POTSDAM · GERMANY

46th European Muscle Conference

SEPTEMBER
19–22, 2017

Program & Abstracts

www.emc2017-potsdam.com

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Welcome Address

Dear colleagues,

Welcome to the 46th European Muscle Conference!

This is the annual scientific meeting of our society, the European Society for Muscle Research (http://www.esmr.org), of which all of you have become a member by attending this conference, or have been a member for some time. This conference has originally been initiated by Professor Marcus Schaub from Switzerland, who sends his best greetings to this year's assembly, but unfortunately, could not anymore attend himself. As we are nearing almost half a century of annual EMC events, muscle biology has never been more exciting. New technologies, targets, and concepts allow novel insight into heart and muscle function, which we could not have dreamed of, sometimes even a few years before. I hope that some of the excitement about scientific progress in the field can be felt during this meeting.

I am delighted that we convene in Potsdam, formerly a residence of the Prussian kings and famous for its beautiful parks and palaces, including Sanssouci, the largest World Heritage Site in Germany. We will visit this park and get a glimpse of some of its architecture during the meeting. I hope you will also have time to explore other areas of Potsdam, including the city center featuring an interesting mix of old and new buildings, or the park area 'Neuer Garten', where the castle 'Schloss Cecilienhof' hosted an important post-WW2 conference in 1945. Our meeting site, the Seminaris SeeHotel, is nestled in between a forest and lake, a few miles from the city center. I hope that the secluded setting next to spectacular historic sights will make it an ideal place to discuss science in the true spirit of the European Muscle Conferences and allow you to gain both personally and scientifically from this meeting.

I would like to thank the many helpful people involved in the organization of this conference, in particular Elisa Antonucci from the K.I.T. Group, the members of the International Advisory Board and Young Investigator Award jury, as well as the industrial sponsors and the German Research Foundation for their generous financial support.

With best wishes for an enjoyable visit to Potsdam,

Prof. Wolfgang Linke

EMC 2017 CHAIRMAN

ACKNOWLEDGEMENT

The European Society for Muscle Research and the organizers of the 46th European Muscle Conference gratefully thank the German Research Foundation (DFG) for the financial support to this meeting.

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European Society for Muscle Research (ESMR)
c/o Karolinska Institutet
Department of Physiology and Pharmacology
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SE 171 77 Stockholm, Sweden
http://www.esmr.org

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Web: www.kit-group.org

Conference Venue

Seminaris SeeHotel Potsdam
An der Pirschheide 40
14471 Potsdam, Germany

Potsdam is located southwest of Berlin and can be easily reached by various means of transport.

By Train

Long-distance trains stop at Berlin's main station 'Hauptbahnhof'. From here you can reach Potsdam's main station (Hauptbahnhof) in 45 minutes with the S-Bahn (city trains) **S7** and **S1**.
From Potsdam-Main Station, you can take a taxi (about 15 €) to the Conference Venue.
If you prefer to reach the hotel by regional train or public transport, the nearest stop is **'Potsdam-Pirschheide'**, situated ca. 800 m (less than 15 min by foot) from the Seminaris SeeHotel (regional train **R22**, tram **#91**, bus **#631**, **#580** and **#695**).

By Plane

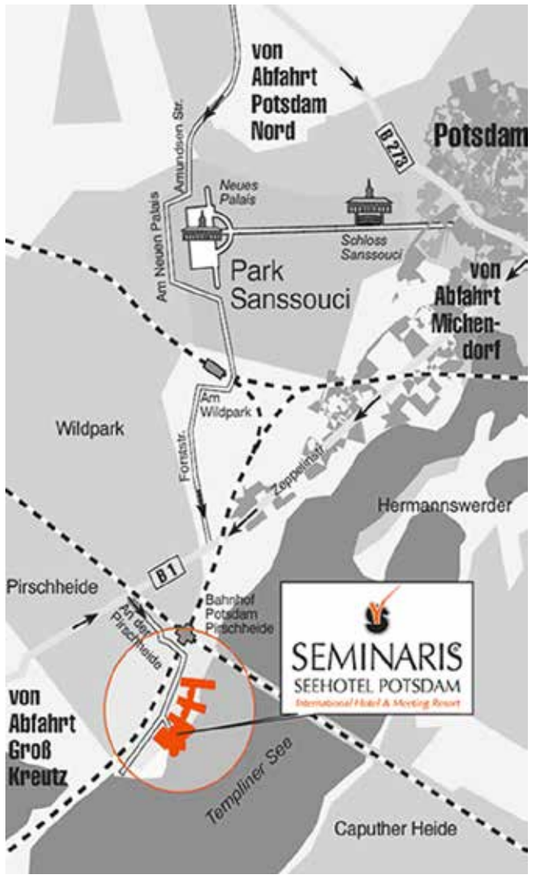
From Berlin-Tegel and the Schönefeld Airport, you can easily reach Potsdam. From Schönefeld, regional trains or buses take you directly to Potsdam. From Tegel, you can take the airport shuttle to the train station **'Charlottenburg'** or to the station **'Zoologischer Garten'** and then the S-Bahn or regional train to Potsdam's main station.

By Car

BY CAR FROM THE NORTH: coming from the A24 (Hamburg/Rostock) to the A10 (Berliner Ring), exit **'Potsdam Nord'**.
FROM THE WEST: coming from the A2 (Hannover) to the A10, exit **'Groß Kreutz'**.
FROM THE SOUTH AND EAST: coming from the A9/A13/A12 (Leipzig, Dresden, Frankfurt/O.) to the A10, exit **'Michendorf'**.

By Water Taxi

REACHING THE SEMINARIS SEEHOTEL BY WATER TAXI
The Seminaris SeeHotel is situated on the shore of Lake Templin and has its own boat landing. A regular water taxi can be taken right to/from Seminaris Hotel from/to downtown Potsdam, Sanssouci, Cecilienhof Castle and other attractions.
From Potsdam Main Station-Haven, it takes 40 minutes to reach the hotel by Water Taxi. Check the time schedule on the website
www.potsdamer-wassertaxi.de



Registration Desk

The registration desk, located in the entrance foyer, will be at your service throughout the duration of the conference.

Opening hours

Tuesday, September 19 //	**13:00–19:00**
Wednesday, September 20 //	**08:00–17:00**
Thursday, September 21 //	**08:00–18:30**
Friday, September 22 //	**08:00–13:00**

On-site Registration Fees

Full Participant	**€580.00**
Young Investigator	**€450.00**

The conference fee includes:
- Admission to all sessions, poster area and exhibition
- Coffee and Lunch breaks
- Final program/abstract book
- Attendance at the Welcome Reception on September 19, 2017
- Sightseeing tour to Sanssouci Park and New Palace on September 20, 2017

Certificate of Attendance

Certificates of attendance will be sent to every participant **by e-mail** after the conference.

Internet Access

Free Wi-Fi is provided throughout the conference venue. You will get the corresponding password at the registration desk.

Social Program

Welcome Reception
Tuesday, September 19, 2017 // 18:00
The EMC 2017 Welcome Reception will take place on Tuesday evening in the lobby of the Seminaris SeeHotel. It states the perfect opportunity to meet old friends and colleagues or start new networks, while enjoying some snacks and drinks.

Sightseeing Tour
Wednesday, September 20, 2017 // 17:15
We invite all conference participants to join us for the Guided Tour through the Sanssouci World Heritage palaces and scenic park.
The attendance is included in the registration fee. Come and don't miss this unique opportunity!

Conference Dinner
Thursday, September 21, 2017 // 19:00
The social highlight of EMC 2017 will be the dinner cruise on board of the beautiful 'MS Sanssouci'.
The ticket price of **€ 50** includes dinner and drinks for the duration of the cruise (ca. 2,5/3 hours).
Tickets can be purchased at the registration desk (upon availability).

Young Investigator Award

Abstracts of Young Scientists participating in the **YOUNG INVESTIGATOR COMPETITION** have been pre-sorted and pre-ranked by the Young Investigator Award Committee and the best 10 abstracts chosen for oral communication. During the meeting, the YIA Committee will rank the quality of the YIA talks and come up with a final listing. The winners will be announced during the Closing Ceremony on Friday, September 22.

Following three money prizes will be awarded:

1st Prize	**€ 500**
2nd Prize	**€ 300**
3rd Prize	**€ 200**

The awards will be co-sponsored by the **Journal of Muscle Research and Cell Motility.**

Springer

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TUESDAY, SEPTEMBER 19, 2017

13:00 **Registration opens**

14:00–14:15 **Welcome & Introduction**

14:15–16:00 **Session 1**

Actin-Myosin Interactions, Novel Structural and Mechanical Aspects

CHAIRS: **Malcolm Irving** London, UK; **Dilson Rassier** Montreal, Canada

14:15–14:35 **S1-1** **Dilson Rassier** Montreal, Canada
Single sarcomere mechanics and inter-sarcomere dynamics in skeletal muscle myofibrils

14:35–14:40 Discussion

14:40–15:00 **S1-2** **Malcolm Irving** London, UK
Regulation of contraction by the thick filaments in skeletal muscle

15:00–15:05 Discussion

15:06–15:18 **S1-3** **Daria Logvinova** Moscow, Russia **YOUNG INVESTIGATOR AWARD COMPETITION**
Nucleotide-induced movements of essential light chain-1 in myosin subfragment 1 as studied by fluorescence resonance energy transfer (FRET)

15:18–15:23 Discussion

15:24–15:36 **S1-4** **Mamta Amrute-Nayak** Hannover, Germany
Regulatory light chain's role in fine-tuning myosin motor function

15:36–15:41 Discussion

15:42–15:54 **S1-5** **Masataka Kawai** Iowa City, USA
Nebulin KO in mice causes a decrease in in-series stiffness of the thin filament and consequential decrease in force / cross-bridge in slow-twitch soleus muscle fibers

15:54–15:59 Discussion

16:00–16:30 **Coffee Break and Visit of Exhibitors**

16:30–17:15 **Debate Session I**

Muscle Contraction: Is Titin Contributing Actively?

DISCUSSANTS: **Vincenzo Lombardi** Florence, Italy; **Julio Fernandez** New York, USA
MODERATOR: **Miklos Kellermayer** Budapest, Hungary

17:15–18:00 **Keynote I** **Stefan Raunser** Dortmund, Germany

The power of cryo-electron microscopy: Structure of a human cytoplasmic actomyosin complex at near-atomic resolution

CHAIR: **Kristina Djinovic-Carugo** Vienna, Austria

18:00 **Welcome Reception,** LOBBY OF SEMINARIS SEEHOTEL

WEDNESDAY, SEPTEMBER 20, 2017

08:30–10:15 **Session 2**

Titin and its Binding Partners

CHAIRS: **Olga Mayans** Konstanz, Germany; **Wolfgang Linke** Bochum / Münster, Germany

08:30–08:42 **S2-1** **Antonio Sponga** Vienna, Austria **YOUNG INVESTIGATOR AWARD COMPETITION**
Structural and biophysical characterization of α-actinin-2 in ternary complex with FATZ-1 and Zq titin

08:42–08:47 Discussion

08:48–09:00 **S2-2** **Robbert van der Pijl** Amsterdam, Netherlands **YOUNG INVESTIGATOR AWARD COMPETITION**
Diaphragm passive stretch induces hypertrophy, which is modulated by titin-based stiffness

09:00–09:05 Discussion

09:06–09:18 **S2-3** **Sandra Swist** Bochum, Germany
Loss of Z-disc anchored titin in adult skeletal muscle cells leads to sarcomere disassembly

09:18–09:23 Discussion

09:23–09:35 **S2-4** **Henk Granzier** Tucson, USA
The giant elastic protein titin regulates the length of the striated muscle thick filament--titin rules

09:35–09:40 Discussion

09:40–09:52 **S2-5** **Zsolt Martonfalvi** Budapest, Hungary
Force generation by titin folding

09:52–09:57 Discussion

09:57–10:09 **S2-6** **Jorge Alegre-Cebollada** Madrid, Spain
Native redox posttranslational modifications as regulators of titin mechanical properties

10:09–10:14 Discussion

10:15–11:30 **Coffee Break, Poster Discussions and Visit of Exhibitors**

11:30–13:15 **Session 3**

E-C Coupling and Neuromuscular Interactions

CHAIRS: **László Csernoch** Debrecen, Hungary; **William Louch** Oslo, Norway

11:30–11:50 **S3-1** **William Louch** Oslo, Norway
Cardiomyocyte dyadic structure and function in health and disease
11:50–11:55 Discussion

11:55–12:15 **S3-2** **László Csernoch** Debrecen, Hungary
Role of surface membrane calcium current in the regulation of sarcoplasmic calcium release in adult skeletal muscle fibres
12:15–12:20 Discussion

12:21–12:33 **S3-3** **Dilyana Filipova** Köln, Germany YOUNG INVESTIGATOR AWARD COMPETITION
Transcriptomic changes during secondary myogenesis in RYR1- and DHPR-deficient limb skeletal muscles
12:33–12:38 Discussion

12:39–12:51 **S3-4** **Richard Ribchester** Edinburgh, UK
Membrane excitability and excitation-contraction coupling at neuromuscular junctions in larval Drosophila
12:51–12:56 Discussion

12:57–13:09 **S3-5** **Arthur Cheng** Stockholm, Sweden
A fast skeletal muscle troponin activator (FSTA), CK-2066260, mitigates the fatigue-induced decline in skeletal muscle contractile force by lowering the metabolic cost
13:09–13:14 Discussion

13:15–14:15 **Lunch**

14:15–16:00 **Session 4**

Stem Cell-derived Myocytes, Experimental Genome Editing, Muscle Tissue Engineering

CHAIRS: **Lucie Carrier** Hamburg, Germany; **Michael Regnier** Seattle, USA

14:15–14:35 **S4-1** **Lucie Carrier** Hamburg, Germany
Inherited cardiomyopathies: disease modeling in iPSC-derived cardiomyocytes and engineered heart tissue
14:35–14:40 Discussion

14:40–15:00 **S4-2** **Michael Regnier** Seattle, USA
Cronos titin in developing human cardiomyocytes: degraded no more
15:00–15:05 Discussion

15:06–15:18 **S4-3** **Natalie Weber** Hannover, Germany YOUNG INVESTIGATOR AWARD COMPETITION
Single cell mapping used to assign mRNA and protein expression of cardiac myosin heavy chain to twitch kinetics of the same human embryonic stem cell derived cardiomyocyte
15:18–15:23 Discussion

15:24–15:36 **S4-4** **Albano Meli** Montpellier, France
Patient-specific induced pluripotent stem cell-derived cardiomyocytes to model, screen drugs and decipher molecular mechanisms of CPVT1 syndrome
15:36–15:41 Discussion

15:42–15:54 **S4-5** **Thomas Iskratsch** London, UK
Alteration of cardiomyocyte mechanosensing through hypertrophic signaling
15:54–15:59 Discussion

16:00–16:45 **Keynote II** **Norbert Hübner** Berlin, Germany

Dissecting the genetic basis of translational regulation in heart failure

CHAIR: **Martina Krüger** Düsseldorf, Germany

16:45–17:15 **Coffee Break and Preparation for Sightseeing Tour**

17:15 **Departure in front of Seminaris hotel for Sightseeing Tour**
(see page 7 for detailed information)
Dinner on your own

THURSDAY, SEPTEMBER 21, 2017

08:30–10:15 **Session 5**

Signaling and Regulatory Mechanisms

CHAIRS: **Anders Arner** Stockholm, Sweden; **Belinda Bullard** York, UK

08:30–08:50 **S5-1** **Anders Arner** Stockholm, Sweden
Using zebrafish larvae to examine striated and smooth muscle function and mechanisms in human muscle disease
08:50–08:55 Discussion

08:55–09:15 **S5-2** **Belinda Bullard** York, UK
Thin filament regulation in insect flight muscle and how it differs in cardiac muscle
09:15–09:20 Discussion

YOUNG INVESTIGATOR AWARD COMPETITION

09:21–09:33 **S5-3** **João Almeida Coelho** Porto, Portugal
Titin phosphorylation by protein kinase G as a novel mechanism of diastolic adaptation to acute load
09:33–09:38 Discussion

09:39–09:51 **S5-4** **Kristina Djinovic-Carugo** Vienna, Austria
Regulation and mechanostability of titin/α-actinin bond
09:51–09:56 Discussion

09:57–10:09 **S5-5** **Pieter de Tombe** Chicago, USA
Impact of titin strain on the cardiac slow force response
10:09–10:14 Discussion

10:15–12:00 **Coffee Break, Poster Discussions and Visit of Exhibitors**

12:00–12:45 **Debate Session II**

Residual Force Enhancement in Muscle – Facts and Fancy

DISCUSSANTS: **Walter Herzog** Calgary, Canada; **Dilson Rassier** Montreal, Canada; **Wolfgang Linke** Bochum/Münster, Germany; **Daniel Hahn** Bochum, Germany
MODERATOR: **Wolfgang Linke** Bochum/Münster, Germany

12:45–13:45 **Lunch**

13:45–15:30 **Session 6**

Muscle Protein Development, Turnover and Repair

CHAIRS: **Julia von Maltzahn** Jena, Germany; **Robert Bryson-Richardson** Melbourne, Australia

13:45–14:05 **S6-1** **Julia von Maltzahn** Jena, Germany
Functional relevance of Klotho for maintenance and regeneration of skeletal muscle
14:05–14:10 Discussion

14:10–14:30 **S6-2** **Robert Bryson-Richardson** Melbourne, Australia
Autophagy as a therapy for myofibrillar myopathy
14:30–14:35 Discussion

14:36–14:48 **S6-3** **Judith Hüttemeister** Berlin, Germany YOUNG INVESTIGATOR AWARD COMPETITION
The role of titin in sarcomere assembly and dynamics
14:48–14:53 Discussion

14:54–15:06 **S6-4** **Frank Schnorrer** Marseille, France
Measuring molecular tension at muscle attachment sites during myofibril assembly
15:06–15:11 Discussion

15:12–15:24 **S6-5** **Elisabeth Barton** Gainesville, USA
Satellite cell migration is regulated by MMP13 and required for skeletal muscle growth and repair
15:24–15:29 Discussion

15:30–16:00 **Coffee break and Visit of Exhibitors**

16:00–17:45 **Session 7**

Plasticity, Metabolism and Energetics

CHAIRS: **Graham Lamb** Melbourne, Australia; **Christoph Handschin** Basel, Switzerland

16:00–16:20 **S7-1** **Christoph Handschin** Basel, Switzerland
The regulation of cell plasticity and tissue-crosstalk by skeletal muscle PGC-1α
16:20–16:25 Discussion

16:25–16:45 **S7-2** **Graham Lamb** Melbourne, Australia
Effects of oxidation and nitrosylation on muscle function in exercise and disease
16:45–16:50 Discussion

16:51–17:03	**S7-3**	Gaia Gherardi Padova, Italy **YOUNG INVESTIGATOR AWARD COMPETITION** **The physiopathological role of mitochondrial calcium uptake in skeletal muscle homeostasis**
17:03–17:08		Discussion
17:09–17:21	**S7-4**	Richard Jaspers Amsterdam, Netherlands **IGF-1 attenuates hypoxia-induced atrophy but inhibits myoglobin expression in C2C12 skeletal muscle myotubes**
17:21–17:26		Discussion
17:27–17:39	**S7-5**	Martin Connolly London, UK **miR-424-5p: a novel negative regulator of ribosomal biogenesis which contributes to muscle wasting**
17:39–17:44		Discussion

17:45–18:30 **Business Meeting** (All conference participants are invited.)

19:00 **Social Dinner / Boat tour on lake** (Departure at pier behind Seminaris hotel, see page 7 for detailed information)

FRIDAY, SEPTEMBER 22, 2017

08:30–10:15 **Session 8**

Heart Failure and Cardiomyopathies

CHAIRS: **Nazha Hamdani** Bochum, Germany; **Simon Sedej** Graz, Austria

08:30–08:50	**S8-1**	Nazha Hamdani Bochum, Germany **The metabolic road of co-morbidities to understanding the pathophysiology of heart failure: The role of inflammatory signaling pathways in obesity and diabetes**
08:50–08:55		Discussion
08:55–09:15	**S8-2**	Simon Sedej Graz, Austria **Cardioprotective mechanisms of dietary spermidine in aging and hypertension**
09:15–09:20		Discussion
09:21–09:33	**S8-3**	Stephanie Carr Newcastle, UK **YOUNG INVESTIGATOR AWARD COMPETITION** **Studying mdx cardiomyocyte hypertrophy in vitro**
09:33–09:38		Discussion

09:39–09:51	**S8-4**	Samantha Harris Tucson, USA **The Spy-C method for in situ replacement of cardiac myosin binding protein-C in sarcomeres**
09:51–09:56		Discussion
09:57–10:09	**S8-5**	Steven Marston London, UK **The molecular defects in Ca^{2+}-regulation due to mutations that cause hypertrophic cardiomyopathy can be reversed by small molecules that bind to troponin**
10:09–10:14		Discussion

10:15–10:45 **Coffee Break and Visit of Exhibitors**

10:45–12:30 **Session 9**

Skeletal Muscle Diseases

CHAIRS: **Lars Larsson** Stockholm, Sweden; **Isabelle Richard** Evry, France

10:45–11:05	**S9-1**	Isabelle Richard Evry, France **Calpain 3 and its partner titin in muscular dystrophies**
11:05–11:10		Discussion
11:10–11:30	**S9-2**	Lars Larsson Stockholm, Sweden **Bench to bedside research on critical illness myopathy (CIM) and ventilator induced diaphragm muscle dysfunction (VIDD): Mechanisms and intervention strategies**
11:30–11:35		Discussion
11:36–11:48	**S9-3**	Catherine Wingate Perth, Australia **YOUNG INVESTIGATOR AWARD COMPETITION** **The skeletal muscle pathophysiology of a novel dystrophin-negative mouse strain which exhibits a decrease in susceptibility to muscle damage**
11:48–11:53		Discussion
11:54–12:06	**S9-4**	Vincenzo Sorrentino Siena, Italy **Mutations in the CASQ1 gene alter calsequestrin properties and cause tubular aggregate myopathy**
12:06–12:11		Discussion
12:12–12:24	**S9-5**	Andreas Unger Münster, Germany **Translocation of molecular chaperones to the titin springs compromises sarcomere function in skeletal myopathy patients**
12:24–12:29		Discussion

12:30–13:00 **Closing Ceremony and Young Investigator Award**
(see page 7 for detailed information)

Muscle Physiology

Aurora Scientific's muscle physiology instruments include dual-mode levers, length controllers, force transducers, stimulators, data acquisition hardware and software and experimental apparatus. All of our products are sold individually or in complete systems.

With our complete systems, muscle physiologists can precisely measure force, length, sarcomere length and ratiometric calcium. Our systems allow all standard muscle physiology protocols to be run such as twitch, tetanus, fatigue, force-frequency, force-velocity, stiffness and work loops. In addition, our systems have the flexibility that allows the researcher to customize experimental protocols to meet their needs.

