mice.

#3 Monday Poster Session

Challenges in characterizing pyruvate dehydrogenase kinase 1 degradation in skeletal myotubes

Blaž Kociper, Katarina Miš, Sergej Pirkmajer

Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Slovenia

Pyruvate dehydrogenase kinase 1 (PDK1) is an important mitochondrial kinase that inhibits the conversion of pyruvate to acetyl-CoA by phosphorylating the pyruvate dehydrogenase complex and thus shifting metabolism toward glycolysis. Mitochondrial proteins are typically degraded through three main pathways: autophagy, ubiquitin-proteasomal system, or intrinsic mitochondrial proteases. However, the role of these pathways in the case of PDK1 remains unclear.

Our previous work showed that inhibition of the proteasome reduces PDK1 levels, and that this reduction is not mediated by increased autophagy. In this follow-up study, we investigated three potential mechanisms underlying the MG132-induced decrease in PDK1: (1) involvement of the mitochondrial protease LONP1, (2) involvement of the mitochondrial protease CLPP, and (3) a potential role of HSP70 proteins, which have been implicated in mediating FOXM1 degradation in response to proteasomal inhibition.

In rat L6 myotubes, immunoblotting revealed that the MG132-induced decrease in PDK1 levels was not prevented by inhibiting the mitochondrial proteases LONP1 or CLPP. LONP1 was targeted using bortezomib or MG262, while CLPP was silenced via siRNA. To assess the potential involvement of HSP70, we used 9-aminoacridine, which successfully rescued FOXM1 levels under proteasomal inhibition, in line with previous findings. However, the same treatment led to a further reduction of PDK1, suggesting that PDK1 degradation occurs through a different mechanism from that of FOXM1.

Although suppression of LONP1, CLPP, or HSP70 did not prevent the MG132-induced reduction of PDK1, these findings help narrow down the potential mechanisms involved.

#4 Monday Poster Session

ALTERED MYOSIN SUPER-RELAXATION IN ATRIAL MYOCYTES FROM PATIENTS WITH ATRIAL FIBRILLATION

Chahida Chaami[1], Funsho E. Fakuade[2], Julius Ryan D. Pronto[2], Niels Voigt[2], Julien Ochala[1]

Affiliations:
1. Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark
2. Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Göttingen, Germany

Atrial fibrillation (AFib) is the most prevalent form of cardiac arrhythmia, affecting over 33 million individuals globally, with its frequency expected to double in the next four decades. Despite significant progress in understanding its molecular pathophysiological and compensatory mechanisms, the exact role of one of the most abundant atrial proteins, myosin, remains largely unexplored. This is surprising considering that protein is crucial for atrial performance and energetics. Hence, in the present study, we aimed to define myosin remodeling that happens in AFib. For that, we took advantage of tissue obtained from right atrial appendages of ten human patients with persistent AFib and of ten sinus rhythm patients (defined as controls). Thin cardiac strips were dissected from these appendages, and Mant-ATP chase experiments were performed. Overall, we did not observe any significant difference in the proportion of myosin heads in their biochemical ATP-preserving super-relaxed states. Nevertheless, and unexpectedly, we found that the ATP turnover time of these super-relaxed myosin molecules is slower for patients with persistent AFib than controls. We are now in the process of defining the structural basis of such findings by characterizing whether differences exist in myosin post-translational modifications (LC/MS peptide mapping) and their potential functional impact (Molecular Dynamics Simulations). Altogether, our preliminary results indicate that myosin faces a remodelling in AFib that can have energetic implications.

#5 Monday Poster Session

IMPACT OF THE REDUCTION OF MITOCHONDRIAL CALCIUM UPTAKE ON THE CONTRACTILE PROPERTIES OF FAST AND SLOW MOUSE SKELETAL MUSCLE WITH CONSTITUTIVE DELETION OF MCU (skMCU-/-)

Beatrice Scellini[2], Marco Caremani[1], Nicoletta Piroddi[2], Gaia Gherardi[3], Cristina Mammucari[3], Massimo Reconditi[2], **Chiara Tesi[2]**

Affiliations:
1. Department of Biology, University of Florence, Florence, Italy
2. Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
3. Department of Biomedical Sciences, University of Padua, Padua, Italy

Mitochondrial dysfunction is recognized as one of the hallmarks of aging and an essential determinant in age-related muscle wasting and weakness, with mitochondrial calcium playing the role of a key signal linking oxidative metabolism to muscle function. Mitochondrial calcium ($mtCa^{2+}$) uptake is ensured by the presence in the inner mitochondrial membrane of the mitochondrial calcium uniporter (MCU) complex, a macromolecular structure composed of pore-forming and regulatory subunits. Loss-of-function mutations in the MICU1 gene associate with myopathy and muscle weakness. In a mouse model, the constitutive deletion of MCU in skeletal muscle (skMCU-/-) causes a reduction in mitochondrial Ca^{2+} uptake and a significant decrease in *in vivo* muscle force and exercise capacity (Gherardi et al, 2019). Here we characterise the impact of the reduction of $mtCa^{2+}$ on muscle performance by classical mechanical measurements in fast (tibialis anterior and EDL) and slow (soleus) intact muscles and isolated myofibrils from skMCU-/- mice vs MCUfl/fl controls. Results of preliminary experiments reported no significant difference in the force-velocity relations and power output in skMCU-/- muscles vs controls, both fast and slow, as well as in the maximal calcium-activated force and kinetics of force development of myofibrils from the same muscle types. These data suggest that reduced mitochondrial calcium uptake has little impact on muscle mechanical properties, corroborating the notion that muscle performance is primarily affected by the metabolic rewiring caused by MCU deletion. Interestingly, characterising the same parameters in aged muscle (24-month-old mice) showed a reduction in muscle performance, with a marked reduction of both the maximal velocity during unloaded shortening and the maximum power in both fast and slow muscles. Further experiments are planned to both improve the statistical significance of the results and assess the impact of decreased $mtCa^{2+}$ uptake in skMCU-/- vs controls fast and slow muscles on muscle fatigue, to evaluate the potential of the skMCU-/- mouse model for studying at the sarcomeric level age-related metabolic alterations responsible for muscle weakness. Supported by PRIN 2022 - 2022B32SCL (MUR- Italy).

#6 Monday Poster Session

PROTECTIVE EFFECTS OF METFORMIN AGAINST STATIN-INDUCED MYOTOXICITY: THROUGH AMPK-MEDIATED PROTECTIVE METABOLIC INHIBITION

Chuqi He [1], Mike Wesselink[1], Jelle Y Huijts[1], Moritz Eggelbusch[1,2], Carla Offringa[1], Richard T Jaspers[1], Rob CI Wüst[1*]

1 Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; 2 Technical University of Munich, Munich, Germany

Objective: Statins are commonly prescribed lipid-lowering drugs that cause myopathy as a significant side effect. Metformin, a first-line antidiabetic medication, is frequently co-administered with statins in patients with metabolic disorders. This study aimed to investigate the cellular and molecular mechanisms of metformin on simvastatin-induced myotoxicity in skeletal muscle cells.

Methods: Differentiated C2C12 myotubes were treated with simvastatin (10 μM) alone or in combination with metformin low (50 μM) or high (1000 μM) concentrations for 24-72 hours. We assessed myotube morphology, myoblast proliferation, expression of genes related to cellular stress, atrophy and metabolism, and mitochondrial respiration and networks.

Results: Simvastatin significantly reduced myotube diameter, induced cellular stress responses, upregulated muscle atrophy genes, and disrupted mitochondrial function and network structure. Co-treatment with metformin partially reversed simvastatin-induced myotube atrophy while activating AMPK signaling (increased p-AMPK/AMPK ratio) and suppressing mTOR activity (reduced p-p70s6k/p70s6k ratio). Notably, metformin further reduced maximal mitochondrial respiration and ATP production beyond simvastatin alone, yet paradoxically preserved mitochondrial network integrity. This metabolic adaptation was accompanied by decreased hexokinase II gene expression, suggesting coordinated downregulation of both oxidative phosphorylation and glycolysis, characteristic of an "energy-saving mode" that prioritizes cellular survival over energy production.

Conclusions: Our findings reveal that metformin protects against simvastatin-induced myotoxicity through protective metabolic inhibition, where sustained AMPK activation induces an energy-conserving state that preserves mitochondrial structure while reducing metabolic demands. This study introduces the concept that deliberate metabolic suppression can be cytoprotective in the context of drug-induced muscle toxicity and suggests new therapeutic approaches for preventing statin-associated myopathy.

#7 Monday Poster Session

Reduced ATP turnover during hibernation in relaxed skeletal muscle

Cosimo De Napoli[1,2], Luisa Schmidt [3], Mauro Montesel[1,2], Laura Cussonneau [1,2], Samuele Sanniti[1] , Lorenzo Marcucci [2] , Elena Germinario[2] , Jonas Kindberg [4,5], Alina Lynn Evans[6] , Guillemette Gauquelin-Koch[7] , Marco Narici[2] , Fabrice Bertile [8,9], Etienne Lefai[10], Marcus Krüger [3] , Leonardo Nogara [1,2,11] & Bert Blaauw [1,2]

Affiliations:

1. Venetian Institute of Molecular Medicine (VIMM), Padova, Italy.
2. Department of Biomedical Sciences, 35131, University of Padova, Padova, Italy. 3. Institute for Genetics, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany. 4. Norwegian Institute for Nature Research, Trondheim, Norway.
5. Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden.
6. Department of Forestry and Wildlife Management, Faculty of Applied Ecology and Biotechnology, Inland Norway University of Applied Sciences, Koppang, Norway.
7. French Space Agency, Centre National d'Etudes Spatiales (CNES), Paris, France. 8. Université de Strasbourg, CNRS, IPHC UMR 7178, 7 Strasbourg, Cedex 2, France.
9. National Proteomics Infrastructure, ProFi, Strasbourg, France.
10. Université Clermont Auvergne, INRAE, UNH UMR 1019, CRNH Auvergne, Clermont-Ferrand, France.
11. Department of Pharmaceutical Sciences, 35131, University of Padova, Padova, Italy

Hibernating brown bears, due to a drastic reduction in metabolic rate, show only moderate muscle wasting. Here, we evaluate if ATPase activity of resting skeletal muscle myosin can contribute to this energy sparing. By analyzing single muscle fibers taken from the same bears, either during hibernation or in summer, we find that fibers from hibernating bears have a mild decline in force production and a significant reduction in ATPase activity. Single fiber proteomics, western blotting, and immunohistochemical analyses reveal major remodeling of the mitochondrial proteome during hibernation. Furthermore, using bioinformatical approaches and western blotting we find that phosphorylated myosin light chain, a known stimulator of basal myosin ATPase activity, is decreased in hibernating and disused muscles. These results suggest that skeletal muscle limits energy loss by reducing myosin ATPase activity, indicating a possible role for myosin ATPase activity modulation in multiple muscle wasting conditions.

#8 Monday Poster Session

MUSCLE NON-SHIVERING THERMOGENESIS INCREASES ENERGY COST OF PHYSICAL ACTIVITY

Kristina Bardova[1], Anna Vavrova[1], Sara Stanic[1], Petr Zouhar[1], Jan Kopecky[1]

Affiliation:
1. Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
Recent research reveals the substantial gaps in our understanding of the processes underlying energy metabolism, concerning both thermal homeostasis and obesity. Our recent study directed our interest to non-shivering thermogenesis (**NST**) in skeletal muscle (ref 1). Prolonged exposure to cold resulted in increased capacity of NST mediated by uncoupling protein 1 (**UCP1**) in brown adipose tissue (**BAT**) in obesity-prone C57BL/6 (**B6**) mice. Surprisingly, obesity-resistant A/J mice failed to activate BAT but showed an adaptive increase in muscle NST; uncoupling of sarco(endo)plasmic reticulum calcium ATPase (**SERCA**) pump activity by sarcolipin (**SLN)** and possibly other mechanisms could be involved. Thus, the absence of BAT-mediated NST was associated with adaptive NST in skeletal muscle. The interaction between the muscle NST and work is not known. We hypothesised that adaptation to muscle NST may affect the efficiency of muscle work, due to prioritizing energy expenditure over ATP supply for work, with severity increasing proportionally to workload. i.e., muscle NST increases the energy cost of muscle work. Similarly to A/J mice, also B6 mice with genetic ablation of UCP1 (**UCP1-KO**) activate the SLN/SERCA muscle thermogenesis in cold (ref 2). Therefore, in this study, we used this well-defined model to verify our hypothesis. UCP1-KO and control mice were gradually adapted to temperatures of 30°C, 22°C, and 8°C for 3 weeks. When exposed to cold (8°C), both mice were cold-tolerant, with cold-preacclimated mice showing higher body temperature in the absence of UCP1. Unlike basal metabolic rate, energy demands for physical activity, measured using treadmills at thermoneutrality were higher in UCP1-KO mice acclimated to 8°C, suggesting that adaptive muscle NST interferes with muscle contraction. Our results elucidated further the mechanism of muscle NST, and they suggest that relatively high energy demands for physical activity in cold-adapted individuals harbouring muscle NST may compromise the development of obesity.

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#9 Monday Poster Session

EXERCISE-INDUCED GLUT4 TRANSLOCATION TOWARD SITES OF HIGH GLUCOSE DELIVERY AND UTILIZATION (2/2)

Jelle Y. Huijts[1], Richie P. Goulding[1], Ignė Kanopaitė[1], Milda Kirvaitytė[1], Wendy Noort[1], Esmee van den Berg[1], Frank W. Bloemers[2], Rob C.I. Wüst[1]

[1] Faculty of Behavioral and Movement Sciences, Vrije Universiteit Amsterdam, The Netherlands
[2] Department of Trauma Surgery, Amsterdam UMC, Amsterdam, The Netherlands

Introduction: Glucose uptake is predominantly facilitated by glucose transporter 4 (GLUT4). Upon muscle contraction or insulin stimulation GLUT4 translocates from intracellular compartments toward the membrane. In this study we will shed more light in to the specific direction of this translocation process within human skeletal muscle.

Methods: Biopsies were taken before and immediately after a 30 minute bout of moderate exercise from the *vastus lateralis* of 22 participants. Biopsy sections were subsequently stained with an immunofluorescent staining to visualize GLUT4, cell membrane and capillaries, mitochondria, t-tubules, and were imaged using widefield microscopy. The images were deconvolved and analyzed with multiple metrics to assess GLUT4 distribution, the co-occurrence, and colocalization to the membrane and capillaries, mitochondria and T-tubules. GLUT4 protein content was assessed via western-blot.

Results: GLUT4 translocation to the cell membrane increased after exercise (Pearson's membrane-GLUT4 colocalization coefficient, rest: 0.39 ± 0.05 *versus* exercise: 0.42 ± 0.04, p=0.01). Post-exercise individual GLUT4 clusters increased in size (rest: 14.89 ± 7.12 *versus* exercise: 20.17 ± 8.36 μm^2, p=0.01). These clusters occupied a smaller fraction of the total membrane (Mander's 1 GLUT4-membrane co-occurrence, rest: 0.48 ± 0.11 *versus* exercise: 0.43 ± 0.11, p=0.04), indicating GLUT4 translocation to specific regions in the membrane. To assess whether GLUT4 translocation followed a physiological directed pattern rather than a random distribution, we examined its translocation towards myonuclei. Following exercise GLUT4 translocated away from the myonuclei (Pearson's nuclei-GLUT4 colocalization coefficient, rest: 0.21 ± 0.06 *versus* exercise: 0.19 ± 0.05, p=0.03). The amount of GLUT4 located near capillaries increased with exercise (GLUT4 near capillaries, rest: 18.08 ± 9.97 *versus* exercise: 24.66 ± 10.07 μm^2, p=0.02), indicating GLUT4 translocated not only to the membrane but specifically towards capillaries. In addition GLUT4 cooccurrence with the mitochondria increased after exercise (Manders's 2 GLUT4-mitochondria cooccurrence, rest: 0.25 ± 0.18 *versus* exercise: 0.30 ± 0.21, p=0.03). Finally, our preliminary analysis showed no significant differences in GLUT4-T-tubules colocalization pre- and post-exercise.

Conclusion: Exercise resulted in GLUT4 cluster formation in the membrane, an effect that is related to the formation of GLUT4 clusters at sites associated with glucose delivery and utilization. GLUT4 translocates away from the myonuclei and towards the membrane,

capillaries and mitochondria, suggesting an coordinated mechanism to improve glucose uptake and metabolic efficiency in skeletal muscle.

#10 Monday Poster Session

A combined experimental and computational analysis of mantATP turnover in skinned muscle fibers

Mauro Montesel1,2*, Cosimo De Napoli1,2*, Luisa Schmidt3, Elena Germinario1, Ulises H. Guzman3, Jesper V. Olsen3, Lorenzo Marcucci1, **Leonardo Nogara**1,2,4

Affiliations:

1. Department of Biomedical Sciences, University of Padua, Padua, Italy
2. Veneto Institute of Molecular Medicine (VIMM), Padua, Italy
3. Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark
4. Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy

Myosin is the primary motor protein in skeletal muscle, responsible for ATP hydrolysis that drives muscle contraction. In addition to force production, resting myosin consumes ATP in futile cycles at two rates, the slower one being associated with the Super Relaxed State (SRX), in contrast to the less inhibited Disordered Relaxed State (DRX). The SRX is typically measured using the mantATP chasing technique, where the decay of a fluorescent ATP analogue is fitted using multi-exponential functions. Recently, significant concerns have been raised regarding the use of this technique, particularly when applied to soluble myosin preparations. While skinned fibers offer the advantage of preserving the native thick filament structure and myosin cooperativity, limited diffusion and non-specific mantATP binding pose challenges. In this study, we combine experimental data and in-silico modeling to dissect the contributions of different components in the mantATP chasing signal. We analyze control skinned fibers and fibers subjected to myosin extraction. Our analysis shows that the non-specific component partially overlaps with the DRX timescale. In contrast, the slow component linked to myosin SRX nucleotide release is characterized by a time constant that significantly differs from those of the non-specific signal and DRX, enabling its reliable estimation using this technique. Our findings indicate that evaluating non-specific mantATP components is necessary to obtain a reliable estimation of both SRX and DRX. We validated our analysis by comparing populations and time constants obtained from chasing with mantATP to mantATPase rates in control conditions and upon piperine-induced SRX destabilization.

#11 Monday Poster Session

BIOMECHANICAL CONSEQUENCES OF GIGANTOMASTIA: SHOULDER DYSKINESIA, NEUROVASCULAR COMPRESSION, AND FUNCTIONAL RECOVERY WITH TARGETED INTERVENTIONS

Lev Kalika[1], Rostyslav Bubnov[2]
Affiliations:
1 New York Dynamic Neuromuscular Rehabilitation & Physical Therapy https://nydnrehab.com/, New York, USA
2 Clinical hospital 'Pheophania' of State Affairs Department, Ukraine

A 37-year-old female presented with right-sided scapular and neck pain, radiating to the fifth digit, accompanied by paresthesia, heaviness, and restricted right shoulder flexion (limited to ~100°). Symptoms began spontaneously in November 2024 and intensified following an allergic reaction to ibuprofen. Notably, passive elevation to 180° became possible when the right breast was manually lifted. MRI showed no pathology, but thoracic outlet syndrome (TOS) tests were positive. High-resolution ultrasound revealed myoaponeurotic tears and fascia bands in the infraspinatus.
Kinematic analysis using Showmotion (Figure 1) showed significantly reduced scapular upward and posterior rotation on the right side, altered scapulohumeral rhythm, and diminished elevation capacity (HumMax 103.62°; NormZoneA 70.19 vs. 100 on the left). These findings suggest impaired early-phase motion and mechanical dysfunction.
Gigantomastia was identified as the primary biomechanical driver of dysfunction via: (1) scapular loading with abnormal anterior tilt and limited upward mobility, (2) neurovascular compression aggravating TOS symptoms, and (3) pain-induced neuromuscular inhibition. Symptom resolution following breast lifting and significant improvement after scapular prolotherapy and nerve-targeted injections support the mechanical and neurogenic etiology.
Conclusion: Gigantomastia causes significant shoulder dysfunction through combined kinematic and neurovascular mechanisms. Breast reduction should be considered alongside ongoing scapular stabilization and neuromuscular therapy.

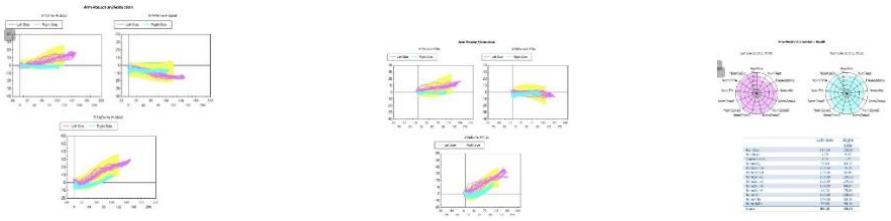

Figure 1: Kinematic and biomechanical assessment using Showmotion reveals right-sided scapular dyskinesia, reduced upward/posterior rotation, and impaired glenohumeral elevation (HumMax 103.62°) associated with gigantomastia. Functional improvement was observed following targeted interventions including prolotherapy and brachial plexus injections. Graphs illustrate motion asymmetry and reduced NormZoneA on the affected side.

#12 Monday Poster Session

DELETION OF A MUSCLE-SPECIFIC PROMOTER IN THE 3' REGION OF THE *ANK1* GENE AFFECTS GLUCOSE HOMEOSTASIS AND THE EXPRESSION OF GENES INVOLVED IN VARIOUS METABOLIC PATHWAYS.

Lucrezia Puccini[1], Federica Fiore[1], Valentina Guardascione[1], Chiara Malerba[1], Annunziatina Laurino[1], Stefano Cagnin[3], Gabriele Sales[3], Daniela Rossi[1,2], Enrico Pierantozzi[1], Vincenzo Sorrentino[1,2].

Affiliations:
1. University of Siena, Department of Molecular and Developmental Medicine, 53100 Siena (Italy)
2. Azienda Ospedaliera Universitaria Senese, 53100 Siena (Italy)

3. Department of Biology, University of Padova (Italy)

The *ANK1* gene encodes Ankyrin1 (ANK1), a structural protein that links membrane proteins to the spectrin-based cytoskeleton in various cell types. In both humans and mice, in addition to the principal promoter (P1), *ANK1* also contains a skeletal muscle-specific promoter (P2), which drives the expression of a small ANK1 isoform, namely sAnk1.5, as well as miR-486. Interestingly, the P2 promoter is located within a genomic region that serves as a muscle-specific stretch/super enhancer, capable of regulating the expression of distant genes.

Previous studies conducted in our laboratory showed that mice carrying a deletion of the P2 region [*Ank1*(P2) KO mice] exhibit increased glycemia and an altered response following an intraperitoneal glucose tolerance test. To investigate the molecular mechanisms underlying the deregulation of glucose homeostasis in this model, we performed RNA sequencing and real-time PCR on extensor digitorum longus (EDL) and soleus muscles from *Ank1*(P2) KO mice to identify differentially expressed genes in *Ank1*(P2) KO mice compared to wild-type littermates.

In an initial analysis, we identified 19 genes that encode for proteins involved in glucose, glycogen, or lipid metabolism. However, their expression is only modestly altered, and the data do not converge on identifying a single specific metabolic pathway. Further analysis revealed that *Nek5*, a gene potentially involved in mitochondrial integrity, is strongly downregulated in skeletal muscles of *Ank1*(P2) KO mice. Interestingly, *Nek5* is located on chromosome 8, approximately 1 Mb from the Ank1 P2 promoter, suggesting a potential long-range effect of P2 deletion. We are extending the analysis of *Nek5* expression in various non-muscle tissues of *Ank1*(P2) KO mice to determine whether the observed downregulation of *Nek5* expression is restricted to skeletal muscle, considering that the P2 deletion affects a muscle-specific enhancer.

We are continuing our work to assess the extent of differential gene expression in *Ank1*(P2) KO mice, aiming to gain a more comprehensive understanding of the involvement of the region around the P2 promoter in regulating gene expression in skeletal muscle and how this is connected to metabolic regulation.

#13 Monday Poster Session

MUSCLE-IN-A-DISH: A NOVEL MODEL TO STUDY THE MICROENVIRONMENTAL CONTRIBUTION TO MUSCLE GLUCOSE METABOLISM AND CONTRACTION

Marta Hanczar,[1] Josine de Winter,[1] Peter Hordijk,[1] Etto C. Eringa[1,2]

Affiliations:
1. Amsterdam UMC, Amsterdam, The Netherlands
2. Maastricht University, Maastricht, The Netherlands

Type 2 diabetes is a disease of global importance due to its rising prevalence, serious comorbidities and high healthcare costs. The first stage of the disease is characterized by impairment of insulin-stimulated glucose uptake into muscle cells, otherwise known as muscle insulin resistance. The causes of this condition are not fully understood and increasingly research suggests that the disturbed muscle microenvironment, including vasculature and adipose tissue, can be an important player.

Previous studies from our group have shown that factors secreted from perivascular adipose tissue (PVAT) have been shown to regulate muscle perfusion, glucose uptake and mitochondrial gene expression in skeletal muscle. Type 2 diabetes also associates with microvascular dysfunction and impaired muscle function, both contributing to majority of patient comorbidity and complaints. Therefore, we hypothesize that endothelial cells and PVAT present in the muscle microenvironment can impact muscle function.

To study this interaction, we are developing an endothelial cell-skeletal muscle co-culture model. This allows for investigating the contribution of vasculature and PVAT on muscle insulin resistance, contractility and mitochondrial function. The preliminary results suggest that endothelial cells can affect muscle cells activation and relaxation time, suggesting the muscle microenvironment aids in its contractile function.

Overall, this project will allow for understanding the pathology of muscle insulin resistance and explore possibilities for therapeutic intervention to prevent or reverse type 2 diabetes and increase patients quality of life.

#14 Monday Poster Session

ENLIGHTEN THE DECAY: MANTATP CHASING ASSAY OPTIMIZATION AND NEW INSIGHT

Mauro Montesel[1,2], Cosimo De Napoli[1,2], Luisa Schmidt[3], Elena Germinario[1], Ulises H. Guzman[3], Jesper V. Olsen[3], Lorenzo Marcucci[1,2], and Leonardo Nogara[1,2]

Affiliations:
1. University of Padua, Padua 35131, Italy
2. Veneto Institute of Molecular Medicine, Padua 35129, Italy
3. University of Copenhagen, Copenhagen DK-2200, DenmarkUniversity

Myosin is among the most abundant proteins in skeletal muscle and is responsible for the basal metabolism of the tissue. Through a futile cycle of ATP consumption, myosin in resting muscle still accounts for a significant percentage of the whole-body resting metabolism. In resting muscle, myosin contributes significantly to whole-body resting energy expenditure through a futile ATP hydrolysis cycle. Two distinct biochemical ATP turnover rates characterize myosin in the resting conditions: the energy-conserving Super Relaxed state (SRX) and the relatively more energy-consuming Disordered Relaxed state (DRX).
Given the growing interest in understanding myosin's resting energy consumption, accurate quantification and interpretation of SRX and DRX populations are critical for advancing both basic research and translational applications such as drug screening and clinical phenotyping. The mantATP chasing assay remain the gold standard for detecting SRX but still presents some critical issues related to the model used. The classical skinned fiber preparation preserves native sarcomere architecture and fiber complexity, closely mimicking physiological conditions, and allows for convenient long-term sample storage. Nonetheless, this model is affected by restricted mantATP diffusion and non-specific binding to fiber components, which can bias the results.
By combining studies in fibers without myosin (ghosted fibers) and in silico modeling, we demonstrate how these critical points lead to an overestimation of DRX and, conversely, an underestimation of SRX. Taking these factors into account, we developed a more accurate method to analyze mantATP chasing data. Applying this improved analysis, we achieved, for the first time, quantitative agreement between ATP turnover rates measured by the NADH coupled enzyme reaction ATPase assay and those derived from mantATP chasing.

#15 Monday Poster Session

NICOTINAMIDE RIBOSIDE SUPPLEMENTATION PREVENTS THE ONSET OF MITOCHONDRIAL DYSFUNCTION IN A MOUSE MODEL FOR NEMALINE MYOPATHY TYPE 6

Rianne Baelde[1], Alexcia Fortes Monteiro[1], Edgar Nollet[1], Ricardo Galli[1], Sultan Bastu[3], Edoardo Malfatti[3], Bauke Schomakers [4], Kasper Vinten [4], Riekelt Houtkooper [4], Jolanda van der Velden[1], Coen Ottenheijm[1,2] and Josine de Winter[1]

1 Dept. of Physiology, Amsterdam UMC, location VUmc, The Netherlands
2 Dept. of Cellular and Molecular Medicine, University of Arizona, Tucson, USA
3 AP-HP, Neuromuscular Pathology Reference Centre, Henri Mondor Hospital, Créteil, France

4 Dept. of Genetic Metabolic Diseases, Amsterdam UMC, location AMC, The Netherlands

Nemaline Myopathy type 6 (NEM6) is caused by variants in Kelch-repeat-and-BTB-(POZ)-Domain-Containing-13 (*KBTBD13*). The majority of the NEM6 patients harbors the Dutch founder mutation *KBTBD13*^{R408C} (c.1222C>T, p.Arg408Cys), resulting in a hypercontractile phenotype caused by sarcomere-based impaired muscle relaxation. Histological characterization of NEM6 patient biopsies by NADH staining show the presence of cores, indicating the absence of complex I (NADH) activity and mitochondrial dysfunction.

In this study, we aimed to perform a natural history study in homozygous *Kbtbd13*^{R408C}-knockin mice (NEM6 mouse model) to investigate whether hypercontractility contributes to mitochondrial dysfunction in NEM6. First, enzymatic NADH staining showed absence of cores at 1 month, onset at 3 and progression of cores at 9 months. Therefore we can conclude that the NEM6 mouse model phenocopies core formation found in NEM6 patients. Second, mitochondrial respiration was investigated by *in vitro* high-resolution respirometry. In line with the presence of NADH cores at 3 and 9 months old, soleus muscle of NEM6 mice showed significant decreased total OXPHOS and NADH-linked respiration. To study the functional consequences *in vivo*, metabolic treadmill experiments were performed. These experiments showed significant impaired running performance, decreased VO_2max and increased respiratory exchange ratio (RER) in NEM6 mice. Next, we studied the pathways underlying mitochondrial dysfunction in NEM6 muscle. Metabolomics and proteomics were performed on soleus muscle, and joint pathway analysis revealed that TCA cycle related pathways are most affected in NEM6 mice. Of special interest, NAD^+ levels in 3 and 9 months old NEM6 mice were significantly decreased. We attempted to prevent the onset of the mitochondrial phenotype with supplementation of nicotinamide ribose (NR), a NAD^+ precursor that is generally considered safe and is currently tested in clinical trials. One month old WT and NEM6 mice received nicotinamide riboside (NR) supplementation for 8 weeks. Our data showed that chronic NR supplementation prevented the onset of both total OXPHOS and NADH-linked respiration in NEM6 mice. To conclude, this study provided insights into the natural history of mitochondrial dysfunction in NEM6 and provides proof-of-concept for the ability of NR to revert the mitochondrial phenotype in NEM6.

#16 Monday Poster Session

Loss of CCDC78 impairs muscle mass and force in a knockout mouse model

Daniela Rossi[1,2], Carlotta Pranzo[1], Federica Fiore[1], Annunziatina Laurino[1], Matteo Serano[1], Sara Roccabianca[1], Andreas Merdes[3], Feliciano Protasi[4], Vincenzo Sorrentino[1,2].