EXERCISE & METABOLISM

Understanding Muscle Performance, Injury and Recovery

From studying how different athletes perform or recover to quantifying an animal model's resistance to metabolic fatigue, the field of exercise and metabolic physiology encompasses a broad range of study. Aurora Scientific has solutions to address these different areas of inquiry whether in murine or other animal models, or even with human samples. Powerful software married with precise instruments for studying whole animal, whole muscle and single fiber samples make Aurora Scientific the leader in providing Exercise Physiologists with the means to understand muscle performance at the highest level.

CARDIOLOGY

The Study of Heart Muscle Tissue and How to Preserve It

For nearly 20 years Aurora Scientific has been at the forefront of cardiac tissue mechanics research. Aurora Scientific equipment is used by many of the world's top researchers studying isolated heart tissue, fibers and even cells. We understand that heart muscle is different from other types of muscle and that its study presents unique challenges. Whether measuring force in cardiac myocytes or quantifying the mechanical properties of iPSC-derived cardiac scaffolds, Aurora Scientific has the expertise and the instruments to support the most demanding of cardiac mechanics experiments.

MUSCLE PATHOLOGY & WASTING

Muscle Atrophy Through Disease, Disuse or Chronic Injury

As the largest generation in history enters their senior years, research on muscle pathologies has come into focus. These can include rare conditions like muscular dystrophy or more commonly, muscle atrophy due to cancer or disuse after injury. To develop drugs and therapies that combat this broad group of myopathies, functional measurements of muscle are often required in animal models. Aurora Scientific has worked with researchers in this community to provide tools that allow the study of muscles like the diaphragm as well as non-invasive assays to perform longitudinal tests on an individual animal as a disease or therapy progresses. We are proud of our long history working with the leading researchers and academics in this field and proud of the role our muscle pathology research instruments have played.

MOLECULAR BIOLOGY & PHARMACOLOGY

Understanding Muscle Biochemistry and Response to Pharmaceuticals

The study of animal models is one of the foundational pillars of molecular biology research. Aurora Scientific understands that your animal model is unique, and that characterizing its molecular phenotype can be challenging. It is important that the mechanism of drug action be understood in order to comprehend complex tissue responses. We have the solutions to study gross whole body physiology or the function of specific muscles to make characterizing the response of your animal model to pharmaceuticals both precise and efficient.

GENERAL PHYSIOLOGY

Studying the Relationship Between Structure and Function in Muscle

Aurora Scientific produces transducers specially designed for measuring force, velocity, sarcomere length and other contractile properties in muscle. Integration with specially designed apparatus and software simplifies complex characterization of muscle properties. No matter what building block of muscle tissue you use as your model, from the myofibril to whole muscle groups and connective tissue, Aurora Scientific has a solution for you.

COMPARATIVE PHYSIOLOGY

Functional Diversity of Muscle in Various Organisms

The study of how living things move and are shaped by their environment often requires specialized tools and assays. Aurora Scientific has proven instruments engineered to the highest standards of accuracy and precision to uncover the diverse functional characteristics of muscle in your unique animal models. Measure complex muscle contractile properties from leeches, zebrafish and even cheetahs with our diverse, high performance muscle physiology equipment.

POSTERS should hang for the duration of the whole conference.

Poster Viewing / Discussions are scheduled during the morning coffee breaks on **Wednesday, September 20** (10:15–11:30) and **Thursday, September 21** (10:15–12:00).

Poster Session Number	Title
1	Contractility and Force Generation
2	Cytoskeletal Proteins Beyond Actin / Myosin
3	EC Coupling
4	Heart Failure and Cardiomyopathies
5	Heart Muscle Structure and Function
6	Muscle Development, Turnover and Repair
7	Muscle Exercise, Metabolism, Energetics and Plasticity
8	Regulation of Muscle Contraction
9	Signalling Mechanisms in Muscle
10	Skeletal Muscle Diseases
11	Skeletal Muscle Structure and Function
12	Stem Cell Derived Myocytes and Experimental Genome Editing

1 Poster Session 1

Contractility and Force Generation

P1-1 **Partial titin degradation increases sarcomere length non-uniformities and reduces absolute residual force enhancement after active stretch in single myofibrils**
Venus Joumaa, Fanny Bertrand, Shuyue Liu, Sophia Poscente, Walter Herzog

P1-2 **The significance of phosphate dependent rate modulation of k_{TR} for the cross-bridge mechanism**
Robert Stehle

P1-3 **The sag response in human adductor pollicis muscle**
Ian C. Smith, Jahaan Ali, Geoffrey A. Power, Walter Herzog

P1-4 **Estrogen affects skeletal muscle force generation in females by modulating post-tetanic potentiation and by altering the super relaxed state of myosin**
Dawn Lowe, Gengyun Le, Lien Phung, Sira Karvinen, David Thomas

P1-5 **On the nature of unloading-induced postural muscle stiffness decline**
Boris Shenkman, Irina Petrova, Sergey Tyganov, Timur Mirzoev

P1-6 **Direct isometric muscle strain analyses using speckle tracking technology. A validation study**
Lars Henrik Frich, Anders Holsgaard Larsen, John Hjarbaek, Jordi Sanchez Dahl, Kate Lykke Lambertsen

2 Poster Session 2

Cytoskeletal Proteins Beyond Actin / Myosin

P2-1 **Filamin actin-binding and titin-binding fulfill distinct functions in Z-disc cohesion**
Nicanor Gonzalez-Morales, Frieder Schock

P2-2 **Interactions between titin and myosin filaments explored with atomic force microscopy**
Miklós Kellermayer, Dominik Sziklai, Zsombor Papp, Brennan Decker, Eszter Lakatos, Zsolt Mártonfalvi

P2-3 **Extraocular muscles (EOM) in desmin knock out and R349P desmin mutant mice**
Maria Angels Rodriguez, Joel Stenow, Arvin Behzadi, Kimmo Parkkonen, Jing-Xia Liu, Rolf Schröder, Christoph S. Clemen, Zhenlin Li, Fatima Pedrosa-Domellöf

P2-4 **Structural and biophysical characterization of FATZ-1, FLNc and α-actinin 2 interactions**
Georg Mlynek, Martin Puchinger, Antonio Sponga, Julius Kostan, Kristina Djinovic-Carugo

3 Poster Session 3

EC Coupling

P3-1 **Electrophysiological characteristics of the neuromuscular apparatus in case of impaired motor activity**
Elvira Yamalitdinova, Arthur Fedianin, Tatyana Baltina, Anton Eremeev

P3-2 **Modulation of the spinal cord motor evoked potentials after spinal contusionin rat during treatment with local hypothermia**
Dinara Silantyeva, Ekatirina Loban, Maksim Baltin, Tatyana Baltina, Igor Lavrov

P3-3 **Influence of reinforcement maneuver on the spinal cord motor evoked responses in calf muscles**
Alena Militskova, Ksenya Spiridonova, Leysan Bikchentaeva, Gusel Yafarova

P3-4 **Evoked potentials in gastrocnemius muscle of rat in condition of gravitational unloading with spinal cord stimulation**
Artur Fedianin, Irina Lvova, Nafis Ahmetov, Anton Eremeev, Igor Lavrov

P3-5 **Theoretical analysis of Ca^{2+}-handling in skeletal muscle fibers of Calsequestrin-null mice**
Lorenzo Marcucci, Carlo Reggiani, Marta Canato, Ger Stienen

4 Poster Session 4

Heart Failure and Cardiomyopathies

P4-1 **Comparison of complete titin deletion versus titin truncation reveals difference in striated muscle phenotype**
Michael Radke, Christopher Polack, Michael Gotthardt

P4-2 **Combined therapy deflazacort/omega-3 ameliorates heart and diaphragm dystrophy in the mdx mouse**
Maria Julia Marques, Marcos Maciel Jr, Camila de Jesus Saqueli, Samara Camaçari de Carvalho, Adriana Fogagnolo Mauricio, Humberto Santo Neto

P4-3 **Pifitrin-α, an inhibitor of p53, ameliorates dystrophic cardiomyopathy in the *mdx* mice model of Duchenne muscular dystrophy**
Humberto Santo Neto, Isabel C. Chagas Barbin, Juliano Alves Pereira, Maria Julia Marques

P4-4 **Intrinsic *MYH7* expression regulation may contribute to tissue level allelic imbalance in hypertrophic cardiomyopathy**
Judith Montag, Mandy Syring, Julia Rose, Anna-Lena Weber, Pia Ernstberger, Anne Mayer, Edgar Becker, Britta Keyser, Jolanda van der Velden, Carolyn Y Ho, Antoni Francino, Bernhard Brenner, Theresia Kraft

P4-5 **Effect of cardiomyopathy-associated mutations of tropomyosin on the calcium regulation of the actin-myosin interaction in atria**
Galina Kopylova, Daniil Shchepkin, Alexander Matyushenko, Sergey Bershitsky

P4-6 **Altered myofilament structure and function in dogs with Duchenne muscular dystrophy cardiomyopathy**
Younss Ait Mou, Alain Lacampagne, Thomas Irving, Valerie Scheuermann, Stephane Blot, Bijan Ghalah, Pieter De Tombe, Olivier Cazorla

P4-7 **Analysis of active transcription sites in single human cardiomyocytes reveals burst-like transcription of *MYH7***
Kathrin Kowalski, Ante Radocaj, Cristobal G. dos Remedios, Antonio Franciono, Francesco Navarro-Lopez, Theresia Kraft, Bernhard Brenner

P4-8 **Development of novel computational biology pipeline for the efficient classification of titin SNPs for clinical use**
Jennifer Fleming, Dan Rigden, Olga Mayans

P4-9 **Identification and functional annotation of E2-activated ERα and ALC-1 regulated genes in human cardiomyocytes based on microarray analysis data**
Cindy Schriever, Anna Greco, Shokoufeh Mahmoodzadeh, Ingo Morano

P4-10 **Diabetes affects adaptive titin modification in response to acute myocardial ischemia/reperfusion**
Malgorzata Kazmierowska, Sebastian Kötter, Dominik Semmler, Joachim P Schmitt, Martina Krüger

P4-11 **Characterization of a minipig model of hypertrophic cardiomyopathy**
Sadie Bartholomew Ingle, Marcus Henze, David Meyerholz, Robert Weiss, Christopher Rogers, Abhay Divekar, Ferhaan Ahmad, Eric Green

P4-12 **The role of Akt/GLUT/HK salvage pathway in the induction of a cardioprotective phenotype in SHR conplastic strains by adaptation to chronic hypoxia**
Jitka Zurmanova, David Kolar, Barbara Elsnicova, Jiri Novotny, Martin Kalous, Michal Pravenec, Jan Neckar, Frantisek Kolar, Iveta Brabcova

P4-13 **Model of independent stochastic burst-like transcription can explain observed functional and transcriptional variability among cardiomyocytes from Hypertrophic Cardiomyopathy patients**
Ante Radocaj, Kathrin Kowalski, Judith Montag, Theresia Kraft, Bernhard Brenner

P4-14 **Distinct signalling pathways mediate phosphorylation of sarcomeric proteins in the right and left ventricle of the failing heart**
Arpad Kovacs, Mark Waddingham, Detmar Kolijn, Judit Barta, Attila Tóth, Zoltán Papp, Sophie van Linthout, Carsten Tschöpe, Wolfgang Linke, Nazha Hamdani

5 Poster Session 5

Heart Muscle Structure and Function

P5-1 **Mathematical model of electromechanical coupling in human cardiomyocytes allowing for cooperative effects of the crossbridge attachment on CaTnC kinetics**
Leonid Katsnelson, Tatiana Sulman, Arseniy Dokuchaev, Olga Solovyova

P5-3 **Cardiological involvement in idiopathic inflammatory myopathies and the diagnosis of cardiac involvement in idiopathic inflammatory myopathy by cardiac magnetic resonance tomography**
Suqiong Ji

P5-4 **Effect of phosphorylation of tropomyosin on the calcium regulation of the actin-myosin interaction in myocardium**
Daniil Shchepkin, Galina Kopylova, Valentina Oshchepkova, Salavat Nabiev, Larisa Nikitina, Alexander Matyushenko, Sergey Bershitsky

P5-5 **Hypobaric Hypoxia Enhances Expression of Connexin 43 in the Rat Left Ventricular Myocardium**
Jana Kohútová, Bára Elsnicová, Ondrej Šebesta, Kristyna Holzerova, Markéta Hlavačková, Oľga Nováková, František Kolář, Ján Neckář, Narcisa Tribulová, Jitka Žurmanová

P5-6 **Structural and biochemical characterisation of human cardiac troponin C mutations associated with genetic cardiomyopathies**
Khawla Kasar

P5-7 **Cold acclimation induces prolonged changes in β-adrenergic response of rat myocardium**
Veronika Tibenská, Aneta Marvanová, David Kolář, Anna Podojilová, Lucie Hejnová, Barbara Elsnicová, Zdeněk Drahota, Jiří Novotný, Stanislav Vybíral, Jitka Žurmanová

P5-8 **A human CSRP3/MLP mutation is methylated, causes mis-splicing and expression of pathological isoforms, ultimately leading to heart failure**
Zaher Elbeck, Valerio Azzimato, Ali Mustafa Tabish, Byambajav Buyandelger, Xidan Li, Akos Vegvari, John.W. Wiseman, Mohammad Bohlooly, Jessica Wahlgren, Karine Enesa, James Hunt, Roy Milner, Ralph Knöll

P5-9 **Dobutamine increases mechanical efficiency in isolated rat papillary muscle by increasing external work without affecting oxygen consumption**
Eva Peters, Duncan van Groen, Ingrid Schalij, Harm Jan Bogaard, Anton Vonk Noordegraaf, Willem van der Laarse

P5-10 **Novex-3 titin as a potential novel signalling node in cardiac and skeletal muscle development**
Lisa Beckendorf, Sandra Swist, Andreas Unger, Wolfgang Linke

P5-11 **The impact of titin oxidation and unfolding on cardiac and skeletal muscle function**
Martin Breitkreuz, Yong Li, Karl Toischer, Lars Leichert, Wolfgang Linke, Nazha Hamdani

P5-12 **Impact of cGMP-PKG pathway modulation on titin phosphorylation and titin-based myocardial passive stiffness**
Melissa Herwig, Soraya Hölper, Marcus Krüger, Doris Koesling, Michaela Kuhn, Wolfgang Linke, Nazha Hamdani

6 Poster Session 6

Muscle Development, Turnover and Repair

P6-1 **The actin-binding properties of *Drosophila* Zasp52 contribute to myofibril assembly**
Kuo An Liao, Frieder Schock

P6-2 **Physiological function of myogenic cells is impaired in piglets with low birth weight**
Katja Stange, Claudia Miersch, Martin Kolisek, Monika Röntgen

P6-3 **Expression and localisation of the 3-hydroxylacyl-coA dehydratase (HACD) enzymes in developing zebrafish embryos**
Rhiannon Morgan, Richard Piercy, Imelda McGonnell, Mandy Peffers, Richard Barrett-Jolley, Gemma Walmsley

P6-4 **Glycerol-induced injury activates fibrosis in rat muscle through upregulation of transforming growth factor-β1**
Mohamed Mahdy, Katsuhiko Warita, Yoshinao Hosaka

P6-5 **Effects of heat treatment on force recovery after fatiguing contraction in rat fast-twitch muscle**
Chihiro Aibara, Daiki Watanabe, Sohta Fukatsu, Masanobu Wada

P6-6 **Bone marrow derived cells contribute to the satellite cell niche during skeletal muscle regeneration**
Dana Cizkova, Zora Komarkova, Ales Bezrouk, Jirina Vavrova, Stanislav Filip, Jaroslav Mokry

P6-7 **Myogenic differentiation of *Pax7-/-* pluripotent stem cells in teratomas**
Anita Helińska, Iwona Grabowska, Maria Ciemerych-Litwinienko

7 Poster Session 7

Muscle Exercise, Metabolism, Energetics and Plasticity

P7-1 **Does the Hfe gene mutation have an effect on physical performance in a hereditary hemochromatosis mouse model?**
Haidar Djemai, Rémi Thomasson, Lotfi Mhamdi, Damien Vitiello, François Desgorces, Jean-François Toussaint, Philippe Noirez

P7-2 **Absence of gonad-related factors alters exercise performance in mice**
Philippe Noirez, Rémi Thomasson, Arnaud Ferry

P7-3 **Eccentric training prevents skeletal muscle wasting in colon 26 tumor-bearing mice**
Daisuke Tatebayashi, Koichi Himori, Ryotaro Yamada, Yuki Ashida, Mitsunori Miyazaki, Takashi Yamada

P7-4 **Lipid peroxidation and antioxidant system activity changes of rat blood and cardiac muscle cells under chronic stress**
Natalia Dachanidze, George Burjanadze, Matrona Chachua, Keti Menabde, Nana Koshoridze

P7-5 **Acute and long-term effects of reduced capillary perfusion on skeletal muscle function and adaptive remodelling**
Peter Tickle, Hans Degens, Stuart Egginton

8 Poster Session 8

Regulation of Muscle Contraction

P8-1 **The chaperone co-inducer BGP-15 alleviates ventilation induced diaphragm dysfunction**
Heba Salah, Meishan Li, Nicola Cacciani, Stefano Gastaldello, Hannah Ogilvie, Hazem Akkad, Arvind Venkat Namuduri, Valeria Morbidoni, Konstantin Artemenko, Gabor Balogh, Vicente Martinez-Redondo, Paulo Jannig, Yvette Hedström, Barry Dworkin, Jonas Bergquist, Jorge Ruas, Laszlo Vigh, Leonardo Salviati, Lars Larsson

P8-2 **Study of the effects of tropomyosin dimers on actin-myosin interaction at molecular level**
Larisa Nikitina, Salavat Nabiev, Oksana Alimpieva, Galina Kopylova, Daniil Shchepkin, Alexander Matyushenko, Sergey Bershitsky

P8-3 **Effects of N202K and R133W mutations in β-chains of tropomyosin on structural and functional properties of its αβ-heterodimers**
Alexander Matushenko, Daria Logvinova, Daniil Shchepkin, Galina Kopylova, Sergey Bershitsky, Dmitrii Levitsky

P8-4 **Study of the effect of cardiomyopathic mutations of Tpm on the stiffness of thin filaments using the optical trap**
Salavat Nabiev, Larisa Nikitina, Margarita Filippova, Oksana Alimpieva, Galina Kopylova, Daniil Shchepkin, Alexander Matyushenko

P8-5 **Titin-mediated thick filament activation, through a mechanosensing mechanism, introduces sarcomere-length dependencies in mathematical models of rat trabecula and whole ventricle**
Lorenzo Marcucci, Takumi Washio, Tashio Yanagida

P8-6 **Myocyte Ca2+ cycling is impaired in the nonischemic remote parts of the heart early after myocardial infarction**
Annette Kronenbitter, Florian Funk, Katarzyna Hackert, Martina Krüger, Joachim P. Schmitt

P8-7 **Molecular markers associated with different time-periods of muscle disuse: role of regulatory proteins**
Laurence Stevens, Laetitia Cochon, Valerie Montel, Bruno bastide

P8-8 **Loss of the endoplasmic reticulum resident antioxidant selenoprotein S (SEPS1) impairs fast twitch contractile function in mouse hindlimb muscles**
Alex Addinsall, Craig Wright, Chris Shaw, Natasha McRae, Leonard Forgan, Chai-Heng Weng, Xavier Conlan, Paul Francis, Zoe Smith, Sofianos Andrikopoulos, Nicole Stupka

P8-9 **Thin filament regulation in insect flight muscle and how it differs in cardiac muscle**
Belinda Bullard, Demetris Koutalianos, Kate English

9 Poster Session 9

Signalling Mechanisms in Muscle

P9-1 **Role of mitoKATP in skeletal muscle**
Giulia Di Marco, Angela Paggio, Cristina Mammucari, Diego De Stefani, Rosario Rizzuto

P9-2 **Muscle *igf1* deletion leads to impaired glucose homeostasis**
Georgios Vasilakos, Katherine Bennett, Tongjun Gu, Julio Ayala, Michael Matheny, Jason Puglise, Elisabeth R Barton

P9-3 **Garlic-derived S-allylmercaptocysteine and chronic aerobic exercise improve insulin sensitivity and modulate Nrf2 and NF-κB/IκBα pathways in the skeletal muscle of a non-alcoholic fatty liver disease animal model**
Qian YU, Zhengyun Xia, Emily Liong, Man Lung Fung, George Tipoe

P9-4 **Exogenous application of La^{3+} upregulates myosin heavy chain type I mRNA through activation of calcineurin in C2C12 cells**
Yoshiaki Mori, Junko Yamaji

P9-5 **Effect of calcineurin activation by organic acids on expression of interleukin-6 and myosin heavy chain class II_b mRNA levels in mouse myocytes**
Junko Yamaji, Yoshiaki Mori

P9-6 **Cav1.1 is involved in eEF2k activity increase in rat m.soleus during hindlimb suspension. NH125 activates eEF2k**
Yulia Lomonosova, Natalia Vilchinskaya, Svetlana Belova, Tatiana Nemirovskaya, Erzhena Altaeva, Boris Shenkman

P9-7 **The effects of two different stretching protocols on skeletal myotubes**
Athanasios Moustogiannis, Anastassios Philippou, Evangelos Zevolis, Antonis Chatzigeorgiou, Michael Koutsilieris

P9-8 **Transcription factors regulating E3-ligases MuRF-1 and MAFbx expression at the early stage of muscle disuse**
Tatiana Nemirovskaya, Ekaterina Mochalova, Svetlana Belova, Natalia Vilchinskaya, Boris Shenkman

P9-9 **A possible role of stretch-activated ion channels in the activation of anabolic signalling in rat soleus muscle during an acute recovery from disuse atrophy**
Timur Mirzoev, Sergey Tyganov, Boris Shenkman

P9-10 **Impact of stretch-activated ion channels inhibition on the transduction of mechanical signal to mTORC1 in rat soleus muscle under hindlimb unloading**
Sergey Tyganov, Timur Mirzoev, Boris Shenkman

P9-11 **Signaling pathways regulating contractility in smooth muscle from the rat aorta and pulmonary artery**
Frank Brozovich, Neslihan Dikmenoğlu-Falkmarken, Alexander Hedfjäll, Young Soo Han, Anders Arner

P9-12 **Enhanced capacity for CaMKII signaling lowers contraction-induced calcium release and slows contraction of fast-twitch muscle and fatigued slow-oxidative muscle**
Martin Flück, Colline Sanchez, Vincent Jacquemond, Christine Berthier, Arnold de Haan, Guus Baan, Richard Jaspers, Wouter Eilers

P9-13 **The effect of myofillar myopathy assocated desmin mutants on protein turnover**
Jody Martin, Stephanie Simon, Florence Delort, Pieter de Tombe, Patrick Vicart, Sabrina Batonnet-Pichon

P9-14 **Mechanically unfolded titin immunoglobulin domains refold more accurately when assisted by chaperone alpha-B-crystallin**
Yong Li, Wolfgang Linke

P9-15 **Ultrastructural and biochemical characterization of the interaction between activator of Hsp90 ATPase protein 1 (Aha1) and titin in cardiac muscle cells**
Christian Winter, Andreas Unger, Wolfgang Linke

P9-16 **Ceramide in skeletal muscle during hindlimb unloading**
Irina Bryndina, Maria Shalagina, Vladimir Protopopov, Alexey Iakovlev

10 Poster Session 10

Skeletal Muscle Diseases

P10-1 **Comparative transcriptome analysis of skeletal muscle in ADSSL1 myopathy**
Hyung Jun Park, Young-Chul Choi

P10-2 **Myofibrillar dysfunction in a rat model of critical illness myopathy is prevented by neuromuscular electrical stimulation**
Takashi Yamada, Ryotaro Yamada, Koichi Himori, Daisuke Tatebayashi, Yuki Ashida, Yoshiki Masuda, Tomihiro Imai

P10-3 **Eccentric exercise prevents impaired contractility and autophagy flux in skeletal muscle from adjuvant-induced arthritis rat**
Koichi Himori, Daisuke Tatebayashi, Ryotaro Yamada, Yuki Ashida, Takashi Yamada

P10-4 **miR-424-5p: a novel negative regulator of ribosomal biogenesis which contributes to muscle wasting**
Martin Connolly, Richard Paul, Roser Farré Garrós, John Wort, Paul Kemp

P10-5 **The inflammatory profile of fast twitch skeletal muscle is amplified by reduced Selenoprotein S (SEPS1) expression**
Craig Wright, Giselle Allsopp, Alex Addinsall, Natasha McRae, Sof Andrikopoulos, Nicole Stupka

P10-6 **25(OH) Vitamin D protects from atrophy *in vitro***
Hana Sustova, Marilisa De Feudis, Simone Reano, Flavia Prodam, Nicoletta Filigheddu

P10-7 **A selective androgen receptor modulator, TEI-SARM2, improves muscle function in rat model of Duchenne muscular dystrophy**
Katsuyuki Nakamura, Masanobu Kanou, Toshie Jimbo, Hiroyuki Sugiyama, Kei Yamana

P10-8 **Oleic acid rescues palmitic acid-induced damage in human skeletal muscle fibroblasts undergoing transdifferentiation into adipocytes**
Oihane Jaka, Chibeza Agley, Norman Lazarus, Stephen Harridge

P10-9 **Effect of AHK2, a novel modulator of ryanodine receptors, in Duchenne muscular dystrophy**
Haizpea Lasa-Fernandez, Garazi Aldanondo, Jaione Lasa-Elgarresta, Aitziber Irastorza, Jose Ignacio Miranda, Jesus Maria Aizpurua, Adolfo López de Munain, Ainara Vallejo-Illarramendi

P10-10 **The effects of m. trapezius latent trigger point vibration on postural stability**
Elvira Mukhametova, Artur Fedianin, Tatyana Baltina

P10-11 **Positive end-expiratory pressure ventilation causes diaphragm fiber shortening in critically ill patients**
Johan Lindqvist, Marloes van den Berg, Robbert van der Pijl, Pleuni Hooijman, Charissa van den Brom, Sylvia Bogaards, Albertus Beishuizen, Marinus Paul, Jeroen Kole, René Musters, Michael Lawlor, Monique de Waard, Henk Granzier, Leo Heunks, Coen Ottenheijm

P10-12 **Characterisation of *MYO9A* as a pre-synaptic CMS gene**
Emily O'Connor, Vietxuan Phan, Isabell Cordts, Andreas Roos, Hanns Lochmüller

P10-13 **Brief prednisolone treatment improves critical illness myopathy in rats**
Hazem Akkad, Nicola Cacciani, Rebeca Corpeño Kalamgi, Lars Larsson

P10-14 **Expanding the phenotype of *BICD2* mutations towards skeletal muscle involvement**
Matthias Vorgerd

P10-15 **The clinical and pathologic findings of Korean patients with *RYR1*-related congenital myopathy**
Ha-Neul Jeong, Seung Woo Kim, Young-Chul Choi

P10-16 ***Drosophila* models of Laing distal myopathy**
Martin Dahl Halvarsson, Montse Olivé, Anne Uv, Homa Tajsharghi

P10-17 **Sarcomere dysfunction in nemaline myopathy caused by a mutation in slow skeletal troponin T (TNNT1)**
Martijn van de Locht, Stefan Conijn, Jose Pinto, Davide Iannuzzi, Michiel Helmes, Simon Edvardson, Coen Ottenheijm

P10-18 **Effects of a chaperone co-inducer (BGP-15) on contractile properties of single fibres from soleus muscle of rats exposed to Intensive Care Unit (ICU) conditions**
Nicola Cacciani, Heba Salah, Hazem Akkad, Anders Backeus, Lars Larsson

P10-19 **Biochemical investigations to unravel myopathological perturbations caused by the Caveolin-3 p.P104L mutation**
José Andrés Coraspe, Denisa Gabriela Hathazi, Hannah Michels, Eva Brauers, Stephanie Carr, Hanns Lochmüller, Erik Freier, Joachim Weis, Andreas Roos

P10-20 **The high Ca^{2+}-sensitivity associated with the Glu139del and Arg91Gly mutations in tropomyosin is caused by freezing of tropomyosin near the closed position**
Olga Karpicheva, Armen Simonyan, Nikita Rysev, Vladimir Sirenko, Charles Redwood, Yurii Borovikov

P10-21 **Turnover studies on DNAJB6 and the CASA pathway proteins**
Jaakko Sarparanta, Sabita Kawan, Per Harald Jonson, Bjarne Udd

P10-22 **Fast skeletal muscle troponin activator *tirasemtiv* improves *in vivo* muscle performance in a nemaline myopathy mouse model harboring the *Acta1*^{H40Y} mutation**
Josine de Winter, Charlotte Gineste, Elisa Minardi, Darren Hwee, David Bendahan, Maria Antonietta Pellegrino, Fady Malik, Roberto Bottinelli, Julien Gondin, Coen Ottenheijm

P10-23 **Injection of botulinum toxin A leads to impaired muscle function, hyperreflexia, increased passive stiffness and damage of the fibrilar and non-fibrilar structures of rat skeletal muscles**
Jessica Pingel, Mikkel Schou Nielsen, Torsten Lauridsen, Kristian Romlund Rix, Martin Bech, Tine Alkjaer, Ida Torp Andersen, Jens Bo Nielsen, Robert Feidenhansl

P10-24 **A patient-mimicking filaminopathy mouse model reveals increased myofibrillar lesion formation as a major pathomechanism**
Julia Schuld, Zacharias Orfanos, Frédéric Chevessier, Lucie Wolf, Alexandra Maerkens, Rudolf A. Kley, Anne-C. Plank, Stephan von Hörsten, Katrin Marcus, Matthias Vorgerd, Peter F. M. van der Ven, Rolf Schröder, Dieter O. Fürst

P10-25 **Characterization of the stromal cell population that interplay in skeletal muscle degeneration**
Cansu Özdemir-Saka, Duygu Akcay, Cetin Kocaefe

P10-26 **In vitro phenotypic comparison between young and aged human myotubes**
Joanne Young, Eve Duchemin-Pelletier, Melanie Flaender, Pauline Poydenot, Mathieu Raul

P10-27 **Muscle inflammation following supraspinatus tears**
Lars Henrik Frich, Henrik Daa Schrøder, Allan Stensballe, Kate Lykke Lambertsen

11 Poster Session 11

Skeletal Muscle Structure and Function

P11-1 **The active force-length relationship of skinned skeletal muscle fibres during extensive eccentric contractions**
André Tomalka, Christian Rode, Jens Schumacher, Tobias Siebert

P11-2 **Proteomic characterization of murine muscle fiber types via lasermicrodissection and LC MS/MS mass spectrometry**
Britta Eggers, Katalin Barkovits, Carolin Berwanger, Rolf Schröder, Maike Ahrens, Christoph Clemen, Katrin Marcus

P11-3 **Assessment of the relation between produced force and way of recruiting muscle fibres to achieve this force in humans**
Else Marie Bartels, Cécyl Claudel, Adrian P Harrison

P11-4 **Structural changes in diaphragm fibers of critically ill patients**
Stefan Conijn, Johan Lindqvist, Marloes van den Berg, Weikang Ma, Leo Heunks, Tom Irving, Coen Ottenheijm

P11-5 **Titin N2A behavior during passive and active stretch in mouse EDL muscle**
Mike DuVall, Kiisa Nishikawa

P11-6 **Rigor bonds under denaturing conditions reduce isometric tension development and lattice spacing of skinned porcine skeletal muscle fibres**
Jiao Liu, Matthias Schwartzkopf, Anders Arner

P11-7 **Immobilization during muscle development reduces muscle strength and lattice spacing**
Jiao Liu, Elisabeth Le, Matthias Schwartzkopf, Anders Arner

P11-8 **Nebulin"s C-terminus regulates muscle structure, function, and growth**
Frank Li, Elisabeth Barton, Henk Granzier

P11-9 **Structural studies of human ZASP in complex with α-actinin 2**
Valeria Stefania, Euripedes De Almeida, Julius Konstan, Kristina Djinovic

P11-10 **Features of tension rise in fast and slow skeletal muscles of the rabbit**
Pavel Kochubei, Galina Kopylova, Natalia Dremina, Daniil Shchepkin, Sergey Bershitsky

P11-11 **The rate of force generation in skeletal muscle is limited by the stress-dependent kinetics of the OFF-ON transition of the myosin-containing thick filament**
Luca Fusi, Elisabetta Brunello, Ziqian Yan, Malcolm Irving

P11-12 **Lack of desmin in normal human extraocular muscle fibers: a complex relation to innervation**
Fatima Pedrosa Domellöf, Jingxia Liu

P11-13 **A role for titin in the activation-dependent shift of the force-length relationship in skeletal muscle**
Anthony Hessel, Venus Joumaa, Walter Herzog, Kiisa Nishikawa

P11-14 **Towards a reconstitution of the sarcomeric Z-body: a possible strategy to assemble a mini Z-disk**
Tobias Thöni, Joan L. Arolas, Kristina Djinovic-Carugo

P11-15 **Structural insight into the myotilin-actin interaction**
Vid Puž, Sibylle Molt, Julius Kostan, Friedel Drepper, Thomas Schwarz, Miha Pavšič, Brigita Lenarčič, Peter F.M. van der Ven, Bettina Warscheid, Robert Konrat, Dieter O. Fürst, Kristina Djinović-Carugo

P11-16 **Mass spectrometry-based protein identification to understand the proteomic signature of human skeletal muscle**
Denisa Hathazi, Laxmikanth Kollipara, Werner Stenzel, René Zahedi, Andreas Roos

P11-17 **Identification of protein interaction pathways and partners of Klf5 in myoblast differentiation program**
Duygu Akcay, Cetin Kocaefe

12 Poster Session 12

Stem Cell Derived Myocytes and Experimental Genome Editing

P12-1 **Do β-Myosin heavy chain isoform-expressing myofibrils within human ESC-derived cardiomyocytes recapitulate the contractile features of adult human ventricular myofibrils?**
Bogdan Iorga, Natalie Weber, Kristin Schwanke, Birgit Piep, Meike Wendland, Stephan Greten, Ulrich Martin, Robert Zweigerdt, Theresia Kraft, Bernhard Brenner

P12-2 **Subdividing porcine satellite cell subpopulations with discontinuous gradient density centrifugation**
Claudia Miersch, Katja Stange, Monika Röntgen

P12-3 **A porcine model for the ß-myosin mutation R723G suggests onset of hypertrophic cardiomyopathy during fetal development**
Judith Montag, Björn Petersen, Anna K Flögel, Edgar Becker, Andrea Lucas-Hahn, Gregory J Cost, Christian Mühlfeld, Theresia Kraft, Heiner Niemann, Bernhard Brenner

P12-4 **Effect of a recombinant protein derived from a 6 EGF-like domain within delta-like 1 homology (DLK1) on inhibition of myogenesis in mouse myoblast cells**
Hyun Lee, Hye Jin Park, Jung Im Yun, Seung Tae Lee

P12-5 **Is myosin VI playing a role in myotube formation *via* its involvement in cell adhesion?**
Malgorzata Suszek, Olena Karatsai, Maria Jolanta Redowicz

P12-6 **The effect of different mechanical loading protocols on differentiated H9C2 cells**
Evangelos Zevolis, Anastassios Philippou, Athanasios Moustogiannis, Antonis Chatzigeorgiou, Michael Koutsilieris

P12-7 **Both autophagy and the ubiquitin-proteasome system contribute to titin turnover in stem cell-derived human cardiomyocytes**
Andrey N. Fomin, Wolfgang Linke

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KEYNOTE LECTURE

... K-1

The power of cryo-electron microscopy: Structure of a human cytoplasmic actomyosin complex at near-atomic resolution

*Stefan Raunser
Max Planck Institute of Molecular Physiology, Structural Biochemistry, Dortmund, Germany

Muscular movement plays an essential role not only in our lives. Muscle contraction is initiated by the release of calcium from the sarcoplasmic reticulum into the cytoplasm of myocytes through ryanodine receptors. Calcium binds to troponin, which releases tropomyosin from its blocking position allowing myosin filaments to move along actin filaments resulting in the contraction of the muscle. In my talk I will present not only our recent cryo-EM structure of a human cytoplasmic actomyosin complex, but also high-resolution structures of the mouse ryanodine receptor 1 in its open and closed state and F-actin in complex with tropomyosin. Together, the structures reveal the mechanisms involved in muscle contraction at an unprecedented level of molecular detail.

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ORAL SESSIONS

Session 1

Actin-Myosin Interactions, Novel Structural and Mechanical Aspects

... S1-1

Single sarcomere mechanics and inter-sarcomere dynamics in skeletal muscle myofibrils

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The sarcomere is the smallest functional unit of striated muscles. Sarcomeres are connected in series in a myofibril through a network of structural and elastic proteins. During myofibril activation, sarcomeres develop forces that are balanced through a complex, dynamic interaction among their structures. The mechanisms that regulate this inter-sarcomere dynamics are unclear, which limits our understanding of fundamental features of muscle contraction. In order to understand the mechanisms that regulate sarcomere contraction and inter-sarcomere dynamics, we developed (i) a system with micro-needles to measure contractility of isolated half-sarcomeres and sarcomeres, (ii) an system with atomic force microscopy to measure the force of isolated myofibrils, and (iii) a system with microfluidic perfusion to point-activate/deactivate and control one half-sarcomere/sarcomere within a myofibril, while measuring the individual behavior of all other sarcomeres. We found that the forces developed by an isolated half-sarcomere or sarcomere can be predicted by the their lengths, taking into account the degree of overlap between myosin and actin filaments and the stiffness of titin. We found that the force produced by myofibrils is more complex, as it also depends on the degree of half-sarcomere/sarcomere length non-uniformities caused by activation and different mechanical perturbations. Finally, we observed that activation and relaxation of one sarcomere within a myofibril leads to adjustments of adjacent sarcomeres, in a mechanism that is dependent on the myofibril length and stiffness. We concluded that the cooperative work of the contractile and elastic elements within a myofibril regulates inter-sarcomere dynamics, with important consequences for muscle contraction.

... S1-2

Regulation of contraction by the thick filaments in skeletal muscle

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Contraction of skeletal muscle is triggered by a calcium-dependent structural change in the actin-containing thin filaments that permits binding of myosin motors from the neighbouring thick filaments. In resting muscle, however, most of the myosin motors are folded back against their tails and packed into helical tracks on the surface of the thick filaments, making them unavailable for actin binding or ATP hydrolysis. These features are largely conserved across muscle types and species, including myosin-regulated muscles. Thick filament-based regulation is highly co-operative, and the regulatory states of the thick and thin filaments are positively coupled. Thick filaments can also be switched ON by mechanical stress (Linari *et al*., *Nature* 2015; Fusi *et al., Nat. Commun.* 2016). During muscle activation, this mechanism relies on a small population of constitutively ON motors outside thick filament control, and this population of motors is sufficient to drive unloaded shortening when the thin filament is ON. At higher load, these motors generate sufficient stress to release the remaining motors from their OFF conformation. This mechano-sensing mechanism seems to determine both the rate of force development and the classic force-velocity relationship of skeletal muscle. The well-known calcium/thin filament pathway provides the START signal for contraction, but the subsequent functional response of the muscle cell, including the rate of force development, adaptation to external load and the metabolic cost of contraction, is largely controlled by thick filament-mediated mechanisms.

... S1-3

Nucleotide-induced movements of essential light chain-1 in myosin subfragment 1 as studied by fluorescence resonance energy transfer (FRET)

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We applied FRET to examine how conformational changes occurring in myosin subfragment 1 (S1) upon formation of S1-ADP-BeFx complex (stable analog of the S1 ATPase intermediate state S1*-ATP) affect the distances between various sites on the essential light chain-1 (LC1) associated with S1 regulatory domain and two sites on S1 motor domain (Cys707 and nucleotide-binding site). Cys707 was labeled with 1,5-IAEDANS as a donor, and TNP-ADP bound in the S1 active site was used as an acceptor. S1 was reconstituted with various recombinant LC1 mutants, each containing a single Cys residue which was fluorescently labeled with either 5-IAF (acceptor) or 1,5-IAEDANS (donor). At physiological ionic strength (150 mM KCl) the distances of 4–5 nm were calculated between Cys707 in the S1 motor domain and different sites on LC1, both in the absence of nucleotides and in the complex S1-ADP-BeFx. On the other hand, the distances to S1 active site containing bound TNP-ADP significantly varied for different sites of LC1. For Cys41 and Cys15 in the LC1 N-terminal extension these distances exceeded 5.5 nm independently of formation of the S1-ADP-BeFx complex, thus indicating that these sites are located too far from the S1 active site. For Cys99, Cys160, and Cys 180 located in LC1 C-terminal part, these distances decreased upon formation of the S1-ADP-BeFx complex; this effect was the most pronounced in the case of Cys99 and Cys160, for which this distance dramatically decreased upon complex formation, from >6 nm to 3.3–3.7 nm. These results testify in favor of nucleotide-induced interaction between this part of LC1 and S1 motor domain. Importantly, the distance between TNP-ADP in the S1 active site and Cys residues located at the very N-terminus of the LC1 N-terminal extension also decreased upon formation of S1-ADP-BeFx complex, from 5 to 4–4.5 nm, suggesting nucleotide-induced interaction of LC1 N-terminus with S1 motor domain. This work was supported by RFBR (grant 15-04-03037).

... S1-4

Regulatory light chain's role in fine-tuning myosin motor function

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Myosin motors drive diverse motile processes, ranging from intracellular cargo transport, cell division to muscle contraction and the whole cell movement. These wide variety functions can be attributed to their precisely regulated chemo-mechanical coupling, particular structural features, and the assembly with specific accessory subunits, such as the light chains. Here, we investigated the functional significance of molecular composition of the native myosins from striated muscles. Combination of ensemble and single molecule investigation methods were used to gain detailed insights into the role of regulatory light chain (RLC) in modulating myosin heavy chain (MHC) properties.

We reconstituted chimeric myosin motors with MHCs isolated from rabbit fast (M. Psoas) and slow (M. soleus) muscles with different regulatory light chains. 'Fast' and 'slow' signifies the speed of unloaded shortening velocity in the muscles, which is primarily determined by the myosin heavy chain isoform expressed in the muscle fibers. For the functional characterization, we examined such reconstituted proteins in an *in vitro* actin filament motility assay to derive the velocity of movement. Furthermore, in-depth analysis was performed to determine the kinetic and mechanical parameters, modulated by RLC during acto-myosin cross-bridge cycle. We employed TIRF-based ATP turnover measurements to study kinetics and, optical trapping of individual myosin molecules to investigate the mechanical features such as, force generation, power stroke and, stiffness of the motors.

Our results revealed a crucial modulatory role of RLC affecting the velocity of motors as a consequence of altered

ATPase, and actin binding affinity. Despite the high degree of homology among different RLCs; they exert diverse impact on MHC function. These findings demonstrate that specifically assembled sub-components determine distinct properties, and consequently, physiological roles of motor proteins. Additionally, precise details of modulatory role of RLC isoform is of particular clinical relevance, since eg., single point mutations in MLC-2V (RLC specific to the slow myosin in heart ventricle) have been linked to causing familial hypertrophic cardiomyopathy (FHC) in humans.

S1-5

Nebulin KO mice causes a decrease in in-series stiffness of the thin filament and consequential decrease in force / cross-bridge in slow-twitch soleus muscle fibers

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To understand nebulin (Nb)s role in contraction, skinned slow-twitch soleus muscle fibers from Nb KO (Nb-) mice were used, and the cross-bridge kinetics and the elementary steps of the cross-bridge cycle were characterized by sinusoidal analysis, and compared the results with those from wildtype (WT, Nb+). Experiments were carried out at 25°C and in the presence of 5 mM MgATP, 1 mM Mg++, pCa 4.6, pH 7.0, and ionic strength adjusted to 200 mM by K-acetate. We observed that Nb increases active tension and rigor stiffness both in the absence and in the presence of phosphate (Pi, 8 mM). There were subtle modifications in elementary steps: the nucleotide binding constants (*K*0, *K*1) are less in Nb- fibers than those of WT, and so is the equilibrium constant of the cross-bridge detachment step (*K*2). The rate constants (*k*m2, *k*4, *k*m4) are generally larger in Nb- fibers, except for forward rate constant of the cross-bridge detachment step (*k*2), which is smaller in Nb- fibers. However, when cross-bridge distributions among the six states were calculated, there were no significant differences in the distribution of the six states. Therefore, force/cross-bridge is reduced in Nb- fibers. This is because active force development is proportionate to in-series stiffness (Eq. 3 of Wang and Kawai, *JMRCM* 34:407-415, 2013), which account for the results. The subtle modifications in cross-bridge kinetics may be related to the reduced force/cross-bridge. We conclude that Nb stiffens the thin filament to cause larger force development, but the number of force-generating cross-bridges is not much different in Nb± fibers. We further observed that the Ca2+ sensitivity (pCa50) or cooperativity (*n*H) does not differ between Nb± fibers, indicating that the thin filament stiffness does not influence the regulatory functions of the thin filament.