Affiliations:
1. University of Siena, Department of Molecular and Developmental Medicine, Via Aldo Moro, 2 53100 Siena (Italy)
2. Azienda Ospedaliera Universitaria Senese, Viale Bracci, 1 53100 Siena (Italy)
3. Universite Paul Sabatier, Centre de Biologie Integrative, 118 route de Narbonne 31062 Toulouse (France)
4. CeSI-Met, Center for Research on Ageing and Translational Medicine & University of Chieti G. d'Annunzio, Dept. of Neuroscience, Imaging and Clinical Sciences, 66100 Chieti (Italy)

Congenital myopathies (CM) are a heterogeneous group of diseases that most commonly present in childhood with a total prevalence estimated around 1:25000. More than 30 genes have been linked to CM; among them, the "coiled-coil domain containing 78" (CCDC78) gene was found to be mutated in a family with autosomal dominant centronuclear myopathy. CCDC78 is localized to the perinuclear compartment, but its function in skeletal muscle is currently unknown. Evidence from Xenopus multiciliated cells suggests a role for CCDC78 in centriole biogenesis, indicating that it may be associated with the centrosome in mammals and potentially involved in microtubule assembly and nuclear positioning. Immunostaining of differentiated C2C12 myoblasts with anti-CCDC78 antibodies showed a perinuclear localization, mirroring that of known centrosome-associated proteins such as pericentriolar material-1 (PCM1), pericentrin, and nesprin-1, as well as Golgi elements like GM130, which also act as microtubule organizing centers (MTOCs) in muscle cells. Treatment of C2C12 cells with Brefeldin A led to disassembly of the Golgi complex and redistribution of both CCDC78 and GM130 to the endoplasmic reticulum, while PCM1 remained associated with the nuclear envelope. To investigate potential interactions between CCDC78 and MTOC-associated proteins, co-immunoprecipitation experiments were conducted using anti-PCM1 and anti-CCDC78 antibodies. These experiments demonstrated that PCM1 co-immunoprecipitates CCDC78 and nesprin-1 in differentiating C2C12 cells. Likewise, CCDC78 was found to interact with nesprin-1, PCM1, and the anchoring proteins AKAP6 and AKAP9—both critical for microtubule nucleation from the nuclear envelope and Golgi apparatus in differentiated muscle cells.

To further evaluate the role of CCDC78 in skeletal muscle we generated a knockout mouse model by using the CRISPR/CAS9 technique. CCDC78 knockout mice are viable and fertile. However, male mice show a significant reduction in body weight; in addition, grip test and hanging wire test performed on two-months old male mice revealed that they display a significant deficit in muscle strength.

In conclusion, results from preliminary localization and co-immunoprecipitation experiments support the idea that CCDC78 is a member of the MTOC in skeletal muscle cells and that lack of its expression significantly affects muscle mass and performance.

#17 Monday Poster Session

Effect of simulated microgravity and proton irradiation on PIEZO1 mechanosensitive ion channel and cytoskeletal Septin 7 in C2C12 myogenic cell line

Eliza Guti[1,2], **Áron Gere**[1,3], Nóra Beatrix Máthé[1], László Szabó[1,2], Brigitta Tillmann[1,3], Máté Szarka[4], Beatrix Dienes[1,2], László Csernoch[1,2], Mónika Gönczi[1,2]

1. Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

2. HUN-REN Cell Physiology Research Group, University of Debrecen, Debrecen, Hungary

3. Doctoral School of Molecular Medicine, University of Debrecen, Debrecen, Hungary

4. HUN-REN Institute for Nuclear Research, Debrecen, Hungary

Mechanical forces that cause changes in the molecular dynamics of cells include stresses and strains on the cell membrane, stretching of the extracellular matrix, or fluid flow across the cell surface. The skeletal muscle, the most mechanosensitive tissue, is highly responsive to mechanical stimuli, including gravitational forces. The skeletal muscle system has evolved to maintain body posture against a constant gravitational load. Both mechanical stimulation and diffusible trophic factors stimulate a pool of myogenic satellite cells, which lie beneath the basal lamina and sarcolemma of the muscle, leading to their proliferation, migration to the sites of injury, and fusion with existing muscle fibers. As a result, the biosynthetic capacity is increased during mechanostimulation. The processes of migration, proliferation, and myotube formation are tightly regulated and require significant cytoskeletal rearrangements. Our previous research has shown that the absence of Septin7 in C2C12 myogenic cells disrupts their ability to divide and nearly halts their fusion into myotubes, highlighting the essential role of cytoskeletal Septin7 in muscle regeneration. The mechanosensitive ion channels, such as Piezo1, which respond to mechanical pressure or tension on the cell membrane, may be crucial for the integrity and function of skeletal muscle. Disruptions to physical strain, such as in bedridden patients, elderly individuals with limited mobility, or astronauts exposed to weightlessness, can significantly impact muscle strength, conditioning, and ultimately, the regenerative capacity of muscle tissue. As altered physical conditions in space have major negative impacts on the human body, we aimed to examine the effects of microgravity and ionizing proton radiation on myogenesis, in addition, the role of Piezo1 and Septin7 proteins in these processes was also determined. The microgravity was simulated using an RPM (Random Positioning Machine) placed in a CO_2 incubator. The viability and cell cycle of the C2C12 cell line were measured using flow cytometry. Changes in the expression of Piezo1, Septin7, and specific markers characterizing myogenesis, were monitored using RT-qPCR, Western blot, and immunocytochemistry. Our results show that simulated microgravity did not affect cell viability, but altered the cell cycle. The cell proliferation was decreased under *in vitro* zero gravity. Based on confocal microscopy analysis which revealed alterations in the arrangement of myotubes, and change in the number of nuclei within the fused myotubes, we imply that microgravity negatively impacts myogenesis. Additionally, we observed a decrease

in Piezo1 mRNA expression as myogenesis progressed, although contrary result was found in Western blot analysis. Septin7 protein appears to play a key role during the early stages of differentiation, as its mRNA expression decreased at later stages of myogenesis. In a subsequent series of experiments designed to simulate cosmic radiation, myogenic C2C12 cells were exposed to proton irradiation at low, medium, and high doses. Following irradiation, a reduced differentiation capacity in irradiated cultures was observed. We also examined the intracellular localization of Piezo1 during differentiation. Furthermore, the role of Piezo1 may be more complex than a simple ion channel. Our experimental results suggest that muscle loss during spaceflight is comparable to muscle atrophy during aging, and thus, its understanding may have a significant role in the health maintenance of an aging population.

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#18 Monday Poster Session

FROM MECHANOSENSATION TO MYOFIBRIL REPAIR: THE REGULATION OF FILAMIN C BY PHOSPHORYLATION

Dieter O. Fürst (1), Lorena Heil (1), Johannes P. Zimmermann (2), Peter F. M. van der Ven (1), Bettina Warscheid (2)

Affiliations:
1. University of Bonn, Bonn, Germany
2. University of Würzburg, Würzburg, Germany

During physical activity cross-striated muscle cells are continuously exposed to mechanical forces high enough to result in the unfolding of proteins. Since such events may ultimately affect the integrity of the contractile apparatus, muscle cells must be equipped with a broad range of physiological mechanisms that range from local stress-sensing to large-scale repair processes. In the myofibrillar Z-disc, the large actin-binding multi adapter protein filamin C (FLNc) seems to be part and parcel of these events: it is a mechanosensitive protein, it is highly dynamic and sensitive to signalling events, as signified by a number of phosphorylations induced by electrical pulse-stimulated physical activity of cultured muscle cells, and it interacts with a plethora of additional ligands, including components of a chaperone-assisted selective autophagy machinery. Focussing on a number of regulated phosphorylation sites in two of the 24 immunoglobulin-like domains of FLNc, we demonstrate direct effects on dimer formation and the binding to several proteins, including small heat shock proteins, SYNPO2 and myotilin. At the cellular level, these changes in protein interactions result in grave alterations in protein dynamics and the capability to repair activity-induced myofibrillar damage. Our findings therefore not only provide novel mechanistic insight into the detrimental consequences of mutations affecting the FLNc-encoding gene, resulting in a variety of myopathies and cardiomyopathies, but they also reveal general mechanisms of mechanosignalling and myofibrillar damage repair.

#19 Monday Poster Session

HSPB7 REGULATES FILAMIN C DIMERISATION

Katja Gehmlich[1,2], Zihao Wang[2], Guodong Cao[2], Miranda P Collier[2], Charlotte[2] Hooper, Amar Azad[1,2], Sophie Broadway-Stringer[1], Kristina Djinovic-Carugo[3], Dieter O Fürst[4], Bettina Warscheid[5], Georg K A Hochberg[6], Justin L P Benesch[2]

Affiliations:
1. University of Birmingham, Birmingham, UK.
2. University of Oxford, Oxford, UK.
3. European Molecular Biology Laboratory, Grenoble, France.
4. University of Bonn, Bonn, Germany.
5. University of Würzburg, Würzburg, Germany.
6. Philipps-University Marburg, Marburg, Germany.

Filamin C is a crucial mechano-signalling and actin crosslinking protein in muscle cells. Its actin cross-linking ability is mediated by two key domains, the amino-terminal actin binding domain and the carboxy-terminal immunoglobulin-like dimerization domain. HSPB7 is a small heat shock protein known to bind filamin C. It has unique properties, e.g. it shows predominantly cardiac expression and it does not form oligomers as other family members.
Aim of this study was to explore the relevance of this interaction in cardiac muscle, using a multi-scale approach.
Our mouse models of biomechanical stress demonstrated co-upregulation of filamin C and HSPB7 at sites of force transduction and increased co-localisation, highlighting the relevance of the interaction for stress signaling.
The structure of the filamin C HSPB7 heterodimer was assessed by X-ray crystallography. It identified that HSBP7 uses the same interface for binding to filamin C as filamin C itself for its dimerization. Biochemical experiments demonstrated that HSPB7 out-competes filamin C at the binding interface and may therefore disrupt the filamin C homo-dimers and hence filamin C's actin cross linking ability.
Two phosphorylation sites in the dimersation domain of filamin C, at T2677 and at Y2683, were shown to have opposing effects on shifting the homo-dimer to hetero-dimer (filamin C–HSPB7) equilibrium in biochemical assays.
Of note, evolutionary, HSPB7 appeared to have co-evolved with filamin C at the time primitive hearts evolved in chordates.
In conclusion, HSPB7's primary role in the heart might be to modulate filamin C dynamics via controlling filamin C dimerisation. These findings might give insight into a common pathway why genetic variants in both genes have been associated as risk factors for cardiomyopathy and heart failure.

#20 Monday Poster Session

TITIN CLEAVAGE IN LIVING CARDIOMYOCYTES INDUCES SARCOMERE DISASSEMBLY BUT DOES NOT TRIGGER CELL PROLIFERATION

Maria Rosaria Pricolo[1], Miguel A. López-Unzu[1], Natalia Vicente[1], Cristina Morales-López[1], Carla Huerta-López[1], Wendy Pérez-Franco[1], Andra C. Dumitru[1,2], Francisco M. Espinosa[3], Mateo I. Sanchez[4], Ricardo Garcia[3], Roberto Silva-Rojas[1], Elías Herrero-Galán[1], Jorge Alegre-Cebollada[1]

Affiliations:

1. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Calle de Melchor Fernández Almagro 3, 28029, Madrid, Spain.

2. Louvain Institute of Biomolecular Science and Technology, Universite catholique de Louvain, Louvain-la-Neuve, Belgium.

3. Instituto de Ciencia de Materiales de Madrid (ICMM), Madrid, Spain.

4. University of Cambridge, Department of Chemistry, Lensfield Road, Lensfield Road CB2 1EW, Cambridge, UK.

Adult mammalian hearts have limited regenerative capacity due to the inability of cardiomyocytes to proliferate. The presence of highly organized, contractile sarcomeres in cardiomyocytes has long been considered a barrier to cell division. Indeed, sarcomere disassembly is a crucial step to complete the cell cycle in the few situations where cardiomyocytes have been observed to proliferate. However, whether sarcomere disassembly can per se favor cell cycle re-entry remains unknown. To examine this possibility, here we have engineered a system to induce sarcomere disassembly in living murine cardiomyocytes through the specific cleavage of the structural protein titin by the exogenous tobacco etch virus protease (TEVp). Our results indicate that isolated neonatal cardiomyocytes with disassembled sarcomeres remain viable and retain contractile function, albeit with reduced amplitude. Notably, we find no evidence of cell proliferation in targeted cardiomyocytes, as indicated by analysis of markers of DNA synthesis and cytokinesis. We obtain equivalent results when titin is cleaved in the adult myocardium in vivo. Hence, while the removal of sarcomere structural barriers is necessary for proliferation, additional factors are probably required to trigger cell cycle re-entry of terminally differentiated cardiomyocytes.

#21 Monday Poster Session

ACTIN ASSEMBLY-MODULATING EFFECT OF TITIN'S DISORDERED PEVK DOMAIN

Áron Gellért Altorjay[1], Hedvig Tordai[1], Ádám Zolcsák[1], Nikoletta Kósa[1], Tamás Hegedűs[1,2] and **Miklós Kellermayer[1,2]**

Affiliations:
[1]Department of Biophysics and Radiaton Biology, Semmelweis University, Budapest, Hungary
[2]HUN-REN-SU Biophysical Virology Research Group, Semmelweis University, Budapest, Hungary

Titin is a giant multi-domain filamentous muscle protein that serves as an elastic template and mechanosensor of the sarcomere. Its PEVK domain varies in size in different muscle types due to splicing, contains PPAK and poly-E motifs and is intrinsically disordered due to the propensity of highly charged residues. PEVK has been shown to bind actin in spite of the fact that the domain does not contain canonical actin-binding motifs. In the present work we investigated whether PEVK may also influence the polymerization of actin. The middle, 729-residue-long segment (called PEVKII) of the full-length PEVK domain was cloned, expressed in *E.coli* and purified by using the His- and Avi-tags engineered to the N- and C-termini, respectively. Polymerization of actin was monitored by the pyrene assay in the presence of different concentrations of PEVKII. The structure of PEVKII-bound F-actin was investigated by atomic force microscopy. PEVKII increased the initial and log-phase rates of actin polymerization and the peak F-actin amount in a concentration dependent manner and without significantly altering the critical concentration of actin polymerization. Thus, PEVK enhances the polymerization of actin by facilitating seed-formation. This feature was manifested in the AFM images of F-actin-PEVKII complexes adsorbed to a supported lipid bilayer, in which radially symmetric complexes of short actin filaments dominated the sample. The actin-polymerization modulating effect of PEVK may in principle play a role in regulating actin turnover in the sarcomere. In sum, the PEVK domain of titin is a non-canonical actin-binding protein that modulates sarcomeric shortening and actin polymerization.

#22 Monday Poster Session

GENOMIC AND PROTEOMIC ANALYSIS OF SEPTIN7 KNOCKDOWN MYOGENIC CELL LINE AND MAMMALIAN SKELETAL MUSCLE SAMPLES

Brigitta Tillmann[1,3], László Szabó[2], Eliza Guti[2], Nóra Beatrix Máthé[1], László Csernoch[1,2], Beatrix Dienes[1,2], **Mónika Gönczi[1,2]**

Affiliations:
1. Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
2. HUN-REN Cell Physiology Research Group, University of Debrecen, Debrecen, Hungary
3. Doctoral School of Molecular Medicine, University of Debrecen, Debrecen, Hungary

Skeletal muscle is not only the most massive organ in the human body, but it also has a considerable regeneration capacity. Regulatory factors and signaling pathways of muscle regeneration are similar to those of myogenesis. We have previously shown that cytoskeletal septins play an important role in the regulation of myoblast proliferation, and their differentiation into myotubes. Muscle regeneration was also severely modified in mice where Septin7 expression was downregulated.

Our next aim was to study the gene expression alterations in Septin7-modified myogenic cell cultures and mammalian skeletal muscle. Using RNA sequencing of control and Septin7 knockdown samples we found significant changes at the expression level of several structural proteins (actin, tubulin, myosin, troponin, tropomyosin, triadin), regulatory factors of myogenesis and cell cycle (myoglobin, myoblast fusion protein, rgcc), and also ion channel- and receptor proteins (voltage-dependent calcium channel, purinergic receptors). Furthermore, by mass spectrometry analysis of co-immunoprecipitated samples, we identified partner proteins of Septin7. Among the Septin7-interaction partners, the expression and intracellular localization were followed by RT-qPCR, western blot, and immunolabeling, respectively. We evaluated the presence of further septins (Septin2, Septin5, Septin9) and other cytoskeletal proteins (actin, tubulin). The localization of the ryanodine receptor, Orai1 and STIM1 (SOCE proteins) relative to Septin7 was also determined on individual skeletal muscle fibers.

These sequencing and proteomic analyses of Septin7-modified myogenic cells and skeletal muscles imply that several signaling pathways and regulatory molecules, which are involved in the differentiation of myoblasts, muscle force generation, and regeneration, are affected by the reduced expression of cytoskeletal Septin7.

This research was funded by grants ELKH-DE 11017 and NKFIH NK-137600.

#23 Monday Poster Session

GLYCEROL STORAGE INCREASES PASSIVE STIFFNESS OF MUSCLE FIBERS THROUGH EFFECTS ON TITIN EXTENSIBILITY

Seong-won Han[1], Justin Kolb[1], Gerrie P. Farman[1], Jochen Gohlke[1], & Henk L. Granzier[1]
Affiliations:
1. Department of Cellular and Molecular Medicine, University of Arizona, Tucson, USA

It is crucial to store muscle in ways that preserve its properties in order to investigate pathological and physiological characteristics of muscle at all structural hierarchies. Glycerol has been commonly used for muscle storage, since it is known to minimize degradation of muscle by slowing down enzymatic activities. However, the effect of glycerol on passive properties of muscle has not been examined, and thus, the purpose of this study was to quantify changes in passive stiffness of muscle induced by glycerol storage and investigate potential mechanism.

Mouse extensor digitorum longus (EDL) muscles were stored either in 50% glycerol/relaxing mixed or relaxing solution for various durations, and then rehydrated in relaxing solution to assess mechanical properties. Glycerol storage increased passive stiffness significantly, compared to the muscle fibers stored in the relaxing solution (Fig A). The increase was most pronounced at >2.8 µm sarcomere lengths, where titin's PEVK segment extension dominates.

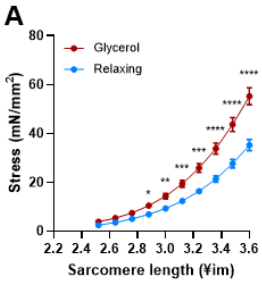

Figure A. *Effect of glycerol storage on peak passive stress-sarcomere length relationship for EDL fibers stored either in 50% glycerol (red curve) or relaxing (blue curve) solution for durations from 1-14 days. The same results were found for elastic and viscous passive stress-sarcomere length relationships. Two-way ANOVA with post-hoc analysis.*

In the second set of experiment, using gelsolin, we determined whether the observed increase in passive stress of the glycerinated fibers requires the thin filament, which is known to interact with titin's PEVK region. Both glycerol-stored fibers with and without thin filament extraction by gelsolin exhibited increased passive stress, suggesting that the underlying mechanism is intrinsic to titin.

Finally, fibers were treated with methylglyoxal (MG), a reactive carbonyl and glycating agent that forms cross-links on lysine residues, which are abundant in the PEVK segment. Mechanical properties of muscle fibers were evaluated before and after the treatment. MG treatment elevated passive stiffness of muscle fibers that were stored in relaxing solution (Fig B), but it did not affect the glycerinated fibers (Fig C). These results suggest that the mechanisms underlying the modification induced by methylglyoxal treatment and glycerol storage are similar. Specifically, the targeted region in titin for both methylglyoxal and glycerol is likely to be the PEVK region.

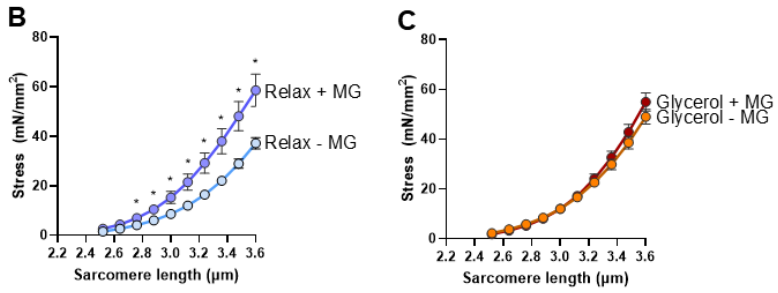

Figure B-C. *Effect of methylglyoxal (MG) on passive stress of fibers stored in relaxing solution (A) or in glycerol (B). All values are shown as mean values of 10 fibers ± SE.*

In conclusion, our study demonstrates that glycerol storage alters the passive mechanical properties of muscle fibers in a titin-specific manners. Therefore, glycerol storage should be avoided especially when outcomes are passive properties of muscle. We further suggest that, for long-term storage of muscle, storing muscle at -80°C following a rapid-freezing in the liquid nitrogen is a viable option.

#24 Monday Poster Session

INVESTIGATING THE EFFECTS OF MAVACAMTEN ON DIAPHRAGM MUSCLE MYOSIN

Ayesha Sarfraz[1], Arnab Nayak[1], Theresia Kraft[1], Mamta Amrute-Nayak[1]

[1]Institute of Molecular and Cell Physiology, Hannover Medical School, 30625, Hannover, Germany

The diaphragm is a primary muscle for respiration, responsible for driving breathing through its contraction and relaxation. Mavacamten (Mava), a small molecule inhibitor of β-cardiac myosin ATPase, is currently in clinical trials for the treatment of Obstructive Hypertrophic Cardiomyopathy (oHCM). Interestingly, β-myosin heavy chain (β-MyHC) expression is not restricted to the ventricular myocardium but is also present in slow fibers that make up nearly 50 % of the total muscle fibers in the diaphragm. Therefore, we hypothesize that Mavacamten used to improve the cardiac function in HCM patients may affect diaphragm muscle activity. Here we investigated the effects of Mavacamten on the diaphragm muscle myosin. We utilized the Oryctolagus cuniculus (rabbit) model for the source of diaphragm muscle. An equal proportion (50:50) of slow and fast muscle fibers in rabbit diaphragm was complemented by the expression of three myosin heavy chains (MyHCs) and six myosin light chains (MLCs) in the rabbit diaphragm as determined by SDS-PAGE analysis. For functional experiments, we employed the *in vitro* motility assay. Using this assay, we evaluated the effect of Mava on diaphragm muscle myosin and observed that increasing drug concentrations resulted in a graded decrease in myosin driven actin filament velocity. Notably, the drug's effect was fully reversible, as mean velocity was restored upon drug washout. Given the diaphragm's mixed composition of slow and fast fibers and thereby myosin II isoforms, we questioned whether Mava specifically affect slow myosin II or both the myosin types, but to a different extent. To investigate this, we studied the drug's impact on pure fast and slow myosin isoforms isolated from rabbit M. psoas and M. soleus. Both myosin isoforms responded to increasing concentration of drugs but to a varying degree, with M. soleus myosin II being 20 fold more sensitive with IC_{50} of 51.68 nM than M. psoas myosin II. We further extended our investigation to the effect of Mava on the motile speeds of Ca^{2+} sensitive native thin filaments isolated from the diaphragm. We found that the native thin filaments were more sensitive to Mava as compared to the unregulated F-actin. Importantly, among all the tested myosin isoforms in M. soleus, M. psoas, diaphragm, and ventricle, Mava exerted the strongest effect on M. soleus myosin II activity. Our studies suggest dual mechanisms by which Mava affects the diaphragm myosin activity, i.e., by influencing the structural state as well as by modulating actomyosin ATPase cycle resulting in myosin inhibition. Altogether, mavacamten treatment may positively influence ventricular myosin function towards normalizing diastolic dysfunction in HCM patients, but its effects should be critically monitored in HCM patients for any respiratory anomaly. Furthermore, the suitability of Mavacamten as a treatment option in HCM patients with underlying respiratory complications needs careful consideration.

#25 Monday Poster Session

Asynchronies related to mechanical ventilation exacerbates Diaphragm Dysfunction in mice model of Duchenne Muscular Dystrophy.

Mohamad Yehya[1#], Haikel Dridi[2#], Eric Estève[1], Johan Moreau[1,3], Steven Reiken[2], Andrew R Marks[2], Alain Lacampagne[1], Stefan Matecki[1,3].

1: PhyMedExp, Montpellier University, INSERM, CNRS, CHRU Montpellier, 34295-Montpellier, France
2: Department of Physiology and Cellular Biophysics, Clyde and Helen Wu Center for Molecular Cardiology Columbia University College of Physicians and Surgeons New York USA
3: Physiological Department, Montpellier University and Montpellier University Health Care Center, 34295-Montpellier, France
#: Authors contributed equally to this work.

Mechanical ventilation (MV) with non-invasive positive pressure (NIVPP) is recommended for patients with Duchenne Muscular Dystrophy (DMD) experiencing daytime hypercapnia or symptoms of alveolar hypoventilation. Nevertheless, the use of mechanical ventilation which unload the diaphragm is associated with a detrimental condition known as ventilator-induced diaphragmatic dysfunction (VIDD) in non-dystrophic patients, which may accelerate the progression of respiratory muscle disease and increase dependence on ventilatory support in DMD patients.
Specifically, the asynchrony between diaphragm contraction in response to the patient's air demand and the air delivery by the ventilator can lead to a mechanical stress resembling eccentric contraction, which is particularly harmful in the context of dystrophic deficiency diaphragm.
In healthy mouse models, MV induces early remodeling of the Ca2+ release channel (RyR1) and disrupts intracellular Ca2+ homeostasis, resulting in VIDD. We hypothesized that VIDD is exacerbated in dystrophic patients due to increased fragility of the diaphragmatic membrane caused by the absence of dystrophin, amplifying mechanical strain and worsening intracellular Ca2+ dyshomeostasis and dysfunction.
To model asynchrony, we imposed eccentric stress on a mechanically ventilated mdx diaphragm. We observed that MV impairs RyR1 function, increases susceptibility to eccentric contraction, and causes injuries and weakness in the diaphragm. This effect can be mitigated by specific RyR1 stabilizers, such as S107, showing comparable efficacy to ebselen treatment, a specific inhibitor of NOX2. Our findings highlight the importance of minimizing asynchrony during NIVPP in DMD patients and pave the way for future preventive treatments targeting RyR1 functional deficits.

#26 Monday Poster Session

Molecular basis of length-dependent activation (LDA) in cardiac muscle

Alice Arcidiacono[1], Emma Smith[1], Luca Fusi[2], Malcolm Irving[1] and Elisabetta Brunello[1].

[1] Randall Centre for Cell and Molecular Biophysics, School of Basic and Medical Biosciences and British Heart Foundation Centre of Research Excellence, King's College London, London, United Kingdom.

[2] Centre for Human and Applied Physiological Sciences, School of Basic and Medical Biosciences, King's College London, London, United Kingdom.

Activation of the cardiac sarcomere is sensitive to changes in sarcomere length (SL), so that longer length results in increased myofilament calcium (Ca^{2+}) sensitivity and, consequently, force of contraction. This phenomenon is known as length-dependent activation (LDA) and is thought to constitute the cellular equivalent of the Frank-Starling law of the heart, a key auto-regulatory mechanism for modulation of cardiac contractility on a beat-to-beat basis. Nevertheless, the molecular mechanisms underlying LDA in the heart remain to be elucidated. The Frank-Starling law and cardiac LDA responses were found to be disrupted in several cardiac diseased states (i.e. heart failure, inherited cardiomyopathies). Understanding of the molecular mechanisms at the basis of cardiac LDA represents therefore an important premise for the development of new target drugs to treat those diseases. The focus of this study is to investigate the role of the thin and thick filament in cardiac LDA responses. Bifunctional rhodamine probes in the N- and C-terminal lobes of cTnC in the thin filament, and in the cardiac regulatory light chain (cRLC) of myosin in the thick filament were exchanged into demembranated rat cardiac trabeculae. Polarised fluorescence emitted from the probes was used to investigate the effects of SL and Ca^{2+} on the regulatory structural changes of cTnC and myosin motors in the thin and thick filament, respectively. To do this, demembranated trabeculae were activated by temperature jump at steady-state [Ca^{2+}], in conditions that preserve the OFF structure of the thick filament at diastolic [Ca^{2+}] (T=27°C, 3% Dextran T-500) (Ovejero *et al*.; *J Gen Physiol*, 2022), at both 2.1 μm and 2.3 μm SL in sarcomere isometric conditions. Increasing SL from 2.1 μm to 2.3 μm increased the Ca^{2+} sensitivity (pCa_{50}) of force by ~ 0.2 pCa units, but it did not affect either the amplitude or the pCa_{50} of the regulatory structural changes in the N- and C-lobes of cTnC. Instead, the Ca^{2+} sensitivity of the structural changes in the cRLC of myosin was increased by ~ 0.2 pCa units at longer SL, without changes in the relaxed (pCa 9.0) or active (pCa 4.7) RLC orientations. Furthermore, the contribution of myosin to the SL- and Ca^{2+}-dependent regulatory structural changes in cTnC was determined using 25 μM of the myosin motors inhibitor Mavacamten (Mava). Mava completely inhibited active force at both 2.1 μm and 2.3 μm SL in demembranated trabeculae, and this was associated with a total inhibition of the OFF-to-ON transition of myosin motors in the thick filament at all [Ca^{2+}], independently of SL. Moreover, Mava did not affect the amplitude of the Ca^{2+}-dependent structural changes of the N-lobe of cTnC at maximal [Ca^{2+}] (pCa 4.7) at 2.1 μm SL, but significantly reduced that of the C-lobe of cTnC by ~ 50% in the same conditions. Moreover, Mava significantly decreased the Ca^{2+} sensitivity of the regulatory structural

changes in the N- and C-lobes of cTnC by ~0.3 and ~0.5 pCa units, respectively. In the presence of Mava, there were no significant changes in either the amplitude or the Ca^{2+} sensitivity of the structural changes in the N- and C-terminal lobes of cTnC between 2.1 μm and 2.3 μm SL. In conclusion, our results show that in cardiac muscle the Ca^{2+} sensitivity of the thin filament is largely dependent on force generating myosin motors attached to actin, and is not altered by changes in SL. Instead, increasing SL induces an increase in the activation of the myosin motors on the thick filament, but only at systolic [Ca^{2+}]. These findings indicate that cardiac LDA is not a diastolic phenomenon, but is a calcium-dependent mechanism, likely involving interfilament signalling pathways.

#27 Monday Poster Session

DEFINING THE STRUCTURAL FUNCTION OF SLOW AND FAST MYOSIN-BINDING PROTEIN C ISOFORMS IN MOUSE EXTENSOR DIGITORUM LONGUS

Anthony L. Hessel[1,2], Nichlas M. Engels[3], Rachel Sadler[3], Christine E. Delligatti[2,4], Khoi Nguyen[2], Samantha P. Harris[3]

Affiliations:
1. University of Muenster, Muenster, Germany
2. Accelerated Muscle Biotechnologies, Boston, USA
3. University of Arizona, Tucson, USA

4. Loyola University, Chicago, USA

In skeletal muscle there are two isoforms of Myosin-Binding Protein C (MyBP-C), so-called slow and fast, although both can exist in any fiber type. Both isoforms attach to the sarcomeric thick filament backbone at its C-terminal domains, while the middle- and N-terminal domains are off the thick filament backbone and interact with various parts of the myosin motors and thin filament. The middle and N-terminal domains are critical to the regulation of force production, with each isoform having somewhat different functions – important because both isoforms are expressed within fibers, and expression levels vary between fiber type, muscle, and during myopathy. To study the importance of each isoform to normal sarcomere function, we used three transgenic mouse models that allow for the rapid and specific cleavage of the middle- and N-terminal domains of either the fast isoform, slow isoform, or both isoforms. We studied passive fiber bundles from mouse extensor digitorum longus (EDL) of all three mouse lines before and after cleavage of the desired MyBP-C isoform using small-angle X-ray diffraction to monitor changes to sarcomeric proteins. Our preliminary results show that cleavage of the fast isoform produces an obvious loss of myosin head order important to control of calcium sensitivity during contraction, as previously reported. Cleavage of the slow-twitch isoform presents a subtle or limited effect to myosin head order, which could be attributed to its relatively low expression in mouse EDL. However, cleavage of both isoforms produces a greater change in myosin head order than anticipated from simply adding the fast and slow cleavage effects, suggesting that some control of myosin head orientation is via a mechanism where slow and fast isoforms are both needed. Ongoing experiments are evaluating if normal myosin head order can be returned using the myosin deactivator mavacamten.