Session 2

Titin and its Binding Partners

S2-1

Structural and biophysical characterization of α-actinin-2 in ternary complex with FATZ-1 and Zq titin

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The sarcomere is the minimal contractile unit in striated muscle cells and is delimited by Z-disks. α-Actinin isoform 2 (208 kDa) is a key protein in Z-disk assembly as it crosslinks antiparallel actin filaments from adjacent sarcomeres [Ribeiro Ede et al. Cell 2014]. It is also a binding platform for a number of other Z-disk proteins such as titin and FATZ-1, which participate in Z-disk formation and regulation. FATZ-1, also known as myozenin-1 and calsarcin-2, is a relatively small (30 kDa) intrinsically disordered protein believed to be an adaptor linking α-actinin-2 to other Z-disk proteins, while titin is a giant multidomain protein (~3,800 kDa; including folded and disordered parts) that spans half the sarcomere and combines elastic, architectural and signaling function. Although both FATZ-1 and titin (via its Zq domain) are reported to interact with the rod domain of α-actinin-2 [Young et al. EMBO J 1998, Faulkner et al. J Biol Chem 2000], very little is known at molecular level about the structures and binding mode of their complexes [Atkinson et al. Nat Struct Biol 2001, Grison et al. Proc Natl Acad Sci USA 2017].

In the last few years, we have comprehensively studied the interaction between α-actinin-2 and FATZ-1 by combining different techniques such as crosslinking/MS, NMR, SAXS, and crystallography (our unpublished data). Importantly, we have managed to solve the crystal structure of a soluble FATZ-1 construct in complex with a half dimer of α-actinin-2 at 3.2Å. Structural analysis shows that FATZ-1 interacts in an extended conformation with spectrin-like repeats 1, 3 and 4 from α-actinin-2 rod and displaces EF hand pairs 1-2 from the position found in the previously reported structure of full length α-actinin-2 [Ribeiro Ede et al. Cell 2014]. More recently, we have focused on addressing the question whether interactions of FATZ-1 and titin are competitive, synergic or independent. To this end, we have produced different variants of titin comprising its Zq domain and assessed their interaction with α-actinin-2 in the absence and presence of FATZ-1. We have characterized the binding affinity and stoichiometry of binary (α-actinin-2/titin and α-actinin-2/FATZ-1) and ternary (α-actinin-2/titin/FATZ-1) complexes using ITC and SEC-MALS. Our next goal is to unravel the molecular determinants of these complexes by using an integrative structural biology approach.

S2-2

Diaphragm passive stretch induces hypertrophy, which is modulated by titin-based stiffness

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BACKGROUND: Titin has been proposed to play a key role in mechanosensing and striated muscle trophicity: spanning half-sarcomeres, single titin molecules bind hypertrophy signaling proteins in its I-bands extensible region. However, there is no conclusive data to support this proposition in skeletal muscle, mainly due to the lack of appropriate tools.

HYPOTHESIS: Titin-based passive tension modulates skeletal muscle hypertrophy.

METHODS & RESULTS: We used unilateral diaphragm denervation (UDD) in mice, a model that induces a transient hypertrophy in the denervated hemidiaphragm. Using ultrasound imaging, we reveal that the denervated hemidiaphragm undergoes cyclic passive stretch (26 ± 2% muscle lengthening), corresponding with a sarcomere stretch from 2.9 to 3.7 um. Mass increase in wet weight of ~48 ± 3% after six days of UDD, resulted from both increased fiber cross-sectional area and increased fiber length through sarcomere addition.

Next, to test whether titin-based passive tension plays a role in the hypertrophy response, we used two mouse models: one with decreased (RBM20ΔRRM; RBM20) and one with increased (TtnΔIAjxn; IA) titin-based passive tension. In RBM20 mice the denervated hemidiaphragm showed a blunted response (20 ± 6% less hypertrophy), whereas the IA mice showed an exaggerated response (18 ± 8% more hypertrophy) relative to wt mice.

Titin-binding proteins implicated in muscle trophicity were induced after UDD, in particular Ankrd1 & 2, FHL1, and MuRF1. Interestingly, Ankrd1 was differentially induced, with a higher induction in the RBM20 mice and a blunted induction in the IA mice. Ankrd2 and FHL1 showed blunted induction in both models, and MuRF1 was similarly induced in both models.

CONCLUSION: In skeletal muscle, titin-based stiffness modulates hypertrophic remodeling. MARP1 might play an important role in titin-based hypertrophy signaling.

S2-3

Loss of Z-disc anchored titin in adult skeletal muscle cells leads to sarcomere disassembly

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BACKGROUND: The giant muscle protein titin (TTN) forms an elastic continuum along the myofibril. Different isoforms are expressed from the *Ttn* gene by alternative splicing. The smallest known TTN isoform, Novex-3, results from the inclusion of *Ttn* exon 48, which contains a premature stop codon. Recently, a new TTN isoform was identified and termed Cronos. This variant is generated by using an alternative transcription start site near the A-band edge; thus, other than the full-length TTN or Novex-3, Cronos lacks the N-terminal Z-disc part of TTN. Here, we asked whether TTN isoforms containing the Z-disc portion of the protein are necessary to maintain the integrity of the sarcomere.

METHODS: We generated a mouse line, which allows the knockout of Z-disc anchored TTN in adult skeletal muscle cells, and analyzed the consequences of TTN loss by western blotting, immunohistochemistry, qRT-PCRs and immunogold electron microscopy.

RESULTS AND CONCLUSIONS: After titin knockout induction in adult skeletal muscle cells we observed a strong reduction of full-length titin expression and ultrastructural analyses revealed the gradual loss of sarcomeres. We detected areas of mild myofibrillar disruptions marked by vacuolization and disappearance of well-shaped Z-discs. However, the TTN epitopes could still be observed in their expected sarcomeric regions. Within the same muscle we observed areas of dramatic myofibrillar destruction, marked by the total loss of sarcomeric periodicity. Remarkably, in those areas we found isolated A-bands which were positive for TTN A-band epitopes, while structures positive for TTN I-band epitopes were missing. Therefore, we speculate that N-terminally shortened isoforms, like Cronos, are able to stabilize the A-band to some degree. Ultimately, however, the shorter titin isoforms are not able to maintain sarcomeric structure. The full-length, Z-disc integrated titin isoforms are crucial for sarcomere integrity in adult skeletal muscles.

S2-4

The giant elastic protein titin regulates the length of the striated muscle thick filament-- titin rules

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The contractile machinery that powers striated muscle has as its most crucial component the thick filament, comprised of the molecular motor myosin. The thick filament is of a precisely controlled length, defining thereby the force level that muscle generates and how this force varies with muscle length. One of the outstanding biological mysteries is the mechanism by which the thick filament length is so exquisitely controlled; it has been speculated that the giant protein titin could be involved. Individual titin molecules span the half thick filament length and contain multiple super-repeats each ~43 nm in length, a distance that coincides with the ~43 nm myosin helical repeat along the thick filament. Hence a popular theory is that in vertebrates titin functions as a thick filament template that is responsible for determining thick filament length, but this

is controversial as deleting a large part of titin at the edge of the A-band does not alter thick filament length. Here we report studies on a mouse model, TtnΔC1-2, in which two of titin"s super-repeats (C1 and C2) were deleted. Structural studies in both cardiac and skeletal muscles of TtnΔC1-2 mice reveal a reduced thick filament length, in line with the concept of a 2x 43 nm shortened titin ruler. Skeletal muscles of TtnΔC1-2 mice generate less force and have a steeper descending limb of their force-sarcomere length relation, supporting the structural finding of shorter thick filaments. The heart generates less pressure and, unexpectedly, has a dilated cardiomyopathy (DCM) phenotype. Thus, thick filament length control is titin-based and is crucial for maintaining muscle health.

S2-5

Force generation by titin folding

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Titin is a giant protein that provides elasticity to muscle. As the sarcomere is stretched, titin extends hierarchically according to the mechanics of its segments. Whether titin"s globular domains unfold during this process and how such unfolded domains might contribute to muscle contractility are strongly debated. To explore the force-dependent folding mechanisms, here we manipulated skeletal-muscle titin molecules with high-resolution optical tweezers. In force-clamp mode, after quenching the force below 10 pN, titin domains refolded through structural fluctuations without resolvable discrete events. In position-clamp experiments, the time-dependent force trace contained rapid fluctuations and a gradual increase of average force, indicating that titin can develop force via dynamic transitions between its structural states en route to the native conformation. In 4 M urea, which destabilizes H-bonds hence the consolidated native domain structure, the force increase disappeared but the fluctuations persisted. Thus, whereas net force generation is caused by the ensemble folding of the elastically-coupled domains, force fluctuations arise due to a dynamic equilibrium between unfolded and an intermediate molten-globule states. Monte Carlo simulations incorporating a compact molten-globule intermediate in the folding landscape recovered all features of our nanomechanics results. Remarkably, the ensemble molten-globule dynamics delivers significant additional contractility that may assist sarcomere mechanics, and it may reduce the dissipative energy loss associated with titin unfolding-refolding during muscle contraction-relaxation cycles.

S2-6

Native redox posttranslational modifications as regulators of titin mechanical properties

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The relevant role of titin in cardiac muscle is evidenced by the existence of mutations in the titin gene that lead to pathologies involving changes in the mechanical function of the heart. The elastic properties of titin depend on the folding/unfolding equilibria of its immunoglobulin (Ig) domains. Strain-induced posttranslational modifications of buried cysteines have been shown to be key regulators of the folding dynamics of titin Ig domains, leading to changes in the elasticity of cardiomyocytes1. However, the identity, extent and specific residues targeted by these modifications *in vivo* remain unexplored. We have optimized a method for in-gel detection of oxidized thiols by fluorescent labeling, which has allowed us to provide the first experimental evidence that a fraction of titin"s cysteines are oxidized in cardiac tissue in basal conditions. By mass spectrometry, high-resolution structure modeling and single-molecule atomic force microscopy, we study the redox state of specific cysteine residues, predict the presence of disulfides in the different Ig domains of titin, and examine the effect of these native modifications in the mechanical properties of the protein. Our approach leads to a better understanding of how the contractility and passive mechanical properties of the heart muscle are modulated in physiology and disease.

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Session 3

E-C Coupling and Neuromuscular Interactions

S3-1

Cardiomyocyte dyadic structure and function in health and disease

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Contraction of cardiomyocytes is dependent on sub-cellular structures called dyads, which are functional junctions between invaginations of the surface membrane (t-tubules) and the sarcoplasmic reticulum. Well-organized dyads enable efficient triggering of Ca^{2+} release during the action potential, and powerful contraction. Dyads are formed gradually during development, with progressive assembly of both t-tubules and sarcoplasmic reticulum and precise trafficking of Ca^{2+} handling proteins including the L-type Ca^{2+} channel and Ryanodine Receptor. During diseases such as heart failure, dyads are broken down with a reversion to an immature phenotype. Our data indicate that these alterations include both disorganization of t-tubules and dispersion of Ryanodine Receptor clusters; changes which reduce the efficiency of Ca^{2+} release. Elevated stress placed on the myocardial wall of the dilated, failing heart is a key trigger of disrupted dyadic structure as it signals reduced expression of the dyadic anchor junctophilin-2. However, other changes that occur during heart failure are compensatory, including the growth of new dyads in the longitudinal axis of the cell. Our data indicate that the membrane-bending protein BIN1 signals such dyadic growth. Thus, interventions which unload the heart and/or exploit the hearts inherent compensatory capacity to grow dyads can benefit heart failure patients.

S3-2

Role of surface membrane calcium current in the regulation of sarcoplasmic calcium release in adult skeletal muscle fibres

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In adult mammalian skeletal muscle the primary function of the surface membrane Ca^{2+} channel (Dihydropyridine receptor; DHPR or Cav1.1) is voltage sensing for excitation–contraction (EC) coupling. Unlike its adult isoform which does not conduct significant Ca^{2+} current during physiological activation, the embryonic splice variant (Cav1.1Δ29) displays normal channel function and gives rise to a considerable Ca^{2+} influx. The isoform shift after birth is essential for proper fibre type specification and healthy mitochondrial function.

The consequences of the preserved Ca^{2+} influx on EC-coupling and on the release of Ca^{2+} from the sarcoplasmic reticulum (SR) through the SR Ca^{2+} release channel (Ryanodine receptor; RyR) were examined in mice expressing only the embryonic isoform. Fibres were enzymatically isolated from the *flexor digitorum brevis* muscles of Cav1.1Δe29 mice and either Ca^{2+} transients evoked by depolarizing pulses under voltage clamp conditions or Ca^{2+} release events (CRE) in intact fibres were detected.

The altered channel function gave rise to otherwise not seen CRE in intact adult fibres with unusual characteristics. Their appearance depended on the presence of extracellular Ca^{2+} and required the activation of RyR, however, type3 RyR were not found to be significantly overexpressed in these muscles. The complex kinetics of these events indicate an opening and closing mechanism other than the generally accepted model in which a few neighbouring RYR act in concert. It was also accompanied by modified SR Ca^{2+} release properties as repeated activation resulted in greater decline in the amplitude of the Ca^{2+} transients (the ratio of the second and first amplitude being 70 ± 3% in control and 42 ± 5% in Cav1.1Δe29 mice).

Our results imply that the Ca^{2+} influx through the DHPR gives rise not only to mitochondrial damage and fibre type disarrangement, but most likely it modifies the structure of the triad hence modulating the regulation of RyR activity.

S3-3

Transcriptomic changes during secondary myogenesis in RYR1- and DHPR-deficient limb skeletal muscles

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On a molecular level excitation-contraction (EC) EC coupling in skeletal muscle can be described as the coordinated functions of two Ca^{2+} channels – the 1,4-dihydropyridine receptor (DHPR), located on the sarcolemma and acting as a voltage sensor; and the type 1 ryanodine receptor (RYR1) – the main Ca^{2+} release unit from the sarcoplasmic reticulum. While multiple studies have examined the electrophysiological properties of both channels, their roles in myogenesis remain obscure. Here, we analyzed the global gene expression changes in mice occurring from the initial (E14.5) to the final stages (E18.5) of secondary embryonic myogenesis in WT, as well as in RYR1-deficient ($RYR1^{-/-}$) and DHPR-deficient ($Cav1.1^{-/-}$)

mouse limb skeletal muscle. At E14.5 we found 61 differentially expressed genes (DEGs) in RYR1$^{-/-}$ and 97 DEGs in Cav1.1$^{-/-}$ compared to WT samples with only 2 DEGs found in both genotypes. The RYR1$^{-/-}$ DEGs were largely involved in innervation-related processes and in Cav1.1$^{-/-}$ muscle contraction was most severely influenced. At E18.5 493 DEGs were found in RYRY1$^{-/-}$ and 1047 – in Cav1.1$^{-/-}$ samples, with a converging enrichment pattern most significantly affecting contraction-related processes, while RYR1$^{-/-}$ samples exhibited multiple specific DEGs for extracellular matrix structure and organization; and those in Cav1.1$^{-/-}$ for lipid metabolism. Furthermore, both RYR1$^{-/-}$ and Cav1$^{-/-}$ samples exhibited alterations in the G1-to-S cell cycle control, failed to upregulate multiple genes involved in the formation and functions of the contractile machinery and did not promote the expression of at least 44 miRNAs, developmentally-regulated in the WT samples. The latter may partly explain the complex changes in various signaling pathways we have previously observed in E18.5 RYR1$^{-/-}$ samples. Taken together, these findings strongly suggest important roles of RyR1 and DHPR and the EC coupling-related Ca^{2+} signaling in the late steps of muscle development.

... S3-4

Membrane excitability and excitation-contraction coupling at neuromuscular junctions in larval Drosophila

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Striated muscle fibres mediating motility of fruit-fly larvae do not require action potentials in order to generate forceful muscle contractions, despite their focal innervation by distinctive motor neurones (PubMedID: 10218160, 11133154). We are examining the relationship between the amplitude and frequency of excitatory junction potentials (EJPs), measured with intracellular microelectrodes, while simultaneously recording intracellular Ca transients, using transgenically-expressed GCaMP(5 or 6f) as a reporter (PMID:24174659, 28285823). These recordings show a generalised increase in intracellular Ca and muscle contraction that lags both postsynaptic depolarisation and focal Ca transients, either in response to single stimuli or repetitive stimulation at 5-50Hz, which is also the normal range of EJP frequency observed in larval preparations with an intact ventral nerve cord. EJP amplitude was attenuated by 50-80% in 500 µM kainate (PMID:19695344), producing a corresponding reduction in the postsynaptic Ca transient and muscle contractile responses. This observation contrasts with GluRIII mutants in which larval motility was indistinguishable from wild type, despite an 80-90% reduction in EJP amplitude (PMID:15685612). However, in recordings made within 5–20 minutes of dissection in HL3.1 saline (1.5 mM Ca; 4 mM Mg) many muscle fibres produced synaptically-driven regenerative responses, with a threshold at about -25 mV, resembling cardiac action potentials recorded from the vertebrate atrioventricular node (PMID: 19843444). These observations suggest that activation of voltage-sensitive Ca channels expressed in larval Drosophila muscle membranes may not always be mitigated by high resting K-permeability (PMID:23959639, 7666192, 27783155, 24671529) and regenerative response threshold may be a pivotal determinant of safety factor for neuromuscular transmission in the intact Drosophila larva, as in vertebrate skeletal muscle.

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... S3-5

A fast skeletal muscle troponin activator (FSTA), CK-2066260, mitigates the fatigue-induced decline in skeletal muscle contractile force by lowering the metabolic cost

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Potential pharmacological treatments that specifically alleviate skeletal muscle fatigue (i.e., decreased strength and endurance) are limited. Here, we investigated whether fatigue could be ameliorated by the FSTA, CK-2066260, a calcium sensitizer that amplifies the force response to subtetanic neural input. To induce fatigue, repeated electrical stimulation was applied to intact single mouse muscle fibers *in-vitro* and whole rat hindlimb muscles *in-situ* in the presence or absence of CK-2066260. At approximately half the stimulation frequency, CK-2066260-treated muscle produced force that was greater or equal to untreated muscle during repeated contractions. CK-2066260-treated muscles also demonstrated lower sarcoplasmic reticulum Ca^{2+} release and less ATP utilization. In healthy rats and rats with peripheral artery insufficiency, CK-2066260 significantly improved treadmill performance *in vivo*. In summary, using *in-vitro*, *in-situ*, and *in-vivo* rodent models of muscle function, we demonstrate that CK-2066260 effectively mitigates skeletal muscle fatigue.

Session 4

Stem Cell-derived Myocytes, Experimental Genome Editing, Muscle Tissue Engineering

... S4-1

Inherited cardiomyopathies: disease modeling in iPSC-derived cardiomyocytes and engineered heart tissue

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All forms of heart failure (HF) are treated the same way, although HF is not only one disease. For example, the diuretic spironolactone has been shown to improve survival in HF patients with reduced ejection fraction, whereas it has no beneficial effect in HF patients with preserved ejection fraction. An example of extreme case of HF is the inherited forms of HF or inherited cardiomyopathies. Mutations in the same gene have been shown to be associated with either hypertrophic cardiomyopathy (HCM) with mainly preserved ejection fraction or with dilated cardiomyopathy (DCM) with reduced ejection fraction. One of the main goals of my lab is to evaluate whether induced-pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) and engineered heart tissues (iPSC-EHTs) help to decipher disease mechanisms of inherited cardiomyopathies and to test molecular therapy options. We used fibroblasts from healthy individuals (control) and patients with hypertrophic cardiomyopathy (HCM) carrying sarcomeric gene mutation. Fibroblasts were reprogrammed into iPSCs and further differentiated into CMs and EHTs. Additionally, we used CRISPR/Cas9 gene editing to correct the mutation in iPSC (=isogenic controls). The iPSC-CMs were cultured in 2D for 7-30 30 days and cell areas were measured by confocal microscopy and the Opera® High Content Screening System. In addition, iPSC-EHTs were generated and paced at 1 Hz in 1.8 mM tyrode solution at 37°C. Measurements of amplitude and kinetics of force and action potential duration with sharp electrodes were performed in iPSC-EHTs. We also evaluated gene expression with a custom-made, 84-gene panel using the nanoString nCounter Elements technology. Data obtained in different cell lines carrying *MYBPC3* or *ACTN2* mutation will be presented and discussed.

... S4-2

Cronos titin in developing human cardiomyocytes: degraded no more

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The giant sarcomere protein titin has numerous important roles in cardiomyocytes, including providing passive tension and facilitating myofibril formation. An internal promoter has recently been identified in the titin gene (TTN) that is predicted to regulate expression of a previously unstudied isoform, Cronos. To study the role of Cronos titin in human hearts, we genetically engineered human induced pluripotent stem cells (hiPSCs) using the CRISPR/Cas9 system to introduce homozygous frameshift mutations in the TTN gene. TTN was mutated upstream of the Cronos start site in Exon 2 (Ex2 KO hiPSCs) to prevent translation of full-length titin but leave the Cronos isoform intact, and downstream of the Cronos start site in Exon 326 to prevent translation of both full-length and Cronos titin (Ex326 KO hiPSCs). Ex2 KO hiPSC-derived cardiomyocytes (hiPSC-CMs) visibly contract, though weakly, and immunofluorescence studies indicate the formation of short, dispersed myofibrils. These myofibrils stain positively for the C-terminal MIR and M8-M10 regions of titin but not the N-terminal Z1Z2 domain, consistent with Cronos titin being the only isoform expressed in these cells. Twitch forces of multicellular engineered heart tissues (EHTs) containing Ex2 KO hiPSC-CMs were ~10% of those with isogenic wildtype hiPSC-CMs ($p<0.01$), and single cell forces on micro-post platforms were reduced by ~20% compared to wildtype ($p=0.03$). Interestingly, Ex326 KO hiPSC-CMs do not visibly beat, form sarcomeres, or produce measurable twitches in EHTs, but they do exhibit regular calcium transients. Based on these data, we conclude that Ex2 KO hiPSC-CMs express predominantly Cronos titin, which is sufficient for rudimentary sarcomere formation but not normal function. Ongoing studies will investigate the role of Cronos titin during normal human heart development and maturation.

S4-3

Single cell mapping used to assign mRNA and protein expression of cardiac myosin heavy chain to twitch kinetics of the same human embryonic stem cell derived cardiomyocyte

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The aims of this work were (i) to gain insight into the variability of α- and β-myosin heavy chain (α,β-MyHC) protein and *MYH6* and *MYH7*-mRNA expression among individual human stem-cell derived cardiomyocytes (hSC-CMs) and (ii) to investigate how MyHC composition of the individual CMs affects their twitch kinetics. To assign different parameters to the same individual hSC-CMs we developed a novel, setup-independent single-cell mapping technique. After cultivation of hSC-CMs for ~35 days either in cardiac bodies (CBs) or plated on glass coverslips, twitch contractions were assessed. Then X-Y-coordinates of the CMs were recorded for remapping of the same CMs at different microscopes after fluorescence *in situ* hybridization using specific probe sets against *MYH6* and *MYH7* mRNAs and again after fluorescent immunostaining of MyHC proteins. Single long-plated CMs showed 84±24% *MYH7* and 16±24% *MYH6*-mRNA expression (n=63). We detected on average 527±529 *MYH7*- and 125±248 *MYH6*-mRNA molecules per cell. Correlation of mRNA to protein expression showed only 1±4% *MYH6*-mRNA of total myosin-mRNA for pure β-MyHC expressing CMs (n=26). hSC-CMs cultivated in CBs showed 62±28% *MYH7* and 38±28% *MYH6*-mRNA expression, with 27±31% *MYH6*-mRNA of total myosin-mRNA in pure β-MyHC CMs (n=9). Pure α-MyHC CMs from CBs had 73±16% *MYH6*-mRNA (n=9). In CB-derived CMs we detected 649±591 *MYH7*- and 372±416 *MYH6*-mRNA molecules per cell. Taking all analyzed CMs together we find highly variable fractions for both *MYH6*- and *MYH7*-mRNAs from cell to cell, ranging from 0% to 100% (n=45-63). Preliminary data so far show no correlation between twitch contraction kinetics and the expressed MyHC-isoform in the individual cardiomyocytes.