#28 Monday Poster Session

REGULAITORY STATES OF THE THICK AND THIN FILAMENT OF FAST TWITCH MOUSE EDL DURING ACTIVE STRETCH

Cameron Hill[1], Michaeljohn Kalakoutis[1], Alice Arcidiacono[1], Yanhong Wang[1], Atsuki Fukutani[1], Theyencheri Narayanan[2], Elisabetta Brunello[1], Luca Fusi[1,3], Malcolm Irving[1]

1. Randall Centre for Cell & Molecular Biophysics and British Heart Foundation Centre of Research Excellence, New Hunt's House, Guy's Campus, King's College London, London, SE1 1UL;
2. European Synchrotron Radiation Facility, 38043 Grenoble, France
3. Centre for Human & Applied Physiological Sciences, Shepherd's House, Guy's Campus, King's College London, London, SE1 1UL

Regulation of contraction in skeletal muscle is mediated by structural changes in the actin-containing thin filaments and myosin-containing thick filaments, and dynamics regulated by the interaction between the two filaments. Much of our understanding of this dual-filament regulatory paradigm is derived from studies of isometric and concentrically contracting skeletal muscle. However, eccentric muscle activation, where the muscle is generating active force during lengthening, is less understood despite its importance for normal locomotor function, such as stair descent. The aim of this study was to use time-resolved X-ray diffraction to measure structural change in the thick and thin filaments of fast-twitch mammalian skeletal muscle when the muscle is active and lengthening.

Whole, intact mouse EDL (n=7) were dissected and mounted at beamline I22 of the ESRF to collect time-resolved, ultra-small-angle and small-angle two-dimensional X-ray diffraction patterns at 28°C. Muscles were set to a starting sarcomere length of 2.56 μm and tetanically activated for 70 ms under fixed-end conditions, followed by a 65 ms ramp of 5% of optimal muscle length (0.73 FL.s^{-1}) provided at the force plateau, and stimulated for a further 38 ms at the longer muscle length. Changes in sarcomere length and X-ray reflections associated with the thick and thin filaments were collected in 160 x 2 ms frames on an Eiger 4M detector at 31m and either 3.2m or 2m.

Muscles shortened to 2.32 μm at the force plateau before the stretch (T_0) and increased to 2.40 μm at the end of the stretch, in which force was 1.4 T_0. Sarcomere length remained at 2.40 μm until the end of activation where force has decreased to 1.2 T_0. After the stimulation, sarcomeres remained at 2.40 μm for 30 ms during isometric relaxation, longer than the ca. 20 ms duration observed in fixed-end conditions as determined by a prolongation of the appearance of a longer population of sarcomeres of length 2.6-2.8 μm that appear during the exponential decay of force. X-ray signals associated with myosin motor attachment ($I_{1,1}/I_{1,0}$, I_{M3}, L_{M3}, I_{AL1}, I_{AL6}, I_{AL7}) increased prior to the stretch as motors leave the helically ordered OFF state and bind to actin. However, the intensities of these reflections decreased by the end of the stretch, but the X-ray signal for the ON state of tropomyosin (I_{AL2}) slightly increased. There was no change in I_{ML1} or I_{ML4} during the stretch, indicating that motors do not form a helically ordered OFF state.

The present results show that whilst force increased as a result of providing a ramp stretch, myosin motors were detaching during this time period, indicating the force per remaining

attached motors increased. This is contradictory to previous studies of frog muscle which show increased cross-bridges causing enhanced force.

#29 Monday Poster Session

Differences in twitch and Mechanical Control of Relaxation under increasing Mavacamten and Aficamten doses

Aram Babakr, **Chalres S Shung**

Affiliations:
1. Wayne State University, Detroit Michigan, USA

Introduction: Myosin inhibitory drugs are showing substantial value in treatment of hypertrophic cardiomyopathy. Both inhibitory drugs and myotropic activators have potential to not only modify contractile properties, but also modify relaxation. This study characterizes how twitch kinetics and the dependence of relaxation to strain rate (Mechanical Control of Relaxation) is modified.

Methods: Intact cardiac trabeculae were isolated from female Sprague Dawley rats. Trabeculae were mounted between a force transducer and motor, superfused with a Tyrodes solution, and paced. In addition to isometric contractions, muscles were interrogated for Mechanical Control of Relaxation by shortening under feedback controlled load clamps and lengthening to various strain rates prior to relaxation. Myosin inhibiting compounds, Mavacamten and Aficamten, were added to separate trabeculae at increasing doses and allowed to incubate before repeating twitch and Mechanical Control of Relaxation protocols.

Results/Discussion: Both myosin inhibitory compounds reduced developed force, but time to peak force was reduced more substantially (~30% vs 15%) in Aficamten treated trabeculae vs Mavacamten treated trabeculae. These data support that myosin inhibitory compounds both suppress force generation, but altered twitch duration indicates a difference in mechanism of inhibition. Neither inhibitor resulted in a clear change in isometric relaxation rate nor Mechanical Control of Relaxation, which is similar to our findings with myosin activator Danicamtiv. In contrast, Omecamtiv Mercarbil slowed isometric relaxation and enhanced Mechanical Control of Relaxation. As Omecamtiv Mercarbil is believed to alter thin filament regulation, in addition to myosin activation, these data support that thin filament off-kinetics may be essential in regulation of relaxation.

#30 Monday Poster Session

PHOSPHATE GROUPS COVALENTLY BONDED TO SERINES-283 OF STRIATED MUSCLE TROPOMYOSIN 1.1 (ALPHA) ALTER THIN FILAMENT STRUCTURE AND FUNCTION. A BIOCHEMICAL STUDY.

1. **David Heeley,** Memorial University of Newfoundland, St. John's, Canada
2. Stephanie Hasan, Memorial University of Newfoundland, St. John's, Canada
3. Madhushika Silva, University of Toronto, Toronto, Canada

Introduction.
Tropomyosin is a constituent of the striated muscle thin filament. A coiled-coil dimer, the major isoform, Tpm1.1 (alpha), contains a phosphorylation site at the penultimate residue, serine-283. Changes in the extent of phosphorylation are age-related: near-stoichiometric in the early-developing heart and decreasing with age (method, charge-sensitive electrophoresis). Herein the problem of tropomyosin phosphorylation is investigated biochemically, by process of isolation and reconstitution.

Methods.
Ion exchange chromatography; sedimentation; viscometry; SDS PAGE; chymotryptic digestion; Edman sequencing; circular dichroism; affinity chromatography; actomyosin-MgATPase and stopped-flow.

Results.
Adult rabbit cardiac muscle (containing 0.2 mole of P-Tpm1.1 per mole) provides a source of protein for biochemical analysis. Chromatography yields tropomyosin containing two phosphate moieties per 66,000 Da molecule and, from the same column, the unphosphorylated control.

In sedimentation assays (sub-optimal binding conditions, eg 4 °C), there is a two-fold difference in affinity for F-actin (P-Tpm > Tpm). The difference is consistent with low ionic strength solutions of P-Tpm being of comparatively higher viscosity (method, capillary flow). Collectively, the observations are explained by ion pairing between the phosphate groups and lysyl side-chains (res. 1 -12) that serve to tighten the merger of contiguous tropomyosins and by a long-range conformational rearrangement that is revealed by limited proteolysis. In such experiments, P-Tpm is more susceptible vs. control to cleavage at the primary chymotryptic site, leucine-169 (SDS PAGE and Edman-based sequencing). The result is consistent with an induced opening of the mid-section of the molecule, a region that coincides with the 5th actin binding period (res. 165 - 188) and also troponin core attachment. The proposal, which is supported by ellipticity measurements, is consistent with the tighter binding of troponin-T fragment C-Tn-T2 (res. 159 – 259) to P-Tpm-Sepharose vs. control.

The functional ramifications of tropomyosin phosphorylation are as follows. Thin filaments (pCa 4) containing P-Tpm are more activating (steady state MgATPase, 2-fold difference in Vmax) than those containing the unphosphorylated protein. The relative increase correlates with a difference in inhibition mediated by the elongated domain of troponin-T (N-Tn-T1, res. 1 - 158) and a small change in myosin binding. However, phosphorylation is not observed to affect thin filament cooperativity, at least in solution. Nor the maximal rate of Pi release (stopped-flow; regAM-ADP-Pi to regAM-ADP + Pi).

Discussion.
Structurally, the results point to a strategy whereby bonded phosphate at both C-terminal serines in tropomyosin help drive thin filament assembly during extensive myofibrillogenesis. Specifically, by stabilization of the end-to-end merger and allosteric connection to a centrally-located ‘chink’, a site of troponin attachment and actin period 5 attachment. Linkage between distant regions, center and end, demonstrates again the relaying nature of tropomyosin’s coiled coil. Functionally, destabilization of the Tn-T ‘bridge’ structure, whereby N-Tn-T extends from the troponin core complex to the next tropomyosin molecule in the polymer, may contribute to a pattern of contractility that is suited to the developing heart.

#31 Monday Poster Session

The myosin in essentail light chain is not essential for muscle function

[1]Bernadette M. Glasheen, [1]Emily Dunn, and **[1]Douglas M. Swank**[1]

Rensselaer Polytechnic Institute, Trou, USA

INTRODUCTION

Myosin, the molecular motor of muscle, is composed of two identical heavy chains and two different light chains, the essential light chain (*Mlc1*) and the regulatory light chain. The light chains are part of the myosin lever arm, which is critical for generating force and motion. *Drosophila* has a single *Mlc1* gene that is alternatively spliced into two isoforms. We created a *Drosophila* null for *Mlc1* to investigate the function of these *Mlc1* isoforms on muscle mechanical properties, such as stretch activation and enabling the fastest known frequency of muscle contraction.

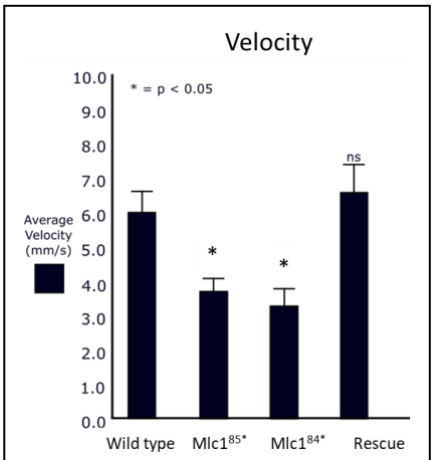

Figure 1: *Mlc1* null larval crawling velocity was reduced by about 50% compared to rescue line and wild-type larvae.

METHODS

The CRISPR/Cas9 system was used to generate three *Mlc1* null lines*, $Mlc1^{29*}$, $Mlc1^{84*}$, and $Mlc1^{85*}$*. Each contains a stop codon in exon 3 of the *Mlc1* gene at amino acids 29, 84, and 85, respectively. An *Mlc1* rescue line was created by transgenically expressing a 4.099 kb DNA fragment containing the genomic locus of *Mlc1* with 5' and 3' UTR and crossing this gene into the $Mlc1^{84*}$null background. All lines were assayed for percent hatched, pupariated and eclosed. Larval locomotion was assayed by filming the larvae on agar plates and computing time active and crawling velocities. Adult flies were assays for flight and jump velocity. IFM fibres were isolated from adult flies, permeabilized, calcium activated on a muscle mechanics apparatus, and power production was measured.

RESULTS AND DISCUSSION

To our surprise, we found that *Mlc1* homozygous null animals hatched, crawled and molted. Sequencing confirmed that we had targeted *Mlc1*. *Mlc1* null larvae crawling velocity was reduced by ~50% compared to wild-type and the rescue line (**Figure 1**). Approximately 50% of *Mlc1* larvae pupariate, of these at least 50% progress through metamorphosis, but only 8% eclose and survive to adulthood. Fiber level IFM and TDT muscle structure appeared normal in *Mlc1* null adults, but they were unable to fly or jump. Permeabilized IFM fibers isolated from 1- or 3-day old null flies did not activate or produce power (**Figure 2**). The rescue line restored adult viability, crawling velocity, and IFM power production to wild-type levels. We are currently performing experiments to determine if another protein is replacing Mlc1 on the myosin lever arm in the null line. We conclude that the essential light chain is not essential for embryonic and larval muscle development and function but is required for IFM force and power production.

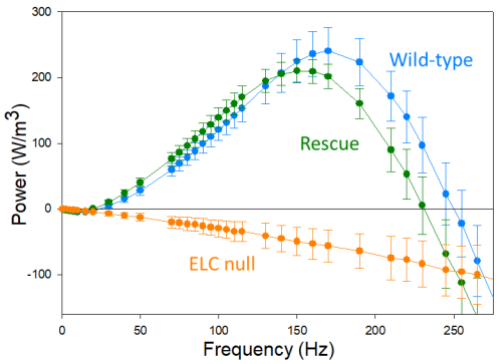

Figure 2: IFM fibres from *Mlc1* null lines were unable to generated power while the fibers from the rescue line produced similar amounts of power as wild-type fibres. Power production was measured by subjecting permeabilized IFM fibres to small muscle length

ACKNOWLEDGEMENTS

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#32 Monday Poster Session

REPLATING STEM CELL-DERIVED CARDIOMYOCYTES ALTERS CONTRACTILE FUNCTION AND ASSOCIATED MYOSIN ISOFORM EXPRESSION

Felix Osten[1], Natalie Weber[2], Meike Wendland[1], Tim Holler[1], Birgit Piep[1], Simon Kröhn[1], Jana Teske[3], Alea K. Bodenschatz[1], Santoshi Biswanath Devadas[3], Kaja S. Menge[2], Shambhabi Chatterjee[2], Kristin Schwanke[3], Maike Kosanke[4], Judith Montag[1], Thomas Thum[2], Robert Zweigerdt[3], Theresia Kraft[1], Bogdan Iorga[1], Joachim D. Meißner[1]

Affiliations:
1. Institute of Molecular and Cell Physiology, Hannover Medical School, Hannover, Germany
2. Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hannover, Germany
3. Leibniz Research Laboratories for Biotechnology and Artifical Organs (LEBAO), Hannover Medical School, Hannover, Germany
4. Research Core Unit Genomics, Hannover Medical School, Hannover, Germany

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) serve as an invaluable tool in cardiac research with significant implications for the development of therapeutic strategies and cardiac disease models. Myosin heavy chain (MyHC) is a key determinant of cardiac contractile function. Human adult ventricular cardiomyocytes predominantly express the β-isoform of MyHC, whereas human atrial cardiomyocytes express mainly α-MyHC. We have previously shown that prolonged culture on a stiff matrix can drive hPSC-CMs towards a predominant β-MyHC expression profile.

In many experimental contexts, hPSC-CMs are replated onto fresh substrates before analysis. We investigated the consequences of disrupting cell-extracellular matrix (ECM) interactions by enzymatic detachment and replating of long-term cultured hPSC-CMs. Myofibrils measured in a micromechanical setup showed accelerated crossbridge cycling kinetics after replating compared to myofibrils from non-replated cardiomyocytes. In line with this, we observed a significantly increased expression of α-MyHC, known for its higher ATPase activity relative to β-MyHC, along with increased *MYH6* mRNA levels in the replated hPSC-CMs. Interestingly, myosin light chain isoform expression did not show substantial changes toward an atrial phenotype.

Notably, both the functional changes and the *MYH*/MyHC expression profile were reversed within the second week following replating. RNA-Seq-based gene enrichment analyses revealed that this shift in *MYH*/MyHC expression correlated with alterations in mechanosensitive signaling pathways, especially those mediated by integrins and downstream focal adhesion kinase (FAK) pathways. Consistently, FAK inhibition resulted in reduced β-MyHC expression on stiff matrix, underscoring the critical role of FAK in regulating MyHC isoform expression.

Overall, these findings highlight the impact of ECM alterations on the hPSC-CM phenotype. The replating-induced shift in MyHC isoform expression and its associated functional effects are critical to consider in downstream applications, as they may influence experimental outcomes.

#33 Monday Poster Session

MYOSIN LIGHT CHAIN KINASE AND PROTEIN KINASE A INDEPENDENTLY AND COOPERATIVELY REGULATE CONTRACTILE FUNCTION VIA MYOFILAMENT PHOSPHORYLATION

Kyrah L. Turner[1], Bertrand C.W. Tanner[2]

Affiliations:
1. School of Molecular Biosciences, Washington State University, Pullman, Washington, USA
2. Department of Integrative Physiology & Neuroscience, Washington State University, Pullman, Washington, USA

Cardiovascular disease is the leading cause of death in the United States, with heart failure contributing to 1 in 7 deaths. Cases of heart failure are multifactorial but remain associated with contractile deficits at the sarcomere level, leading to cardiac dysfunction. The regulatory function of thick-filament proteins, cardiac myosin binding protein-C (cMyBP-C) and regulatory light chain (RLC), is altered by their phosphorylation state; protein kinase A (PKA) phosphorylates cMyBP-C and myosin light chain kinase (MLCK) phosphorylates RLC. Phosphorylation levels of both proteins decrease during heart failure, highlighting their potential role in regulating contractility in the healthy heart. While the individual effects of phosphorylation on cMyBP-C and RLC are partially defined, it is not clear how cMyBP-C and RLC interact with each other in a phosphorylation-dependent and/or length–dependent manner to regulate cardiac contractility.

PKA also phosphorylates the thin-filament regulatory protein troponin I (TnI), making it difficult to isolate the sole effects of cMyBP-C phosphorylation on contractile function. To circumvent this, we created a transgenic mouse model (S2A-TnI) wherein the canonical PKA targets on TnI, serines 23 and 24, were mutated to alanines via CRISPR site-directed mutagenesis. We utilized skinned papillary muscle strips from wild-type (WT), cMyBP-C knockout (KO), and S2A-TnI mice to assess the individual and combined effects of PKA and MLCK on Ca^{2+}-activated isometric contraction at short (1.9 μm) and long (2.2 μm) sarcomere length. All strips underwent phosphatase treatment prior to PKA or MLCK treatment to start with a basal, dephosphorylated myofilament profile.

In WT strips, at a short sarcomere length, Ca^{2+}-sensitivity of contraction (i.e. pCa_{50}) decreased (ΔpCa_{50}=0.05) with PKA treatment, increased (ΔpCa_{50}=0.03) with MLCK treatment, and increased (ΔpCa_{50}=0.01) with PKA+MLCK treatment. In cMyBP-C KO strips, at a short sarcomere length, decreased (ΔpCa_{50}=0.03) with PKA treatment, pCa_{50} increased (ΔpCa_{50}=0.07) with MLCK treatment, and increased (ΔpCa_{50}=0.1) with PKA+MLCK treatment. In S2A-TnI strips, at a short sarcomere length, pCa_{50} increased with all treatments (ΔpCa_{50}=0.02 for PKA, ΔpCa_{50}=0.03 for MLCK, and ΔpCa_{50}=0.05 for PKA+MLCK). These effects were even further amplified at a long sarcomere length. Additional passive stress, Ca^{2+}-activated stress, and viscoelastic measurements suggest altered cross-bridge cycling kinetics may be driving these changes in Ca^{2+}-sensitivity.

These findings show that isolated effects of cMyBP-C and RLC phosphorylation both alter pCa_{50}. Moreover, the increase in pCa_{50} from the combined influence of cMyBP-C and RLC phosphorylation was blunted in WT, but not cMyBP-C KO strips, compared to either protein being phosphorylated individually. This suggests there may be coupled, phospho-dependent

regulatory interactions between cMyBP-C and RLC. We anticipate this will provide a strong foundation to launch future studies to restore contractile dynamics in diseased myocardium, where these thick-filament regulatory protein interactions are likely disturbed.

#34 Monday Poster Session

ACTIVATION OF CARDIAC MYOSIN FILAMENT DURING SYSTOLE WITH DIFFERENT AFTERLOADS

I. Morotti[1], M. Caremani[1], G. Sautariello[1], M. Balzano[1], M. Marcello[3], I. Pertici[1], P. Bianco[1], T. Narayanan[2], G. Piazzesi[1], V. Lombardi[1], M. Reconditi[1], **M. Linari**[1]

[1]PhysioLab, University of Florence, Florence, Italy; [2]ESRF, Grenoble, France ;
[3]Généthon, Évry-Courcouronnes, France

Using X-ray diffraction on intact trabeculae and papillary muscles from rat ventricle it has been shown (Morotti *et al*. 2024, PNAS 121:e2410893121) that at near-physiological temperature (27 °C), at which most of the myosin motors in diastole lie on the surface of the thick filament folded back on their tails (OFF state), unable to hydrolyze ATP and attach to actin, there is a relation between the twitch peak force (T_p) developed during the systole and the region of thick filament from which motors attach to actin. At low T_p (<40 kPa), actin attached motors are limited to the central region of the half-thick filament (htf), the C-zone characterized by the presence of the Myosin Binding Protein C (MyBP-C), suggesting a role of the MyBP-C N-terminus in promoting attachment. Instead at these low T_p the loss of folded OFF state of motors, marked by the drop in intensity of the first myosin layer line (IML1), spreads starting from the periphery of the htf and is preceded by a titin-mediated structural change, marked by the early loss of intensity of forbidden reflections. Increase in T_p above 40 kPa is accounted for by progressive spread of attachments beyond the C-zone towards the periphery of htf (D-zone). Considering that motor activation in the D-zone is almost complete at 40 kPa, this progression in motor attachment can be explained with the near-neighbor cooperative activation of the thin filament (McKillop and Geeves. 1993, *Biophys J* 65:693; Caremani *et al*. 2022, *Commun Biol* 5:1266), operating at the low intracellular Ca^{2+} of the cardiac systole.
Here, we tested these hierarchical regionalizations of thick filament activation and motor attachment during a more physiological systolic performance, combining sarcomere level mechanics and X-ray diffraction to measure the structural signals during shortening under three different afterloads (T): 0.25, 0.5 and 0.75 T_p (where T_p is 80 ± 3 kPa, diastolic sarcomere length 2.34 ± 0.01 μm; external Ca^{2+} concentration 2.5 mM; first twitch from quiescence; temperature 27°C).
We find that myosin motors recruitment from the OFF state progresses from the periphery of the htf with the increase in T, while motor attachment at low afterload is limited to the C-zone and spreads toward the periphery of the thick filament for $T > 0.5T_p$, according to cooperative thin filament activation. Comparison of the mechanical output of the afterloaded contraction to that of the isotonic-release contraction (T superimposed to T_p) shows that for $T< 0.5\ T_p$ the shortening velocity and power of the afterloaded contraction are lower, while at higher T they are the same as in the isotonic release contraction. We conclude that both the thick filament activation and the mechanical performance are tuned to the afterload by the energetically convenient mechanism that integrates thick filament mechanosensing and cooperative thin filament activation. At the organ level these results confirm that, even under the condition that

best reproduce cardiac cycle mechanics, the aortic pressure, rather than the diastolic filling, determines the systolic performance.
Supported by MUR (Italy) and ESRF.

#35 Monday Poster Session

ORIENTATION CHANGES OF A C-TERMINAL REGION OF TROPONIN I DURING CONTRACTION OF CARDIAC MUSCLE

Kalakoutis, M[1]., Smith, E[1]., Arcidiacono, A[1]., Brunello, E[1]., Fusi, L[2]., & Irving, M[1]. Affiliations:

1. Randall Centre for Cell and Molecular Biophysics and British Heart Foundation Centre of Research Excellence, King's College London, London, United Kingdom.
2. Centre for Human and Applied Physiological Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom.

Introduction: Contraction of heart muscle is triggered by calcium binding to troponin C (TnC) leading to a structural change that allows it to bind the switch peptide of troponin I (TnI), followed by movements of the C-terminal region of TnI, the N-terminal region of troponin T (TnT), and tropomyosin, that expose the myosin binding sites on actin in the thin filament and permit the myosin-actin interaction that drives contraction. The initial structural changes in this signalling pathway have been monitored in heart muscle cells using probes on TnC (Sevrieva et al., 2024). Here, we extended this approach to a helical region of TnI between the switch peptide and the C-terminus that is roughly parallel to tropomyosin when calcium is not bound to troponin in isolated thin filaments, and becomes mobile when calcium binds (Risi et al., 2024; Yamada et al., 2020).

Methods: Bifunctional rhodamine (BR) probes were introduced into TnI by cross-linking cysteines in the expressed A171C, E179C variant. The BR-labelled reconstituted troponin complex was exchanged into demembranated trabeculae from the right ventricle of rat heart, and the orientation of the BR probe was measured by polarised fluorescence. Trabeculae with an initial sarcomere length of 2.15μm were activated by temperature jump to 27ºC in solutions containing 3% Dextran with different calcium concentrations.

Results: Introduction of BR-TnI did not affect maximum calcium activated force, which was 53 ± 9 kPa (n = 6, SEM). The order parameter $\langle P_2 \rangle$ that determines the orientation of the BR-TnI probe dipole with respect to the filament axis was 0.50 ± 0.02 in diastolic conditions (pCa 7). Calcium activation produced a decrease in $\langle P_2 \rangle$, showing that the probe dipole became less parallel to the fibre axis. At maximal calcium activation $\langle P_2 \rangle$ was 0.28 ± 0.03. Force and $\langle P_2 \rangle$ at different pCa were fitted with the Hill equation, with Ca^{2+} sensitivities (pCa_{50}) that were not significantly different for each other. 25μM Mavacamten (n = 3) abolished active force without changing $\langle P_2 \rangle$ for the BR-TnI probe in diastolic conditions. However, the decrease in $\langle P_2 \rangle$ associated with maximal calcium activation was reduced to about half of that in the absence of Mavacamten, which also significantly reduced the calcium sensitivity of the orientation change.

Conclusion: The region 171-179 of TnI, C-terminal to the switch peptide, is relatively parallel to the filament axis in diastolic heart muscle, as seen in cryo-EM structures of isolated thin filaments in the absence of calcium. It becomes less parallel during calcium activation, with a calcium sensitivity similar to that of active force. The movement of this region of TnI was

reduced but not abolished in the presence of Mavacamten. These preliminary data suggest that the orientation of this region of TnI is sensitive to both calcium and myosin binding to the thin filament.

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#36 Monday Poster Session

THE M-DOMAIN OF CARDIAC MYOSIN BINDING PROTEIN-C IS NECESSARY TO DAMP SPONTANEOUS OSCILLATORY CONTRACTIONS (SPOC) IN MOUSE CARDIOMYOCYTES

Dylan L. Rechert[1], Nichlas M. Engels[1], and **Samantha P. Harris[1]**

Affiliations:
1. University of Arizona, Tucson, Arizona, USA

Cardiac myosin binding protein-C (cMyBP-C) is a regulatory protein necessary for normal cardiac contraction and relaxation and for increased inotropy in response to β-adrenergic agonists that phosphorylate cMyBP-C. Conversely, mutations in *MYBPC3*, the gene encoding cMyBP-C, are among the most common causes of hypertrophic cardiomyopathy (HCM), a disease typically characterized by cardiac enlargement and impaired diastolic relaxation. Most mutations in *MYBPC3* introduce premature termination codons which ultimately lead to under-expression of cMyBP-C in patients with HCM (i.e., haploinsufficiency). To better understand the functional effects of phosphorylation and reduced expression of cMyBP-C, we used a "cut and paste" approach to first eliminate cMyBP-C from cardiac sarcomeres of gene-edited $SpyC_3$ mice followed by stoichiometric replacement with recombinant cMyBP-C proteins localized to the thick filament. We previously reported using the "cut and paste" methodology that loss of cMyBP-C causes a reversible decrease in Ca^{2+} sensitivity of tension, an increase in cross-bridge cycling kinetics, and faster rates of relaxation. Notably, loss of cMyBP-C also induces a distinctive wave-like pattern of spontaneous contractions and subsequent relaxations in detergent-permabilized cardiomyocytes at constant activating [Ca^{2+}]. This behavior – known as spontaneous oscillatory contractions (SPOC) – occurs independently of Ca^{2+} cycling from the sarcoplasmic reticulum, indicating that it is intrinsic to the sarcomere. To investigate the ability of cMyBP-C to suppress the occurrence of SPOC, we replaced the N'-terminal domains of endogenous cMyBP-C (C0-C7) with exogenous recombinant proteins containing selective domain deletions. Results showed that the C0-C1-M-C2 domains effectively damp SPOC, whereas the C3-C4-C5-C6-C7 domains did not. To assess the role of the regulatory M-domain, we tested a recombinant construct lacking the M-domain (ΔM). Preliminary results with this construct showed that there is minimal supression of SPOC, suggesting the M-domain of cMyBP-C is mainly responsibly for the ability of cMyBP-C to damp sarcomeric oscillations. Furthermore, PKA-mediated phosphorylation of the C0-C7 domains reduced the ability of cMyBP-C to suppress SPOC compared to unphosphorylated C0-C7. Taken together, these results indicate that the M-domain is necessary to damp SPOC and that the occurrence of SPOC may be subject to regulation by phosphorylation of cMyBP-C. These results suggest that cardiomyocytes may be predisposed to SPOC specifically under conditions of increased ß-adrenergic drive or when cMyBP-C expression is reduced as in HCM.

#37 Monday Poster Session

SPATIAL CONTROL OF MYOSIN REGULATORY LIGHT CHAIN PHOSPHORYLATION MODULATES CARDIAC THICK FILAMENT MECHANO-SENSING

Caterina Squarci[1], Daniel Koch[2], Paul Aanaya[1], Kenneth S. Campbel[l1] and **Thomas Kampourakis[1,3]**

Affiliations:
1. Division of Cardiovascular Medicine, Internal Medicine, College of Medicine, University of Kentucky, Lexington KY 40536, USA
2. Max Planck Institute for Neurobiology of Behavior — CAESAR, Ludwig-Erhard-Allee 2, D-53175 Bonn
3. Randall Centre for Cell and Molecular Biophysics, King's College London, London SE1 1UL, United Kingdom

The heart can adapt its performance in response to changing metabolic demands of the rest of the body. A central mechanism intrinsic to the heart is to modulate the function of the cardiac contractile proteins via post-translational modifications. Although phosphorylation of the cardiac myosin motor-associated regulatory light chain (RLC) by cardiac myosin light chain kinase (cMLCK) has been recognized as a key signaling pathway to increase myocardial contractile function, little is known about its molecular mechanism of action. We show that phosphorylation of RLC is not a stochastic process but a spatially tightly controlled mechanism. Myosin motors in the region of the thick filament associated with cardiac myosin binding protein-C (cMyBP-C) are the primary target for phosphorylation by cMLCK (**Fig. 1**). Moreover, phosphorylation of RLC likely only leads to activation of one of the two myosin motors of the cardiac myosin molecule and increases their force-dependent recruitment. We propose that RLC phosphorylation exerts its functional effects via increasing the gain of the mechano-signaling between different zones of the thick filament. A better mechanistic understanding of the role of RLC phosphorylation likely underpins the development of therapeutic interventions for both heart disease and heart failure.