In conclusion, the large variability of copy numbers per individual hSC-CM of both mRNAs suggests that transcription of the two genes occurs in a burst-like fashion. Surprisingly, twitch contraction kinetics seem to be dominated by parameters other than the MyHC-composition.

S4-4

Patient-specific induced pluripotent stem cell-derived cardiomyocytes to model, screen drugs and decipher molecular mechanisms of CPVT1 syndrome

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a highly lethal inherited arrhythmogenic disorder predominantly caused by mutations in the cardiac ryanodine receptor gene (*RYR2*). Human induced pluripotent stem cells (hiPSCs) offer a unique opportunity for disease modeling. We have identified a 25 years-old athletic woman with exercise-induced syncope associated with ventricular tachycardia (VT), harboring the novel single mutation RyR2-D3638A. The ECG revealed no improvement on the b-blocker metoprolol (METO) while flecainide treatment did. We aimed at deriving functional cardiomyocytes (CMs) from the proband via hiPSCs and investigating the response to standard therapy administrated to CPVT patients as well as to new Rycal compounds.

When focusing on the intracellular calcium (Ca2+) handling in CPVT hiPSC-CMs, we observed no preventing effect of METO on the aberrant Ca2+ transients under isoproterenol (ISO), a b-adrenergic receptor agonist, which was consistent with the clinical data. However, both S107 and flecainide applications preceding ISO were able to suppress abnormalities of the Ca2+ transients in CPVT hiPSC-CMs with reduced RyR2 Ca2+ leak and increased Ca2+ release velocity and amplitude. Co-immunoprecipitation of the RyR2 macromolecular complex showed that CPVT hiPSC-CMs exhibited higher basal RyR2 PKA phosphorylation at Ser2809 and less PP2A bound to RyR2 when compared to healthy control (HC) hiPSC-CMs. Unlike METO, S107 treatment was able to prevent the depletion of calstabin2, a stabilizing RyR2 partner under stress conditions which is likely associated with its stabilizing effect on the Ca2+ transients.

This work provides new evidence of CPVT modeling, drug screening and molecular mechanism deciphering, using patient-specific hiPSC-CMs.

S4-5

Alteration of cardiomyocyte mechanosensing through hypertrophic signalling

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Cardiac diseases, such as hypertrophic cardiomyopathies are frequently associated with myocardial fibrosis, resulting a stiffening of the cellular microenvironment. Previous studies have established a direct relationship between matrix rigidity and the contractile forces of healthy cardiomyocytes. However, it is still unclear how cardiomyocytes sense matrix stiffness. Moreover, the combined effects of abnormal matrix stiffness and disease signalling pathways remain elusive.

Recent work has highlighted the exceptional temporal and spatial resolution of nanopillar arrays, which enabled the identification of the molecular machinery behind mechanical sensing in fibroblast cells. Here we use nanopillar arrays, together with molecular tension sensors and custom made multi-rigidity plates to uncover a novel cardiomyocyte rigidity sensing mechanism. Moreover we characterize the impact of hypertrophic stimulation on cardiomyocytes in different mechanical environments and identify how hypertrophic pathways alter mechanical sensing and downstream signalling to affect cardiomyocyte morphology and function.

Session 5

Signaling and Regulatory Mechanisms

S5-1

Using zebrafish larvae to examine striated and smooth muscle function and mechanisms in human muscle disease

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Zebrafish (Danio Rerio) is an important model organism in developmental biology since most organ systems, including muscle, are developed and established during the larval stage. The genome is characterized, and orthologues of human genes can be identified. Gene expression can be manipulated and several mutated strains exist. We have developed physiology techniques for studies of zebrafish larval smooth and striated muscle, with a focus on understanding muscle function and human muscle disease. Zebrafish larval muscles (<6 dpf) are isolated and analysed with mechanical measurements (active force, shortening velocity) in combination with structural studies (microscopy, small angle x-ray diffraction). Using knock-down strategies we have examined the function of the intermediate filament protein desmin, and shown that knock down results in a desminopathy with impaired muscle force, increased interfilament spacing and resistance to stretch-induced injury. Following knock down of the fast skeletal myosin binding protein C, a myopathy was identified with affected force and muscle structure. The Sapje strain is a zebrafish model of Duchenne muscular dystrophy, with a missense mutation resulting in a stop codon in the dystrophin gene. These animals have impaired force generation and can be successfully treated using novel read-through compounds (Ataluren/Translarna). Since the larvae are not dependent of food intake, in vivo experiments can be performed to examine effects of interventions not easily performed in mammals, e.g. immobilization. Smooth muscle isolated from the gut and examined in vitro reveal the main signalling pathways and provides a model for examining effects on visceral muscle. In conclusion muscle physiological studies can be performed on zebrafish larval muscle, and this fast, relevant and economical model enable examination of structure/function of muscle with normal and mutated muscle proteins and for developing novel treatment strategies.

S5-2

Thin filament regulation in insect flight muscle and how it differs in cardiac muscle

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The indirect flight muscle of insects (IFM) and cardiac muscle have mechanical properties in common. Both contract rhythmically and both show length-dependent activation

(LDA). The high frequency contractions of IFM that power the rapid wing beats are produced by periodically stretching opposing muscles (stretch activation). Cardiac muscle is also activated by a rapid stretch at each beat. Both LDA and stretch activation are more pronounced in IFM than in cardiac muscle. In Lethocerus (water bug) IFM, troponin bridges between thick and thin filaments may transmit force between the filaments on stretch (Perz-Edwards et al, 2011). We have investigated the interaction between tropomyosin-troponin (Tm-Tn) and thick filaments. Unlike cardiac muscle, IFM has a Tm-Tn complex with two isoforms of TnC (TnCF1 and TnCF2) and two isoforms of Tm (Tm1 and Tm2). TnCF1 regulates stretch activation and there is no homologue in cardiac muscle. Force production in IFM with TnCF2 has the same calcium sensitivity and cooperativity as cardiac trabeculae, suggesting similar regulation by TnCF2 and cardiac TnC. In pulldown experiments with IFM thick filaments or filaments assembled from pure myosin, we found that the Tm-Tn complex with both Tm isoforms binds to thick filaments, and the interaction is not calcium sensitive. Unexpectedly, Tm1 alone binds to thick filaments but Tm2 alone does not. Two regions of sequence differ in the isoforms: one some way from the N-terminus and one at the C-terminus. Tm1 is predicted to have less stable end-to-end association that Tm2. The two isoforms isolated from IFM do not form heterodimers. Tm1 binds to skeletal myosin S1, showing that the interaction between Tm and thick filaments is specific to Tm1, not to IFM myosin, and that the interaction is in the myosin head region. These results suggest troponin bridges activate the thin filament by pulling directly on Tm.

... **S5-3**

Titin phosphorylation by protein kinase G as a novel mechanism of diastolic adaptation to acute load

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QUESTIONS: Titin is the main determinant of myocardial passive tension (PT) and its distensibility is increased via phosphorylation by protein kinase G (PKG), activated by nitric oxide (NO) and natriuretic peptides (NP). We tested the role of these pathways in the acute response to myocardial stretch and whether this leads to decreased stiffness, optimizing diastolic filling.

METHODS: Isolated rat hearts, left ventricular (LV) and atrial strips of cardiac surgery patients and rabbit right ventricular papillary muscles were acutely stretched for 15 minutes. Passive tension (PT) was measured in skinned cardiomyocytes extracted from non-stretched (nSt) and stretched (St) LV before and after PKG or phosphatases incubation. Rabbit muscles were incubated with a PKG inhibitor (PKGi) or, simultaneously, a NO synthase inhibitor, a NO scavenger, a NP receptor A antagonist. Titin and vasodilator-stimulated phosphoprotein (VASP) phosphorylation and cGMP levels were assessed. Response to VO was assessed invasively in sham and TAC rats and in cardiac surgery patients, and echocardiographically in healthy volunteers.

RESULTS: After acute stretch/VO, diastolic pressure/PT decreased throughout 15 minutes in all species and experimental preparations, which was blunted by PKGi and NO/NP pathway inhibition and absent in TAC animals. Skinned cardiomyocytes from St hearts showed decreased PT which was abrogated by phosphatase while PKG decreased PT in those from nSt. St samples had higher cGMP levels, and VASP and titin phosphorylation, which was blunted in PKGi and TAC animals. Healthy volunteers and cardiac surgery patients showed E/E and end-diastolic pressure decrease after VO, respectively.

CONCLUSIONS: The progressive decrease in myocardial stiffness after acute hemodynamic overload is preserved at myofilamental level and seems to depend on PKG activity. The mechanism was translated to human physiology and may be abolished in the hypertrophic heart.

... **S5-4**

Regulation and mechanostability of titin/α-actinin bond

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In striated muscle cells, the Z-disk represents a highly organized three-dimensional assembly containing a large directory of proteins orchestrated in a multi-protein complex centered on its major component α-actinin. The cross-linking of actin and myosin at the boundaries of their filamentous structures is essential for the muscle integrity and function. In the Z-disks – the lateral boundaries of the sarcomere machinery – α-actinin cross-links antiparallel actin filaments from adjacent sarcomeres, and additionally serves as a binding platform for a number of other Z-disk proteins as well as to the Z-disk portion of titin. Stable anchoring of titin within the muscle Z-disk is essential for preserving muscle integrity during passive stretching. One of the main interactions that anchor titin in the Z-disk is between differentially spliced titin Z-repeats and the calmodulin-like domain α-actinin.

Using a combination of structural, biochemical and cell biophysics approaches we provided insight into molecular architecture of α-actinin and into phosphoinositide-based mechanism controlling titin Z-repeat/α-actinin interaction (Ribeiro et al, 2014, Cell). However, the mechanical and kinetic properties of this important interaction remained unknown. We subsequently used an optical tweezers assay to study the mechanics of this interaction at the single-molecule level. Surprisingly, a single interaction of α-actinin and titin is weak if force is applied, but depending on the direction of force application, the unbinding forces can more than triple. Our results suggest a model where multiple titin Z-repeat/α-actinin interactions cooperate to ensure long-term stable titin anchoring while allowing the individual components to exchange dynamically (Grison et al, 2017, PNAS).

... **S5-5**

Impact of titin strain on the CARDIAC slow force response

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Stretch of myocardium, such as occurs upon increased filling of the cardiac chamber, induces two distinct responses: an immediate increase in twitch force followed by a slower increase in twitch force that develops over the course of several minutes. The immediate response is due, in part, to modulation of myofilament Ca2+ sensitivity by sarcomere length (SL). The slowly developing force response, termed the Slow Force Response (SFR), is caused by a slowly developing increase in intracellular Ca2+ upon sustained stretch. A blunted immediate force response was recently reported for myocardium isolated from homozygous giant titin mutant rats (HM) compared to muscle from wild-type littermates (WT). Here, we examined the impact of titin isoform on the SFR. Right ventricular trabeculae were isolated and mounted in an experimental chamber. SL was measured by laser diffraction. The SFR was recorded in response to a 0.2 µm SL stretch in the presence of [Ca2+]o=0.4 mM, a bathing concentration reflecting ~50% of maximum twitch force development. Presence of the giant titin isoform (HM) was associated with a significant reduction in diastolic passive force upon stretch, and ~50% reduction of the magnitude of the SFR; the rate of SFR development was unaffected. SL strain was identical in both muscle groups. Therefore, our data suggest that cytoskeletal strain may underlie directly the cellular mechanisms that lead to the increased intracellular [Ca2+]i that causes the SFR, possibly by involving cardiac myocyte integrin signaling pathways.

Session 6

Muscle Protein Development, Turnover and Repair

... **S6-1**

Functional relevance of Klotho for maintenance and regeneration of skeletal muscle

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The hypomorphic Klotho (delta-Klotho) mouse is a common model for accelerated ageing. Beginning at an age of three weeks, these mice develop premature ageing phenotypes including a progressive decline in muscle mass and grip strength and a severely reduced life span. Here, we investigated the function of Klotho in skeletal muscle maintenance and address the question which form of Klotho (secreted or membrane-bound) is responsible for the muscle-specific phenotype. Delta-Klotho mice demonstrate several characteristics of sarcopenia including reduced myofiber diameters and a depletion of the muscle stem cell (satellite cell) pool. To investigate the regeneration process, we injured the tibialis anterior muscles of Klotho-hypomorphic mice with cardiotoxin. Delta-Klotho mice showed regeneration deficits, which were more pronounced with increasing age. Satellite cells contribute essentially to muscle regeneration. Therefore, we analysed the functionality of satellite cells on isolated myofibers where the satellite cells are still in their endogenous niche, but are cultured independently of systemic influences. This ex vivo analysis demonstrated a perturbed function of satellite cells from delta-Klotho mice whereas in vitro assays with isolated myoblasts showed no influence of Klotho expression on the differentiation potential. These results support the hypothesis that the soluble Klotho rather than the membrane-bound form of Klotho influences the regeneration potential in skeletal muscle.

... **S6-2**

Autophagy as a therapy for myofibrillar myopathy

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In order to better understand the mechanism of disease in myofibrillar myopathy we generated zebrafish models for FLNC and BAG3 myofibrillar myopathy. From transgenic overexpression of the dominant disease causing variants we have demonstrated the formation of protein aggregates within the muscle. However, these transgenic fish

do not exhibit the fibre disintegration characteristic of this group of diseases.
In the BAG3 myofibrillar myopathy model we have demonstrated that expression of the disease causing form BAG3^{P209L} results in formation of protein aggregates and that these aggregates also contain both wild type BAG3 and binding partners including FLNC. Sequestration of BAG3 in the aggregates may therefore result in a loss of function phenotype. In both FLNC and BAG3 mutant fish we observe the fibre disintegration characteristic of the disease, suggesting that the aggregates that result from the expression of the mutant protein, lead to the loss of the wildtype product.
We therefore investigated the potential for stimulation of autophagy to remove the protein aggregates, which would be predicted to reduce or prevent loss of the wild-type product. Stimulation of autophagy via rapamycin and other known stimulators successfully remove d protein aggregates. We therefore carried out a screen of 71 known autophagy promoting compounds and identified 9 that were highly effective in remove BAG3^{P209L}-eGFP aggregates. Of these 2 were FDA approved and utilised in follow up studies. Of these the most effective was not only able to remove protein aggregates but also reduce fibre disintegration and rescue swimming impairment in the BAG3 mutant fish. As such the drug we have identified is highly promising for the treatment of BAG3 myofibrillar myopathy.
I will describe the work examining the zebrafish myofibrillar myopathy models and demonstrating the potential of the drug as a therapy.

... S6-3

The role of titin in sarcomere assembly and dynamics

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The giant striated muscle protein titin determines sarcomere structure and elasticity. It spans the half-sarcomere with the N-terminus integrated into the Z-disc and the C-terminus into the M-band.
To visualize sarcomere assembly and dynamics we generated titin knockin mice with GFP fused to the M-band region of titin and dsRed to the Z-disc region, respectively. Cardiomyocytes and myotubes derived from these animals provide an opportunity to follow titin mobility and turnover in living cells.
During the de novo assembly of sarcomeres in cells derived from double-heterozygous GFP/dsRed mice titins N- and C-terminus integrate synchronously and not stepwise into the sarcomere. Photobleaching experiments reveal that sarcomeric titin is not a rigid backbone, but is actively exchanged between sarcomeres. Interestingly, we find differences in titin kinetics comparing Z-disc and M-band. Understanding the role of titin in sarcomere assembly and -dynamics could help improve cardiac remodelling and skeletal muscle regeneration as well as contractile and passive properties of muscle with old age and in disease.

... S6-4

Measuring molecular tension at muscle attachment sites during myofibril assembly

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Muscle fibers require to assemble myofibrils spanning the entire cell in order to produce active forces. Before myofibril formation starts, muscle fibers establish integrin-mediated attachments to tendon cells that allow the generation of mechanical tension across the developing fibers. This tension is essential for myofibrillogenesis and the formation of periodic sarcomeres. However so far, tension at the molecular level could not be directly measured in the living organism.
Therefore, we adapted a foerster resonance energy transfer (FRET)-based molecular tension sensor from cell culture and introduced it into the *Drosophila* genome by CRISPR/Cas9-mediated genome engineering. By inserting different sensor modules into the endogenous locus of the integrin adaptor Talin, which localizes to muscle attachment sites, we ensure proper expression levels and timing in all tissues, including the flight muscles. These knock-in flies are homozygous viable and fly, which shows that the Talin tension sensor fusion protein is fully functional.
We established a protocol for fluorescence lifetime imaging (FLIM) and data analysis, which enables us to measure FRET in a reproducible manner in developing muscles of living pupae. We tested three different sensor modules to identify the best-suited sensor for the range of forces present in the muscle-tendon system. We then applied this sensor to determine how molecular tension across Talin at muscle attachment sites changes during attachment formation and maturation, the latter coinciding with myofibrillogenesis, in the living organism.
Currently, we are quantifying the effects of genetic perturbations on tension levels and myofibrillogenesis during muscle development. This will allow us to determine how force is transduced molecularly at muscle attachment sites to forming myofibrils and provide insights into the molecular mechanism of how tension build-up is functionally linked to myofibrillogenesis during muscle morphogenesis.

... S6-5

Satellite cell migration is regulated by MMP13 and required for skeletal muscle growth and repair

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Skeletal muscle regeneration requires coordinated remodeling of extracellular matrix (ECM), and matrix metalloproteinases (MMP)s play a critical role. MMP-13, a collagenase, was shown to have high expression during regeneration and regulate C2C12 myoblast migration, yet MMP-13"s function in skeletal muscle in vivo has not been studied. We utilized an MMP13-/- mouse to determine the necessity of MMP13 for muscle growth and repair. Skeletal muscles from MMP13-/- mice were smaller than age- and sex-matched controls, although muscle force, capillary density, or ECM area were not different. Following cardiotoxin injection, muscle repair was delayed, where regenerating MMP13-/- mice had 25% smaller peripherally nucleated fibers, 44% reduced capillary density, and a presence of IGG+ fibers indicating fiber degeneration at 2 weeks. Live cell imaging of single fiber cultures was conducted to directly measure satellite cell migration. MMP13-/- satellite cells had 33% lower velocity than controls. In invasion assays through basement membrane extract (BME) or collagen, MMP13-/- cells had a 53% reduced ability to migrate through BME but neither group migrated through collagen. Finally, using satellite cell specific ablation of MMP13, we observed similar effects on cell migration as well as impaired growth of skeletal muscle, as found in MMP13-/- mice. These data extend evidence that MMP-13 is a critical component of myoblast migration, and supports that this migration defect slows growth and repair.
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Session 7

Plasticity, Metabolism and Energetics

... S7-1

The regulation of cell plasticity and tissue-cross-talk by skeletal muscle PGC-1α

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Skeletal muscle has an enormous capacity to dynamically adapt to different external stimuli, for example by initiating a pleiotropic plastic response to endurance or resistance exercise. In endurance training, the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) represents a key regulatory nexus that integrates different inputs and coordinates the control of a complex transcriptional network ultimately resulting in a slow-twitch, high endurance, oxidative muscle phenotype. As part of this biological program, PGC-1α confers potent anti-stress and anti-inflammatory events in the muscle cells. In addition, by modulating the production and secretion of several so-called myokines, auto-, para- or endocrine hormones originating from skeletal muscle tissue, muscle PGC-1α can also affect other cell types and organs. In contracting muscle, the coordinated recruitment and activation of macrophages is essential for proper repair and regeneration. We found that by regulating the myokine BNP (brain natriuretic peptide), PGC-1α affects macrophage polarization and hence tissue repair. The relevance of these findings transcend the context of exercise and pertain to our understanding of the pathological events leading to, but hopefully also potential novel therapeutic avenues for the treatment of muscle diseases.

... S7-2

Effects of oxidation and nitrosylation on muscle function in exercise and disease

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Reactive oxygen and nitrogen species (ROS/RNS) are produced in skeletal muscle in normal exercise, and at excessive levels in various disease states. They may potentially affect muscle function by perturbing many of the excitation-contraction coupling steps, though the predominant sites affected appear to be the contractile apparatus and the ryanodine receptors (RyRs) in the sarcoplasmic reticulum. Acute exposure to moderate levels of ROS, such as hydrogen peroxide and superoxide, increase the Ca^{2+}-sensitivity of the contractile apparatus in fast-twitch mammalian fibres via S-glutathionylation of Cys134 on fast TnI. In contrast, RNS, such as GSNO, reduce Ca^{2+}-sensitivity by competitive action at the same site, preventing the effects of S-glutathionylation. Both reactions protect that labile

site from irreversible damage by more excessive oxidation treatments. It appears that in normal exercise, S-glutathionylation predominates, because both brief intense cycling exercise in humans and 12 weeks of nightly wheel running in rats results in increased Ca^{2+}-sensitivity in fast-twitch fibres that can be reversed by treatment with the reducing agent dithiothreitol. Slow-twitch fibres show no such reversible oxidation-dependent effect. Stronger or more prolonged exposure to ROS/RNS, however, results in irreversible decreases in maximum force production in fast-, slow- and cardiac muscle, and this is likely an important factor in muscle dysfunction in a range of diseased and abnormal states. ROS/RNS seem not to greatly affect the action potential-induced Ca^{2+} release mechanism, but nevertheless increase Ca^{2+} leakage through the RyRs. This can potentiate force responses by raising resting cytoplasmic [Ca^{2+}], resulting in increased Ca^{2+} occupancy of TnC. In the long term, however, such RyR Ca^{2+} leakage can decrease net releasable SR Ca^{2+} in muscle fibres, as seen with ageing and limb immobilization in humans, potentially adversely affecting force production.