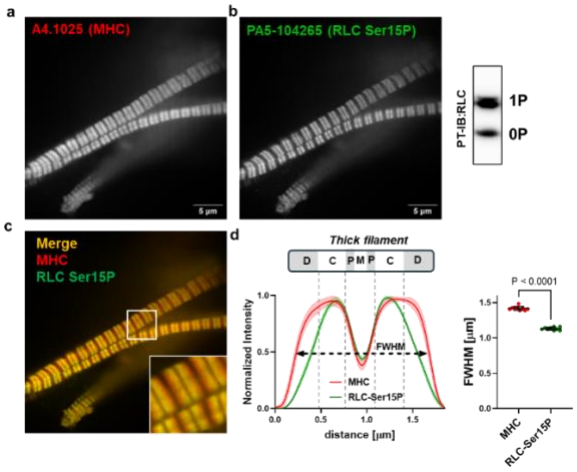

Figure 1. Spatial RLC phosphorylation distribution in human ventricular myofibrils under native conditions. (a) Stain for myosin heavy chain (MHC, red). (b) Left: Stain for Serine 15 phosphorylated RLC (RLC Ser15P, green). Right: Phostag™-Western-blot analysis of RLC phosphorylation levels in human ventricular myofibrils. (c) Merge of (a) and (b). (d) Left: The normalized averaged intensity

profiles. Continuous lines denote average profiles and shaded areas indicated 95% confidence intervals. The structure of the thick filament is shown to scale above the plot and the different filament zones are labelled accordingly.

#38 Monday Poster Session

MECHANOSENSING DYSFUNCTION FOLLOWING N2B DELETION IN HUMAN IPSC-DERIVED CARDIOMYOCYTE

Maral Azodi[1,2], Pragati Parakkat[2,3,4], Anca Margineanu[5], Narasimha Swamy Telugu[6], Sebastian Diecke[2,6], Michael Gotthardt[2,3,4], Claudia Crocini[1,2]

1. Max Rubner Center for Cardiovascular Metabolic and Renal Research, **Deutsches Herzzentrum der Charité, Charité-Universitätsmedizin Berlin, Germany.**
2. German Center for Cardiovascular Research (DZHK), partner site Berlin, Berlin, Germany
3. Department of Translational Cardiology and Functional Genomics, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.
4. Department of Cardiology, Charité-Universitätsmedizin Berlin, Germany.
5. Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Advanced light Microscopy departrment, Berlin, Germany.
6. Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Technology Platform Pluripotent Stem Cells, Berlin, Germany.

Titin is a protein critical for maintaining cardiac elasticity and passive stiffness. In the heart, titin is expressed as three major isoforms: fetal cardiac titin, N2BA, and N2B. All cardiac isoforms contain the unique N2B segment, which contributes to titin's elastic function and harbors key post-translational modification sites that regulate titin-based stiffness and mechanotransduction. Mice lacking the N2B region on titin develop normally but have smaller hearts, elevated ejection fraction and diastolic dysfunction compared to controls.

To investigate the specific role of the N2B region of titin in human cardiomyocytes, we generated N2B-deficient cardiomyocytes (N2BKO) from human induced pluripotent stem cells (hiPSC-CMs) using CRISPR/Cas9 genome editing. N2BKO hiPSC-CMs differentiated efficiently and showed normal morphological development relative to their isogenic controls. Titin expression and localization appeared comparable between N2BKO and control cells, as confirmed by titin gel electrophoresis and immunofluorescence analysis.

To further explore N2B-mediated mechanosensing, we measured nuclear stiffness with a live cell membrane tension probe and fluorescence lifetime imaging microscopy (FLIM). N2BKO hiPSC-CMs showed reduced nuclear tension as compared to isogenic controls. Finally, we used ATAC-seq and RNA-seq to identify N2B-mediated gene expression and regulation. Gene Ontology (GO) analysis of both RNA-seq and ATAC-seq data revealed significant enrichment of genes involved in extracellular matrix (ECM) organization, PI3K-AKT signaling, MAPK signaling, and calcium signaling pathways. Furthermore, TOBIAS footprinting analysis identified reduced activity of AP-1 and TEAD4 transcription factors in N2BKO hiPSC-CMs. These findings contribute to a deeper understanding of titin function in the heart.

#39 Monday Poster Session

POSSIBLE ROLES OF TROPONIN, TROPOMYOSIN AND CA^{2+} IN TRANSCRIPTIONAL REGULATION IN STRIATED MUSCLE MYOCYTE NUCLEI: COULD THEY MODULATE RNA POLYMERASE II ACTIVITY IN CONJUCTION WITH NUCLEAR ACTIN?

Amelia J. Koopman[1*], Alexandra J. Martin[1*], Lauren G. Moore[1*], Michelle Rodriguez[1*] and P. Bryant Chase[1]

Affiliations:
1. Florida State University, Tallahassee, Florida, USA

*** Equal contributions**

Actin and its association with actin-binding proteins in the eukaryotic nucleus can influence transcription, the activity of RNA polymerases that is an essential step in gene expression, and processes related to maturation of eukaryotic RNA (Kyheröinen et al. 2024; Philimonenko et al. 2004; Serebryannyy et al. 2016; Visa and Percipalle 2010). RNA polymerases are the macromolecular machines that catalyze the synthesis of RNA, the sequences of which are coded for by the sequences of regions of DNA in the nucleus of eukaryotic cells—nuclei in the case of many mature striated muscle cells, or myocytes, which are in many cases polynucleated. Herein, we begin by reviewing the evidence for the role(s) of nuclear actin's influence on transcription. We furthermore hypothesize that the troponin complex and tropomyosin—which bind actin to form thin filaments in the myofilament lattice of striated muscle myocytes (Risi et al. 2024; Risi et al. 2025; Risi et al. 2021), but which can also be found in the nuclei of striated muscle myocytes and some cancerous cells (Asumda and Chase 2012; Johnston et al. 2018; Zhang et al. 2013a; Zhang et al. 2013b)—could modulate the influence of nuclear actin on transcription when present in a nucleus. Interestingly, troponin and tropomyosin could confer Ca^{2+}-dependence to transcriptional modulation by nuclear actin, a mechanism that would complement Ca^{2+}-dependent modulation of posttranslational modifications that influence gene expression.

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#40 Monday Poster Session

BENCHMARKING FUNCTIONAL MATURATION AND PHARMACOLOGICAL RESPONSIVENESS IN HIPSC-DERIVED ENGINEERED HEART TISSUES FOR PRECLINICAL APPLICATIONS

Ayesha Arefin[1], **Hamed Ghazizadeh**[1], Neda Kabi[1], Katharina Gees[1], Kelly Kernan[1], Ellie Sullivan[1], Shawn Luttrell[1], Thomas Leahy[1], Christal Worthen[1], Greg Luerman[1], and Nicholas A. Geisse[1]

Affiliations:
1. Curi Bio; Seattle, Washington, USA

Introduction: Human induced pluripotent stem cell (hiPSC)-derived engineered heart tissues (EHTs) are a powerful *in vitro* model for cardiotoxicity screening and mechanistic toxicology. However, their immature functional phenotypes and the absence of standardized benchmarks for their fidelity to clinically relevant pharmacology limit their widespread adoption as a preclinical platform. For example, EHTs typically fail to recapitulate a physiologically relevant positive force frequency relationship (FFR), likely due to immature calcium handling and ion channel expression. Therefore, our objective was to conduct a comprehensive assessment of EHT maturation and pharmacological fidelity, which we hypothesized would be promoted by continuous electrical pacing during tissue maturation.

Methods: Our study utilized a commercially available EHT platform (Curi Bio) and three independent hiPSC-cardiomyocyte lines, including one proprietary Curi Bio line. Beginning on day 4, EHTs were subjected to continuous electrical pacing with frequencies ramping from 0.5 Hz to 4 Hz by day 10 and maintained at 4 Hz through day 18 (**Fig 1A**). Unstimulated EHTs were used as controls (n = 7-8/group). Functional maturation and pharmacological responses were quantified using a FFR protocol, graded calcium challenge, inotropic agents (Isoproterenol, Omecamtiv, Verapamil, Blebbistatin), and known cardiotoxins (Digoxin, Doxorubicin, Sunitinib; Aspirin as negative control).

Results: All EHTs exhibited consistent compaction (~40% width reduction) by day 4 and stabilized spontaneous contractions by day 12, generating peak twitch forces up to 200 μN. Notably, chronically paced EHTs displayed robust functional adaptation, with an 80% increase in twitch force at 2.75 Hz relative to 1 Hz (i.e., positive FFR), followed by expected force suppression at higher frequencies (**Fig 1B**). In contrast, untrained controls exhibited a 23% decrease in twitch force across the same frequencies (t-test; $p<0.001$). Chronically paced tissues also showed faster relaxation kinetics across all stimulation frequencies relative to controls (**Fig 1C**). Calcium and inotropic dose-response curves were reproducible across hiPSC lines (graded calcium challenge data in **Fig 1D**), whereas cardiotoxin treatment revealed inter-line differences in the onset and reversibility of functional impairment (digoxin treatment data in **Fig 1E**), highlighting the importance of genetic background.

Discussion: This study provides evidence-based protocols and reproducibility metrics to benchmark EHT maturation and pharmacologic responsiveness. The combination of electrical conditioning during maturation with robust functional assays enhances the translational utility of EHT platforms and supports their integration into regulatory-aligned pharmacological studies.

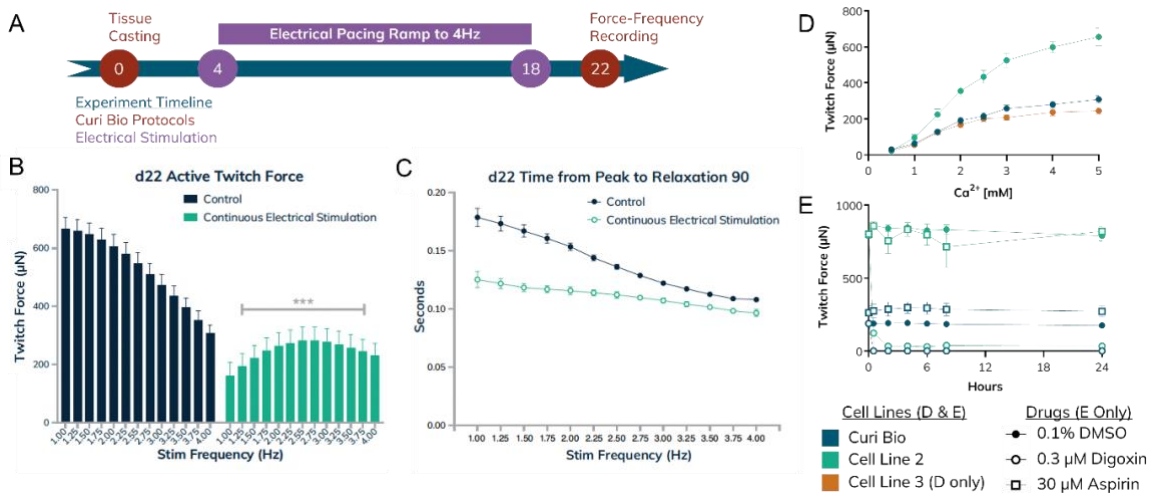

Figure 1. A) Study design for continuous electrical stimulation and force-frequency analysis. B) Twitch forces and C) relaxation times across increasing stimulation frequencies demonstrate functional adaptation in continuously stimulated EHTs. D) Increased calcium concentration and E) digoxin treatment elicit expected pharmacological effects in EHTs generated from multiple cell lines.

#41 Monday Poster Session

SKELETAL MUSCLE METABOLITE ALTERATIONS IN LONG COVID PATIENTS REVEALED BY AN OPTIMIZED UNTARGETED METABOLOMICS PIPELINE

Po Lin (Brian) Chen[1], Braeden T Charlton[1], Anouk Slaghekke[2], Brent Appelman[2], Frank W Bloemers[3], Paul van Amstel[3], Jelle J Posthuma[4], Michèle van Vugt[5], Bauke Schomakers[6], Michel van Weeghel[6], Rob C I Wüst[1]

Affiliations:

1 Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, The Netherlands
2 Center for Experimental and Molecular Medicine, Amsterdam UMC, The Netherlands
3 Department of Trauma Surgery, Amsterdam UMC, The Netherlands
4 Division of Surgery, Flevoziekenhuis, Almere, The Netherlands
5 Department of Internal Medicine, Amsterdam UMC, the Netherlands
6 Core Facility Metabolomics, Amsterdam UMC, the Netherlands

Introduction Long COVID (LC) poses a substantial risk to healthcare demands in the coming years. Patients show metabolic shifts toward glycolysis, increased glycolytic fibers, necrosis, atrophy, reduced capillary-to-fiber ratios, and leukocyte infiltration in skeletal muscle, potentially contributing to exercise intolerance and muscle pain. However, many metabolites within skeletal muscle involved in these changes remain unexplored.

Objective This study aims to establish an optimized untargeted metabolomics pipeline to identify novel metabolites in skeletal muscle associated with muscle abnormalities in long COVID. The objective is to address metabolite heterogeneity among individuals by refining the analysis protocol and selecting long COVID patients most representative of muscle abnormalities for analysis.

Method Vastus lateralis biopsies were collected and analyzed for metabolite abundance using liquid chromatography–mass spectrometry. Principal component analysis and K-Medoids were performed to cluster subjects based on their metabolite profiles, yielding 14 LC patients and 12 healthy. Metabolomic data was analyzed using MetaboAnalystR incorporating databases such as HMDB, KEGG, and RaMP to investigate pathway-specific metabolite.

Results Untargeted metabolomics analysis identified 274 metabolites with unadjusted $p < 0.05$, showing distinct patterns of up- and downregulation in LC patients (Fig. 1). Only 6 metabolites (citric acid, pyruvic acid, methyl hydrogen fumarate, FAD, sorbitol, and ADP-ribose 1-2 cyclic phosphate) were significantly downregulated in LC (FDR < 0.05). Our results show attenuated metabolite level in tricarboxylic acid cycle and polyol pathway, while those in glycolysis pathway increased in LC (Fig. 2). Moreover, reduced tRNA splicing activity in skeletal muscle of LC patients is suggested by notably low levels of ADP-ribose 1-2 cyclic phosphate, a unique molecular marker of this process.

Conclusions Our newly developed untargeted metabolomics pipeline allows for the identification of long COVID patients' phenotype based on the metabolites profile in skeletal muscle. The analysis reveals that increased glycolytic activity may be a compensatory mechanism for reduced polyol pathway activity, which might partially explain the metabolic shift in LC skeletal muscle. Notably, the reduction of metabolites linked to the end products of tRNA splicing suggests a possible decrease in protein synthesis in Long COVID patients, although further validation is needed. These findings highlights new directions to investigate cellular pathways that underlie the physiological change in skeletal muscle.

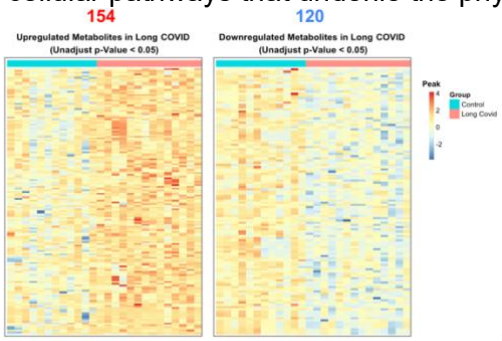

Figure 1. Heatmap illustrating distinct patterns of upregulated and downregulated metabolites in long COVID group

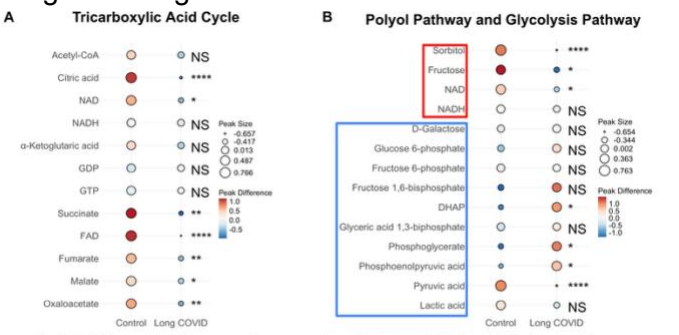

Figure 2. A) TCA metabolites are downregulated in long COVID. B) Polyol pathway metabolites were reduced (red frame), while glycolysis metabolites (blue frame) were increased. Statistical significance for each metabolite is indicated by unadjusted p-values.

#42 Monday Poster Session

DISORGANIZATION OF MICROTUBULE CONTRIBUTES TO PATHOLOGICAL PHENOTYPE OF DCM IN LMNA-MUTATED HIPSCs

Wollenhaupt, J.[1,2], , Knosalla, C.[1,2], Diecke, S.[2,3] , Crocini, C.[1,2]

Affiliations:
1. Max Rubner Center for Cardiovascular Metabolic and Renal Research, Deutsches Herzzentrum der Charité, Charité-Universitätsmedizin Berlin, Germany
2. Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK), Berlin, Germany
3. Max-Delbrück-Center Stem Cell Facility, Berlin, Germany

Dilated cardiomyopathy (DCM) is a common heart muscle disease affecting approximately 1 in 250–500 individuals, characterized by a reduction in left ventricular ejection fraction and dilation. Mutations in the LMNA gene encoding lamin A/C, a nuclear protein, are a leading cause of DCM, yet the cellular mechanisms linking nuclear envelope defects to impaired cardiomyocyte function remain incompletely understood. We hypothesized that cytoskeletal disorganization leads to impaired communication of the nucleus and the sarcomere, leading to the severe phenotype of LMNA DCM.

To examine the role of the nucleus in the pathogenesis of DCM, we used patient-derived induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) expressing the heterozygous K117-framshift (K117fs) mutation. We also employed hiPSC-CMs engineered to express either wild-type lamin A/C (isogenic control) or homozygous K117fs mutation. Contractility assays using IonOptix revealed a reduced contraction amplitude in mutant hiPSC-CMs compared to controls, despite preserved calcium handling.

Fluorescence lifetime imaging (FLIM) with the membrane tension probe Flipper-TR showed increased nuclear stiffness in mutant cells, consistent with elevated nuclear envelope rupture risk, in line with previous studies. Notably, treatment with sarcomere modulators failed to rescue contractile deficits and further increased nuclear membrane tension and rupture incidence.

Immunofluorescence microscopy analysis confirmed nuclear abnormalities and cytoskeletal disorganization, including disorganization of alpha-tubulin and an increased nuclei per cell.

In summary, this study highlights the impaired interplay between different cellular compartments and their potential impact on disease manifestation, paving the way for safe personalized targeted therapeutic interventions in DCM.

#43 Monday Poster Session

Investigation of z-disc and cytoskeletal remodeling in Hypertrophic Cardiomyopathy using hiPSC-derived cardiomyocytes

Laura Lechner[1,2], Girish Ramesh[1,2], Diederik W.D.Kuster[3], Claudia Crocini[1,2]
Affiliations:

1. **Max Rubner Center for Cardiovascular Metabolic Renal Research, Deutsches Herzzentrum der Charité, Charité-Universitätsmedizin Berlin, Germany**
2. DZHK (German Centre for Cardiovascular Research), Partner Site Berlin, Berlin, Germany
3. **Department of Physiology,** Vrije Universiteit Medical Center, Amsterdam, Netherlands

Cardiomyopathies are a diverse group of diseases affecting the heart muscle, often leading to altered contractility and structural changes at cellular and molecular levels. Hypertrophic cardiomyopathy (HCM) is characterized by hypercontractility, asymmetric myocardial thickening and alterations of the cytoskeleton and commonly caused by mutations in sarcomeric proteins. Studies suggest that changes in cytoskeletal organization may alter membrane tension, affecting mechanotransduction pathways and can thereby contributing to pathological remodeling in in HCM. Despite its prevalence, the underlying disease mechanisms and the structural changes that affect downstream cellular architecture remain poorly understood.

Using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from wild-type controls and HCM patients, we aim to assess structural and functional consequences of Z-disc alterations. We aim to investigate changes in cytoskeletal organization, nuclear membrane tension, and contractile performance. To explore potential therapeutic avenues, we also plan to test the effects of cytoskeletal and sarcomere modulators on these phenotypes. Data from these ongoing experiments will be presented, highlighting structural and functional differences between wild-type and HCM hiPSC-CMs, and their modulation by targeted interventions.

Our work aims to establish how Z-disc dysfunction in HCM drives broader mechanical and signaling changes within the cardiomyocyte, offering new insight into disease mechanisms and potential.

#44 Monday Poster Session

NUCLEAR ABNORMALITIES AND DNA DAMAGE IN HYPERTROPHIC CARDIOMYOPATHY

Linda R. Micali[1], Inez Duursma[1], Kenneth B. Margulies[2], Stephan A.C. Schoonvelde[3], Michelle Michels[3], Jolanda van der Velden[1], Diederik Kuster[1], Tyler J. Kirby[1]

[1] Department of Physiology, Amsterdam Cardiovascular Sciences, Amsterdam UMC, Amsterdam, the Netherlands.

[2] Department of Medicine, University of Pennsylvania, Philadelphia, USA

[3] Department of Cardiology, Erasmus MC, Rotterdam, the Netherlands

Introduction. Hypertrophic cardiomyopathy (HCM) is characterized by increased left ventricular wall thickness, diastolic dysfunction and the onset of potentially fatal arrhythmias. About half of the HCM cases is associated with a pathogenic genetic mutation (genotype positive, G^+) of sarcomeric proteins, while a growing cohort of HCM patients do not carry a known pathogenic genetic mutation (genotype negative, G^-). However, regardless of the etiology, hallmarks of HCM are cardiomyocyte hypertrophy and cytoskeletal remodeling. The close interaction between sarcomeric z-disks and the non-sarcomeric cytoskeleton allows mechanical cues to be relayed from the sarcomere to the nucleus, through the “linker of nucleoskeleton and cytoskeleton” (LINC) complex. Mechanical signals eventually regulate gene expression through the interaction between the LINC complex and chromatin.

The integrity of the LINC complex is crucial in maintaining cardiomyocyte health, and its aberrations have already been linked to the onset of cardiomyopathies. However, the connection between the LINC complex and disease pathogenesis in HCM patients remains unknown. We hypothesize that the hypertrophic and hypercontractile state of HCM cardiomyocytes stresses the LINC complex, ultimately leading to abnormal nuclear morphology and DNA damage.

Objective. We aim to investigate whether nuclear morphology and DNA integrity are compromised in HCM.

Methods. We performed immunofluorescence on paraffin-embedded heart sections of non-failing (NF) donors and HCM tissue from G^+ and G^- patients. Cardiac troponin T and WGA staining were used to differentiate cardiomyocytes from other cell types. To assess nuclear morphology and DNA damage, we stained for lamin A/C and gH2AX, respectively. Images were acquired using the Nikon X-Light V3/DeepSIM Spinning Disk Confocal laser-scanning microscope (63x magnification) and analyzed on Fiji. Nuclear segmentation, morphological assessment, and invagination analysis were performed based on the lamin A/C signal. DNA damage was assessed by measuring the γH2AX signal area normalized to the nuclear area.

Results. While NF and HCM nuclei have comparable area and aspect ratio, G^+ and G^- HCM cardiomyocytes show more nuclear roughness ($p = 0.03$ and $p= 0.001$, respectively) and tend to have more nuclear invaginations compared to NF cardiomyocytes. Moreover, although the

nuclei of G^+ patients show more DNA damage compared to NF nuclei ($p=0.03$), we found no correlation between nuclear roughness and DNA damage.

Conclusion. HCM nuclei show more nuclear abnormalities and DNA damage than NF donors, but these aberrations do not explain the increased DNA damage. Future experiments will focus on assessing nuclear morphology in *in vitro* models of HCM and manipulating the LINC complex to investigate whether the hypertrophic phenotype can be reversed.

#45 Monday Poster Session

STUDYING OF MODULATORS OF CARDIAC MYOSIN USING SINGLE MOLECULE METHODS SPPLIED TO BASAL AND ACTIN-ACTIVATED ATP-TURNOVER

Albin E Berg , Lok Priya Velayuthan, Alf Månsson, Marko Ušaj*

* **Corresponding author**

Marko Ušaj (marko.usaj@lnu.se)

Affiliations:
1. Linnaeus University**,** Kalmar, Sweden

Therapeutic myosin modulators are rapidly being developed with the aim of treating a range of debilitating diseases including cardiac conditions, cancer, malaria etc. The effects of the new compounds also give detailed insights into individual processes of the normal chemo mechanical cycle as well as effects of pathologic mutations, allowing us to further identify and understand important steps required for force production. This process to gain insight is greatly aided by the rapid growth of new, easily accessible, computational tools based on artificial intelligence and similar approaches. However, to make best use of these methods it is essential to also have access to key experimental information about how the myosin motor properties change in response to the modulators. Traditional assays such as NADH-coupled, stopped-flow and in vitro motility assays contribute valuable information, but the µg-mg quantities of myosin required for such assays hampers effective studies. To overcome this challenge, we have developed a miniaturized kinetic assay, that requires only 5-10ng (10 nM in 5-10µl) of myosin motor domains. The experiments utilize total internal reflection fluorescence (TIRF) microscopy that allows us to observe binding of single fluorescent Alexa 647-ATP/ADP to myosin. In addition to previous use of this method to characterize basal ATP turnover by myosin we have developed the approach to allow determination of maximum catalytic activity, k_{cat} of the actin-activated myosin ATPase by cross-linking single human cardiac myosin motor domains (subfragment 1) to actin filaments. The motor domains used were expressed in C2C12 cells employing a non-viral transfection method. Using the described single molecule methods, we found that the myosin-active compounds Omecamtiv mecarbil (OM) and Mavacamten (MAVA) at saturating concentrations of 30 and 100 µM respectively both promote a slow actin-activated turnover pathway. This pathway with k_{cat} of 1-2 s^{-1} makes up approximately 50 % (OM) and 30% (MAVA) of the turnover at saturating modulator concentrations compared to <10 % without modulator. In the absence of a modulator k_{cat} was about 10 s^{-1} for > 90 % of the turnover. In the absence of F-actin, basal ATP turnover was ≥4-times slower in the presence of the compounds than in the absence.

#46 Monday Poster Session

COOPERATIVITY IN THERMODYNAMICALLY EQUILIBRATED MYOSIN POPULATIONS GENERATES A MANTATP BIPHASIC DECAY

Lorenzo Marcucci

Affiliations:
1. Padova University, Padova, Italy

Myosin is the skeletal muscle motor protein responsible for converting the energy of ATP into mechanical work. At rest, myosin ATPase activity is limited compared to muscle contraction, and two biochemical states with different ATP turnover have been observed: the slower was named “Super Relaxed State” (SRX), while its disordered counterpart was named “Disordered Relaxed State” (DRX). Myosin resting states play a critical role in regulating muscle contractility and basal metabolism.
The mantATP chasing technique has been widely used to assess the nucleotide turnover of resting myosin associated with the SRX. However, recent theoretical arguments have raised questions about the reliability of results obtained using this technique across various models. If SRX and DRX myosin are in thermodynamic equilibrium, the fluorescent decay should describe a single exponential despite the existence of two distinct populations. Yet, a biphasic decay occurs in mantATP assay when myosin heads are assembled in native thick filaments, possibly due to a cooperativity among motors.
Here, a Monte-Carlo approach is used to simulate how different intra- and intermolecular cooperativity hypotheses affected the hydrolyzation of the mantATP in a sarcomere. Coherently with theoretical arguments, when each myosin head was stochastically fluctuating between SRX and DRX states with no cooperativity between heads, the simulated fluorescence decay could be fitted by a single exponential. However, a model in which the SRX and DRX populations were completely out of equilibrium is not strictly needed to generate a two-exponential decay. In fact, the Monte-Carlo model based on different inter- and intraproteins cooperativity, reproduced the multiexponential decay even when the motors were individually in thermodynamic equilibrium. The multiexponential decay can be observed when at least two sets of motors with different SRX and DRX turnovers are considered, a configuration possibly not available in a purified myosin S1 preparation. Instead, an intradimer cooperativity, where the rates of the blocked head are affected by the state of the free head in the same dimer, can generate a multiple decay signal.
In this hypothesys, the native thick filaments maintain a fluid equilibrium of myosin heads distributed across a continuum of biochemical states, influenced by structural features and cooperative interactions. In this complexity, the coherence of the chasing parameters with the ATPase hydrolysis rate must be verified to ensure that the simplification of considering only two independent populations still preserves enough details to describe the system.

#47 Monday Poster Session

DISRUPTION TO THE TWISTING AND UNTWISTING OF ACTIN AND TROPOMYOSIN FILAMENTS DURING MUSCLE CONTRACTION CAN LEAD TO MUSCLE DYSFUNCTION

Tishkova M.V.[1], Karpicheva O.E.[2], Borovikov Y.S.[1]
Affiliations:
1. Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia
2. Department of Pharmacology, Physiology and Biophysics, Boston University Chobanian and Avedisian School of Medicine, Boston, MA, USA

Polarized fluorescence microscopy provided new insight into the role of actin and tropomyosin filaments in muscle force generation. Experiments were performed on single “ghost” muscle fibres reconstructed with FITC-phalloidin-labelled F-actin, troponin, 5-IAF-labelled β-tropomyosin, and 1,5-IAEDANS-labelled myosin subfragment 1 (S1). Data analysis showed that adding troponin to actin-tropomyosin at low Ca^{2+} induces actin overtwisting and increases its bending stiffness. Conversely, tropomyosin filaments untwist, decreasing their bending stiffness. As the calcium concentration increases, the actin filament untwists, facilitating the binding of myosin heads; meanwhile, the tropomyosin twists. S1 and nucleotides also caused changes in filament twisting during the ATPase cycleaccompanied by the twisting or untwisting of actin and tropomyosin filaments, resulting in a change in their bending stiffness. Thus, modelling weak binding (AM*•ATP) showed actin twisting and an increase in rigidity, accompanied by tropomyosin filament untwisting. During the transition to the AM•ADP state, the strongly bound myosin heads induce sharp untwisting of the actin filaments, while the tropomyosin filaments assume a rigid and twisted conformation. We suggest that the untwisting of supertwisted actin filaments, combined with myosin head tilting towards actin and increased tropomyosin twist and stiffness (Fig. a,b), causes thin filaments to slide along thick filaments.

Point mutations in β-tropomyosin (R91G, E117K and Q147P) significantly altered the ability of actin and tropomyosin to change their twist pattern and stiffness, thereby affecting force generation. All substitutions were associated with increased Ca^{2+} sensitivity but had different effects on tropomyosin helix ordering. Consequently, the presence of R91G-tropomyosin increased actin stiffness at high Ca^{2+} concentrations (Fig. e,f), whereas E117K- and Q147P-tropomyosin decreased it (Fig. c,d). In both cases, the changes in stiffness prevented actin from passing effectively through the stages of the ATPase cycle, thereby affecting the amplitude of its untwisting during ATP hydrolysis. These changes in actin filament untwisting and bending stiffness correlated with changes in actomyosin ATPase activity in thin filaments containing mutant tropomyosin: activity increased in the presence of the R91G substitution and decreased in the presence of the Q147P substitution. These findings suggest that actin and tropomyosin torques are involved in the molecular mechanisms of force generation and that their disruption may cause contractile dysfunction.

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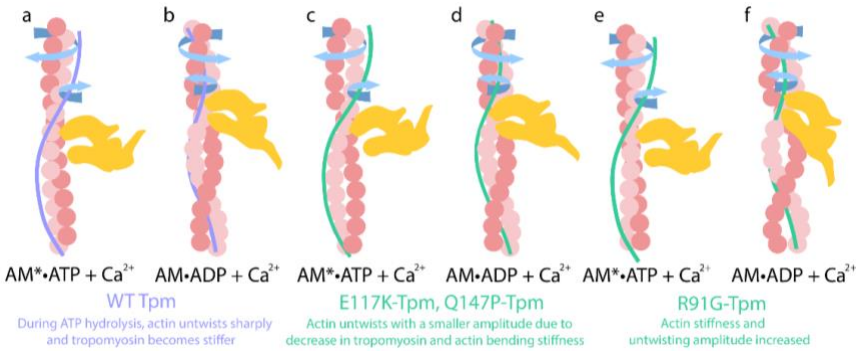
a
b
c
d
e
f
AM*•ATP + Ca2+
AM•ADP + Ca2+
AM*•ATP + Ca2+
AM•ADP + Ca2+
AM*•ATP + Ca2+
AM•ADP + Ca2+
WT Tpm
During ATP hydrolysis, actin untwists sharply and tropomyosin becomes stiffer
E117K-Tpm, Q147P-Tpm
Actin untwists with a smaller amplitude due to decrease in tropomyosin and actin bending stiffness
R91G-Tpm
Actin stiffness and untwisting amplitude increased

#48 Monday Poster Session

FUNCTIONAL AND STRUCTURAL PROPERTIES OF CARDIAC MUSCLE BUNDLES IN RESPONSE TO A HIGH FAT AND SUCROSE DIET IN FEMALE RATS

Venus Joumaa[1], Bharat Duggal[1], Abdul-Samad Ahmed[1], Mauricio Delgado[1,2], Hannah Smith[1], Nada AbuGhazaleh[1], John Holash[1], and Walter Herzog[1]

1. University of Calgary, Calgary AB, Canada
2. Pontificia Universidad Católica de Chile, Santiago, Chile

Introduction Obesity has been identified as an epidemic that affects over 650 million individuals globally (WHO, 2024). This complex disease is characterized by excessive lipid accumulation in tissues creating chronic low-level systemic inflammation and exposing them to deleterious changes (e.g., Collins et al., Front Physiol 2018).

Obesity is a major risk factor for developing cardiovascular diseases. Knowing that fat metabolism differs between males and females, obesity pathways and obesity-related comorbidities including heart diseases are likely sex-specific. While there is substantial literature investigating the alterations in the mechanical and structural properties of the heart at the cellular level in obese males (e.g., Boldt et al., Phys Act Nutr 2021), there remains a significant gap in research focused on females. This disparity stems from historical biases about female subjects in research, including concerns about hormonal fluctuations and other supposed confounding factors. This gender bias in research has resulted in a limited understanding of how obesity specifically affects female heart at the cellular level.

Purpose The aim of this study is to investigate the effect of diet-induced obesity on the functional and structural properties of the papillary muscle in female rats.

We hypothesize that obesity compromises the mechanical properties of skinned papillary bundles, and results in decreased myofibrillar proteins, increased fat and collagen accumulation, and disrupted mitochondrial function in the papillary muscle.

Methods Twelve-week-old female Sprague Dawley rats were subjected to a standard chow (n=7) and a high fat and sucrose HFS (n=7) diet for 12 weeks. At the end of the intervention, the animals were sacrificed, and the papillary muscle was used for the mechanical, structural, and mitochondrial function tests.

Mechanical properties: Skinned papillary bundles were tested for their maximal active stress and passive stress at slack length (L0)+5%, L0+10% and L0+15%, and calcium sensitivity (EC50) at L0.

Structural properties: BCA, hydroxyproline, and triglycerides assays were used to determine the myofibrillar protein, collagen and fat content respectively, in the papillary muscle. SDS-PAGE was used to determine MHC and actin content.

Mitochondrial function: Markers of mitochondrial function (COX I-IV) were assessed by western blot.

Results No significant difference was observed in maximal active stress (24.0±6.5 vs. 27.0±6.3 kPa for chow and HFS, respectively), passive stress at L0+5% (4±2 vs. 4±2 kPa), L0+10% (11±8 vs. 8±5 kPa) and L0+15% (15±8 vs. 14±9 kPa), and EC50 (1.4±0.7 vs. 1.2±0.2 μM) between groups.

Biochemical analysis revealed no significant difference in protein (92.7±18.7 vs. 89.1±15.5 µg/mg muscle) and MHC/actin content (HFS/chow=0.82±0.26 and 0.99±0.23) between the groups. Analysis of fat and collagen content and mitochondrial proteins are underway.

Discussion Unlike male rats, where diet-induced obesity disrupts the mechanical properties of the cardiac muscle (Boldt et al., Phys Act Nutr 2021), female rats appear to have preserved mechanical properties and myofibrillar protein content. This highlights potential sex-specific protective mechanisms. Further research is warranted to investigate these mechanisms.

#49 Monday Poster Session

NOVEX-3 TITIN CONNECTS THE MYOFILAMENT TO MITOCHONDRIA AND INTERACTS WITH THE CALCIUM-HANDELING SYSTEM IN SKELETAL MUSCLE

Robbert van der Pijl[1], Dalma Kellermayer[1], Justin Kolb[1], Paola Tonino[1], Balasz Kiss[1], Jochen Gohlke[1], John Smith 3rd [1], Henk Granzier[1]

Affiliations:
3. University of Arizona, Tucson, United States of America

Alternative splicing of the *Titin* gene (*Ttn*) produces isoforms that contribute to passive tension development in striated muscle. One rare isoform incorporates exon 48, which encodes a unique carboxy-terminus, known as Novex3. This variant results in a truncated titin isoform (~638 kDa; Nvx3) implicated in calcium-handling and cardiomyocyte development. Here, we investigated the role of Nvx3 in skeletal muscle function. To study Nvx3, we generated a knockout mouse lacking *Ttn* exon 48 ($Ttn^{\Delta Nvx3}$). Western blot analysis across developmental stages revealed modest Nvx3 expression in adult (6-month) cardiac and fast-twitch skeletal muscle, with approximately 1/14 titin molecules consisting of the Nvx3 isoform. In contrast, slow-twitch soleus muscle exhibited ~65% lower expression (~1/40 titin molecules). Developmentally, Nvx3 was highly expressed across all muscle types tested at embryonic day 18, postnatal day 7, and day 30, with reduced expression by adulthood. To assess functional effects of Nvx3 deletion, we performed contractility measurements in intact extensor digitorum longus (EDL) and soleus muscles from 6-month-old mice. Nvx3 deletion modestly reduced the force-frequency (FF) response in soleus muscle, accompanied by a rightward shift and delayed relaxation. In EDL muscle, FF was enhanced, but relaxation kinetics were also slowed. Muscle mass of EDL, tibialis, gastrocnemius and quadriceps were reduced at 6-months-old, indicating onset of atrophy. Immunoelectron and super-resolution microscopy localized Nvx3 primarily to the I-band in a sarcomere length-dependent manner, with additional localization at the myofibril periphery. RNA sequencing of EDL and diaphragm muscle revealed downregulation of mitochondrial genes in $Ttn^{\Delta Nvx3}$ mice. RNA sequencing also indicated increased expression of Per3, a circadian gene with roles in glucose regulation. Transmission electron microscopy showed generally preserved sarcomere structure, though some regions exhibited hypercontracted sarcomeres, Z-disk streaming, and increased autolytic mitochondria. These findings suggest that Nvx3 titin might influence both calcium and metabolic regulation in skeletal muscle, potentially through interactions with mitochondria.

#1 Tuesday Poster Session

Short and sweet – crosslinking glycation stiffens diabetic titin

Alejandro Clemente-Manteca[1], Agata Bak[1], Diana Velázquez-Carreras[1], Inés Martínez-Martín[1,2], Enrique Calvo[1], Andrea Laguillo[1], Roberto Silva-Rojas[1], Elías Herrero-Galán[1], Jesús Vázquez[1], Miquel Adrover[2], Jorge Alegre-Cebollada[1].

Affiliations:

1. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

2. Department of Physics, King's College London, London, UK.

3. Universitat de les Illes Balears, Palma de Mallorca, Spain.

Myocardial stiffening is an early hallmark of diabetes, leading to the development of diabetic cardiomyopathy and heart failure. Although the mechanism driving extracellular matrix stiffening is known, the molecular basis of cardiomyocyte stiffening remains poorly understood. To address this question, we investigate the mechanical alteration of cardiomyocytes produced by glycation, a highly prevalent posttranslational modification subsequent to high glucose levels. This modification generates non-crosslinking and crosslinking modifications in biomolecules. Our work focuses on the effect of glycation on the nanomechanical properties of titin, a protein that plays an essential role in cardiomyocyte's mechanical response.

To explore the extent of this modification in titin and in cardiomyocyte mechanics, we first demonstrate increased levels of non-crosslinking modifications in titin extracted from Ob/Ob mice (leptin KO obese mice) using mass spectrometry. Furthermore, we detect for the first time crosslinking glycations in native titin, by developing an *ad hoc* strategy based in titin domain's three-dimensional structure and mass spectrometry. We further support our mass spectrometry findings using single-molecule force spectroscopy magnetic tweezers. For this, we first demonstrate the efficacy of this technique to detect crosslinking glycations using recombinant titin domains. Then, using the features of the Halo mice (Halo-Tag titin KI mice), we measure an increase of crosslinking modifications in the native titin of Ob/Ob-Halo mice compared to their healthy counterparts. Finally, we test mutant recombinant titin using single molecule force spectroscopy AFM to reveal the spatial distribution of residues undergoing crosslinking modifications.

Our observations suggest that glycation reactions produce crosslinking modifications that stiffen titin and contribute to the altered mechanical landscape of the heart in diabetic patients.

#2 Tuesday Poster Session

ACUTE LOSS OF TITIN-BASED STIFFNESS TRIGGERS FIBROSIS AND LEFT VENTRICULAR STIFFENING

A.J. Klotz[1], P. Hartmann[1], J.K. Freundt[1], A. Unger[1], C.M. Loescher[1], F.Koser[1], L. Wachsmuth[2], R. Holtmeier[2], V. Hörr[2], C. Faber[2], O.J. Müller[3], W.A. Linke[1]

[1] University of Münster, Institute of Physiology II, 48149 Muenster, Germany; [2] University of Münster, Clinic of Radiology, 48149 Muenster, Germany; [3] University of Kiel, Department of Internal Medicine III, 24105 Kiel, Germany

Introduction: Titin, the largest human protein, is the major contributor to myocardial passive stiffness. Elevated proteolytic activity targeting the elastic I-band region of titin has been linked to common heart disease conditions, including atrial fibrillation and ischemic or chemotherapy-induced cardiac injury. Moreover, increased proteolytic activity in cardiomyocytes has been suggested to initiate reactive fibrosis and a subsequent decline in cardiac function; however, the specific role of titin cleavage in this process is not understood. Here, we use the titin cleavage (TC) mouse model to investigate the mechanical effects of in vivo titin I-band cleavage and the interplay between loss of titin-based stiffness and fibrosis.

Methods: The TC mouse contains a knock-in tobacco etch virus protease (TEVp) recognition site in the titin I-band. Cardiac specific in vivo titin-cleavage was induced via AAV9-mediated TEVp overexpression under a troponin T- promotor, delivered through tail-vein injections; AAV9-GFP served as control. Mice were sacrificed on day 6 (D6) or day 13 (D13) post-injection. Passive stress-strain curves were obtained by exposing surgically isolated left ventricular (LV) wall trabeculae to a five-step stretch protocol up to 20% strain. Fibrosis and cell proliferation were assessed by staining LV sections with anti-Ki67, anti-Periostin, and Picrosirius-red.

Results: In D6 TEVp-injected samples, about 40% of titin was cleaved, increasing to 55% at D13, while no cleavage was observed in GFP controls. At D6, passive stress was significantly reduced relative to controls, particularly at higher strains (20% strain: -44%), reflecting the expected loss of titin-based stiffness. In contrast, D13 TEVp-injected samples showed a pronounced increase in passive stress at intermediate to high stain levels (20% strain: +46%). Fibroblast proliferation peaked at D6, with $6.05 \pm 0.56\%$ Ki67-positive nuclei, declining to $1.70 \pm 0.34\%$ at D13; GFP controls remained below 1%. Co-staining with Periostin identified the cycling cells as myofibroblasts confined to interstitial regions. Picrosirius-red staining revealed minimal fibrosis at D6, but extensive interstitial fibrosis at D13, explaining the increase in passive stiffness observed on D13.

Conclusion: Our results suggest that the acute loss of titin-based passive stiffness, as induced by proteolytic cleavage in several heart disease conditions, triggers fibroblast activation, leading to fibrosis and culminating in a rebound increase in LV stiffness.

Keywords Titin – cardiac mechanics – passive stiffness – extracellular matrix – fibrosis

#3 Tuesday Poster Session

METHYLGLYOXAL GLYCATION COMPETES WITH UBIQUITINATION, DISRUPTING SARCOMERE FUNCTION

Christine E. Delligatti[1], Michaela M. Door[1], Henry M. Gong[1], Devin Nissen[2], Alexandra Pena[1], Maria Papadaki[1], Thomas G. Martin[1], Maicon L. Vieira[2], Seby Edassery[1], Weikang Ma[2], Thomas Irving[2], David Y. Barefield[1], Jonathan A. Kirk[1]

Affiliations:
1. Loyola University, Chicago, USA
2. IIT, Chicago, USA

Methylglyoxal is a reactive carbonyl species that modifies lysine residues via a process called glycation and is elevated in high-risk conditions for heart failure, like diabetes and aging. Glycation is non-enzymatic and irreversible, thus modified proteins must be removed via degradation. We hypothesized methylglyoxal competes with and blocks protein lysine ubiquitination in a dangerous feed-forward cycle of decreasing protein turnover and increasing glycation. Indeed, mass spectrometry analysis of human and mouse cardiomyocytes confirm these two PTMs share target lysine residues on sarcomeric proteins, including β-myosin heavy chain. To determine whether methylglyoxal and ubiquitination directly compete, we exposed C2C12 myoblasts and neonatal rat ventricular myocytes to methylglyoxal (100μM; 10μM respectively for initial supraphysiological, and more physiological perspectives respectively); both exhibited reduced protein ubiquitination. To test whether reduced protein turnover results in accruing glycation, we utilized BAG3 KO mice (hetero-and-homozygous), which have decreased sarcomere protein turnover. Sarcomere protein glycation was increased in BAG3 KO mice, and residues overlapped with those glycated in diabetic humans, supporting these PTMs' competition. Further, literature suggests SRX supports myosin turnover, reduces contraction, and increases relaxation. Our data (x-ray; MANT-ATP; cell culture, microscopy) shows methylglyoxal increases SRX (MANT-ATP), but decreases filament proximity (X-Ray), and impairs autophagy (cell culture; microscopy), suggesting methylglyoxal prevents myosin turnover by preventing myosin ubiquitination and interaction with chaperones. We previously showed sarcomere glycation reduced contractile function, so this feed-forward mechanism has the potential to worsen function. Thus, we tested whether increased glycation impacted sarcomere function in BAG3 KO mice. Force-calcium measurements were performed pre- and post-100μM methylglyoxal treatment. As previously, methylglyoxal caused a significant decrease in maximum calcium activated force (Fmax) in wild-type mice, but this effect was reduced in BAG3 KO mice, Indicating elevated protein glycation already impacted these mice. These results underscore the possibility that glycation/ubiquitination crosstalk impacts sarcomere function across multiple high-risk conditions. Breaking this loop therapeutically may be efficacious in treating diseases with increased glycation.

#4 Tuesday Poster Session

THE BEHAVIOUR OF PHYSIOLOGICAL PARAMETERS OF ISOLATED PERFUSED RAT HEART IN EXPERIMENTALY INDUCED CARDIAC HYPERTROPHY WITH ISOPROTERENOL

Cristian Romeo Revnic1,Flory Revnic,2

1.UMF"Carol Davila", Cardiology Department, Bucharest,Romania, 2.National Institute of Gerontology and Geriatrics"Ana Aslan" Biology of Aging Department, Bucharest,Romania

To investigate the effect of hormone treatment Testosteron (T), Hydrocortisone (Hy), estradiol (Es) associated with Isoproterenol (ISO) on cardiovascular system in rats of different genders.

The study was conducted on 40 Wistar rats aged 8 months old :20 male and 20 female divided into 4 groups of 10 rats each (5male and 5 female): A. Controls treated with 0.9% saline, B. treated for 20 days with ISO i.m. injections in order to induce cardiac hypertrophy, C. treated with (Hy) (1,8mg/kg), D. male rats treated with (T) 6,75 mg/kg, female rats treated with (Es) 0,45 mg/kg i.m. injections. Since the 8th day of treatment, the animals have received also treatment with ISO (0.5 mg/kg. Coronary flow (C.F.), Heart rate (H.R.) and Left venticle developed pressure (LVDP) were determined in Langendorff retrograde perfusion system for all groups.

Following hormone treatment there is an increase in GGT in male versus controls. Hormone treatment associated with ISO lead to amplification of enzymatic activity but not to the levels of Controls .In female rats, Hy administration lead to a decrease of enzyme activity while estradiol lead to amplification both for controls and (I) administration.GST behaviour in male treated with (ISO) does not influence values of enzymatic activity, while hormone association lead to a decrease in levels in (C) and (I) treated. In female, hormone treatment lead to a decrease in enzyme activity. Association (H)+(I) has no effect. Synthesis and utilization of GSH for inhibition of ROS and for free O radicals is limited in male rats treated with Isoproterenol or in association with (T). Redox potential of myocardium is intensified in female treated with(I) alone or in association with Estradiol.

Isoproterenol induced marked endocardial injury, associated with hypertrophy of surviving myocytes.

The hearts from isoproterenol-treated rats demonstrated decreased LV compliance, as evidenced by an upward shift in the diastolic pressure–volume relationship, with normal LV systolic function.

Our data have pointed out that myocardial insufficiency has biochemical particularities related to gender and etiologic factors.

REdox potential is expressed in both genders, male having an increased SH reserve while in female enzymatic activity is amplified being engaged in inhibition of deleterious effects of oxidative stress. Our data may have a clinical significance .

Isoproterenol administration provides a simple, non invasive tool means to induce endocardial injury and diastolic dysfunction without significant impairment of systolic function.

This experimental model has a low incidence of mortality and may be useful to assess the effects of gene or stem cell therapy on cardiac dysfunction without the potential confounding effects of invasive procedures.

#5 Tuesday Poster Session

CARDIAC PROTEOMIC AND PHOSPHOPROTEOMIC SCREENING OF HUMAN HEART FAILURE WITH PRESERVED VERSUS REDUCED EJECTION FRACTION

F Koser[1], M Abdellatif[2], T Bock[3], M Krüger[3], S Sedej[2], WA Linke[1]

Affiliations:
1. Institute of Physiology II, University Hospital Münster, Münster, Germany
2. Division of Cardiology, Medical University of Graz, Graz, Austria
3. Institute for Genetics, University of Cologne, Cologne, Germany

Introduction: The prevalence of heart failure with preserved ejection fraction (HFpEF) is anticipated to rise in tandem with the prolongation of our lifespan and the increasing burden of comorbidities. Despite the significant public health implications, evidence-based therapeutic interventions for HFpEF are limited and the pathomechanism remains elusive. However, low-grade systemic inflammation and endothelial dysfunction have been postulated to contribute. Here, we aimed to identify a cardiac proteomic and phosphoproteomic signature that distinguishes HFpEF from heart failure with reduced ejection fraction (HFrEF), compared to nonfailing donor hearts.
Methods & Results: Left ventricular myocardial tissues of explanted or donor human hearts (Medical University Graz) were classified into nonfailing Control, HFpEF or HFrEF (mean EF (%): 63 (Ctrl), 62 (HFpEF), 24 (HFrEF); mean age 58.1±9.5; mean BMI 26.6±2.7) and were analysed by quantitative mass spectrometry (N=6-7/group). Cardiac proteome analysis identified 49 significantly regulated proteins (fold-change >1.5, $p<0.05$) in HFpEF and 161 in HFrEF versus Control hearts, out of 1813 cardiac proteins detected. Gene Ontology (GO) enrichment analysis of significantly regulated proteins in HFpEF versus Control hearts revealed only two biological process terms, "complement activation" (e.g., C6, C4B) and "innate immune response" (e.g., S100A8, STING1). However, these terms also appeared in the analysis of HFrEF versus Control hearts indicating low-grade systemic inflammation as a potential disease factor in both HFpEF and HFrEF hearts. The presence of low-grade systemic inflammation in either heart failure syndrome was confirmed by immunoblot analyses and immunohistochemistry, which demonstrated substantially increased ICAM1 and S100A8 expression, respectively, in failing versus Control hearts. Phosphoproteome analysis revealed 83 phosphosites in cardiac proteins that were differentially expressed (fold-change >1.5, $p<0.05$) in HFpEF and 181 in HFrEF versus Ctrl hearts, out of 1045 different phosphosites mapped in cardiac proteins. Phosphoproteomic alterations in HFpEF and HFrEF versus Control hearts primarily concerned sarcomeric proteins (e.g. titin, desmin, troponin I), which were predominantly hyper-phosphorylated. Moreover, gene ontology enrichment analysis revealed that proteins associated with “protein kinase binding“ (e.g., protein phosphatase 1 regulatory subunits 12A, 12B and 12C) were hyper-phosphorylated in HFpEF versus Control.
Conclusion: Low-grade systemic inflammation is present in both HFpEF and HFrEF patient hearts. There is no distinct HFpEF inflammatory phenotype in this cohort that could be used for diagnostics or HFpEF-specific therapy development. However, our data suggest a link between systemic inflammation and sarcomeric protein phosphorylation, which we will further investigate in follow-up studies.

#6 Tuesday Poster Session

CONTRACTILITY MEASUREMENTS COMBINED WITH SINGLE CELL PERFUSION REVEAL KINETICS AND VARIABILITY OF ISOPRENALINE RESPONSE IN MOUSE CARDIOMYOCYTES

Ilse S. Altenburg, Daan Hoomoedt, Valentijn Jansen, Michiel Helmes, Jolanda van der Velden, Diederik W.D. Kuster

Affiliations:

1. Amsterdam University Medical Center, Amsterdam, The Netherlands
2. Cytocypher BV, Amsterdam, The Netherlands

Contractility measurements combined with single cell perfusion reveal kinetics and variability of isoprenaline response in mouse cardiomyocytes

The heart has to generate pressure through contraction and allows filling through relaxation of cardiomyocytes. However in heart diseases such as heart failure and cardiomyopathies, impaired contraction and relaxation are observed. Therefore, there is a strong need for compounds that can modulate contraction and relaxation. Drug discovery and hit-to-lead development of new compounds is hampered by three main challenges: (1) large variation in contractile behavior of cardiomyocytes under control conditions, (2) poorly understood variability in drug response between cells from the same patient or mammal, and (3) large sample volumes are required for drug testing. The aim of this project is to develop a stable and reproducible methodology to measure single cell drug response in very small volumes. We have integrated contractility measurements in single CMs (MultiCell, CytoCypher) with single cell perfusion (Biozone, Fluicell). We have performed paired measurements of β-adrenergic agonist isoprenaline (15 nM) on individual adult mouse cardiomyocytes (n=22 at t=0). Expected effects of increasing contractility and speeding up relaxation were seen, but with different time profiles (Fig 1 A and B). Variation in drug response was seen between cells, but these effects appeared smaller than in traditional measurements where the drug is applied to the dish directly. The perfusion system was also adapted to allow lysing and collecting of individual cells can for downstream molecular analysis. This will enable correlation between contractile response and the underlying signaling pathways. With this approach we will accelerate cardiac drug discovery by reducing compounds usage and providing insights into drug response at single cell level.

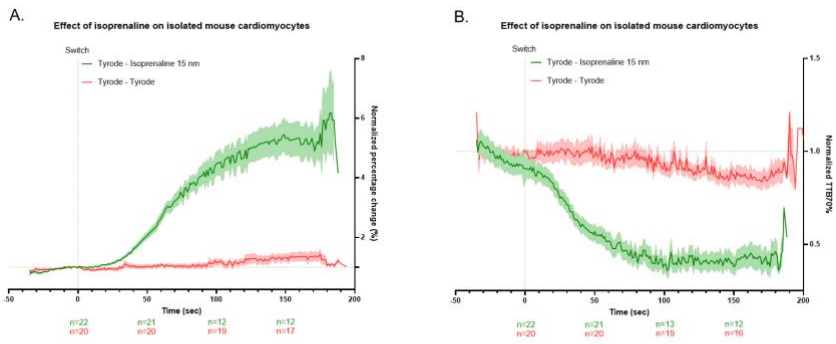

Figure 1. Increased contractility and accelerated relaxation in individual adult mouse cardiomyocytes after perfusion with β-adrenergic agonist isoprenaline. Each transient was normalized by mean of first 5 transients before switching solution. A) Increased percentage change (%) was seen in individual adult mouse cardiomyocytes (n=22 at t=0) perfused with isoprenaline (15nm) compared to individual adult mouse cardiomyocytes perfused with tyrode (n=20 at t=0). B) Decreased TTB70% was detected in individual adult mouse cardiomyocytes (n=22 at t=0) after isoprenaline treatment (15nm) relative to individual adult mouse cardiomyocytes (n=20 at t=0) perfused with tyrode.

#7 Tuesday Poster Session

SPECIFIC CLEAVAGE OF CARDIAC TITIN IN VIVO DEMONSTRATES THE IMPORTANCE OF TITIN IN THE GENERATION OF RESTORING FORCES DURING DIASTOLE.

Johanna K. Freundt[1], Paulina Hartmann[1], Andreas Unger[1], Lydia Wachsmuth[2], Cornelius Faber[2], Oliver J. Müller[3], Wolfgang A. Linke[1]

Affiliations:
1. University of Münster, Institute of Physiology II, Muenster, Germany
2. University of Münster, Clinic of Radiology, Muenster, Germany
3. University of Kiel, Department of Internal Medicine III, Kiel, Germany

The giant sarcomere protein titin provides passive stiffness to cardiomyocytes. Pathological changes in cardiac stiffness impair heart function, particularly in heart failure with preserved ejection fraction. To investigate the impact of titin stiffness loss in vivo, we used a transgenic mouse model enabling specific cardiac cleavage of elastic titin via a TEV protease (TEVp) site inserted into the titin spring region, the titin cleavage (TC) mouse. Cardiac-specific titin cleavage was induced by AAV9-mediated overexpression of TEVp under a cTnT promoter; AAV9-eGFP served as control.
Two weeks post-injection, 54.4±2.8% (N=17) of titin were cleaved in homozygous TC-mice. Cardiac MRI analyses of homozygous TC mice revealed significantly reduced left ventricular internal diameters (diastole and systole), cardiac output, and volumes in AAV9-TEVp–injected mice (N=11), compared to GFP controls (N=6). Systolic septum diameter was significantly increased 6 days post-AAV9-TEVp-injection and the outer diameter of the heart was reduced. Microscopic analyses of cardiomyocytes revealed a "streaming" at the intercalated discs of cells with cleaved titin. Proliferating myofibroblasts appeared in interstitial niches, and histology revealed a sixfold increase in fibrosis by day 13. Ex vivo analysis of single cardiomyocytes demonstrated impaired sarcomere length recovery in activation-relaxation cycles following titin cleavage.
These findings highlight titin's essential role in cardiomyocyte structure, mechanical integrity, and heart function. Cleavage of more than 30% of elastic titin disrupts elastic recoil, impairs diastolic filling, induces concentric remodeling, and leads to diastolic heart failure.

#8 Tuesday Poster Session

FUNCTIONAL IMPACT OF CELL-TO-CELL VARIABILITY IN CMYBP-C EXPRESSION IN A HUMAN IPSC MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Karina Ivaskevica[1], Kathrin Kowalski[1], Birgit Piep[1], Jana Teske[2], Joachim Meißner[1], Maike Kosanke[3], Ante Radocaj[1], Judith Montag[4], Robert Zweigerdt[2], Theresia Kraft[1], Sarah Konze[1]

Affiliations:
1. Institute of Molecular and Cell Physiology, Hannover Medical School (MHH), Hannover, Germany
2. Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Clinic for Cardiac, Thoracic, Transplantation and Vascular Surgery, MHH, Hannover, Germany
3. Research Core Unit Genomics, Hannover Medical School (MHH), Hannover, Germany
4. Medical School Berlin (MSB), Berlin, Germany

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disorder characterized by left ventricular hypertrophy, diastolic dysfunction, and an increased risk of sudden cardiac death. Approximately 40% of sarcomeric mutations associated with HCM occur in the *MYBPC3* gene, which encodes cardiac myosin-binding protein C (cMyBP-C). Mutations in *MYBPC3* gene are predominantly heterozygous, presenting with highly variable clinical phenotypes. Previous studies based on heart tissue sections from *MYBPC3* mutation carriers have shown burst-like transcription of *MYBPC3* and variable cMyBP-C protein distribution from cardiomyocyte to cardiomyocyte. This phenotype was accompanied by functional heterogeneity in calcium sensitivity and force generation.

We hypothesize that cell-to-cell variability in cMyBP-C expression destabilizes myocardial syncytium and contributes to HCM progression. To study the phenotype progression *in vitro*, we developed a human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) model carrying the patient-specific heterozygous truncating mutation *MYBPC3* c.927-2A>G. This mutation was identified in a patient who progressed to end-stage heart failure and underwent heart transplantation at age 45. Mutant and isogenic control hiPSC-CMs were cultured on laminin-coated and micropatterned substrates for over 70 days and analyzed longitudinally for protein expression, sarcomere structure, contraction, and transcriptomic profiles.

Immunofluorescence analysis revealed a variable cMyBP-C expression pattern from cell to cell in mutant CMs, closely recapitulating the phenotype observed in patient heart tissue. Functionally, *MYBPC3* c.927-2A>G hiPSC-CMs demonstrated reduced contraction velocity. Calcium transients were slightly accelerated in mutant CMs, suggesting an imbalance between calcium handling and contractile function. Transcriptomic analysis revealed upregulation of HCM-relevant signaling pathways, including those involved in calcium handling, sarcomeric contraction, and profibrotic MAPK and TGF-β signaling. To further explore the impact of cell-to-cell variability of cMyBP-C expression on cardiac tissue function, we are employing a novel titin-NeonGreen-tagged hiPSC-CM line for real-time sarcomere visualization and single-cell contractility analysis within multicellular clusters. This advanced system enables direct correlation of sarcomeric protein expression with contractile performance, offering new insights into the cellular mechanisms of HCM progression and potential targets for intervention.

In conclusion, our patient-specific hiPSC-CMs model robustly recapitulates key aspects of the *MYBPC3* c.927-2A>G HCM phenotype and indicates that variable cell to cell cMyBP-C expression may drive myocardial dysfunction in HCM patients.

#9 Tuesday Poster Session

INVESTIGATING VCP-RELATED CARDIOMYOPATHIES:

FROM ZEBRAFISH MODEL TO IPSC-DERIVED CARDIOMYOCYTES

Larissa Hartmann[1], Federica Diofano[1], Philipp Voisard[1], Steffen Just[1]

[1]Uniklinik Ulm, Germany

Valosin Containing Protein (VCP) is an ATPase playing a critical role in various cellular processes, including protein degradation, organelle function, and maintenance of cellular homeostasis. In humans, mutations in the *VCP* gene are linked to multisystem proteinopathies, particularly cardiomyopathy and neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) and inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD).