... S7-3

The physiopathological role of mitochondrial calcium uptake in skeletal muscle homeostasis

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Muscle activity leads to major swings in mitochondrial [Ca^{2+}] which control aerobic metabolism, survival pathways and cell death. Recently, we showed that mitochondrial Ca^{2+} uptake positively modulates skeletal muscle trophism by impinging on two major pathways, PGC-1alpha4 and IGF1-AKT/PKB thanks to the use of AAV vectors. Here, we aimed to discern the metabolic route regulated by mitochondrial Ca^{2+} uptake that is responsible for muscle trophism. For this purpose, we generated a skeletal muscle specific *Mcu* knockout mouse (mlc1f-Cre-*Mcu*-/-), by crossing a *Mcufl/fl* mouse with a line expressing the Cre recombinase under the control of the myosin light chain 1f (*mlc1f*) promoter. Our preliminary data confirm that PGC-1alpha4 and IGF1-AKT/PKB signaling pathways are negatively regulated in skeletal muscle specific Mcu knockout animals. In addition, we also observed a slight decrease of fibre size in mlc1f-Cre-*Mcu*-/- skeletal muscles. Most importantly, when these mice were exercised on a treadmill using different training protocols, an impaired running capacity became evident, indicating that mitochondrial Ca^{2+} accumulation is required to guarantee skeletal muscle performance. Finally, a clear metabolic alteration is present in mlc1f-Cre-*Mcu*-/- animals. Specifically, mlc1f-Cre-*Mcu*-/- mice show decreased glucose, increased lactate, free fatty acids and ketone bodies, suggesting an impaired crosstalk between skeletal muscle and liver. Taken together, these data indicate that mitochondrial Ca^{2+} uptake plays a pivotal role in the control of skeletal muscle trophism. Further investigations of MCU-dependent effects on skeletal muscle homeostasis will represent an important task for the future. Indeed, this research will provide new possible targets for clinical intervention in all diseases characterized by muscle loss, such as dystrophies, cancer cachexia and aging.

... S7-4

IGF-1 attenuates hypoxia-induced atrophy but inhibits myoglobin expression in C2C12 skeletal muscle myotubes

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Chronic hypoxia is associated with muscle wasting and decreased oxidative capacity. By contrast, training under hypoxia may enhance the hypertrophic response to training, oxidative capacity and improve oxygen transport to the mitochondria by increasing myoglobin (Mb) expression. The latter may be further enhanced by lipid supplementation. We investigated individual and combined effects of hypoxia, insulin-like growth factor (IGF)-1 and lipids, in C2C12 myotubes. We hypothesized that IGF-1 induced hypertrophy is attenuated under lower oxygen tensions but is enhanced by lipids since expression of mRNAs for Mb and oxidative enzymes are enhanced by hypoxia and lipids. Differentiated C2C12 myotubes were cultured under 20%, 5% and 2% oxygen with or without 24h of IGF-1 and/or lipids. In culture under 20% oxygen, IGF-1 induced 51% hypertrophy. Hypertrophy was only 32% under 5% and abrogated under 2% oxygen. This was not explained by changes in expression of genes involved in contractile protein synthesis or degradation, suggesting a reduced rate of translation rather than transcription. Myoglobin mRNA expression increased by 75% under hypoxia but decreased by 50% upon IGF-1 treatment in normoxia, compared to control. Inhibition of mammalian target of rapamycin (mTOR) activation using rapamycin restored Mb mRNA expression to control levels. Lipid supplementation had no effect on Mb gene expression. Thus, IGF-1 induced anabolic signaling can be a strategy to improve muscle size under mild hypoxia, but lowers Mb expression.

... S7-5

miR-424-5p: a novel negative regulator of ribosomal biogenesis which contributes to muscle wasting

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The loss of skeletal muscle mass is a common co-morbidity in a number of chronic diseases and in older individuals which worsens quality of life and increases mortality. The loss of muscle mass is linked to a shift in the balance of protein turnover in favour of catabolism. Ribosomes are molecular machines that are central to protein synthesis, so defective ribosomal biogenesis is likely to impact this balance. We found that miR-424, a microRNA located in a cluster on the X-chromosome, was significantly upregulated and associated with disease severity in the quadriceps of patients with chronic obstructive pulmonary disease (COPD), intensive care unit-acquired weakness, sarcopenia and those requiring aortic surgery. Furthermore, pre-surgery levels of miR-424 in the quadriceps of patients undergoing aortic surgery was proportional to quadriceps muscle loss over the following 7 days. In silico studies predicted that miR-424 targeted components of the pre-initiation complex (PIC) required to synthesise ribosomal RNA (rRNA), including RNA polymerase I (POLR1A), upstream-binding transcription factor (UBTF) and RRN3. Transfection of the miRNA into a myoblast cell line reduced the expression of these mRNAs as well as rRNA expression and protein synthesis consistent with the predictions. Over-expression in mice tibialis anterior muscles caused rapid fibre atrophy, with 21% muscle mass loss, and reduced rRNA and UBTF expression. In conclusion, we put forward miR-424 as a novel negative regulator of ribosomal biogenesis and propose that it is likely to contribute the inhibition of protein synthesis in muscle wasting patients.

Session 8

Heart Failure and Cardiomyopathies

... S8-1

The metabolic road of co-morbidities to understanding the pathophysiology of heart failure: The role of inflammatory signaling pathways in obesity and diabetes

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The development of effective treatments for heart failure (HF) with left ventricle (LV) diastolic dysfunction (DD) is currently limited by poor understanding of the underlying pathophysiology. Abnormal diastolic LV function with impaired relaxation and increased diastolic stiffness is characteristic of heart failure with preserved ejection fraction; (HFpEF). HFpEF accounts for more than 50% of all cases of HF in Western societies and is closely associated with co-morbidities and gender. To date, all large multicentre trials of HFpEF treatments have produced disappointing results. This outcome suggests that a “one size fits all” approach to HFpEF may be inappropriate and supports the use of tailored, personalized therapeutic strategies with specific treatments for distinct HFpEF phenotypes. Recent evidence suggests that co-morbidities common to HFpEF promote a systemic inflammatory state that contributes to endothelial dysfunction, cardiomyocyte dysfunction, altered extracellular matrix, reactive oxygen species production, nitrosative stress, all of which affect the pathophysiology of HFpEF by modulation of LV stiffness, at least partly the giant protein titin and extracellular matrix. Titin isoform transitions and post-translational modifications such as phosphorylation and oxidation are major modulators of titin-based stiffness and contribute to diastolic stiffness. I will cover novel evidence for the development of HFpEF, which may help to assess specific treatment strategies in an attempt to develop tailored HFpEF therapy.

... S8-2

Cardioprotective mechanisms of dietary spermidine in aging and hypertension

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Aging is a major risk factor for cardiovascular disease and death. One of the paradigms of aging proposes that autophagy, a major cellular repair process associated with rejuvenation, is downregulated over the course of aging. We discovered that delivery of the potent autophagy inducer spermidine as a dietary supplement to old mice extends median lifespan for 10% and exerts cardioprotective effects through reduction of cardiac hypertrophy and preservation of diastolic function. Mice fed spermidine show enhanced cardiac autophagy, mitophagy, mitochondrial

respiration and mechano-elastical properties of cardiomyocytes *in vivo*, coinciding with increased titin phosphorylation and suppressed subclinical inflammation. Age-related effects on subcellular cardiomyocyte composition are reversed by the natural polyamine spermidine. Spermidine feeding phenocopies the age-related cardioprotective effects and lowers arterial blood pressure in a model of hypertension-induced cardiac remodeling and heart failure. This antihypertensive effect in Dahl salt-sensitive rats fed a high-salt diet is associated with greater bioavailability ratio of global arginine - the only source for the vasodilator nitric oxide. In humans, higher spermidine intake correlates with lower blood pressure and lower risk of cardiovascular disease (e.g. heart failure). Our study suggests dietary intake of spermidine as a novel and feasible strategy against aging-associated cardiovascular disease and salt-induced hypertension.

S8-3

Studying *mdx* cardiomyocyte hypertrophy *in vitro*

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QUESTIONS: Several therapies to treat Duchenne Muscular Dystrophy (DMD) are under development; unfortunately many lack efficacy in the heart. In addition, there is increasing need to treat dystrophin-deficient cardiomyopathy due to extending patient life expectancy resulting in a prevalence of cardiac symptoms. Despite the primary genetic defect being identical in skeletal and cardiac muscle, the symptoms and severity differ suggesting the involvement of secondary organ-specific pathways that are yet to be fully understood. We have developed an *in vitro* model of cardiomyocyte hypertrophy using cardiomyocytes isolated from *mdx* (mouse model of DMD) hearts. Using this model, we have been able to utilise various therapeutic approaches to lessen the phenotype. This model could be a fast, efficient way to screen new and existing pharmaceutical and gene-based approaches for therapeutic efficacy. Additionally, by employing transcriptomic and proteomic approaches, this model can be used elucidate the secondary organ-specific pathways involved.

We aim to deduce the cellular mechanisms underlying dystrophin-deficient cardiomyopathy and to identify and target novel therapeutic avenues.

METHODS: Cardiomyocytes are isolated from murine embryos and exposed to serum starvation to induce hypertrophy. RNA-sequencing of hypertrophic *mdx* cardiomyocytes identified differential regulation of genes. Using data generated from transcriptomics, differentially regulated transcripts were targeted for investigation on a protein level. Using a whole proteomic approach to observe expression on a global scale within these cells we will be able to identify novel therapeutic targets that can subsequently be targeted within the model.

RESULTS AND CONCLUSIONS: We have shown restoring the dystrophin protein to these cells can rescue the hypertrophic phenotype in addition to pharmaceutical compounds. RNA-Seq data implicated the involvement of pathways such as angiogenesis, fibrosis and calcium handling which have been further investigated on a protein level. The cellular phenotype has been rescued with pharmaceutical and gene-based approaches. In addition, we have further investigated mechanisms contributing to the pathology using a proteomic approach.

S8-4

The Spy-C method for in situ replacement of cardiac myosin binding protein-C in sarcomeres

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Cardiac myosin binding protein-C (cMyBP-C) is a critical regulator of cardiac contraction that is necessary for normal heart function and for increased contractility in response to inotropic agonists. The importance of cMyBP-C is further highlighted because mutations in the gene encoding cMyBP-C are a leading cause of hypertrophic cardiomyopathy (HCM). However, despite realizing its central importance to cardiac muscle function, the molecular mechanisms by which cMyBP-C affects remain poorly understood. This is in part due to the complex and dynamic interactions of cMyBP-C with its binding partners in the sarcomere as well as to the difficulty of manipulating large thick filaments such as myosin, titin, and myosin binding protein-C in muscle cells. Here we report a novel method to overcome the challenge of manipulating cMyBP-C in sarcomeres. The method uses a protein engineering approach to replace N-terminal domains of cMyBP-C in permeabilized myocytes from Spy-C mice. Spy-C mice were created using CRISPR/Cas9 gene editing to insert a protease recognition site followed by a short SpyTag sequence into the middle of the cMyBP-C, thus allowing for the N-terminus of cMyBP-C to be cut and removed. The technique then relies on the exposed SpyTag sequence on the remaining C-terminal segment of cMyBP-C (which remains anchored to the thick filament) being recognized by its partner protein, SpyCatcher. Together SpyTag and SpyCatcher combine to form a spontaneous covalent bond. By fusing the SpyCatcher sequence to new recombinant cMyBP-C N-terminal sequences, we are able to reconstitute full length cMyBP-C at the precise position of native cMyBP-C in the sarcomere. Thus, by first cleaving cMyBP-C in permeabilized myocytes from Spy-C mice and then adding new recombinant cMyBP-C N-terminal domains fused to the SpyCatcher sequence we are able to efficiently cut and paste cMyBP-C in situ to introduce virtually any desired modification (e.g., point mutations, FRET probes, etc) to study cMyBP-C interactions with its binding partners in sarcomeres. Successful application of the Spy-C method will not only provide new insights into cMyBP-C function, but is expected to have broader applicability to other large sarcomeric proteins that have been difficult or impossible to manipulate by standard methods.

S8-5

The molecular defects in Ca^{2+}-regulation due to mutations that cause hypertrophic cardiomyopathy can be reversed by small molecules that bind to troponin

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Mutations in contractile proteins can cause familial hypertrophic cardiomyopathy (HCM) or familial dilated cardiomyopathy (DCM). HCM has been linked to a higher myofilament Ca2+-sensitivity. In addition we have identified a molecular level dysfunction common to both HCM and DCM-causing mutations. This is an uncoupling of the relationship between troponin I (TnI) phosphorylation and modulation of myofilament Ca2+-sensitivity, essential for normal responses to adrenaline. Adrenergic response is blunted *in vivo* which predisposes to heart failure under stress. We have identified compounds that can specifically reverse these abnormalities *in vitro* and therefore have potential for treatment.

The first compound studied was Epigallocatechin-3-Gallate (EGCG). EGCG is capable of both Ca2+desensitisation and re-coupling of HCM mutant. We have since identified a further 25 similar compounds; many of these can re-couple independent of Ca2+-desentisisation.

We have mapped EGCG binding to whole troponin by molecular dynamics simulations and find that it is located between the N-terminal phosphorylatable peptide of TnI and the N-terminal Ca2+ regulatory domain of unphosphorylated TnC. In this position it could stabilise and influence the N-terminal TnI interaction with TnC that is involved in modulation of Ca2+-sensitivity by phosphorylation.

Furthermore, we have established a biological assay platform for screening EGCG and related analogues in intact cardiomyocytes to study their effects on contractile regulation in vivo. Using an E99K ACTC heterozygous-mutant HCM mouse model, our preliminary findings suggest that two functionally distinct populations of cells exist within the heart: cells that respond to the beta1 agonist dobutamine and cells that do not. EGCG is an inotrope in wild-type cells. In E99K non-responders treatment with EGCG appears to restore the adrenergic response. We are investigating other re-coupling compounds in this assay.

Session 9

Skeletal Muscle Diseases

S9-1

Calpain 3 and its partner titin in muscular dystrophies

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Genetic defects in Calpain 3 leads to Limb-Girdle Muscular Dystrophy type 2A, a disease of the skeletal muscle that affects predominantly the proximal limb muscles. There is no treatment for this disease to date. In a attempt to define a therapeutic strategy, We evaluated the potential of recombinant adeno-associated virus (rAAV) vectors for gene therapy in a murine model for LGMD2A. Efficient and stable transgene expression was obtained in the skeletal muscle after intramuscular and loco-regional administration. Moreover, its presence resulted in improvement of the histological features and in therapeutic efficacy at the physiological levels, including correction of atrophy and full rescue of the contractile force deficits. However when intravenous administration was used, death of some animals was observed in relation with cardiac toxicity. This observation led us to develop new vectors with skeletal muscle restricted expression. Results with this new generation of vectors will be presented.

Besides the primary defect in LGMD2A, secondary deficiency in calpain 3 has been observed in a number of muscle diseases, including muscular dystrophies due to mutations in the giant titin, a known calpain 3 partner. Indeed, mutations at the end of titin can lead to the allelic disorders: Tibial Muscular Dystrophy (TMD) and Limb Girdle Muscular Dystrophy 2J (LGMD2J). Both clinical presentations have been initially identified in a large Finnish family and linked to a mutation (commonly referred to as FINmaj) in the last exon of titin. When this mutation is present on only one allele, it leads to the late onset mild distal TMD and on both alleles to the early onset severe LGMD2J. To study the pathophysiology of these two diseases, a mouse model carrying the FINmaj mutation was created. This model shows a myopathology with high similarity to the human situation, both at the homozygous and heterozygous state. As seen in LGMD2J, the mutation leads at molecular level to a loss of the very end of titin C-terminus and to secondary protein instability of calpain 3, a known partner of titin. This model is currently utilized as a tool to decipher, at molecular level, the pathophysiological mechanisms underlying the two clinical presentations, to understand the functional relationship between titin and calpain 3 and finally to define therapeutic strategies. Latest results will be presented.

... S9-2

Bench to bedside research on critical illness myopathy (CIM) and ventilator induced diaphragm muscle dysfunction (VIDD): Mechanisms and intervention strategies

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Significant improvements in modern critical care related to technological advances, improved understanding of the pathogenesis of disease process, new therapies and the removal of inefficient/harmful interventions have led to improved survival from critical illness. However, the improved survival is associated with an increased number of patients with complications related to modern critical care. Severe muscle wasting and impaired muscle function are frequently observed in immobilized and mechanically ventilated intensive care unit (ICU) patients. Approximately 30% of mechanically ventilated and immobilized ICU patients for durations of 5 days and longer develop generalized muscle paralysis of all limb and trunk muscles, a condition known as CIM. Mechanical ventilation is a lifesaving treatment in critically ill ICU patients; however, the being on a ventilator creates dependence, and the weaning process occupies as much as 40% of the total time of mechanical ventilation. Furthermore, 20–30% of patients require prolonged intensive care due to VIDD, resulting in poorer outcomes, and greatly increased costs for health care providers. CIM and VIDD in ICU patients may be related to the primary disease, but there is heterogeneity of underlying disease and pharmacological treatment among patients exhibiting similar outcomes. Thus, it is highly likely that a common component of ICU treatment per se is directly involved in the progressive impairment of muscle function and muscle wasting during long-term ICU treatment. The specific mechanisms underlying the muscle wasting and impaired muscle function associated with the ICU intervention are poorly understood in the clinical setting. There is, accordingly, compelling need for experimental animal models closely mimicking the ICU condition, including long-term exposure to mechanical ventilation and immobilization. In this project, the muscle dysfunction, which by far exceeds the loss in muscle mass in limb and respiratory muscles in patients with CIM and VIDD have been investigated in detail at the cellular and molecular levels in rodent and porcine experimental ICU models, allowing detailed studies in immobilized and mechanically ventilated animals for long durations. Results demonstrate that the motor protein myosin is highly involved in the pathogenesis of both CIM and VIDD, but mechanisms are different. In CIM there is a preferential loss of myosin due to transcriptional down-regulation and enhanced degradation while post-translational modifications of myosin play a significant role for the diaphragm muscle dysfunction in VIDD. Specific intervention strategies targeting the mechanisms underlying CIM and VIDD will be presented and the translation of these interventions to the clinic.

... S9-3

The skeletal muscle pathophysiology of a novel dystrophin-negative mouse strain which exhibits a decrease in susceptibility to muscle damage

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Duchene muscular dystrophy (DMD) is a debilitating X-linked disease caused by mutations in the gene encoding the protein dystrophin. The lack of dystrophin leads to progressive skeletal muscle damage and wasting, which significantly decreases locomotory ability. DMD ultimately results in death from respiratory or cardiac failure, as no cure is currently available. Mdx mice, an animal model of DMD, exhibit lower levels of voluntary exercise than control mice due to their impaired skeletal muscles. In this study, we bred mdx mice with a mouse strain with a naturally high voluntary exercise capability ('CC' mouse). Dystrophin-deficient progeny of this cross (mdx/CC mice) display significant improvements in daily voluntary running distance ($P<0.001$) and maximum running velocity ($P<0.01$) compared to mdx mice. Therefore, we assessed the pathophysiology of the skeletal muscles from mdx and mdx/CC mice to investigate the physiological mechanisms responsible for the increased exercise ability of mdx/CC mice. Six-week-old mdx controls (n=14) and mdx/CC (n=15) mice were anaesthetised, the extensor digitorum longus (EDL) and soleus hind-limb muscles surgically removed and attached to an *in vitro* muscle test system, muscles frozen for histology and sera taken. EDL and soleus muscles from mdx/CC mice showed a significant increase in resistance to eccentric-contraction induced damage ($P<0.05$) along with a decrease in muscle necrosis ($P<0.05$) compared to mdx controls. Sera was analysed for creatine kinase levels, an indicator of muscle damage particularly in DMD pathology, and was significantly lower in mdx/CC mice compared to mdx controls ($P<0.01$). These novel dystrophin-negative mice show enhanced running ability compared to mdx, which could be attributed to their improved resistance to muscle damage. Therefore, elucidating the genes responsible for this improved performance of dystrophin-deficient skeletal muscle, could lead to novel therapeutic targets for DMD patients.

... S9-4

Mutations in the CASQ1 gene alter calsequestrin properties and cause tubular aggregate myopathy

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Tubular aggregate myopathy is a rare muscle disease characterized by the presence of regular arrays of membrane tubules in a fraction of fibers from patients' muscle biopsies. To date, two genes, *STIM1* or *ORAI1*, which encode key proteins for the mechanism of Store Operated Ca2+ Entry (SOCE) have been found in almost half of the patients with tubular aggregate myopathy. We recently identified three novel missense mutations in the calsequestrin-1 (*CASQ1*) gene in four patients with tubular aggregate myopathy. Exome sequencing did not reveal pathogenic changes in other genes, indicating that *CASQ1* is the third gene associated to tubular aggregate myopathy.A set of functional studies were then performed to test the effect of these mutations on calsequestrin properties. Turbidity and dynamic light scattering measurements at increasing Ca2+ concentrations showed a reduced Ca2+-dependent aggregation for two of these mutant CASQ1 proteins. Accordingly, limited trypsin proteolysis assay showed that they were more susceptible to trypsin cleavage in presence of Ca2+ in comparison to wild-type CASQ1. An increased Ca2+-dependent aggregation was detected with a third mutant, which however was lower than that of the p.Asp244Gly mutation that we previously identified in patients with aggregate vacuolar myopathy, a distinct form of rare myopathy. Analysis of single muscle fibers from the muscle biopsy of one of these patients with tubular aggregate myopathy showed a significant reduction in the amount of releasable Ca2+ compared to normal control fibers. Expression of CASQ1 variants in eukaryotic cells revealed a reduced ability of all these CASQ1 variants to contribute to the intracellular Ca2+ store of transfected cells. Interestingly, two CASQ1 mutants showed a reduced inhibitory effect on Store Operated Ca2+ Entry, SOCE. Altogether, these findings widen the spectrum of skeletal muscle diseases associated to mutations in *CASQ1*, indicate that these CASQ1 variants exhibit changes in properties critical for correct Ca2+ handling in skeletal muscle fibers, and improve the understanding of the mechanisms underlying tubular aggregate myopathy.