Despite the importance of VCP in cellular function, modeling complete VCP deficiency *in vivo* remains challenging due to its essential role during early development (as full knockout often results in embryonic lethality, such as in mice). This developmental constraint complicates the investigation of VCP dysfunction and, thus, our general understanding of VCP's molecular functions in postnatal tissues like the heart.

To overcome this limitation, we employed two complementary experimental systems to study *VCP* deficiency in cardiac cells. Therefore, we used a constitutive VCP knockout zebrafish line generated *via* CRISPR/Cas9 technology as *in vivo* model. For a complementary *in vitro* model, we employed shRNA-mediated knockdown of *VCP* in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) – marking the first application of this approach in human cardiac cells.

Together, our deficient models reveal insights into the molecular pathways disrupted by VCP deficiency and provide powerful tools for advancing our understanding of VCP-related cardiomyopathies.

#10 Tuesday Poster Session

HYPERTROPHIC CARDIOMYOPATHY-LINKED MISSENSE VARIANT IN ALPHA-ACTININ-2: STRUCTURAL AND FUNCTIONAL CHARACTERISATION

Maya Noureddine[1], Fiyaz Mohammed[1], Halina Mikolajek[2], Nathan Coweison[2], Paul Robinson[3], Alexandre Slater[1], Siobhan Loughna[4], Chris Denning[4], Sophie Broadway-Stringer[1] and Katja Gehmlich[1,3]

[1] University of Birmingham, UK
[2] Diamond Light Source, UK
[3] University of Oxford, UK
[4] University of Nottingham, UK

Introduction: Alpha-actinin 2 (ACTN2) is a key protein at the Z-disk of the sarcomere. It is critical for stabilizing the contractile muscle apparatus and organizing actin filaments. Genetic missense variants of *ACTN2* are associated with hypertrophic cardiomyopathy (HCM), a disease characterized by sudden cardiac death. In 2014, a study identified an *ACTN2* missense variant, M228T, in a family of 11 patients. In addition, our previous *in vivo* study showed that mice homozygous for M228T were embryonically lethal. The mechanism by which the M228T variant impacts ACTN2 is poorly understood. Herein, we examine the changes occurring at the structural level of the mutant protein using structural modelling and biophysical characterization techniques. The functional impact of the *ACTN2* M228T variant was further assessed in cellular models.

Methods: To assess the structural consequences of the *ACTN2* M228T variant, it was expressed in *E. coli,* purified, and analysed using a range of biophysical assays, e.g. mass photometry, X-ray crystallography, small-angle X-ray scattering, actin-binding and thermal denaturation assays. Functional and molecular assessments of the *ACTN2* M228T variant were performed using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM).

Results: Structural modelling predictions revealed that the ACTN2 M228T variant adversely impacts on the regulatory function of the actin-binding domain. This hypothesis was confirmed by higher affinity to F-actin in binding assays. The mutant protein exhibited increased aggregation identified through mass photometry and size exclusion chromatography coupled with either multi-angle light scattering or small-angle X-ray scattering (SAXS). Moreover, the mutant protein demonstrated reduced solubility and structural stability based on solubility and enzymatic digestion assays. The thermal stability of mutant protein was also compromised, as assessed through SAXS and thermal denaturation assays.

To correlate the identified structural alterations with disease mechanisms, the functional implications of *ACTN2* M228T variant were assessed using an iPSC-CM model. Mutant cardiomyocytes showed increased ACTN2 aggregate formation and upregulation of hypertrophy, fibrosis, and autophagy markers. Furthermore, significant degradation of ACTN2 was observed through biochemical fractionation assays. ACTN2 destabilization was further evaluated using proteasome and protease inhibitors targeting the ubiquitin-proteosome system and the autophagy-lysosomal pathway, to elucidate their potential roles in the protein

degradation process. In addition, mutant cells demonstrated impairement in autophagy, which might imply the involvement of additional mechanisms.

Conclusion: Collectively, this study provides valuable insights into the effects of *ACTN2* M228T variant on protein structure and function. The integration of structural and functional approaches represents a crucial step towards understanding disease pathways for cardiomyopathy-linked *ACTN2* variants.

#11 Tuesday Poster Session

PRECISE GENOME EDITING OF INHERITED TITINOPATHIES IN HIPSCS-DERIVED CARDIOMYOCYTES

Natalia S. Rojas-Galvan[1], Mathias Gautel[1]

Affiliations:
1. Randall Centre of Cell & Molecular Biophysics, Kings College London, United Kingdom

Titin is the largest protein in the human body, spanning a whole half sarcomere, the basic contractile unit of striated muscle. Here, it fulfils crucial roles as a molecular scaffold, providing mechanical integrity during muscle contraction and relaxation, as a ruler, determining the sarcomere length, and as a signalling hub. Mutations in the gene encoding titin (TTN) are strongly associated with various (cardio)myopathies, in particular truncating variants (TTNtv), which are a major risk factor for the development of dilated cardiomyopathy (DCM). Nonetheless, TTNtv are also found in 1-3% of general population who present no symptoms, which poses an issue to the establishment of a clear diagnosis criteria. More recently, it has been hypothesised that destabilising missense variants (TTNdmv) could act as a genetic second hit when co-inherited with TTNtv that influences their phenotypic penetrance. In this context, we aim to study this hypothesis using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). We combined precise genome editing with adenine base editors (ABE) and CRISPR-Cas9 to introduce patient-specific missense and nonsense mutations in TTN. Then, we followed a small-molecule differentiation protocol to generate a monolayer of cardiomyocytes from our wild-type and edited hiPSC-lines to study the pathological molecular pathways that lead to a DCM-like phenotype. To investigate the molecular and functional consequences of these patient-specific mutations, we performed immunofluorescence staining of sarcomeric proteins (α-actinin and titin), evaluated functional contractile parameters and calcium handling using live-imaging of Cal520-loaded hiPSC-CMs, and investigated alterations in the protein quality control pathways. Presence of mono- and biallelic TTNdmv led to modest but significant decrease in sarcomere organisation. Ongoing work focuses on identifying specific molecular pathways and mechanistic insights that may drive these pathological changes. Our findings contribute to understanding the interplay between TTNdmv and TTNtv in DCM and may improve genetic risk stratification and potentially personalized therapeutic approaches.

#12 Tuesday Poster Session

TIME RESOLVED MODULATION OF FAST SKELETAL AND SLOW CARDIAC CONTRACTILE PROPERTIES BY AFICAMTEN IN THE 12-25 °C TEMPERATURE RANGE

Nicoletta Piroddi[1], Beatrice Scellini[1], Marianna Langione[1], Marica Dente[1], Cecilia Ferrantini[1], Josè Manuel Pioner[2],Corrado Poggesi[1],Chiara Tesi[1]

[1]University of Florence, Department of Experimental and Clinical Medicine DMSC, Florence, Italy.
[2]University of Florence, Department of Biology, Florence, Italy.

Aficamten is a Mavacamten-based small molecule acting as an allosteric inhibitor of myosin, designed to restore cardiac sarcomere contractility to treat obstructive Hypertrophic CardioMyopathy (HCM) patients (Chuang et al, 2021). Aficamten is currently being tested in phase II and III clinical trials and has demonstrated improved safety, efficacy and usability as expected in the development of next generation cardiac myosin inhibitors (Davis et al, 2025). Although Mavacamten and Aficamten induce a similar depression of myosin ATPase and contractility within the cardiac sarcomere, the binding sites of the two drugs to the myosin motor have been shown to be different, leading to the hypothesis that different mechanisms of action converge in the ultimate effect of reducing the number of functionally available motors on the thick filament (Hartman et al, 2024). Here we characterised the effect of Aficamten on maximal isometric force development in single or small bundles of myofibrils isolated from fast skeletal (rabbit psoas) and slow cardiac (minipig ventricle) muscle, calcium activated and relaxed by rapid solution switching. Myofibrils after mounting in relaxing solution (pCa 9; [Pi] ~ 200 μM, 15 or 25 °C) were fully activated (pCa 4.5) and then suddenly moved to and from a second flux of activating solution containing selected μM concentrations of Aficamten ("jumps"; time for solution change ~10 ms). Relaxation from force was achieved by returning myofibrils to the relaxing solution. When subjected to Aficamten jumps, both myofibril types responded with a slow and temperature-dependent decrease in force. The rate of Aficamten-induced force decrease in fast skeletal or slow ventricular myofibrils correlated with the difference in resting ATPase rate associated with the different myosin isoforms present (MyHC-1 or MyHC-7). The effect of Aficamten was found to be fully reversible. Dose-response curves confirmed a higher sensitivity of beta cardiac muscle (IC50 ~1 μM) compared to fast skeletal (IC50 ~10 μM), as previously reported for the pCa50-activated ATPase of the same myofibrillar systems and intact and skinned adult rat ventricular samples (Hartman et al, 2024). Interestingly, Aficamten also reduced the rate of force development in fast skeletal muscle and, to a lesser extent, in the slow ventricle, similar to that reported for Mavacamten (Scellini et al, 2021). These preliminary results suggest that Aficamten strongly affects the regulatory state mediated by the availability of strong actin-binding heads, mainly acting on slow-cycling motors, without inducing fast cross-bridge dissociation as in the case of Mavacamten.

#13 Tuesday Poster Session

INVESTIGATING THE PROTEASOMAL REGULATION AND SARCOMERIC PROTEIN COMPOSITION OF MYOPATHY-ASSOCIATED AGGREGATES

Tom P. Mitchell[1], Mathias Gautel[1]

Affiliations:
1. King's College London, London, UK

Protein aggregates are observed in both in clinical observations and experimental models of cardiac and skeletal myopathies. Such aggregates can contain mutant sarcomeric proteins, established binding partners, poly-ubiquitin chains components of proteasomal machinery. However, their contents, etiology and contribution to disease are poorly established, compared to aggregates found in neurological disorders such as Parkinson's disease. A recent finding (Eibach *et al.* 2025) that increasing the activity of the deubiquitinating enzyme UPS5 is associated with better outcomes for aggregating myopathies in mouse models suggest that aggregate sequestration of ubiquitin may contribute to disordered proteostasis in the muscle. The presence of established binding partners may also suggest a role in sequestering sarcomere components. I will Present preliminary investigations into aggregate formation and regulation in myopathy, with a focus on adapting established techniques from neurology.

#14 Tuesday Poster Session

A SKELETAL MUSCLE–SYMPATHETIC NEURON AXIS CONTROLS CARDIAC FUNCTION

V. Di Mauro[1,2,3], Marco Ronfini [1,2], Valentina Prando [1,2], Laura Poli [1,2], Anais Franco-Romero [1,2], Induja perumal-Vanaja [5], Lolita Dokshokova [1,2], Roberta Sartori [1,2], Mattia Albiero [6], Laura Ventura [7], Gianni Sorarù [8], Michele Guescini [9],Daniele Catalucci [4], Marco Mongillo [1,2,3], Marco Sandri [1,2,3,§], Tania Zaglia [1,2,3,§]

1, Department of Biomedical Sciences, University of Padova, Via U. Bassi 58/b, 35131 Padova, Italy;
2, Veneto Institute of Molecular Medicine (VIMM), Via G. Orus 2, 35129 Padova, Italy;
3, Interdepartmental Research Center of Myology (cirMYO), Via Ugo Bassi 58/B, 35131, Padova, Italy;
4, Humanitas Research Hospital, Via Manzoni 113, 20089 Rozzano, Milan, Italy;
5, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova, Via Giustiniani 2, 35128 Padova, Italy;
6, Department of Medicine, University of Padova, Via Giustiniani 2, 35128 Padova, Italy;
7, Department of Statistical Sciences, University of Padova, Padova, Italy;
8, Department of Neurosciences, University of Padova, via Belzoni 160, 35121 Padova, Italy;
9, Department of Molecular Sciences, University of Urbino Carlo Bo, Via I. Maggetti, 26/2, 61029 Urbino, Italy.

Background:
Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disorder characterized by progressive motor neuron (MN) degeneration and severe skeletal muscle atrophy[1] . While traditionally considered a neurocentric disease, recent evidence supports a multisystemic pathophysiology in which non-neuronal cells actively influence disease onset and progression. Particularly, skeletal muscle is increasingly recognized as an active player rather than a passive target of MN loss[2].

Methods: Using ALS mouse models and models of impaired skeletal muscle autophagy, we investigated inter-organ communication between muscle and the heart. Circulating microRNAs and extracellular vesicles (EVs) were profiled, and cardiac sympathetic innervation and function were assessed. Findings were validated in ALS patient samples and correlated with autonomic markers.

Results: We identified a pathological muscle-to-heart signaling axis mediated by the microRNA miR-206. In ALS and autophagy-deficient muscles, miR-206 is released in EVs, enters the circulation, and accumulates in cardiac sympathetic neurons. There, it represses $p75^{NTR}$ expression, destabilizing NGF–TrkA signaling, leading to sympathetic denervation, autonomic imbalance, and increased arrhythmogenic susceptibility. ALS patients exhibited similar elevations of circulating miR-206 and autonomic dysregulation.

Conclusion: Skeletal muscle actively drives cardiac dysautonomia in ALS *via* a miRNA-based inter-organ communication axis. These findings uncover a novel pathogenic mechanism linking neuromuscular and cardiovascular systems and may open new avenues for biomarker development and therapeutic interventions targeting inter-organ signaling in ALS and related disorders.

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2. Martin, L. J. & Wong, M. Skeletal Muscle-Restricted Expression of Human SOD1 in Transgenic Mice Causes a Fatal ALS-Like Syndrome. *Front Neurol* **11**, (2020).

#15 Tuesday Poster Session

THE STRUCTURE AND FUNCTION OF ZEBRAFISH NEBULETTE

Yaniv Hinits[1] and Mathias Gautel[1]

Affiliations:
1. Randall Centre for Cell and Molecular Biophysics, School of Basic and Medical Biosciences and British Heart Foundation Centre of Research Excellence, Faculty of Life Sciences and Medicine, Guy's Campus, King's College London, London SE1 1UL, UK

The nebulin-family protein Nebulette is expressed in cardiac muscle and is located to the Z-disk in heart sarcomeres. It binds actin and interacts with other thin filament and Z-disk proteins. Mammalian *Nebl,* is thought to be involved in myofibril assembly and in stabilizing actin filaments, like nebulin. *NEBL* mutations in human have been linked to dilated cardiomyopathy and endocardial fibroelastosis, and transgenic mice expressing these mutations develop severe cardiac phenotypes. *Nebl* KO mice have normal cardiac function, but show Z-disk widening and up-regulation of cardiac stress markers.

To further investigate the role of Nebulette in the sarcomere, we conducted a bioinformatic search and RT-PCR and found the yet undiscovered zebrafish *nebl* gene in a newly unannotated release of the zebrafish genome. Zebrafish *nebl* gene shows similar genomic structure to the mammalian gene, coding for both Nebl protein and for LIM-Nebl (Lasp2). The C-terminal zebrafish Nebl protein has a SH3 domain and a serine-rich linker domain, both highly homologous to the mammalian NEBL but contains 40 nebulin-repeats with the predicted α-helical secondary structure and a central conserved S(D/E)XXY(K/R) motif, compared with 23 repeats in mammals. The linker domain is subject to alternative splicing, and in some isoforms is longer and differs from the mammalian one. In situ hybridisation reveals that zebrafish *nebl* is expressed exclusively in cardiac tissue from early heart development, whereas LIM-*nebl* show no distinct expression pattern. Conservation in Nebl genomic structure and protein sequence suggest a conserved role for Nebl during cardiac development and maintenance. To test that, we have used CRISPR/Cas9 to obtain *nebl* loss-of-function model.

#16 Tuesday Poster Session

THE SLOW DELAYED RECTIFIER POTASSIUM CURRENT (IKS) IS DIFFERENTLY REGULATED UNDER BASELINE CONDITIONS AND FOLLOWING β-ADRENERGIC STIMULATION IN CANINE VENTRICULAR CARDIOMYOCYTES

Zsigmond Máté Kovács1,4, Balázs Horváth1, Csaba Dienes1, József Óvári1, Norbert Szentandrássy1,2, János Magyar1,3, Tamás Bányász1, Péter P. Nánási1

Affiliations:
1. Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
2. Department of Basic Medical Sciences, Faculty of Dentistry, University of Debrecen, Debrecen, Hungary
3. Division of Sport Physiology, Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
4. HUN-REN-DE Cell Physiology Research Group, University of Debrecen, Debrecen, Hungary

Sympathetic activation robustly increases the slow delayed rectifier K^+ current (I_{Ks}) in the mammalian ventricular myocardium, however, exact downstream pathways involved in the β-adrenergic regulation of the current are not fully elucidated yet.

This study examined the Ca^{2+} sensitivity of I_{Ks} and the contribution of the protein kinase A (PKA) and the calcium/calmodulin kinase II (CaMKII) pathways in regulating I_{Ks} in isolated canine ventricular myocytes. Experiments were carried out under ß-adrenergic receptor activation (10 nM isoproterenol) and under baseline conditions (without isoproterenol). I_{Ks} was measured as an HMR-1556 sensitive current with the action potential voltage clamp technique under the physiological intracellular calcium homeostasis of the cells.

Reducing intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) with 1 μM nisoldipine decreased the peak and mid-plateau densities of I_{Ks}, reduced the current integral, and increased the time-to-peak value. In contrast, ß-adrenergic receptor activation by isoproterenol resulted in larger I_{Ks} densities and integral, and shorter time-to-peak value. These effects of isoproterenol on I_{Ks} were significantly smaller when the CaMKII inhibitor 1 μM KN-93 was present in the cells, but the PKA inhibitor 3 μM H-89 did not exert such effect. Importantly, all effects of isoproterenol on I_{Ks} have fully developed even in the presence of 1 μM nisoldipine.

Under baseline conditions the mid-plateau density of I_{Ks}, was significantly smaller in the presence of KN-93, H-89 or nisoldipine, while peak I_{Ks} density and the current integral were significantly smaller only in nisoldipine.

In conclusion, many different signaling pathways are involved in regulating I_{Ks}. Under baseline conditions the regulation is strongly $[Ca^{2+}]_i$-dependent, with PKA and CaM-CaMKII involved, whereas during ß-adrenergic stimulation it is $[Ca^{2+}]_i$-independent and supposes a pivotal role of EPAC-mediated activation of CaMKII.

#17 Tuesday Poster Session

TENSION TRANSIENTS IN MUSCLE UPON SUDDEN CHANGES IN ORTHOPHOSPHATE CONCENTRATION – A CRITICAL ASSESSMENT BASED ON A THEORETICAL ANALYSIS

Alf Månsson[1]

Affiliations:
1. Linnaeus University, Kalmar, Sweden

The relative timing of the force-generating power stroke and release of the ATP-hydrolysis product ortophosphate (Pi) in actomyosin energy transduction is debated. It has bearing on the tension response to sudden changes in [Pi] during isometric muscle contraction (Pi-transients; rate constant k_{Pi}) and the initial rate of force rise (k_{tr}) at varied [Pi]. Simple kinetic schemes, ignoring the range of elastic strains of actin-attached myosin cross-bridges, are frequently used to interpret such experiments. The only successful scheme identified has force-generation coincident with actin-myosin attachment. This accounts for single exponential Pi-transients and evidence that $k_{tr}=k_{Pi.}$ However, it is not consistent with the high power output of muscle. We therefore turned to a mechanokinetic model, allowing consideration of the varying elastic cross-bridge strains. We assume Pi-release between cross-bridge attachment and the power stroke but power strokes occurring only if cross-bridges attach in a pre-power-stroke state with zero or negative elastic strain (counteracting shortening). This model suggests two Pi-transient components: one attributed to slow cross-bridge detachment from the pre-power-stroke state at positive elastic strain and the other due to Pi-induced shifts in equilibrium with rapid power stroke reversal. The slow component dominates but the fast component is ubiquitous for all parameter values tested, predicting a biphasic Pi-transient in disagreement with experiments. However, some parameter values give a fast phase of only very low amplitude We also show that the assumption of secondary Pi-binding sites on myosin outside the active site removes the fast component albeit predicting that $k_{tr} \neq k_{Pi}$.

#18 Tuesday Poster Session

NOVEL PLATFORM FOR BIOENGINEERING SKELETAL MUSCLE FOR HIGH RESOLUTION IMAGING AND FUNCTIONAL ANALYSIS TO STUDY DUCHENNE MUSCULAR DYSTROPHY

Arne Hofemeier[1], Eric Schoger[1,2], Malte Rinn[1], Branimir Berecic[1], Oscar Gutiérrez-Gutiérrez[2,3], Lukas Cyganek[2,3], Timo Betz[4], Malte Tiburcy[1,2], Wolfram Zimmermann[1,2]

Affiliations:
1. Department of Pharmacology and Toxicology, University Medical Center Göttingen, Göttingen, Germany
2. German Center for Cardiovascular Research (DZHK), partner site Lower Saxony, Germany
3. Stem Cell Unit, University Medical Center Göttingen, Göttingen, Germany
4. Third Institute of Physics, University of Göttingen, Göttingen, Germany

Contractile function of skeletal muscle is assumed to be tightly linked to muscle structure. Combining tissue level contractility measurements with myotube or even sarcomere level imaging would confirm this hypothesis and investigate in more detail how and under what conditions function follows form. Recently introduced *in vitro* engineered culture models allow for creating macroscale muscle tissue from primary cells (myoblasts) and induced pluripotent stem cells (iPSCs) from healthy and diseased patients. Although highly versatile in their application, available culture platforms are incompatible with high resolution live microscopy due to unfavorable object-lens distances. Here, we present a new platform that allows high-resolution 3D microscopy in living human engineered skeletal muscle (ESM) with parallel non-invasive quantification of tissue level contractility. Using immortalized myoblast cell lines or iPSC differentiation protocols, we demonstrate the engineering of millimeter-sized (4.92±0.02 mm length, 0.28±0.01 mm^2 cross-section; n=38) ESMs between two elastic poles (39 µN/µm bending constant) positioned directly above a thin glass coverslip. Tissue to coverslip distance is 80±9 µm. By this design, we can perform deep tissue imaging down to the sarcomere level (in a model of stable expression of ACTN2-Citrine). By integration of electrodes, we can apply electrical stimuli to introduce controlled contractions (up to 100 Hz). By video-optical pole deflection analyses we report tetanic contractile forces at 100 Hz of up to 0.6 mN. Finally, we report that ESMs from patients suffering from Duchenne muscular dystrophy develop overall thinner myotubes (13.1±0.44 µm; n=24) and show weaker tetanic forces (0.098±0.01 mN / 0.2±0.02 mN/mm^2; n=80) compared to ESMs from healthy patients (14.7±0.3 µm myotube diameter, n=8 and 0.24±0.03 mN / 0.41±0.05 mN/mm^2 (n=42); $p<0.05$ unpaired, two-sided t-test). When comparing ESMs from a Duchenne patient iPSC line to its isogenic genetically corrected version, we were able to show, both, significant increase in myotube diameter (6.8±0.28 mN to 8.7±0.3 mN) and tetanic force generation (0.03±0.004 mN to 0.32±0.05 mN), accordingly. Taken together, the combination of standard pole bending analysis with tissue imaging creates new opportunities to discover muscle structure-contractile function relationships. More specifically, live deep tissue imaging can be applied to study developmental or pathological processes as well as the consequences of novel therapies such as for example genome editing in muscle dystrophies.

#19 Tuesday Poster Session

FROM MOLECULAR PUSH TO FILAMENT PULL IN ZERO LENGTH THEORY

(L0T) ***Frans van Kaam***. Free researcher. Veldhoven. The Netherlands.

famvkaam@upcmail.nl

The atomic structure of the myosin head S1 is very complex, but contains mechanically relevant functional domains: base, lever arm, motor domain (1) to conduct the force developed therein to the filaments in the sarcomere. From a mechanical point of view these domains could be treated in relaxed state as passive elastic structures, obeying Hooke's law (2). Their length depends on external load Fe which then will elongate with L- L0 = Fe / S. The stiffness S of each domain only can be a bulk stiffness due to the numerous bonds it contains.

In pre-power, relaxed state, the conformation of the sub-fragment S1 (the head) is schematized in simple 2D plane by a rectangular triangle ABC. Its side AB represents the base of S1; the hypothenuse BC represents the motor domain; AC a lever arm segment and AD the total lever arm which hinges at vertex D in connection with sub-fragment S2, the tail of the crossbridge. All sides AB,BC, CA and AD and sub-fragment S2 have their own zero length in this relaxed state as determined by their natural externally unloaded geometry.

The motor domain must be distinguished from other domains in that it can become active elastic and will elongate its zero length (BC) when it absorbs exclusively proper energy upon activation. Within the zero length theory, " active elastic" means that its zero length L0 can change (4,5), and in this case of its overall end to end length BC. The net effect of its zero length *change* also will be a compressive force acting upon the vertices B and C along its work line BC .

Apart from a generally expected increase in kinetic energy of its constituting vibrating atoms, all of the zero lengths of the individual bonds within the motor domain are assumed to change as well, thereby possibly increasing their particular potential energy content. Although the elongation of individual bonds are small order of picometer, the sum of them could displace the lever arm at its vertex C in the order of nanometers resulting into a rotation of the lever arm at maximum in the externally unloaded case. In this way of an unloaded case the motor domain only stores conformational change energy.

In case of isometric load, when the vertex D and base B remains fixed, the atoms and bonds in the motor domain will develop a pressing force on the vertices B and C by both their stored kinetic and potential energy increase. A storage of at least potential energy is necessary to explain developed steady isometric force in a single crossbridge head.

Reaction forces are to be expected in the base at A and in B as well as a bending moment in the lever arm AD loaded in A, D and C. In the 2D-case as considered here, the base need to come in contact at only 2 positions with the actin filament. To react with a proper bending moment on actin, only one contact needs to be a chemical bond enabling it to react with a pulling force. The other contact need to react with a pushing force for which no chemical bond is required. This also allows the base to be free to become stretched by the compressive force of the motor domain at B.

When by nature, energy absorption can only result into an increase of the zero length of the motor domain structure in myosin heads, irrespective its detailed mechanism behind it, then its developed pushing force will be only redirected via a lever arm into pulling forces in both filaments. Only one chemical bond is required to conduct the reaction forces and bending moments properly.

From a mechanical point of view sliding filaments (5,6) therefore are a natural mechanism to convert structural expansion of the zero length in a motor domain into sarcomere shortening. The zero length theory could therefore serve as a general frame work, at any structural length scale in muscle.

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#20 Tuesday Poster Session

C2C12-CTE – A VERSATILE MYOTUBE MODEL FOR C-TERMINAL TITIN STUDIES

Jaakko Sarparanta[1,2], Per Harald Jonson[1,2], Anna Vainio[1,2], Helena Luque[1,2], Peter Hackman[1,2] & Bjarne Udd[1,2,3]

Affiliations:
1. Folkhälsan Research Center, Helsinki, Finland
2. Medicum, University of Helsinki, Helsinki, Finland
3. Neuromuscular Research Center, Tampere University Hospital, Tampere, Finland

Mutations in the extreme C-terminus of titin—located in the M-band of the sarcomere—cause a range of myopathies with different clinical pictures and modes of inheritance. These so-called C-terminal titinopathies include the dominant late-onset distal myopathy Tibial Muscular Dystrophy (TMD), the severe recessive Limb-Girdle Muscular Dystrophy R10 Titin-related (LGMDR10), and the Juvenile-onset Recessive Distal Titinopathy. The genotype-phenotype correlations, and the molecular mechanisms underlying the diseases are only partly understood. We have previously demonstrated that the TMD/LGMDR10-causing Finnish founder mutation FINmaj, although located in titin's ultimate C-terminal domain M10, sensitizes the mutant protein to a secondary proteolytic cleavage removing several domains from titin C-terminus. We believe that this so-called pathological cleavage is a key event in the pathogenesis of TMD and LGMDR10, and that the cleavage propensity of titin may explain the different phenotypic effects of the variants.

The huge size of titin sets limitations to experimental analyses of the pathological cleavage and other functional studies of titin variants in the native sarcomeric context. To overcome this challenge, we have developed the C2C12-CTE (C-terminal Titin Exchange) platform, which allows efficient introduction of disease variants, tags or any other desired modifications to C-terminal titin. The system is based on murine C2C12 myoblasts in which heterospecific lox recombination sites were inserted flanking the last four exons of *Ttn*. The thus targeted titin region can be replaced with a gene cassette from a donor plasmid through recombinase-mediated cassette exchange (RMCE): To this end, cells are transfected with the donor plasmid and the Cre recombinase mRNA, and selected for G418 resistance. While unspecific donor integration turned out to hamper the use of recombinant cells as polyclonal pools, recombinant clones can be isolated with good efficiency (20–30% of viable G418-resistant clones) and screened to identify clones with best retained myogenic properties. Creating new derivative cell lines takes approximately 6 weeks from donor transfection to freezing of expanded clones.

We have utilized the C2C12-CTE system to create cells with fluorescently tagged wild-type and FINmaj mutant titin, and are currently using them to model the pathological cleavage of mutant titin in myotubes. We expect our system to have a wide range of additional applications in C-terminal titin studies, and the same principle could also be applied to other regions of interest in titin.

#21 Tuesday Poster Session

MUTLISCALE SIMULATIONS EXHIBIT VENTRICULAR WALL THICKENING WHEN cMYBP-C DOES NOT SUPPRESS MYOSIN CYCLING

[1] Kerry S. McDonald, [2] Julian E. Stelzer, [3] **Kenneth S. Campbell**

Affiliations:
1. University of Missouri, Columbia MO, USA
2. Case Western Reserve University, Cleveland OH, USA
3. University of Kentucky, Lexington KY, USA

Multiscale models of the cardiovascular system can provide insights into both physiological and pathophysiological processes. MyoVent is a computer model that bridges from molecular to organ-level function and that simulates a left ventricle pumping blood around a systemic circulation (PMID 36107358). In prior work, we adapted MyoVent to allow the ventricle to grow concentrically (adding / removing myofibrils in parallel) and eccentrically (adding / removing sarcomeres in series). Simulations in which concentric growth was regulated by the intracellular ATP concentration and eccentric growth was regulated by the passive stress in titin molecules mimicked ventricular growth patterns associated with hypertension, aortic valve insufficiency, and mitochondrial dysfunction.

In this work, we enhanced the multiscale MyoVent framework by replacing the original contractile module (which predicted contraction using Huxley-type cross-bridge distributions) with the spatially-explicit FiberSim framework for half-sarcomeres (PMID 34932957). This allowed us to predict how modifying the function of cardiac myosin binding protein-C (cMyBP-C) molecules influenced growth.

In the new model termed FiberVent, cMyBP-C molecules were localized to the C-zone of half-sarcomeres and assumed to transition between: (1) a null state (no effect), (2) an actin-attached state (which increased cooperative activation of the thin filament and exerted a drag force during interfilamentary movement), and (3) a myosin-suppressing state (which biased nearby myosin heads towards a super-relaxed / interacting-heads motif-like state where they could not interact with actin).

When the cMyBP-C molecules were prevented from entering the myosin-suppressing state, half-sarcomere contractility and the ventricular ATPase both increased. This initiated concentric growth and subsequent wall thickening. These simulations mimic aspects of organ-level growth seen in patients who develop hypertrophic cardiomyopathy linked to variants in cMyBP-C.

These results demonstrate how computer modeling can help bridge between molecular and organ-level scales. Ongoing computing work is investigating non-cMyBP-C mechanisms which can be perturbed to reverse abnormal growth.

#22 Tuesday Poster Session

Computational analysis of the effect of electroporation on action potentials in isolated adult rat cardiomyocytes

Lea Rems[1], Tina Turk[1]

Affiliations:
1. University of Ljubljana, Faculty of Electrical Engineering, Ljubljana, Slovenia

Electroporation—increased membrane permeability induced by high-intensity pulsed electric fields—has become a cornerstone of various biomedical applications including electrochemotherapy, gene electrotransfer, and irreversible electroporation for tumor ablation. Irreversible electroporation has recently been adapted for the treatment of cardiac arrhythmias, where it is known as pulsed field ablation, and represents a major innovation in interventional electrophysiology. One crucial aspect that remains incompletely understood is how electroporation affects the generation and propagation of action potentials (APs) in the myocardium, as evidenced by altered intracardiac electrograms recorded near the ablation site.

To investigate how electroporation affects APs, we first employed a circuital model of an isolated rat ventricular cardiomyocyte and extended the model by including an additional ionic current due to electroporation, I_{ep}. We modeled I_{ep} as a nonselective current through a certain number of electroporation-induced pores N_p. We found that this model effectively captured the spectrum of responses observed in experiments with isolated cardiomyocytes. However, N_p was manually selected to match experimental data, since the model was not designed to predict N_p based on the applied pulse parameters.

To overcome this limitation and enable predictive modeling, we are now developing a three-dimensional finite element model of a cardiomyocyte positioned between electrodes to simulate full electric pulse exposure. This model will couple electric potential calculations around and within a cardiomyocyte with computations of ionic currents across voltage-gated channels and additional voltage-dependent ionic currents caused by electroporation. We will examine how pulse parameters affect the threshold electric fields for electrostimulation and electroporation, and how pulse duration and cardiomyocyte orientation influence the post-electroporation AP shape. Results will be compared against existing optical measurements of APs in adult rat cardiomyocytes.

#23 Tuesday Poster Session

SCALABLE GENERATION OF MUSCLE SPHEROIDS FOR TISSUE ENGINEERING OF HUMAN MUSCLE

Malte Tiburcy[1,2], Malte Rinn[1,2], Mandy Winterhoff[1,2], Mina Shahriyari[1,2], Branimir Berecic[1,2], Wolfram-Hubertus Zimmermann[1,2,3,4]

Affiliations:
1. Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Göttingen, Germany
2. German Center for Cardiovascular Research (DZHK), partner site Lower Saxony
3. Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Germany
4. Fraunhofer Institute for Translational Medicine and Pharmacology (ITMP), Göttingen, Germany

Background: Directed differentiation of human pluripotent stem cells (hPSCs) into muscle lineage mimics the key stages of embryonic myogenesis. Human induced pluripotent stem cells (hiPSCs) offer a potentially unlimited source of human muscle progenitors, providing a valuable platform for regenerative medicine and tissue engineering. In this study, we hypothesized that human muscle spheroids can be generated in a quantitatively robust manner by recapitulating embryonic muscle development within a scalable bioreactor system.

Methods and Results: To direct pluripotent stem cells into muscle, pluripotent cells were expanded in a bioreactor (volumes between 50-300 ml) to obtain homogenous populations of aggregates with a mean diameter of 140±40 µm. Pluripotency was confirmed by detection of NANOG/OCT4/Tra1-60 using flow cytometry. Characteristic stages of muscle embryonic development were then induced by modulation of Wnt/BMP and Notch signaling. Transcriptome analyses and immunostainings confirmed progression from a neuro-mesodermal progenitor (*SOX2+/TBXT+)* to paraxial mesoderm and formation of somite progenitors. Of note, migratory progenitor cells that delaminate from the dermomyotomal somite compartment in the embryo were identified by LBX1 and MET expression. Progenitor spheroids at this stage of development were optimally suited to fuse into compact muscle tissue. Following maturation in serum-free conditions, spontaneously contracting, force generating muscle was obtained.

Conclusion: The development of human muscle spheroids provides a scalable method to derive regenerative progenitor cells as well as muscle building blocks to generate quantitative amounts of skeletal muscle.

#24 Tuesday Poster Session

DYSTROPHIN-DEFICIENCY STIFFENS SKELETAL MUSCLE AND IMPAIRS ELASTICITY – AN *IN VIVO* RHEOLOGICAL EXAMINATION

Angus Lindsay[1-4], Pavithran Devananthan[5], Rebecca Craven[5], Kellie Joe[5], Gretel S. Major[1,3], Jiayi Chen[1], Natalia Kabaliuk[3,5]

Affiliations:
1. School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
2. Department of Medicine, University of Otago, Christchurch, New Zealand
3. University of Canterbury Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand
4. Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand
5. Department of Mechanical Engineering, University of Canterbury, Christchurch, New Zealand

Loss of dystrophin, a protein that provides structural stability to skeletal muscle cells, causes degeneration and loss of biomechanical functionality – commonly associated with Duchenne muscular dystrophy. Dystrophin-associated destabilization of a muscle cell stiffens the muscle architecture, observations driven almost exclusively by isolated *ex vivo* characterizations of whole muscle. Inexplicably, the role of dystrophin in muscle cell viscoelasticity *in vivo* remains to be elucidated because of the challenges of *in vivo* constraints (e.g., the murine skeletal system, the isolation of live muscle for controlled load application, and the alignment of muscle fiber and fascicular organization with the directionality and characteristics of mechanical loading). Here, we developed a platform, termed myomechanical profiling, that enables the *in vivo* biomechanical assessment of skeletal muscle viscoelasticity using rheology. Tibialis anterior muscle of live mice was probed for its viscoelastic properties in response to compressive strain and rotational shear in a custom apparatus designed to accommodate inter-mouse anatomical variability. Dystrophin-deficiency caused increased stiffness in tibialis anterior skeletal muscle in both static compression and compression strength testing and was associated with total collagen content of the muscle. Additionally, dystrophin-deficient tibialis anterior muscles were less elastic and more capable of dissipating mechanical energy than dystrophin-positive tibialis anterior muscles. When exposed to a series of damaging eccentric contractions, dystrophin deficient tibialis anterior muscles lost greater strength (85% vs. 30%), which further increased the stiffness of the muscle. These data indicate that the loss of dystrophin compromises the viscoelastic properties of skeletal muscle *in vivo* and that myomechanical profiling represents an accurate, repeatable, and sensitive technology capable of assessing the biomechanical properties of skeletal muscle.

#25 Tuesday Poster Session

INTERLEUKIN-6 SIGNALLING AND ANTI-IL-6 AUTOANTIBODIES IN IDIOPATHIC INFLAMMATORY MYOPATHIES

Anja Srpčič[1,2], Manca Ogrič[1], Saša Čučnik[1,3], Sergej Pirkmajer[2], Katja Lakota[1], Katja Perdan Pirkmajer[1,2]

Affiliations:
1. University Medical Centre Ljubljana, Ljubljana, Slovenia
2. Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
3. Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Introduction Idiopathic inflammatory myopathies (IIM) are systemic autoinflammatory diseases characterised by immune cell infiltration in skeletal muscle tissue, resulting in inflammation-induced muscle damage. While myositis-specific and myositis-associated autoantibodies are well described, little is known about the presence and function of anti-cytokine autoantibodies (ACAAs) in these patients. ACAAs have been reported in several other autoimmune diseases and even in healthy individuals, with evidence suggesting potential immunomodulatory roles. In this study, we measured serum levels and explored possible biological implications of ACAAs targeting interleukin-6 (anti-IL-6) in IIM patients.

Methods Serum samples were collected from 49 IIM patients treated at the Department of Rheumatology, University Medical Centre Ljubljana, and 39 healthy controls (HCs). Patients were stratified by treatment status (treatment-naive vs. treated subgroups) and IIM subtype (dermatomyositis, polymyositis, immune-mediated necrotising myopathy, antisynthetase syndrome, and overlap syndrome). ELISA was used to quantify serum levels of IL-6 (BioLegend), anti-IL-6 (in-house ELISA) and soluble IL-6 receptor (sIL-6Rα, Proteintech). Serum samples with added recombinant IL-6 (rhIL-6) were applied to a reporter gene assay (RGA, Svar) responsive to IL-6 to assess the potential of sera to modulate IL-6 signalling. Additionally, RGA was performed on sera without rhIL-6 to exclude assay interference of endogenous IL-6 or sIL-6Rα.

Results IIM patients had higher serum IL-6 levels than HCs ($p<0.0001$). They also showed a trend towards lower anti-IL-6 levels ($p=0.07$) and higher siIL-6Rα levels ($p=0.06$). RGA levels revealed significantly enhanced IL-6 signalling activity in IIM sera ($p<0.0001$). IL-6, anti-IL-6, sIL-6Rα, or RGA levels were similar among the IIM subtypes or treatment groups. In IIM patients and HCs, lower anti-IL-6 levels were associated with higher RGA activity. As assessed by manual muscle testing, muscle strength was negatively correlated with RGA levels in treatment-naive IIM patients.

Conclusions Our study showed markedly higher IL-6 signalling activity in IIM patients, which was independent of their serum IL-6 or siIL-6R levels, treatment status or disease subtype. Since higher RGA levels were associated with lower anti-IL-6 levels, we propose these autoantibodies may have a neutralising function. Additionally, a negative correlation between RGA levels and muscle strength measured by manual muscle testing suggests a possible association between dysregulated IL-6 signalling and muscle dysfunction in IIM patients.

#26 Tuesday Poster Session

IMPACT OF R405W MUTATED DESMIN ON MURINE SKELETAL MUSCLE

Sabrina Batonnet-Pichon[1,2], Florence Delort[1], Alain Lilienbaum[1], Carolin Berwanger[3,4], Dorothea Schultheis[5], Ursula Schlötzer-Schrehardt[6], Andreas Schmidt[7,8], Rolf Schröder[5], **Christoph S. Clemen**[3,4]

1 Basic and Translational Myology, Unit of Functional and Adaptive Biology, Université Paris Cité / CNRS UMR 8251, Paris, France
2 Institut Cochin, Université Paris Cité, INSERM U1016, CNRS, Paris, France
3 Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany
4 Institute of Vegetative Physiology, Medical Faculty, University of Cologne, Cologne, Germany
5 Institute of Neuropathology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany
6 Department of Ophthalmology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany
7 CMMC, Medical Faculty, and CECAD, University of Cologne, Cologne, Germany
8 Present address: Bruker Daltonics GmbH & Co KG, Bremen, Germany

To elucidate the pathophysiology of the human heterozygous p.R406W desmin mutation, which causes severe heart disease, we generated orthologous p.R405W desmin knock-in mice. In our previous work, we showed that p.R405W desmin exerts its cardiotoxic potential by inducing disorganisation of desmin filaments and uncoupling them from intercalated disks. The aim of this study was to comprehensively characterise the skeletal muscle pathology in heterozygous and homozygous p.R405W desmin knock-in mice.

Heterozygous and homozygous p.R405W desmin knock-in mice showed classic myopathological features of myofibrillar myopathy with desmin-positive protein aggregation, degenerative changes of the myofibrillar apparatus, increased autophagic accumulation and mitochondrial alterations. Muscle weakness was present only in homozygous animals. Analyses of RNA sequencing and proteomic data from the soleus muscle of 3-month-old mice revealed 59 up- and 3 down-regulated mRNAs and 101 up- and 18 down-regulated proteins that were shared between the heterozygous and homozygous genotypes in the respective omics datasets. Combined analysis of the omics data revealed down-regulation at both mRNA and protein levels for a large number of mitochondrial proteins, including essential proton gradient-dependent carriers. Up-regulation at both omics levels was present for the transcription factor Mlf1, which is a binding partner of the protein quality control related Dnajb6. Down-regulated at the mRNA level but up-regulated at the protein level was the sarcomeric lesion marker Xirp2 (xin actin-binding repeat-containing protein 2), while Ces2c (acylcarnitine hydrolase) was regulated in the opposite direction.

This work shows that expression of the p.R405W mutant desmin leads to myofibrillar myopathy in heterozygous and homozygous R405W desmin knock-in mice. The results from heterozygous mice further demonstrate that the presence of desmin-positive protein aggregates is not necessarily associated with muscle weakness. Transcriptomic and proteomic data delineate a pathogenetic link between the myeloid leukemia factors 1 and 2 and the myofibrillar myopathy and protein quality control related proteins Dnajb6 and Bag3 in the context of desminopathies. A key finding is that extensive secondary mitochondrial damage

and proton gradient-dependent carrier-related dysfunction are important pathogenetic steps and therefore potential pre-clinical therapeutic targets in R405W/R406W and also R349P/R350P desminopathies. Integrated morphological, transcriptomic and proteomic data analysis is a powerful approach to identify and validate early stages of disease in desminopathies and other forms of myofibrillar myopathies.

#27 Tuesday Poster Session

NOVEL INSIGHTS INTO MOLECULAR MECHANISMS LEADING TO CONGENITAL FIBRE-TYPE DISPROPORTION (CFTD)

Fanny Rostedt[1,2], Robert AE Seaborne[3,4], Roosa Harmo[1,2], Milla Laarne[1,2], Teemu Turku[1,2], Harri Kangas[5], Thomas Krag[6], John Vissing[6], Julien Ochala[4], Katarina Pelin[1,7], Jenni Laitila[1,2]

Affiliations:
1. Folkhälsan Research Center, Helsinki, Finland
2. Department of Medical Genetics, Medicum, University of Helsinki, Helsinki, Finland
3. Centre for Human & Applied Physiological Sciences, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom
4. Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark
5. Institute for Molecular Medicine Finland (FIMM), Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland
6. Copenhagen Neuromuscular Center, Department of Neurology Rigshospitalet, University Hospital Copenhagen, Copenhagen, Denmark
7. Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

The diagnosis of congenital fibre-type disproportion (CFTD) is given when (1) a patient presents with clinical features consistent with congenital myopathy, (2) an unusual pattern of fibre-size and fibre-type proportion is observed in the muscle biopsy and (3) other congenital myopathies have been excluded. The molecular and subsequent cellular mechanisms underlying CFTD, or fibre-type disproportion as a phenotypic feature in other congenital myopathies, remain poorly understood.

To get insights into the mechanisms, we collected nine biopsies from patients with CFTD, caused by pathogenic variants in different genes, with varying levels of fibre-size and fibre-type disproportion. For three of the patients, genetic diagnosis with PromethION P2 solo™ nanopore sequencing targeting myopathy genes is underway. We then assessed the function and energetic properties of the most abundant muscle protein, myosin, by performing Mant-ATP chase experiments on individual relaxed muscle fibres. Our preliminary data suggests a trend towards myosin dysregulation in CFTD patients, as attested by an increase in the proportion of myosin heads in the highly energy-consuming state (DRX). This finding is in line with what has previously been observed in patients with *NEB*- and *ACTA1*-related nemaline myopathy. The next experiments include correlating these results with fibre-size or fibre-type heterogeneity and performing single-fibre proteomics.

In conclusion, we believe that the findings of this study will help pave the way for targeted therapeutic strategies benefiting a larger group of affected individuals.

#28 Tuesday Poster Session

LIPOFUSCIN AS A MARKER FOR SKELETAL MUSCLE BIOLOGICAL AGEING (1/2)

Jelle Y Huijts[1], Braeden T Charlton[1], Anouk Slaghekke[1], Per W.B. Larsen[3], Anita E Grootemaat[3], Brent Appelman[4], Wendy Noort[1], Frank W Bloemers [2], Paul van Amstel [2], Jelle J Posthuma [5], Nicole N van der Wel[3], Michèle van Vugt[6], Richie P Goulding [1], Rob C I Wüst[1]

[1] Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, The Netherlands
[2] Department of Trauma Surgery, Amsterdam UMC, The Netherlands
[3] Electron Microscopy Centre Amsterdam, Amsterdam UMC, The Netherlands
[4] Center for Experimental and Molecular Medicine, Amsterdam UMC, The Netherlands
[5] Division of Surgery, Flevoziekenhuis, Almere, The Netherlands
[6] Department of Internal Medicine, Division of Infectious Diseases, Amsterdam UMC, the Netherlands

Aim: Aging reduces skeletal muscle mass and function, but different people age at different rates. Lipofuscin is an intracellular accumulation of lipid-containing residues of lysosomal digestion. It is formed under conditions of impaired autophagy or proteasomal function. Lipofuscin accumulates progressively in neurons and cardiomyocytes with chronological age, but whether this also happens in skeletal muscle is unclear. The aim is to investigate the accumulation of lipofuscin in skeletal muscle as a marker of biological age in health, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and post-COVID patients.

Method: In this study, we studied the accumulation of lipofuscin in the vastus lateralis muscle in relation to the biological age of 16 healthy participants and compared this with patients with chronic diseases, associated with oxidative stress, including post-COVID (n=8), and ME/CFS (n=4). Immunohistochemistry and electron microscopy were used to assess lipofuscin accumulation in skeletal muscle.

Results: Lipofuscin accumulation in skeletal muscle of healthy controls is increased with chronological age ($P=0.002$, $R^2=0.50$, age range: 18-63years). Lipofuscin accumulation was higher in patients with post-COVID (1.66±0.68, $P<0.001$) and ME/CFS (3.94±0.48, $P<0.001$) compared to healthy controls (0.56±0.57). The age-dependent relation was absent in patients with post-COVID (n=8, $P=0.33$) and ME/CFS (n=4, $P=0.28$).

Conclusion: These results suggest that lipofuscin accumulates in skeletal muscle with increasing age, and its autofluorescence complicates immunohistology experiments. Skeletal muscle of post-COVID and ME/CFS had more lipofuscin accumulation. These results suggest lipofuscin-linked accelerated aging of skeletal muscle in post-COVID and ME/CFS and shows the potential of lipofuscin as a physiological biomarker of muscle aging.

#29 Tuesday Poster Session

DEFINING PATHOGENETIC MECHANISMS IN *CASQ1* DISORDERS THROUGH SINGLE-FIBRE STUDIES

Rostedt F[1,2], Laarne M[1,2], Jokela M[3], Sarparanta J[1,2], Zhao F[4], Udd B[1,2,5], Johari M[1,2,6], Huovinen S[7], Reimann J[8], Kornblum C[8], Hackman P[1,2], Lehtokari VL[1,2], Pelin K[1,9], **Laitila J**[1,2]

Affiliations:

1. Folkhälsan Research Center, Helsinki, Finland
2. Department of Medical Genetics, Medicum, University of Helsinki, Helsinki, Finland
3. Division of Clinical Neurosciences, Turku University Hospital and University of Turku, Turku, Finland
4. Department of Pathology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
5. Neuromuscular Research Center, Tampere University Hospital and Fimlab Laboratories, Tampere, Finland
6. Harry Perkins Institute of Medical Research, Centre for Medical Research, University of Western Australia, Nedlands, WA, Australia
7. Department of Pathology, Fimlab Laboratories, Tampere University Hospital, Tampere, Finland
8. Department of Neuromuscular Diseases, Center for Neurology, University Hospital Bonn, Bonn, Germany
9. Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

Calsequestrin-1 (CASQ1) is the most crucial calcium-binding protein in the fast twitch fibers of skeletal muscle, where it facilitates rapid Ca2+ release to initiate muscle contraction. Pathogenic variants in *CASQ1* are rare and cause dominant vacuolar myopathy with CASQ1 aggregates. The typical symptoms include muscle weakness, myalgia, exercise intolerance, cramps, and fatigue.

Here, we aimed to identify the molecular biochemical and biophysical mechanisms underlying the disorder caused by *CASQ1* in two families. The patients underwent a thorough neurological examination, and the muscle biopsies were analysed by routine histopathological methods and electron microscopy. A missense variant in *CASQ1*, p.(Glu89Lys) segregated in three generations with muscle symptoms in Family 1, presenting with exercise intolerance, cramps, myalgia and miosis. In electron microscopy, there were changes in the triads and the SR-feet in all patients. Additionally, the proband had muscle weakness and her muscle biopsy showed nemaline bodies, explained by compound heterozygosity for two pathogenic variants in *NEB*. In Family 2, there were three patients in two generations. The main symptom was progressive muscle weakness, and the biopsy showed heavy accumulation of connective tissue and fat. A frameshift variant p.(Gly383Alafs*39) in *CASQ1* was found in all three patients. The variant extends the protein beyond the stop codon by 24 residues. Western blotting confirmed the expression of the extended protein in patient muscle.

We isolated skeletal myofibres from the patients and healthy controls and performed a combination of structural and functional assays, including loaded Mant-ATP chase experiments on individual relaxed muscle fibres. Our preliminary data suggests a shift of myosin metabolic states towards highly energy-consuming conformations in patients

harbouring pathogenic variants in *CASQ1*. Our ongoing project will continue assessing the calcium sensitivity and muscle mechanics in the muscle fibres.

#30 Tuesday Poster Session

Characterization of NEB pathogenic variants in patients reveals novel nemaline myopathy disease mechanisms and omecamtiv mecarbil force effects

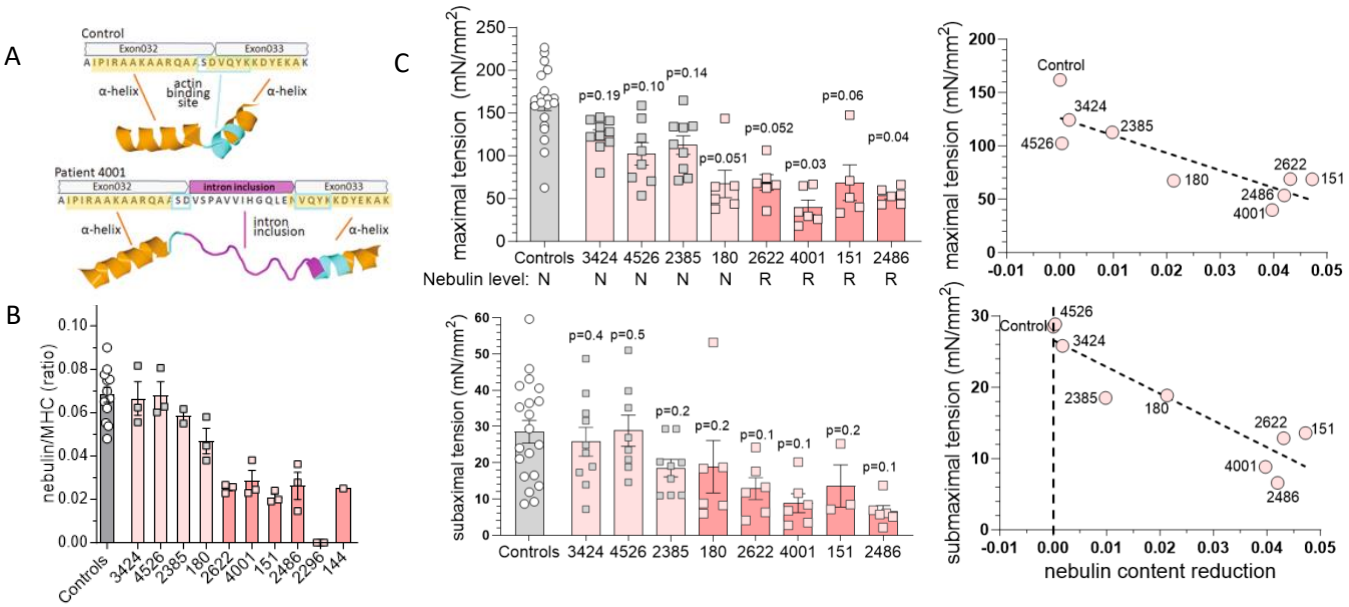
Jochen Gohlke[1], Esmat Karimi[1], Mila van der Borgh[1], Johan Lindqvist[1], Zaynab Hourani[1], Justin Kolb[1], Stacy Cossette[2], Michael W. Lawlor[2,3], Coen Ottenheijm [1,4], Henk Granzier[1]

1. Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ, USA.
2. Department of Pathology, Medical College of Wisconsin, Milwaukee, WI, USA.
3. Diverge Translational Science Laboratory, Milwaukee, WI, USA.
4. Department of Physiology, Amsterdam UMC (location VUMC), Amsterdam, Netherlands.

Nebulin, a critical protein of the skeletal muscle thin filament, plays important roles in physiological processes such as regulating thin filament length (TFL), cross-bridge cycling, and myofibril alignment. Pathogenic variants in the nebulin gene (NEB) cause NEB-based nemaline myopathy (NEM2), a genetically heterogeneous disorder characterized by hypotonia and muscle weakness, currently lacking curative therapies. In this study, we examined a cohort of ten NEM2 patients, each with unique pathogenic variants, aiming to understand their impact on mRNA, protein, and functional levels.

Results show that pathogenic truncation variants affect NEB mRNA stability and lead to nonsense-mediated decay of the mutated transcript. Moreover, a high incidence of cryptic splice site activation was found in patients with splicing pathogenic variants that are expected to disrupt the actin-binding sites of nebulin by insertion of intronic sequences (Fig. A). Determination of protein levels revealed patients with either relatively normal or markedly reduced nebulin (Fig. B). We observed a negative relation between the reduction in nebulin and TFL, or tension (both maximal and submaximal tension, Figure C). Interestingly, our study revealed a duplication pathogenic variant in nebulin that resulted in a four-copy gain in the triplicate region of NEB and a much larger nebulin protein and longer TFL. Additionally, we investigated the effect of Omecamtiv mecarbil (OM), a smallmolecule activator of cardiac myosin, on force production of type 1 muscle fibers of NEM2 patients.

OM treatment substantially increased submaximal tension across all NEM2 patients ranging from 87-318%, with the largest effects in patients with the lowest level of nebulin. In summary, we propose that the pathomechanism of NEM2 involves not only shortened but also elongated thin filaments, along with the disruption of actin-binding sites resulting from pathogenic splice



variants. Significantly, our findings highlight the potential of OM treatment to improve skeletal muscle function in NEM2 patients, especially those with large reductions in nebulin levels.

#31 Tuesday Poster Session

Exploring BAG3P209L Myofibrillar Myopathy: In Vitro Models for Targeted Therapy

Kerstin Filippi[1], Stefan Rupp[2], Michael Peitz[3], Luke M. Judge[4], Bruce R. Conklin[4], Bernd K. Fleischmann[1], Michael Hesse[1]

Affiliations:
1. Institute of Physiology I, University of Bonn, Medical Faculty, Bonn, Germany
2. Pediatric Heart Center, Justus Liebig University Giessen, Giessen, Germany
3. Cell Programming Core Facility, University of Bonn, Bonn, Germany.
4. Gladstone Institutes, San Francisco, USA

Patients carrying a missense mutation in the *BAG3* gene (p.Pro209Leu; c.626C>T) suffer from severe myofibrillar myopathy (OMIM: MFM6) and polyneuropathy, manifesting as skeletal muscle dystrophy and restrictive cardiomyopathy. As part of the chaperone-assisted selective autophagy complex, BAG3 plays a crucial role in maintaining muscle protein homeostasis as part of the protein quality control system.
To gain mechanistic insights into BAG3-associated muscle pathology and explore potential therapeutic strategies, we utilize multiple induced pluripotent stem cell (iPSC) lines, two of which were derived from MFM6 patients, a BAG3 knockout (BAG3-/-) iPSC line and corresponding isogenic controls. All these iPSC lines were successfully differentiated into contractile skeletal myotubes. Both, the BAG3P209L mutation and the loss of BAG3, led to altered myotube morphology, disrupted sarcomeric organization, and upregulation of protein quality control markers. These defects were further enhanced by induction of mechanical stress (by electrical stimulation), proteotoxic stress (by Bortezomib, MG132), and autophagy inhibition (by Bafilomycin).
To assess both muscular and neuronal aspects of MFM6, neuromuscular organoids (NMOs) were generated. The deletion of BAG3 and the BAG3P209L mutation led to pronounced morphological changes in NMOs that manifested upon electrical stimulation and were associated with a markedly impaired contractile response. Consistent with the 2D model, stimulation enhanced sarcomeric disorganization and stress response activation in this 3D context. We are currently investigating whether these BAG3-related pathomechanisms in NMOs are driven by defective neuromuscular innervation.

#32 Tuesday Poster Session

A NOVEL KBTBD13 G67R KNOCK-IN MOUSE MODEL OF NEMALINE MYOPATHY TYPE 6 SUGGESTS A MILD CONTRACTILE PHENOTYPE

Leander A. Vonk[1], Rianne Baelde[1], Ricardo A. Galli[1], Mara Nieuwendijk[1], Josine de Winter[1], Coen A.C. Ottenheijm[1]

Affiliations:
1. Amsterdam University Medical Center, Department of Physiology, Amsterdam, The Netherlands

Introduction: Nemaline Myopathy Type 6 (NEM6) is caused by dominant mutations in the KBTBD13 gene. Patients with NEM6 present with muscle weakness, stiffness and slowness of movement, and histological analysis shows a core-rod myopathy. Clinical severity varies among patients and variants, and the molecular mechanisms underlying this variability remain poorly understood.

Purpose: Previously, we studied the disease process caused by the Dutch founder variant Arg408Cys (R408C) using a homozygous knock-in mouse model. In this model, we found impaired maximum force production and relaxation with a disease onset between 1-3 months and a plateau at 9 months of age. To better understand the function of KBTBD13 and the disease process in different variants of NEM6, we recently developed a knock-in mouse model based on an Italian patient harboring a Gly67Arg (G67R) variant, who presents with a mild NEM6 phenotype.

Methods: We assessed intact muscle function in homozygous G67R knock-in mice at 3, 9 and 18 months of age, measuring maximal force production and the relaxation kinetics of EDL and Soleus muscle. Alongside this, we measured maximum force production, relaxation kinetics and calcium handling of single muscle fibers isolated from the patient. We are also performing histological analyses to study nemaline rods, mitochondrial cores and fiber type composition.

Results: At all ages, G67R knock-in mice showed no reduction in maximal force production and relaxation kinetics compared to wildtype controls. These results are in line with *in vitro* single fiber force measurements performed on the patient biopsy, which suggests only a mild force impairment. Preliminary histology on the knock-in mouse model also revealed no overt abnormalities.

Conclusion: Our findings indicate that the G67R variant in KBTBD13 produces a mild phenotype, with no significant functional impairment observed *in vivo* in our mouse model and only a mild functional impairment at the muscle fiber level in the patient. These results also suggest that force production may be maintained in mice by a compensatory, yet unknown mechanism. Ongoing histological analyses and future *in vitro* studies of protein localization, muscle fiber function and calcium dynamics will help to clarify the pathophysiological effects of KBTBD13 variants and their role in modulating disease severity.

#33 Tuesday Poster Session

ADVANCED HUMAN TISSUE MODELS TO INVESTIGATE COLLAGEN-VI RELATED MUSCULAR DYSTROPHY

Malte Rinn[1,2], Mina Shahriyari[1,2], Lukas Cyganek[2,3], Arne Hofemeier[1,2], Wolfram-Hubertus Zimmermann[1,2], Malte Tiburcy[1,2]

Affiliations:
1. Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Georg-August University, Göttingen, Germany
2. DZHK (German Center for Cardiovascular Research), partner site Lower Saxony Germany
3. Stem Cell Unit, Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany;

Background: Collagen-VI muscular dystrophy is a congenital muscular dystrophy caused by mutations in the *COL6A1*, *COL6A2*, or *COL6A3* genes. Collagen VI is a critical component of the extracellular matrix in skeletal muscle (myomatrix). The disease presents along a clinical spectrum, from mild muscle weakness (Bethlem myopathy) to severe phenotypes characterized by profound weakness, loss of ambulation, and respiratory failure (Ullrich congenital muscular dystrophy, UCMD). Notably, collagen VI is primarily expressed by interstitial fibroblasts rather than myocytes. How collagen VI mutations impair skeletal muscle development and contribute to hallmark symptoms such as weakness and contractures is largely unknown.