... S9-5

Translocation of molecular chaperones to the titin springs compromises sarcomere function in skeletal myopathy patients

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Myopathies encompass a wide variety of acquired and hereditary disorders. The pathomechanisms include structural and functional changes affecting, e.g., myofiber metabolism and contractile properties. In this study, we observed increased passive tension (PT) of skinned myofibers from patients with myofibrillar myopathy (MFM) caused by *FLNC* mutations (MFM-filaminopathy) and limb-girdle muscular dystrophy type-2A due to *CAPN3* mutations (LGMD2A), compared to healthy control myofibers. Because the giant protein titin determines myofiber PT, we measured its molecular size and the titin-to-myosin-heavy-chain ratio, but found no differences between myopathies and controls. All-titin phosphorylation and site-specific phosphorylation in the PEVK region were reduced in myopathy, which would be predicted to lower PT. Electron microscopy revealed extensive ultrastructural changes in myofibers of various hereditary myopathies and also suggested massive binding of proteins to the sarcomeric I-band region, presumably heat shock proteins (HSPs), which can translocate to elastic titin under stress conditions. Correlative immunofluorescence and immunoelectron microscopy showed that two small HSPs (HSP27 and aB-crystallin), which were upregulated in myopathic versus control muscles, and ATP-dependent HSP90 associated with the titin springs in all hereditary myopathies analysed. This binding pattern of chaperones was regularly observed in Duchenne muscular dystrophy (DMD), LGMD2A, MFM-filaminopathy, MFM-myotilinopathy, titinopathy, and inclusion body myopathy due to mutations in valosin containing protein, but not in acquired sporadic inclusion body myositis. The three HSPs also associated with elastic titin in mouse models of DMD and MFM-filaminopathy. Mechanical measurements on skinned human myofibers incubated with exogenous small HSPs suggested that the elevated PT seen in myopathy is caused, in part, by chaperone-binding to the titin springs. Whereas this interaction may be protective in that it prevents sarcomeric protein aggregation, it also has detrimental effects on sarcomere function, with clinical implications. Thus, we identified a novel pathomechanism common to many hereditary muscle disorders, which involves sarcomeric alterations.

DEBATE SESSIONS

Debate Session I

Muscle Contraction: Is Titin Contributing Actively?

... DS1-1

Protein folding as a major source of mechanical work in Physiology

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The most important discovery of force-spectroscopy over the past 20 years is that proteins do mechanical work when they fold against an opposing mechanical force (1). For example, the amount of mechanical work done by a folding immunoglobulin like domain can be 2–3 times larger (~120 zJ) than that of a chemically powered motor (e.g. myosin II; ~38 zJ; ref 2). Folded proteins store mechanical energy by unfolding end extending under force. Elastic energy is stored this way by stretching caused by gravitational pulling during locomotion, inertia, chemical modifications, and ATP powered sources to name a few. Protein unfolding occurs at varying rates over a very wide range of forces above >8 pN. By contrast, most of the stored mechanical energy is delivered back only over a small range of forces where the folding probability rapidly increases from 0 to 1 (10pN to 4 pN respectively; refs 2,3) and the folding protein does large amounts of mechanical work. Thus, protein folding/unfolding is likely to operate as a sort of mechanical battery where different types of energy sources are stored, and then converted back into mechanical work in a highly regulated form over a very small force range. This novel mechanism is likely to be widespread in Physiology.

I will discuss two specific systems: titin folding as a major driver of muscle contraction, and protein folding in general as the driving force of directional protein transport (3).

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Debate Session II

Residual Force Enhancement in Muscle – Facts and Fancy

... DS2-1

Residual force enhancement in muscle – Facts and fancy

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QUESTION: Residual force enhancement (RFE) is a property of skeletal muscle observed on all structural levels, including single, mechanically isolated sarcomere preparations. However, RFE cannot be explained within the cross-bridge theory without making assumptions that are hard to justify. Therefore, the question becomes: How is RFE produced and might it affect how we think about muscle contraction and force regulation?

METHODS: RFE refers to the property of muscle where the isometric steady-state force after active muscle stretching (eccentric contraction) is greater than the purely isometric force at the same length and same activation. We confirmed that this property exists on all structural levels of muscles, from single, mechanically isolated sarcomeres up to the level of in vivo human voluntary muscle contraction.

RESULTS: RFE increases with the magnitude of stretch but is largely independent of the speed of stretch. Force in the enhanced state is metabolically cheaper than the corresponding purely isometric reference force; RFE persists for minutes (in myofibrils and skinned fibres), but can be abolished instantaneously by deactivating a muscle just long enough for force to drop to zero. RFE is associated with an increase in passive force that is likely arising (at least in part) from the molecular spring titin.

DISCUSSION AND CONCLUSION: There is good evidence that titin plays a significant role in the increased forces observed in RFE. It has been shown that titin can change its stiffness in the presence of calcium and active force production. We speculate that titin might contribute to the RFE property by changing its stiffness in two distinct ways: by binding of calcium upon activation and by binding some of its proximal segments to actin thereby decreasing its inherent spring length thus increasing its stiffness and force when stretched.

POSTER PRESENTATIONS

Poster Session 1

Contractility and Force Generation

... P1-1

Partial titin degradation increases sarcomere length non-uniformities and reduces absolute residual force enhancement after active stretch in single myofibrils

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QUESTIONS: Titin plays a key role in stabilizing sarcomeres and controlling the physiological sarcomere length (SL) range during muscle passive and active force generation. However, no in-depth investigation of the role of titin in preventing the development of SL non-uniformities after passive and active stretch and following activation has been performed. Our aim was to determine the effects of partial titin degradation by a low dose of trypsin on SL non-uniformities and force in passive and active myofibrils.

METHODS: Titin-intact (Pas TI) and trypsin-treated (Pas Tryp) myofibrils were passively stretched from a SL of 2.4 µm to SLs of 3.4 and 5.4 µm. In the active condition, titin-intact (Act TI) and trypsin-treated (Act Tryp) myofibrils were activated at a SL of 2.8 µm and actively stretched to a SL of 3.6 µm. The extent of SL non-uniformities was calculated for each sarcomere as the absolute difference between sarcomere length and the mean sarcomere length of the myofibril.

RESULTS AND CONCLUSIONS: Pas TI showed greater passive stresses than Pas Tryp. SL non-uniformities increased after passive stretches but were similar between Pas TI and Pas Tryp (65±6nm, 119±19 nm and 273±42 nm at SLs 2.4, 3.4 and 5.4 µm respectively, vs. 80±11nm, 161±22 nm and 303±47nm). Act TI produced greater stresses at the steady-state following activation and active stretch and showed greater absolute residual force enhancement compared to Act Tryp (108±13, 154±15 and 51±9 kPa vs. 66±10, 84±17 and 18±8 kPa). SL non-uniformities increased to the same extent in Act TI and Act Tryp after activation (83±9 nm to 363±45 nm vs. 85±6 nm to 502±77 nm). However, while active stretch did not further increase SL non-uniformities in Act TI, it resulted in a significant rise in SL non-uniformities in Act Tryp (426±50 nm vs. 737±96 nm). These results suggest that titin is essential for stabilizing actively stretched sarcomeres as well as for active and passive force generation and residual force enhancement.

... P1-2

The significance of phosphate dependent rate modulation of k_{TR} for the cross-bridge mechanism

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The reversible release of inorganic phosphate (P_i) from the active site of cross-bridges is coupled to their transition from non-force-generating to force-generating states. However, the kinetics of the P_i release in this transition remains unclear. During isometric muscle contraction, cross-bridges can rebind P_i and reverse the transition, a process that reduces isometric force and accelerates kinetics of isometric force redevelopment induced by a slack-restretch maneuver. Force redevelopment kinetics is determined by the redistribution of cross-bridges between non-force-generating and force-generating states. The rate constant k_{TR} of force redevelopment increases with $[P_i]$ and reports the sum of rate-limiting transitions in the cross-bridge ATPase cycle. The present study will provide evidence that the extent of rate modulation of k_{TR} by the $[P_i]$ strongly depends on the type of the cross-bridge model. Rate modulation requires a close coupling of P_i release and P_i rebinding with rate-limiting steps for forward transition of cross-bridges to force-generating states and its backward transition to non-force-generating states reflected by the apparent rate constants f and f^-, respectively. Separation of P_i release/rebinding from f and f^- results in a flat k_{TR}–$[P_i]$ dependence, in contrast to the observed steep k_{TR}–$[P_i]$ relation in experiments on muscle fibers and myofibrils. In particular the view implied by many models of a fast force-generating step coupled to rapid P_i release results in strong loss of $[P_i]$-dependent rate-modulation of k_{TR}. Strongest rate modulation of k_{TR} by $[P_i]$ is obtained when P_i release and P_i rebinding are directly coupled the rate-limiting transitions f and f^- in the cross-bridge cycle. Experimental force and k_{TR} data at different $[P_i]$ of cardiac myofibrils from guinea pig indicates a coupling factor close to 1, suggesting that the force-generating mechanism linked to P_i release is intrinsically coupled to the rate-limiting transition f.

... P1-3

The sag response in human adductor pollicis muscle

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QUESTIONS: The mechanisms which cause tension to decline (sag) during unfused tetanic contractions are not well understood. To better understand how sag might occur, we ask: how do muscle length and stimulation rate affect unfused tetanic contractions and sag?

METHODS: Ten healthy participants (4 male, 6 female, 24.4±1.7 years of age, 64±3.8 kg) were recruited. Con-

tractions were evoked in the adductor pollicis via percutaneous electrical stimulation of the ulnar nerve, at short (S) and long (L) muscle lengths (40° difference in thumb adduction angles). Each contraction consisted of 16 pulses delivered at fixed intervals varying between 1.0x and 5.0x the twitch contraction time (CT), which varied between participants and with muscle length.
RESULTS: Sag increased (P<0.05) as the inter-pulse interval was increased from 1.0xCT (L: 0.7±0.2% peak force (P_o) vs S: 2.0±0.5% P_o) to 3.0xCT (L: 15.8±2.6% P_o vs S: 22.4±2.3% P_o). Sag was significantly greater (P<0.05) at S than L in all unfused tetani. Summation, a non-linear process, was assessed by the active tension generated by pulse 2 of unfused tetani. We found pulse 2 active tension increased the most at short inter-pulse intervals, and that these increases were 6.8±5.7% higher (P<0.05) at S than at L across stimulation rates. Contractions evoked at 5.0xCT resulted in a series of twitch contractions. After 16 twitches, twitch duration was significantly reduced (S: 10.8±1.1% and L: 11.6±1.3%; S vs L not significant) as was twitch tension (S: 10.7±2.3% and L: 7.5±2.2%; S vs L P<0.05).
CONCLUSIONS: These results indicate that declines in activation level and contraction duration likely contribute to sag in the adductor pollicis. Moreover, sag exhibits length-dependence which appears related to length-dependent changes to summation and activation level. A more detailed analysis of the summation process seems warranted. Changes in sarcomere length during unfused contractions may also influence sag, but are currently untested.

P1-4
Estrogen affects skeletal muscle force generation in females by modulating post-tetanic potentiation and by altering the super relaxed state of myosin

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Estrogen deficiency impairs skeletal muscle force generation in aged women and ovariectomized rodents. Myosin has been implicated and here we test the hypotheses that in vivo potentiation of force and the super relaxed state (SRX) of myosin contribute to the estrogenic effects.
METHODS: To test post-tetanic potentiation (PTP) of force, a stimulating nerve cuff was surgically implanted on the common peroneal nerve of ovary-intact (Control) or ovariectomized (Ovx) mice. PTP of the anterior crural muscles was measured immediately before and 1 h after treatment with vehicle, 17beta-estradiol (E2), an estrogen receptor agonist (G1), or an estrogen receptor antagonist (G15). The population of myosin in the SRX state was measured in chemically skinned skeletal muscle fibers from adult ovary-intact (Control), Ovx, and Oxv+E2 mice, as well as aged ovarian senescent mice. Quantitative confocal microscopy of fluorescent ATP turnover was used to detect and quantitate myosin SRX in the fibers.
RESULTS: Potentiated twitches generated 22–102% more force than unpotentiated twitches. However, the extent of PTP depended on the presence of an estrogen (p=0.022). PTP of muscles from control (54±5%), Ovx+E2 (66±9%), and control+G15 (57±3) mice were greater than that of Ovx mice (39±3%). Fibers from Ovx mice had less SRX than controls (exhibited faster ATP turnover and loss of the slow decay phase; p=0.01). When Ovx mice were treated with E2, SRX and the slow decay phase was rescued. The population of fibers in SRX state from aged female mice did not differ from those of adult, control mice (23±2% vs 25±2%; p=0.52).
CONCLUSIONS: Estrogenic compounds rescue Ovx-induced low PTP. Estrogen deficiency in female mice via ovariectomy but not aging impact myosin SRX in skeletal muscle fibers. Current experiments are determining the extent to which these results of impaired force generation are due to a common mechanism, namely phosphorylation of the myosin regulatory light chain.

P1-5
On the nature of unloading-induced postural muscle stiffness decline

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At the end of XXth century it was shown that stiffness of isolated muscle and muscle fibers is determined by the active component (cross-bridges formation during stretching and contraction) as well as series elastic component i.e. cytoskeletal proteins capable to resist mechanically to muscle stretching or/and contraction. At present it is known that functional unloading induces a significant decline in passive as well as active, transverse as well as longitudinal stiffness of isolated muscle and muscle fibers [Canon, Goubel, 1995; McDonald, Fitts, 1995; Toursel et al., 2002; Ogneva, Shenkman et al, 2011].
However it is unclear, which molecular machinery determines this stiffness loss. We demonstrated that the passive stiffness of the isolated rat soleus muscle was 20% declined after 3 days of simulated gravitational hindlimb unloading. The treatment of actomyosin inhibitor blebbistatin (75 μM) induced the almost similar 22% stiffness decline in muscles from suspended as well as cage control animals. These data gave evidence that at the early stage of unloading the contribution of the reduced cross-bridges to the stiffness decline was slight. In order to analyze the contribution of μ-calpain-dependent degradation of the cytoskeletal proteins to the muscle stiffness loss, we used the selective calpain inhibitor PD150606. The level of the passive tension of muscle from the PD150606-treated animals did not differ significantly from the cage control level. Thus, the calpain activation break-down of cytoskeletal proteins may sufficiently influence the muscle passive stiffness decline at the early stage of gravitational unloading.

The work was supported by grant of the Russian Foundation of Basic Research #16-04-00529.

P1-6
Direct isometric muscle strain analyses using speckle tracking technology. A validation study

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INTRODUCTION: Muscle contraction is the activation of tension-generating sites within muscle fibers. Diseased, injured, or dysfunctional skeletal muscles may demonstrate abnormal function such as compromised contractility. Therefore, evaluation of mechanical properties of muscles, including passive (volume) and active (strain) properties, has important clinical applications. Real-time in vivo measurements of skeletal muscle contractility during isometric submaximal contractions have not been reported previously. We used Ultrasound speckle tracking (UST) to determine the contractility capacity (strain pattern) of the supraspinatus (SS) muscle under standardized isometric load conditions.
METHODS: UST analysis was performed with the test persons in sitting position and the transducer placed over the SS fossa. Using a linear probe and a GE Vivid 9 ultrasound apparatus tracing of the SS muscle was performed by longitudinal track analysis during standardized submaximal isometric load conditions (40-60-80% of maximal voluntary contraction).Testing was performed for shoulder elevation-external rotation (SS function). Data was analyzed blinded using Q-analysis and Echopacs (GE Healthcare) speckle-tracking software.
RESULTS: We found that strain (shortening of muscle fibers) in the SS muscle varied depending on load condition (p<0.01, One-way Anova). We found a mean negative strain rate of -10.5 ± 1.1 (SEM) at 40% max load, -12.8 ± 1.1 at 60% max load and -17.5 ± 1.5 at 80% max load, with significant differences between 40% and 80% strain (p<0.01, Tukeys *post hoc* test) and between 60% and 80% strain (p<0.05, Tukeys *post hoc* test).
CONCLUSION: We found that UST is able to track and differentiate muscle strain (i.e. muscle contractility) during different submaximal isometric conditions. This non-invasive technique has perspective to be used in daily clinical practice to objectively time treatment and rehabilitation of diseased patients and to monitor effects of individualized medical treatment in neuromuscular diseases.

Poster Session 2

Cytoskeletal Proteins Beyond Actin / Myosin

P2-1
Filamin actin-binding and titin-binding fulfill distinct functions in Z-disc cohesion

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Many proteins contribute to the contractile properties of muscles, most notably myosin thick filaments, which are anchored at the M-line, and actin thin filaments, which are anchored at the Z-discs that border each sarcomere. In humans, mutations in the actin-binding protein Filamin-C result in myopathies, but the underlying molecular function is not well understood. Here we show using *Drosophila* indirect flight muscle that the filamin ortholog Cheerio in conjunction with the giant elastic protein titin plays a crucial role in keeping thin filaments stably anchored at the Z-disc. We identify the filamin domains required for interaction with the titin ortholog Sallimus, and we demonstrate a genetic interaction of filamin with titin and actin. Filamin mutants disrupting the actin- or the titin-binding domain display distinct phenotypes, with Z-discs breaking up in parallel or perpendicularly to the myofibril, respectively. Thus, Z-discs require filamin to withstand the strong contractile forces acting on them.

P2-2
Interactions between titin and myosin filaments explored with atomic force microscopy

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Titin is a giant protein spanning between the Z- and M-lines of the sarcomere. In the A-band titin is associated with the myosin thick filament. It has been speculated that titin may serve as a ruler for thick-filament formation due to the super-repeat structure of its A-band domains. Accordingly, titin might provide a layout that determines the length and structural periodicity of the thick filament. However, the accessibility of A-band anti-titin antibody epitopes and structural reconstructions from electron-microscopic images suggest that titin runs on the thick filament surface, raising important questions about how the two filaments determine or affect each other's structural arrangement. Here we tested the titin ruler hypothesis by mixing titin and myosin at in situ stoichiometric ratios (300 myosins per 12 titins) and dialyzing the sample against buffers of different filament-forming ionic strength (KCl concentration range 50–150 mM). The formed filamentous complexes were investigated with atomic force microscopy. We found that the samples contained distinct populations of titin oligomers and myosin thick filaments.

Complexes in which myosin molecules were associated to titin filaments could not be identified. Occasionally we observed myosin thick filaments with titin oligomers attached to their surface. Thus, the electrostatically driven self-association is stronger in either myosin and titin than their binding to each other. Conceivably, associated proteins, such as myosin-binding protein C, and additional mechanisms are required to modulate and regulate the in situ interactions between titin and the myosin thick filament.

... P2-3

Extraocular muscles (EOM) in desmin knock out and R349P desmin mutant mice

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Extraocular muscles (EOM) are classified as a separate muscle group given their distinct gene and protein expression profiles when compared with other skeletal muscles. EOM show different physiological properties and are selectively spared in several muscular dystrophies. We have shown that a subgroup of myofibers in the normal adult and fetal human EOM partially or completely lack desmin. Data on the EOM in desminopathies are lacking.

Here, we investigated the effect of the lack of desmin as well as the presence of the most common human desminopathy mutation R350P on the structure and composition of the cytoskeleton of the EOM using immunofluorescence and a battery of antibodies against desmin, nestin, dystrophin, plectin, syncoilin, synemin, MyHC fast IIa, MyHC slow, and laminin.

A total of nine desmin knock-out mice and seven R349P desmin knock-in mice, which harbor the ortholog of the human R350P desmin mutation, were compared respectively to eleven and six wild type mice. The EOM in both desmin mice models were remarkably unaffected in contrast to weight bearing muscles. There were no signs of loss of myofiber integrity or altered subsarcolemmal morphology, tested with antibodies against laminin and dystrophin. There were an increased fiber size variability and significantly larger muscle fiber areas in the global layer of desmin knock-out mice. Some sporadic myofibers in the global layer showed aggregations of nestin in both the desmin knock-out and R349P desmin knock-in mice.

The EOM, in spite of being highly active muscles, appeared very well preserved in both desmin knock-out and R349P desmin knock-in mice, in contrast to other skeletal muscles. Studies are ongoing to determine the patterns of distribution of additional cytoskeletal proteins and the fate of the different fiber types in these two mouse models.

... P2-4

Structural and biophysical characterization of FATZ-1, FLNc and α-actinin 2 interactions

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Z-discs are required for the transmission of mechanical strain along the serially arranged sarcomeres, and ultimately along the length of the muscle. In the Z-discs antiparallel F-actin filament from adjacent sarcomeres are crosslinked by major Z-disc protein α-actinin. They are also a phosphorylation hotspots underscoring their functions in signaling and disease-related processes. Filamin C (FLNc), an actin-crosslinking homodimer, is essential for Z-disc assembly and maintenance. It is composed of one actin binding domain, followed by 24 immunoglobulin-like domains with Ig-like domain 20 (Ig-d20) containing an intrinsically unstructured 81 amino acid insertion, which targets FLNc to the Z-disc of striated muscle. In this insertion, two FLNc phosphosites are located (S2233 and S2236) [Reimann L et al. 2017]. FATZ-1 is a 30 kDa adaptor protein, which was shown to interact with FLNc, α-actinin and several other Z-disk proteins [Frey N et al. 2002].

To understand molecular layout of FATZ-1, FLNc and α-actinin interactome, we set for a comprehensive structural, biochemical and biophysical analysis of their interactions. We mapped and quantified interactions between various fragments of these proteins using limited proteolysis, fluorescence correlation spectroscopy (FCS) and microscale thermophoresis. In addition, we compared the affinities of wild type FLNc fragment (Ig-d19-21) to those of three phosphomimetic (S2233D, S2236D, S2233D_S2236D) and two cardiomyopathy causing mutations (W2164C, I2160L). FATZ-1 was found to contain two binding sites for FLNc Ig-d19-21, of which the one located at the C-terminus of FATZ-1 showed higher affinity. Further FCS experiments showed that α-actinin-2 competes with FLNc for the C-terminal FATZ-1 binding site, leading to formation of a ternary α-actinin-2-FATZ-1-FLNc complex crucial for Z-disk assembly through FATZ-1 N-terminal region. We observed a lower affinity of N-terminal FATZ-1 fragment to both phosphomimetic mutants in comparison to the wild type and no interaction to the doubly phosphorylation mimicking mutant. Thermal melting (Tm) experiments displayed no change in the Tm for FLNc wild type and the phosphomimetic mutants, but a decrease in the Tm for both “disease-causing” ones, suggesting a structural destabilization compared to wild type.

Finally, we determined the crystal structures of FLNc Ig-d18-19, and Ig-d21 with a C-terminal FATZ-1 peptide, the latter shedding the first light on the molecular basis of FATZ-1-FLNc interaction.

Poster Session 3

EC coupling

... P3-1

Electrophysiological characteristics of the neuromuscular apparatus in case of impaired motor activity

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The functional state of the neuromuscular apparatus of the gastrocnemius muscle (MG) was analyzed in cases of motor damage caused by cutting of the Achilles tendon and antiorthostatic unloading. The study was conducted on laboratory rats with full observance of the rules of biomedical ethics. After 7 days of experimental conditions the reflex excitability of the corresponding motor center was assessed by the method of testing the H-response of MG. The M-response was used to analyze the condition of the peripheral link of the motor system. It was measured the maximum amplitude and the threshold of potential. The ratio of the maximum amplitudes of the reflex and motor responses was calculated. The data obtained from the study of intact animals was control; the results were processed by conventional statistical methods.