Methods and Results: Using CRISPR/Cas9 gene editing, we introduced a common *COL6A1* mutation associated with UCMD (COL6A1:c.930+189C>T), as well as a complete gene knockout, into a wild-type induced pluripotent stem cell (iPSC) line. To model skeletal muscle development and function, we generated skeletal muscle organoids, which closely recapitulate human muscle development. After 50 days of differentiation, organoids exhibit robust contractility and can be stimulated to tetanic contractions. Organoids harboring collagen VI mutations display reduced contractile force (WT: 0.72±0.09 mN, n=10; mutated: 0.64±0.08 mN, n=12; KO: 0.51±0.07 mN, n=15, p=0.0359).

To further explore the role of fibroblasts in disease pathology, we isolated FAP^+, $PDGFRA^+$ fibroblast populations from skeletal muscle cultures using magnetic-activated cell sorting (MACS). To investigate the fibroblast function in a 3D tissue, engineered connective tissues (ECTs) were generated in a specialized 48-well platform (myrPlate). Tissue contraction was optically quantified by pole deflection over 13 days. ECTs with COL6A1 mutations showed a significantly reduced tissue contraction compared to wild-type controls (WT: 40.6%±0.03% pole deflection, n=26; mutated: 16.3%±0.01% pole deflection, n=26; KO: 5.9%±0.01% pole deflection, n=26, p<0.0001).

Conclusion: Advanced tissue engineering platforms provide powerful tools to investigate the pathophysiology of collagen-VI muscular dystrophy and offer novel possibilities for disease modeling and therapeutic testing.

#34 Tuesday Poster Session

DISTINGUISHING PATHOGENIC FROM BENIGN MISSENSE VARIANTS IN TITIN DOMAIN M10

Rees M[1], Kho AL[1], Pfuhl M[1], Holt M[1], Muirhead RCJ[1], Occhipinti L[1], Savarese M[2], di Feo MF[2,3,4], Hackman P[2,3], Udd B[2,3,5,6] and Gautel M[1]

Affiliations:
[1]Randall Centre for Cell and Molecular Biophysics, King's College London, United Kingdom
[2]Folkhälsan Research Center, Helsinki, Finland
[3]University of Helsinki, Finland
[4]University of Genova, Italy
[5]Department of Neurology, Vaasa Central Hospital, Vaasa, Finland.
[6]Tampere Neuromuscular Center, University Hospital, Tampere, Finland.

Titin is a massive, multidomain protein expressed in striated muscle that traverses half a sarcomere, the basic contractile unit of muscle. The final domain of titin, M10, is the 169th immunoglobulin-like (Ig) domain of the protein and localises to the M-band of the sarcomere, where it is tethered by interactions with the first Ig domain of obscurin or its homologue obscurin like-1. Missense variants in M10 have previously been linked to skeletal myopathies and been shown experimentally to either render the domain unfolded when expressed in bacteria, reduce its thermal stability and/or prevent its binding to obscurin. Twenty additional rare missense variants in M10 in a cohort of people with and without a skeletal myopathy have been identified, and we analysed these using a combination of biophysical and cellular assays. The variants segregate into behaving like either the wild-type domain or known disease-causing variants in assays that measured the domains' thermal stability and propensity to aggregate, and distinguish between individuals with and without a skeletal myopathy, suggesting a way to reliably classify benign from pathogenic variants. Additionally, one non-destabilising variant located at the M10-obscurin interface disrupts their binding, demonstrating a stability-independent way a missense variant could affect the function of titin M10.

#35 Tuesday Poster Session

FBXO30/MUSA1 IS A NOVEL CRITICAL REGULATORY ELEMENT FOR Z-LINE HOMEOSTASIS AND SKELETAL MUSCLE FUNCTION

M. Esposito[1,2], A. Armani[1,5], D. Steffan[1,2], L. Nogara[1,2], B. Blaauw[1,2], P. Grumati[3], L. Cendron[4] and M. Sandri[1,2]

1. Veneto Institute of Molecular Medicine (VIMM), Padova, Italy
2. Dept. of Biomedical Sciences, University of Padova, Padova, Italy
3. Telethon Institute of Genetic and Medicine (TIGEM), Pozzuoli (NA), Italy
4. Dept. of Biology, University of Padova, Padova, Italy
5. Institute of Neuropathology, UZH, Switzerland

Skeletal muscle health and function is guaranteed by a finely tuned balance between synthesis of new proteins and degradation of damaged structures and organelles. In the search for new players involved in muscle mass maintenance, in the past years we identified Fbxo30/MUSA1, a previously uncharacterized E3 ubiquitin ligase that sits at the crossroads of two fundamental pathways controlling muscle mass; indeed, MUSA1 expression is driven by FoxO3 transcription factor during catabolic conditions, while it is suppressed by the BMP signaling. To better characterize MUSA1 role in skeletal muscle physiology, we generated a muscle-specific knock-out murine model. While young 6-month-old knock-out mice do not show a significant phenotype, in aged animals MUSA1 prolonged deletion leads to progressive sarcomere disorganization. In particular, histological and electron microscope analyses reveal the presence of wide areas within aged knock-out muscle fibers characterized by undigested sarcomere components, as well as the aggregation of Z-line structures resembling nemaline rods, a feature typical of nemaline myopathies. As a consequence, the ultrastructural alteration affecting myofibrils organization leads to muscle weakness in aged knock-out animals. Moreover, knock-out muscles proteomes reveal progressive accumulation of proteins important for sarcomere and cytoskeleton assembly and stabilization, together with Z-line components. For this reason, we speculate that MUSA1 could be involved in these proteins turnover. Indeed, we demonstrated by *in vitro* ubiquitination assay that MUSA1 is responsible for actinin 3 (ACTN3) poly-ubiquitination when coupled with the E2 conjugating enzyme UbcH2. Concluding, our data support MUSA1 as a new critical player in controlling Z-line homeostasis together with muscle function. However, further investigations are needed to better characterize MUSA1 substrates and to precisely dissect the molecular pathogenic mechanisms involved in the perspective of therapeutic intervention.

#36 Tuesday Poster Session

CHARACTERISING A MOUSE MODEL FOR PYROXD1 MYOPATHY – PATHWAY TO THERAPY DEVELOPMENT.

Gregory Dziaduch[1,2,3ł], **Michaela Yuen**[1,2,3ł], Joe Yasa[1,2,3ł], Leon Kiriaev[4], Peter Houweling[4], Emma Kettle[5], Ignatius Pang[6], Mark Graham[7], Frances Lemckert[1,2,3], Sandra T Cooper[1,2,3*] & Frances Evesson[1,2,3*]

ł contributed equally to this work

*contributed equally to this work

Affiliations:

8. Kids Neuroscience Centre, Kids Research Institute, The Children's Hospital at Westmead, Westmead, Australia
9. Functional Neuromics, Children's Medical Research Institute, Westmead, Australia
10. Neuroscience, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, Australia
11. Murdoch Children's Research Institute, Department of Paediatrics, University of Melbourne, Melbourne, Australia
12. Westmead Research Hub Electron Microscope Core Facility, The Westmead Institute for Medical Research, Westmead, Australia
13. Bioinformatics Group, Children's Medical Research Institute, Westmead, Australia
14. Synapse Proteomics, Children's Medical Research Institute, Westmead, Australia

Recessive variants in *PYROXD1* cause PYROXD1 myopathy, an ultra-rare congenital or adult-onset disorder affecting skeletal muscle and connective tissue. Clinical features include generalised muscle weakness, respiratory and feeding difficulties, distal joint laxity, hypernasal speech, blue sclerae, and osteopenia. Fewer than 30 individuals have been reported worldwide.

PYROXD1 encodes pyridine nucleotide-disulphide oxidoreductase domain 1 (PYROXD1), a ubiquitously expressed oxidoreductase enzyme essential for cell viability; complete loss of PYROXD1 causes cell death in vitro and is embryonically lethal in mice. Although PYROXD1 is thought to contribute to mitochondrial respiration and regulation of the tRNA ligase complex, its precise biological function remains unclear. The pathomechanism underlying PYROXD1 myopathy is also poorly understood, and no targeted therapies are currently available.

To advance understanding and support therapy development, we generated a mouse model harbouring the *PYROXD1* variant p.N155S (*Pyroxd1*$_{N155S}$), found on at least one allele in 80% of reported cases. *Pyroxd1*$_{N155S}$ mice faithfully replicate the human myopathy, displaying marked skeletal muscle pathology—including fibre atrophy, internalised nuclei, disrupted sarcomeres, and abnormal mitochondrial distribution. We also identified a significant bone phenotype, with reduced cortical thickness and bone mineral density. Functional assessments revealed that *Pyroxd1*$_{N155S}$ mice exhibit decreased skeletal muscle strength, particularly in fast-twitch fibres, as shown by *in vivo* grip strength and *ex vivo* contractile testing. However, muscle performance during force-frequency stimulation, fatigue, and eccentric contraction protocols was comparable to wild-type controls. Proteomic analysis of mutant muscle further supports PYROXD1's role in essential cellular processes, including mitochondrial function and protein synthesis.

In summary, we have established a robust and well-characterised mouse model of *PYROXD1* myopathy that recapitulates the human phenotype. This model is now being used to evaluate therapeutic strategies including PYROXD1 replacement and voluntary exercise.

#37 Tuesday Poster Session

Mechanically-induced nuclear damage leads to global transcriptional heterogeneity across Lmna KO myonuclei

Nienke ten Cate[1,2], Tyler J. Kirby[1,2,3]
Affiliations:
1. Department of Physiology, Amsterdam UMC, Amsterdam, The Netherlands
2. Amsterdam Cardiovascular Sciences, Heart Failure and Arrhythmias, Amsterdam UMC, Amsterdam, The Netherlands
3. Amsterdam Movement Sciences, Tissue Function and Regeneration, Amsterdam UMC, Amsterdam, The Netherlands

Mutations in the *LMNA* gene, encoding for nuclear envelope proteins lamin A and C, gives rise to a group of diseases known as laminopathies. These diseases primarily impact striated muscle, including heart and skeletal muscle. Previous studies have indicated that mechanical stress leads to nuclear abnormalities and DNA damage in *Lmna* mutant muscle cells, potentially contributing to cell dysfunction and death. However, the precise molecular mechanisms linking these nuclear defects to myofiber dysfunction remain uncertain. Skeletal muscle cells contain hundreds of nuclei and transcriptional output must be coordinated across myonuclei to maintain proper muscle cell function. Thus, one possible mechanism where nuclear damage leads to muscle dysfunction is through alterations in the transcriptional output of individual nuclei, for example, through mislocalization of transcription factors or increased DNA damage. This study aimed to gain more insight into the transcriptional dynamics in *Lmna* knock-out (*Lmna* KO) and mutant (*Lmna* N195K and H222P) skeletal muscle cells, focusing on the relationship between lamin A/C, nuclear damage, and transcriptional dynamics.

In this study, we used *in vitro* differentiated myofibers from primary myoblasts, and a sophisticated real-time nascent RNA labeling approach to examine differences in transcription rates and nuclear RNA export. We first compared *Lmna* KO myotubes to wild-type myotubes to investigate potential differences in transcription and/or RNA export. Here, *Lmna* KO myotubes revealed both increased and uncoordinated transcription compared to wild-type myotubes. This same trend was also observed in the more severe *Lmna* N195K mutant cell line, whereas it was not present in the less severe H222P model, indicating that the degree of transcriptional miscoordination correlates with the severity of the disease model. Using a reporter for nuclear rupture (cGAS-mNG), we examined the impact of these nuclear ruptures on transcriptional regulation, and found no clear association between nuclear rupture and transcriptional output. Lastly, to investigate the direct role of mechanical stress, we expressed a DN-KASH construct in *Lmna* KO myotubes, which blocks force transmission to the nucleus and prevents nuclear damage. This approach successfully rescued the transcriptional differences observed in *Lmna* KO myotubes, suggesting a mechanotransduction pathway linking nuclear integrity to gene expression.

Collectively, our results suggest that mechanically induced nuclear damage can lead to aberrant transcriptional dynamics across *Lmna* KO myonuclei, independent of nuclear rupture, which could affect local biosynthetic output within the myofiber. This transcriptional dysregulation was reversed by blocking mechanical stress via the DN-KASH model,

implicating mechanotransduction as a key mediator. Importantly, a trend towards increased and uncoordinated transcription was also observed in the more severe *Lmna* N195K mutant cell line, but not in the less severe H222P model, indicating a possible correlation between the extent of transcriptional miscoordination and disease severity. Future studies will focus on the molecular mechanisms underlying uncoordinated transcription in skeletal muscle nuclei. What remains unclear is which components of the cytoskeleton contribute to nuclear damage. We hypothesize that microtubule dynamics affects nuclear integrity, and we aim to understand the role of the microtubule network in driving the distinct phenotypic differences seen in laminopathic skeletal muscle disease.

#38 Tuesday Poster Session

Impact of *TPM2* Myopathy-Linked Mutations on Thin Filament Length Regulation

Recep Küçükdoğru[1], Katarzyna Robaszkiewicz[1], Małgorzata Siatkowska[1], Joanna Moraczewska[1]

Department of Biochemistry and Cell Biology, Faculty of Biological Sciences, Kazimierz Wielki University, Bydgoszcz, Poland

Introduction In striated muscle, tropomyosin (Tpm), a key regulatory protein of the thin filament, works in concert with troponin (Tn) to control actin-myosin interactions in a calcium-dependent manner. Beyond its well-established regulatory role, Tpm is also thought to contribute to the maintenance of thin filament length, a critical factor for proper muscle function. Thin filament length can vary between muscle fiber types, influencing the number of myosin heads that can engage with actin and, consequently, the force generated during contraction. Several actin-binding proteins contribute to the regulation of thin filament length, including cofilin-2 (Cof-2), an actin-severing protein whose dysregulation has been linked to muscle pathology. Because Tpm spans the entire length of the thin filament, it likely modulates Cof-2 activity. Mutations in the *TPM2* gene, which encodes the muscle-specific tropomyosin isoform Tpm2.2, are associated with congenital muscle disorders characterized by either reduced (hypocontractile) or excessive (hypercontractile) muscle contraction. While these mutations are known to disrupt muscle function, their potential impact on Cof-2–mediated regulation of thin filament length remains unclear.

Aim of the Work In this study, we hypothesized that *TPM2* mutations disrupt the molecular mechanisms that regulate thin filament length, thereby contributing to disease pathogenesis. Our research focuses on how Tpm2.2 and myopathy-associated Tpm2.2 variants influence actin polymerization, filament stability, and the regulation of actin filament dynamics by Cof-2.

Methods To investigate the effects of *TPM2* mutations, we used skeletal muscle-derived proteins (actin, myosin, and troponin) along with recombinant wild-type and mutant Tpm2.2 isoforms, including hypercontractile (D20H, E181K) and hypocontractile (E41K, N202K) variants. Cof-2 affinity for unregulated and regulated actin filaments was assessed by co-sedimentation assays. Actin polymerization dynamics were monitored via light scattering and pyrene-actin fluorescence. Filament length was analyzed by fluorescence microscopy using rhodamine–phalloidin-labeled actin.

Results All Tpm2.2 variants slowed actin polymerization from both the barbed and pointed ends. When the barbed end was capped with CapZ to isolate pointed-end growth, Tpm2.2 similarly inhibited polymerization, and this effect was not altered by the mutations. Fluorescence microscopy of rhodamine–phalloidin-labeled filaments attached to myosin HMM-coated coverslips revealed mutation-specific effects on filament length: hypercontractile mutants (D20H, E181K) had little or modest shortening effects, while hypocontractile mutants (E41K, N202K) significantly increased filament length.

Cof-2 bound to Tpm2.2-decorated filaments with slightly lower affinity than to bare actin. All Tpm2.2 variants were removed from filaments by increasing Cof-2 concentrations, although D20H showed the highest resistance. Interestingly, in the presence of troponin and absence of Ca^{2+}, Cof-2 affinity increased, yet it was unable to displace Tpm2.2, suggesting co-occupancy of all three proteins on actin. Cof-2 markedly accelerated actin polymerization, an effect attenuated by all Tpm2.2 variants, with E41K showing the weakest inhibition.

These findings suggest that *TPM2* mutations modulate thin filament length but do not substantially impair Cof-2 function.

#39 Tuesday Poster Session

VARIANT IN MYOSIN BINDING PROTEIN C1 (MYBPC1) CAUSES SPONTANEOUS OSCILLATORY CONTRACTIONS IN MICE

Stefan Conijn[1], Daan Hoomoedt[1], Rajvi Balesar[1], Josine de Winter[1], Edoardo Malfatti[2], Coen Ottenheijm[1].

Affiliations:
1. Amsterdam UMC, Amsterdam, The Netherlands
2. Centre de Référence de Maladies Neuromusculaires, Paris, France

Slow skeletal myosin binding protein C is a sarcomeric protein that is encoded by MYBPC1. Variants in MYBPC1 have been linked to myogenic tremors, however the underlying contractile abnormalities remain poorly understood. We investigated the impact of a recessive MYBPC1 variant (G132R) on the contractile function and the presence of spontaneous oscillatory contractions (SPOCs) in skeletal myofibers from a patient, alongside a newly developed G132R knock-in mouse model.

Myofibers were isolated from the patient's biopsy, permeabilized, and activated with solutions containing calcium. Data was compared to that of control subjects. The cross-sectional area (CSA) of patient's myofibers (0.0031 mm2) was significantly smaller compared to controls (0.0051 mm2). Patient's myofibers showed a reduced maximum tension (at pCa 4.5) compared to controls (155 mN/mm2 vs. 252 mN/mm2). The calcium sensitivity of force did not differ. Interestingly, both slow- and fast-twitch myofibers displayed spontaneous oscillatory contractions (SPOCs), with the highest occurrence at pCa 6.2. Slow-twitch myofibers displayed an average frequency of 8.9 SPOCs/min, and fast-twitch myofibers 37.3 SPOCs/min. Physiological conditions mimicked by 4% dextran and 30°C enhanced SPOC amplitude and frequency. Treatment with 2.5 µM Mavacamten significantly attenuated both SPOC frequency and amplitude. During the oscillations the sarcomere length varied between 2.23 µm and 2.60 µm for slow-twitch myofibers and 2.40 µm and 2.54 µm for fast-twitch myofibers. No SPOCs were observed in myofibers from healthy controls. Preliminary analyses in the G132R mouse model revealed similar SPOCs in soleus muscle fibers, recapitulating the patient's phenotype.

In summary, this study highlights the contractile dysfunction in myofibers from a patient with a recessive variant in MYBPC1. The suppression of SPOCs by Mavacamten suggests a role for a higher DRX state of myosin in the patient's biopsy. Our mouse model might provide a valuable tool for further mechanistic studies and drug testing, targeting MYBPC1.

#40 Tuesday Poster Session

The effects of myositis-specific autoantibodies in Immune-Mediated Necrotizing Myopathy on muscle fiber contractility.

Tom J. Kerkhoff[1,2,3], Sanna Luijcx[1,2], Buram Ardiç[1,2], Patrycja Stachurska[1], Anneke J. van der Kooi[4], Eleonora M. Aronica[5], Joost Raaphorst[4] & Coen A.C. Ottenheijm[1,2]

t.j.kerkhoff@amsterdamumc.nl, j.raaphorst@amsterdamumc.nl, c.ottenheijm@amsterdamumc.nl

[1]Amsterdam UMC location Vrije Universiteit, Physiology, De Boelelaan 1117, Amsterdam, The Netherlands.
[2]Amsterdam Cardiovascular Sciences, Pulmonary Hypertension & Thrombosis, Amsterdam, The Netherlands.
[3]Amsterdam Movement Sciences, Musculoskeletal Health, Amsterdam, The Netherlands.
[4]Amsterdam UMC location University of Amsterdam, Neurology, Meibergdreef 9, Amsterdam, The Netherlands.
[5]Amsterdam UMC location University of Amsterdam, Pathology, Meibergdreef 9, Amsterdam, The Netherlands.

Background – Immune-mediated necrotizing myopathy (IMNM) is the most severe myositis subtype in terms of muscle weakness. Immunosuppressive therapies are still insufficient and there is a need for better and personalized therapies. IMNM is associated with myositis-specific autoantibodies (MSAs) against signal recognition protein-54 (SRP) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR), which have been suggested to play a pathogenic role. We have previously shown that the force generating capacity of muscle fibers from IMNM patients is impaired, however we do not know the cause of this impairment. Since these patients have elevated MSAs (up to 1000x fold), this raises the question whether it is caused by the MSAs.

Methods – Either anti-SRP$^+$, anti-HMGCR$^+$ or seronegative serum was drawn from IMNM patients and healthy controls and stored in our biobank. Subsequently, healthy murine intact muscle fibers from the Flexor Digitorum Brevis (FDB) muscle were isolated for our *ex-vivo* culture model. After 1 day of culture (to allow the muscle fibers to recover from isolation), the fibers are exposed to the serum for 2 hours. Then the fibers were assessed for contractility in two different set-ups. The first set-up is an innovative high throughput set-up, in which muscle contractility and calcium handling was measured during electrical stimulation. In the second set-up we permeabilized the muscle fibers and mounted them between a force transducer and length motor. Fibers were exposed to solutions with incremental Ca^{2+}-concentrations and the force was measured (without potential confounding effects of Ca^{2+} cycling by the sarcoplasmic reticulum). Afterwards, the fiber was stored in a buffer for gel electrophoresis to fiber type.

Results – Our data suggest that muscle contractility is altered after 2h exposure to serum of IMNM patients compared to healthy controls: during electrical stimulation, sarcomere shortening was higher after exposure to anti-SRP$^+$, anti-HMGCR$^+$ and seronegative serum, indicating altered contractility. Preliminary data suggest that exposure to anti-HMGCR$^+$ serum lowers the maximum force of healthy muscle fibers, but Ca^{2+}-sensitivity of force was not altered.

Conclusion – Serum of IMNM patients has a direct impact on muscle contractility. It remains unclear which serum component causes the impact. Therefore, experiments are ongoing with the IgG and IgG-depleted serum of these patients to further investigate the role of the MSAs.

#41 Tuesday Poster Session

CAPILLARY RAREFACTION AND ASSOCIATED ALTERATIONS IN VEGF/VEGFR IN HUMAN DUCHENNE MUSCULAR DYSTROPHY

Xueying Huang*, Jodie Finn and Karl J.A. McCullagh.

Affiliations:
1. University of Galway, Galway, Ireland.

Background: Duchenne muscular dystrophy (DMD) is a devastating X-linked recessive disease of musculoskeletal system. It is caused by a genetic mutation which results in the absence of the large sarcolemma associated dystrophin protein. The absence of dystrophin leads to compromised muscle stability and progressive muscle weakness through decreased myofibre integrity. Dystrophin is predominantly expressed in all muscles, but it is expressed in other tissues including the smooth and endothelial cells of the blood vessels. Studies on rodent models of DMD have suggested that altered vascularisation is a contributing factor to the pathology of DMD. However, few studies have examined this in humans. Therefore, in the present study we examine the vascularization of human DMD muscles. Myofibre dimensions, capillary density, capillary-to-fibre ratio and VEGF/VEGFR factor mRNAs, in control and DMD human muscle tissues.

Methods: This study included muscles from 9 Control and 8 DMD human patients (aged 12-17) from the Myobank-AFM†. (1) Muscle samples were stained by H&E, trichrome, and triple stained with fluorescent tagged lectins, followed by semi-automated quantitative analyses via ImageJ. (2) RNA was extracted from human muscle tissues, followed by the assessment of VEGF/VEGFR factors mRNA expression by quantitative RT-qPCR.

Results: (1) Clear visualisation of fibrosis was observed in H&E and fluorescent stained DMD muscle tissues. (2) Mean muscle fibre area and minimum Feret's diameter were significantly decreased in DMD tissues compared to control. (3) Capillary density, and capillary to fibre ratio of muscle tissue were diminished in DMD samples compared to control. (4) VEGF/VEGFR factors such as VEGFR1 and VEGFR2 decreased in DMD compared with that in control tissues.

Conclusion: The findings reveal the abnormal myofibre structure, muscle composition, and tissue vascularisation in DMD. Decreased capillarization and the VEGF receptors in dystrophic muscle remains a further therapeutic avenue in the treatment of DMD.

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Keywords: Duchenne muscular dystrophy; vascularisation; VEGF; capillaries.

#42 Tuesday Poster Session

Static skeletal muscle exosomes inhibit osteogenic differentiation of BMSCs via the miRNA6363/S100A8 axis

Yongyong Yan[1,2†], Haiyan Wang[1,2†],Sihua Liang[1], Yuxin Zhang[1], Nan Fang[1], Zihan Hao[1], Janak L. Pathak[1*] ,Richard T. Jaspers[1,2*]

[1]School and Hospital of Stomatology, Guangdong Engineering Research Center of Oral Restoration and Reconstruction & Guangzhou Key Laboratory of Basic and Applied Research of Oral Regenerative Medicine, Guangzhou Medical University, Guangzhou, 510182,China

[2]Laboratory for Myology, Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, 1081 BT Amsterdam, The Netherlands

Introduction: Long-term bedridden patients often experience muscle atrophy and bone density loss. While a diet rich in calcium and protein may help mitigate muscle loss during periods of bedrest, it has limited efficacy in preventing bone degeneration. Exosomes, which are rich in proteins and nucleic acids, play a crucial role in muscle-bone crosstalk. Preliminary experimental results indicate that Static skeletal muscle exosomes (Ssm-Exos) can inhibit the osteogenic differentiation of Bone Marrow Stromal Cells (BMSCs). Hence, we propose the scientific question: What is the mechanism by which Ssm-Exos inhibit BMSCs osteogenic differentiation? **Purpose**: We aim to clarify the inhibition mechanism of Ssm-Exos on BMSC osteogenic differentiation through the miRNA/key gene axis. **Methods:** 1. Exosome extraction via differential centrifugation and identification using TEM, nano-flow cytometry, and western blotting. 2. Analysis of the effect of exosomes on BMSC osteogenic differentiation using RT-qPCR, ALP staining, and Alizarin Red staining(ARS). 3. Identification of key inhibitory genes (S100A8) and verification of miRNA6363 enrichment in Ssm-Exos through high-throughput sequencing, miRNA detection, and dual-luciferase assays. 4. Overexpression of S100A8 in BMSCs, with subsequent analysis of BMSC osteogenic differentiation, S100A8 expression and intracellular calcium ion levels. **Results**: Ssm-Exos were isolated and characterized. Ssm-Exos inhibited osteogenic differentiation of BMSCs by RT-qPCR and ARS. High-throughput sequencing results showed that S100 calcium binding protein A8(S100A8), was robustly downregulated in Ssm-Exos-treated BMSCs. Downregulated S100A8 decrease intracellular Ca^{2+} concentration and inhibited osteogenic differentiation of Ssm-Exos -treated BMSCs. The overexpression of S100A8 in BMSCs rescued Ssm-Exos-inhibited osteogenic differentiation. Furthermore, our experimental results demonstrated that miRNA6363 is significantly enriched in Ssm-Exos and can bind to S100A8, thereby inhibiting its expression. **Conclusion**: *In vitro* experiments show that Ssm-Exos reduce S100A8 expression in BMSCs through miRNA6363, decrease intracellular Ca^{2+} concentration, and inhibit BMSCs osteogenic differentiation. This study is the first to report that static skeletal muscle can inhibit BMSCs osteogenic differentiation through the miRNA6363/S100A8 axis. This study offers a novel insight into the mechanisms underlying bone loss resulting from prolonged bed rest and muscle inactivity, while also pinpointing potential new therapeutic targets.

#43 Tuesday Poster Session

BROAD THERAPEUTIC BENEFIT OF MYOSIN INHIBITION IN HYPERTROPHIC CARDIOMYOPATHY

Laura Sen-Martín[1], Ángel Fernández-Trasancos[1], Miguel Á. López-Unzu[1], Agata Bak[1], Divya Pathak[2], Alessia Ferrarini[1], Verónica Labrador-Cantarero[1], David Sánchez-Ortiz[1,3], María Rosaria Pricolo[1], Natalia Vicente[1], Diana Velázquez-Carreras[1], Lucía Sánchez-García[1], Jose Ángel Nicolás-Ávila[1], María Sánchez-Díaz[1], Saskia Schlossarek[4,5], Lorena Cussó[1,6,7], Manuel Desco[1,6,7,8], María Villalba-Orero[1,9], Gabriela Guzmán-Martínez[10,11], Enrique Calvo[1], Roberto Barriales-Villa[12,13,14], Jesús Vázquez[1,14], Fátima Sánchez-Cabo[1], Andrés Hidalgo[1], Lucie Carrier[4,5], James A. Spudich[2], Kathleen M. Ruppel[2], Olivier Cazorla[15], Annika Klotz[16], Michel N. Kuehn[16,17], Khoi D. Nguyen[16], Anthony L. Hessel[16,17], Jorge Alegre-Cebollada[1]

Affiliations:
1. Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain
2. Department of Biochemistry and Stanford Cardiovascular Institute, Stanford University School of Medicine
3. Servicio de Cardiología, Hospital Puerta de Hierro de Majadahonda, Madrid, Spain
4. Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
5. 2DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Hamburg, Germany
6. Laboratorio de imagen para pequeño animal de experimentación, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain
7. CIBER de salud mental, Instituto de salud Carlos III, Madrid, Spain
8. Departamento de Bioingeniería, Universidad Carlos III de Madrid, Madrid, Spain
9. Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain
10. Department of Cardiology, La Paz University Hospital, IdiPaz, Madrid, Spain
11. Department of Medicine, Faculty of Biomedical and Health Sciences, Universidad Europea de Madrid, Spain
12. Unidad de Cardiopatías Familiares, Servicio de Cardiología, Complexo Hospitalario Universitario de A Coruña, Servizo Galego de Saúde (SERGAS)
13. Instituto de Investigación Biomédica de A Coruña (INIBIC), Universidade da Coruña, A Coruña, Spain
14. CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain
15. PhyMedExp, University of Montpellier, INSERM, CNRS, Montpellier, France
16. Accelerated Muscle Biotechnologies Consultants, Boston, MA, USA
17. Institute of Physiology II, University of Muenster, Muenster, Germany

Many myocardial pathologies are caused by inherited genetic mutations that result in anatomical alterations and compromised cardiac function. In humans, the missense variant R502W in cardiac myosin-binding protein C (cMyBP-C) is the most frequent mutation leading to hypertrophic cardiomyopathy (HCM). However, the molecular mechanisms sustaining the pathogenicity of the R502W variant remain unknown, since both cMyBP-C's mRNA and protein structure have been proposed not to be perturbed by the mutation. Using CRISPR/Cas9-based genetic engineering, we have generated a knock-in mouse model that harbors the R502W mutation in murine cMyBP-C and characterized the resulting cardiac phenotype. R502W mice exhibit progressive myocardial remodeling without alterations in cMyBP-C levels or

localization. In addition, while we observe changes in myosin structural states (ON/OFF) in accordance with increased calcium sensitivity, passive force, and slightly decreased binding affinity between mutant cMyBP-C and myosin. Despite these changes, the myosin conformational states (super-relaxed (SRX) and disordered-relaxed (DRX)), which are defined by the rate at which myosin heads consume ATP, remain unaltered. Although the knockout (KO) and R502W cMyBP-C animals present distinct pathomechanisms, with no affected SRX/DRX ratio in the R502W model, mavacamten effectively attenuates HCM myocardial remodeling in both. Furthermore, R502W mice exhibited enhanced exercise tolerance following treatment. We observed that mavacamten increases the proportion of myosin heads in the OFF state, which could explain the mechanism of action underlying the beneficial effects of myosin inhibition in our R502W model.