In all the experimental series it was observed the decrease of the threshold and the increase of the amplitude of the H-response of MG. The increase of Hmax/Mmax ratio indicates the rising the reflex excitability of the motoneurons of the corresponding motor center and the increasing in the number of motorneurons, responsible for afferent stimulation. It was found that the threshold of the M-response of MG decreased and its amplitude increased which indicating the functional changes in the peripheral structures of the neuromuscular apparatus. It is known that the support afferentation play in important role in motor control [Grigoriev et al., 2004]. We assume that the changes in the state of the neuromuscular apparatus detected in our experiments are initiated by the reorganization of the motor activity, primarily related to the restriction of signaling from the receptors of the support.

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... P3-2

Modulation of the spinal cord motor evoked potentials after spinal contusionin rat during treatment with local hypothermia

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Motor evoked potentials induced by epidural spinal cord stimulation can be used for evaluation of the spinal cord functional state after spinal cord injury (SCI) (Lavrov et al. 2006). In this study, the early response (ER) and the middle response (MR) of spinal cord evoked potentials were studied during a month after applying of local hypothermia right after SCI. The contusion SCI was performed on laboratory Wistar rats, the hypothermia was applied immediately at the site of SCI for 20 min. The motor evoked potentials induced by epidural stimulation of S1 spinal cord segment were recorded from m. gastrocnemius, m. soleus, and m. tibialis anterior on 3, 7, 14, 21 and 30 days after SCI. All procedures were made in accordance to bioethics norms and data were processed statistically with one-way ANOVA.

We found that in m.gastrocnemius the maximum amplitude and threshold of ER and MR were not significantly different during month after SCI, but after applying of local hypothermia threshold of MR significantly increase on 14 day and return to control values on 30 day. In m. soleus the maximum amplitude of ER and MR decreased until 30 day in group with SCI, and recovered back to control values in group of hypothermia treatment. In m. tibialis anterior the maximum amplitude of MR significantly decreased on 21 day after SCI and on 3 day in a group of hypothermia. The threshold of ER and MR significantly increased on 14 day only after hypothermia treatment. Previously it was shown that local hypothermia after spinal cord contusion delay development of excitability of the motoneurons in dogs for a week (Iafarova et al. 2014). Our results confirmed that hypothermia treatment decreased the excitability in the spinal circuitries of hindlimbs muscles during 21 days after SCI and recover at 30 days.

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... P3-3

Influence of reinforcement maneuver on the spinal cord motor evoked responses in calf muscles

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The transcutaneous electrical stimulation of the spinal cord (tSCS) is one of the most promising noninvasive methods in investigation of the spinal neuronal chains. The aim of

the work was to develop a methodology for conditioning of spinal evoked responses by using Jendrassik maneuver and determining the time of dependence of facilitating of reflex responses to tSCS. The amplitude characteristics of evoked potentials in m. Soleus and m. Tibialis anterior induced by tSCS at the level of Th11-Th12 were investigated in 6 healthy men aged 23 ± 2 years in control and with Jendrassik maneuver with increasing maneuver duration of 1s to 10s. The stimulation was carried out by Neurosoft Neuro MVP-4 stimulator (Russia). Registration of muscles potentials was recorded by bipolar self-adhesive electrodes. Statistical data was processed with using of WilcoxonT-test. Differences were considered as significant at p <0.05. Changes in the responses parameters to tSCS while performing Jendrassik maneuver were investigated. At the delay of 0 to 3 seconds the amplitude in m. Tibialis anterior on average increased by 154.9 ± 10.2%, in m. Soleusby 168.2 ± 12.2% (p <0.05). At the delay of 4 to 7 seconds the amplitude increased by 141.6 ± 14.2% and 146.3 ± 15.4%, respectively (p <0.05). At the delay of 8 to 10 seconds, the most obvious relief of the motor responses was observed. The amplitude of the m. Tibialis anterior responses increased by 164.9 ± 8.7%, the amplitude of m. Soleus responses increased by 170.7 ± 12.3% (p <0.05). Facilitating effect of Jendrassik maneuver was more obvious in m. soleus. Thus, the Jendrassik maneuver increases the amplitude of the response reflex component. The Jendrassik maneuver effect is more evident in m. soleus. This technique extends the prospect of using the tSCS for investigation of the locomotion regulation. This work was supported by the Russian Science Foundation (Project № 15-15-20036).

... **P3-4**

Evoked potentials in gastrocnemius muscle of rat in condition of gravitational unloading with spinal cord stimulation

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The main goal of this study was evaluation of reflex excitability of the spinal motor centers related to gastrocnemius muscle (GM) in conditions of gravitational unloading when combined with spinal cord stimulation. The experiments were conducted on male Wistar rats. The gravitational unloading was modeled by hanging animals in the antiorthostatic position [Morey-Holton, Globus, 2002]. For electromagnetic stimulation of the spinal cord we used a magnetic stimulator «Neuro-MS» («Neurosoft, Russia»). The stimulation was provided using coil at the vertebra level L1-L4 everyday, with duration of the stimulation 10 min with 10 min breaks during 1.5 hours with the frequency 3 Hz [Gorodnichev et al., 2010]. Intensity of the stimulus was adjusted based on threshold for the appearance of motor responses. The group of intact animal was control. After 7 days of experimental conditions, the direct motor (M) and reflex (H) responses of GM induced by stimulation of the sciatic nerve were recorded. We found that the in group treated with spinal cord stimulation the thresholds of M-response was 40 ± 3% (p<0,05), of H-responses was 22 ± 2% (p<0,05); max amplitude of M-response was 118 ± 6% (p<0,05), of H-responses was 160 ± 23% (p<0,05); Hmax/Mmax ratio was 130 ± 15% (p<0,05) compare with 100% in a control group. Thus, our results show the increase of the reflex excitability of the spinal motor center of GM and narrowing of the subthreshold border. We have concluded that the gravitational unloading when combined with electromagnetic stimulation of the spinal cord may facilitate reflex excitability of the spinal motor centers of GM. Probably, the stimulation of the spinal cord increases the intensity of the adaptive compensatory processes in hypogravity.

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... **P3-5**

Theoretical analysis of Ca^{2+}-handling in skeletal muscle fibers of Calsequestrin-null mice

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Muscle contraction and relaxation is a complex phenomenon finely regulated by the Ca^{2+} ions movements between muscle cells compartments and Ca^{2+} binding proteins. Calsequestrin (CSQ) is the most abundant Ca^{2+}-binding protein in the sarcoplasmic reticulum, and allows for storing of 20 mM or more of Ca^{2+}, still maintaining the free calcium concentration to 1mM or less. Surprisingly, contractile functions in muscle fibers of mice carrying null mutations of the gene coding for CSQ1 (CSQ-KO), the CSQ predominant isoform in fast skeletal muscles, are close to those of wild type mice (WT), both in twitch and tetanic peak force, as well as free [Ca^{2+}] concentrations in SR and cytosol in quiescent fibers.

Various modification in CSQ-KO muscle cells have been observed, among which an increase in store-operated Ca^{2+} channels (SOCE), a modified permeability of RyR at which CSQ polymers are anchored, and an increase in mitochondrial density and their coupling with calcium release units. However, whether, and to what extent, these are primary modifications, functional to maintain a near-normal contractile behavior, or they are secondary adaptations of the modified conditions, is not well-known yet.

We explore the issue theoretically, through a mathematical model which include diffusional term in Ca^{2+} movements within compartments, to exploit the crucial position of mitochondria close to calcium release units and the presence of microdomains. We simulate the predicted [Ca^{2+}] in the main compartments (SR, cytosol and mitochondria) and buffers (CSQ, Troponin and Parvalbumin) fitting model parameters on WT from our experimental data on (FDB) in mice, then we predict the behavior after ablation of CSQ avoiding any other modification and compared it with experimental data, to appreciate the differences between the two cases.

The results of the model simulation underline the relevance of the changes in RyR permeability and of the contribution of SOCE to maintain the contractile performance.

Poster Session 4

Heart Failure and Cardiomyopathies

... **P4-1**

Comparison of complete titin deletion versus titin truncation reveals difference in striated muscle phenotype

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Titin is the largest mammalian protein and important for sarcomere assembly and function. An ever increasing number of titin mutations found in patents with striated muscle disease make it an attractive target for translational research. This includes its role in trophic signaling.

To distinguish the effects of a truncated protein versus complete loss of titin, we generated a complete titin knockout (deletion of translation start/E2-KO) and compared this mouse model to a mouse deficient in the titin M-Band integration site only (M-KO). We used a conditional knockout approach with the Mck-Cre to overcome embryonic lethality and to obtain a striated muscle specific phenotype.

At 2 weeks of age homozygous deficient mice of both strains fail to gain weight with reduced strength endurance. Both models developed severe skeletal muscle atrophy with disassembly of the sarcomere and die at an age of 30-40 days. Unlike the similarities in the skeletal muscle phenotype, we find striking differences upon cardiac titin deletion: The heart of the complete (E2) knockout results in a DCM-like phenotype, while the M-Band knockouts develop an atrophic heart. Hypertrophy marker are upregulated in both strains. On the molecular level, the titin anchoring protein T-cap is upregulated only in the complete knockout, while the M-band binding protein Myomesin is downregulated in both strains. The I-band signaling molecule FHL2 that binds to the cardiac specific elastic N2B domain is increased in the enlarged E2 KO heart- while reduced in the atrophic M-KO heart.

Titin is an essential sarcomeric protein that does not only serve as an elastic scaffold, but also contributes to trophic signaling and thus regulates striated muscle size. The deletion or absence of titin leads to a similar skeletal muscle phenotype, but distinct cardiac phenotypes associated with changes in titin´s I-band signaling via FHL2.

... **P4-2**

Combined therapy deflazacort / omega-3 ameliorates heart and diaphragm dystrophy in the mdx mouse

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In Duchenne muscular dystrophy (DMD), lack of dystrophin leads to muscle degeneration and cardiorespiratory failure. In the *mdx* mice model of DMD, cardiac and diaphragm (DIA) muscles are severely affected. Corticoids retard the progression of the disease but show severe side effects. We investigated the effects of a combined therapy of the corticoid deflazacort and omega-3 on dystrophy progression, compared to DFZ monotherapy, at a later stage (13 months of age) of the disease. *Mdx* mice (8 months old) received DFZ alone or combined to omega-3 (DFZ/O) by gavage for 5 months. Untreated-*mdx* mice received mineral oil. DFZ/O was more effective than the monotherapy in reducing serum levels of CK (untreated: 1169 ± 252 U/L; DFZ/O: 241 ± 82* U/L; DFZ: 443 ± 98* U/L of CK;*p<0.05 compared to untreated, Anova) and in improving anterior limb muscle force (grip strength after therapy: untreated: 1.4 ± 0.1 g/g; DFZ: 1.4 ± 0.1 g/g; DFZ/O: 1.5 ± 0.1 g/g*; *p<0.05 compared to before therapy: 1.3 ± 0.1). In DIA, only DFZ/O reduced 4-HNE, a marker of oxidative stress (17 ± 0.6 arbitrary units of 4-HNE in untreated-mdx; 15 ± 1.5 with DFZ; 11 ± 2.2* with DFZ/O; *p<0.05 compared to untreated). Fibrosis area was equally decreased by both treatments in DIA and heart. In DIA, DFZ/O was better than DFZ alone in reducing fibronectin (22 ± 7.5 arbitrary units of fibronectin in DFZ *x* 16 ± 4.8 arbitrary units of fibronectin in DFZ/O). In heart, only DFZ/O decreased TGF-β (30% decrease) and fibronectin (46% decrease). DFZ/O was more effective than monotherapy in decreasing Q wave amplitude in electrocardiogram (normal: 9.6 ± 2.7; mdx: 94.8 ± 20; DFZ: 133 ± 39; DFZ/O: 52 ± 44). In dystrophic DIA and heart, pro inflammatory (TNF-α and NF-kB) markers were significantly reduced by both therapies (43% reduction of TNF-α in DIA and 37% reduction of TNF-α in heart). It is suggested that the effects of DFZ in ameliorating mdx dystrophy can be improved by omega-3 co-administration, in skeletal and cardiac muscles, at later stages of dystrophy.

SUPPORT: FAPESP, CNPq, CAPES, Faepex-Unicamp.

... P4-3

Pifitrin-α, an inhibitor of p53, ameliorates dystrophic cardiomyopathy in the *mdx* mice model of Duchenne muscular dystrophy

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In Duchenne muscular dystrophy (DMD), and in the *mdx* mice model of DMD, lack of dystrophin leads to progressive cardiomyopathy. While cardiomyocyte damage is primarily caused by increased influx of calcium, the inflammatory response also contributes to cardiomyopathy. Corticoids are used to treat DMD but given to their side effects, identifying pharmacological alternatives that slow the progression of cardiomyopathy is of importance. Pifithrin-α, an inhibitor of p53, affects the inflammatory process and delays the progression of dystrophy in skeletal muscles of mdx mice. We examined whether pifithrin-α ameliorates cardiac dystrophy in the mdx mouse. *Mdx* mice (8 months old) received pifithrin-α during 3 months. Match control mdx mice were untreated. Histopathological (fibrosis area) and biochemical (cardiac CK; zimography) parameters and molecular markers (TGF-β, MMP-9, TNF-α, NF-kB) of dystrophy were evaluated by Western blot. We found that the level of cardiac CK was 49.5% lower ($p<0.05$ compared to untreated-mdx) indicating decreased cardiac damage in mice treated with pifithrin-α. The area of cardiac fibrosis, expressed as a percentile of the total area of histological section, decreased 36% in pifithrin-α-treated- (9.2±1.02% of fibrosis) compared to untreated- (14.4±1.4% of fibrosis) mdx mice. This was accompanied by a 41% decrease in the activity of MMP-9 evaluated by zimography (6.8±1.6 AU in untreated vs. 4.0±0.3 AU in pifithrin-α -treated mdx mice). TNF-α, NF-κB, MMP-9 and TGF-b, markers of inflammation and fibrosis, were also dramatically reduced in hearts from pifithrin-α treated *mdx* mice. This finding is of importance because inflammation and fibrosis play a role in cardiac hypertrophy and remodeling in many other conditions. In conclusion, pifithrin ameliorates dystrophic cardiomyopathy in the *mdx* mice, being a potential therapy to be tested in future clinical trials in DMD. Supported by: FAPESP, CNPq, CAPES, Faepex-Unicamp.

... P4-4

Intrinsic *MYH7* expression regulation may contribute to tissue level allelic imbalance in hypertrophic cardiomyopathy

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QUESTIONS: HCM, the most common inherited cardiac disease, is mainly caused by mutations in sarcomeric genes. More than a third of the patients are heterozygous for mutations in the *MYH7* gene encoding for the ß-myosin heavy chain (ß-MyHC). In HCM-patients the mutant and wildtype alleles can be expressed imbalanced, namely leading to unequal levels of mutant and wildtype mRNA and protein. This so-called allelic imbalance was detected in whole tissue samples but also in individual cells. The relative amount of the mutant allele, the extend of allelic imbalance in tissues and the variation of allelic imbalance from cell to cell has been associated with the severity of HCM.
RESULTS: Allelic imbalance has been shown to occur in a broad range of genes. Therefore, we aimed to examine whether the *MYH7*-alleles are intrinsically expressed imbalanced or whether the allelic imbalance is solely associated with the disease. We compared the expression of *MYH7*-alleles in non-HCM donors and in HCM-patients with different *MYH7*-missense mutations. In the HCM-patients, we identified imbalanced as well as balanced allelic expression. Also at the protein level, allelic imbalance was determined. Most interestingly we also discovered allelic imbalance and balance in non-HCM donors.
CONCLUSIONS: Our findings therefore strongly indicate that next to mutation-specific mechanisms also non-HCM associated allelic-mRNA expression regulation may account for the allelic imbalance of the *MYH7* gene in HCM-patients. Since the relative amount of the mutant mRNA or the extend of allelic imbalance has been associated with the severity of HCM, an individual analysis of the *MYH7*-allelic expression may provide deeper insights into the prognosis of each patient.

... P4-5

Effect of cardiomyopathy-associated mutations of tropomyosin on the calcium regulation of the actin-myosin interaction in atria

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INTRODUCTION: In cardiomyopathies, pathological changes in the heart include atrial enlargement and fibrillation (Prabhakar *et al. J Mol Cell Cardiol* 2001). Atrial enlargement can increase cardiovascular risk when left ventricle hypertrophy is present (Anwar *et al. J Am Soc Echocardiogr*. 2007). Little is known about the molecular basis of atrial remodeling at HCM and DCM.
AIM: To study effect of mutations of tropomyosin (Tpm) molecule in regions of its interaction with troponin T on the Ca^{2+} regulation of the actin-myosin interaction in atria. One of these regions is the area of residues 170–195, and another region is the overlap junction between N- and C-terminal ends of Tpm molecules.
METHOD: We analyzed Ca^{2+} dependence of the sliding velocity of reconstructed thin filaments containing α-Tpm with HCM (L185R, E180V, E180G, I172T, I284V, and M281T) and DCM (E40K, E54K, M8R, K15N) mutations, cardiac troponin and skeletal F-actin over pig atrial myosin in an *in vitro* motility assay.
RESULTS: Mutations E40K and E54K decreased the maximal filament sliding velocity and did not affect pCa_{50} of *p*Ca-velocity relation. Mutation E180G increased Ca^{2+}-sensitivity of *p*Ca-velocity relation. Mutations I284V and M281T increased the maximal velocity and the Ca^{2+}-sensitivity of the velocity. Ca^{2+} sensitivity estimated by the ratio pCa_8 of *p*Ca-velocity relation was higher for thin filaments containing Tpm with mutations E40K, E54K, E180V, E180G, I172T, I284V, and M281T as compare to those with WT Tpm. Mutations L185R, M8R, and K15N did not affect significantly *p*Ca-velocity relation.
CONCLUSIONS: In general, these mutations have less pronounced effect on the Ca^{2+} regulation of the actin-myosin interaction in atria as in the ventricles (Matyushenko *et al. Biochemistry* 2017; Matyushenko *et al. J Muscle Res Cell Motil.* 2017). Thus, molecular mechanism of atrial and ventricular remodeling at HCM and DCM can be differ. Supported by RFBR (grants 15-34-20136 and 15-04-01558).

... P4-6

Altered myofilament structure and function in dogs with Duchenne muscular dystrophy cardiomyopathy

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AIMS: Duchenne Muscular Dystrophy (DMD) is associated with progressive depressed left ventricular (LV) function. However, DMD effects on myofilament structure and function are poorly understood. Golden Retriever Muscular Dystrophy (GRMD) is a dog model of DMD recapitulating the human form of DMD. The objective of this study was to evaluate myofilament structure and function alterations in this dog model with spontaneous cardiac failure.
METHODS AND RESULTS: We have employed synchrotron x-rays diffraction to evaluate myofilament lattice spacing at various sarcomere lengths (SL) on permeabilized LV myocardium. We found a negative correlation between SL and lattice spacing in both sub-epicardium (EPI) and sub-endocardium (ENDO) LV layers in control dog hearts. In the ENDO of GRMD hearts this correlation is steeper due to higher lattice spacing at short SL (1.9 mm). Furthermore, cross-bridge cycling indexed by the kinetics of tension redevelopment (ktr) was faster in ENDO GRMD myofilaments at short SL. We measured post-translational modifications of key regulatory contractile proteins. S-glutathionylation of cardiac Myosin Binding Protein-C (cMyBP-C) was unchanged and PKA dependent phosphorylation of the cMyBP-C was significantly reduced in GRMD ENDO tissue and more modestly in EPI tissue.
CONCLUSIONS: We found a gradient of contractility in control dogs myocardium that spreads across the LV wall. Interestingly, this gradient is negatively correlated with myofilament lattice spacing. At slack length, increased myofilament lattice spacing in GRMD correlates with improved cross-bridge kinetics, which might explain the improved myofilament sensitivity to calcium when compared to CTRL. Consequently, the gradient of contractility across the LV wall is reduced. In conclusion, at short SL, reduced phosphorylation levels of contractile regulatory proteins and increased lattice spacing improved contraction kinetics that result in GRMD induced myofilament dysfunction.

... P4-7

Analysis of active transcription sites in single human cardiomyocytes reveals burst-like transcription of *MYH7*

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One in 200 individuals is affected by Hypertrophic Cardiomyopathy (HCM), which is mostly caused by mutations in sarcomeric proteins. 30–40% of the patients carry mutations in the β-myosin heavy chain (β-MyHC) gene (*MYH7*). A common mechanism leading to HCM is unknown.
From our previous work on *MYH7*-mutations we hypothesize that HCM-typical hypertrophy, myocyte disarray, and fibrosis could be a consequence of cell-to-cell functional imbalance due to cell-to-cell variation in the expression of mutated vs. wildtype β-MyHC. Here we ask whether stochastic, independent burst-like transcription of mutant and wildtype *MYH7*-alleles could cause such cell-to-cell variability.
Active transcription sites of *MYH7* were analyzed in cardiomyocytes of a patient with β-MyHC-mutation R723G and of a donor heart. We used fluorescence in situ hybridization on 16 µm thick sections of cardiac tissue to check whether the *MYH7*-alleles are indeed transcribed in a discontinuous, burst-like manner. To visualize intronic and exonic sequences of *MYH7*-pre-mRNA in active transcription sites we used two probe sets. The exonic probes also bind to the cytoplasmic *MYH7*-mRNA and allow to identify cardiomyocytes.
Active transcription sites were identified by co-localization of both probe sets inside the nuclei of cardiomyocytes. In 27% and 33% of nuclei of patient and donor, respectively, no active transcription sites were detected. The absence of active transcription sites is inconsistent with continuous transcription but is expected for random burst-like transcription of the two *MYH7*-alleles.
A numerical model of independent, burst-like transcription of the mutant and wildtype allele revealed a very close match between model response and our functional and single cell *MYH7*-mRNA data. Therefore, we suggest that such burst-like transcription induces the observed cell-to-cell variability in mutant *MYH7*-mRNA and functional imbalances among cardiomyocytes, and thus may trigger development of the HCM-phenotype.

... P4-8

Development of novel computational biology pipeline for the efficient classification of titin SNPs for clinical use

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Muscle dysfunction is a major health burden of the 21st century. In this respect, genetic alterations in the giant intrasarcomeric muscle protein titin (>3MDa) are becoming established as common causative agents of myopathies (particularly cardiac) in populations. Enabled by modern screening technologies, a large catalogue of missense single nucleotide polymorphisms (mSNPs, >15,000) in titin is been produced. Yet, there are no easy means of determining whether the revealed mSNPs are linked to clinical pathologies. To date, linkage protocols remain lengthy, costly and labour intensive. Bioinformatic tools are quick and economic, but are only a modest assistance in the linkage process as they can be highly unreliable. To improve the reliability of computational tools, we are developing a titin-centric computational/structural-biochemistry pipeline for the rapid medium-throughput classification of titin mSNPs at the protein level. Our approach exploits the use of positional reoccurrence along the chain that allow titin"s size to be used as an asset instead of a hindrance in mSNP assessment. We also make use of various protein stability calculators to create a protein mutability landscape of titin. Ultimately, we aim to develop a "hot-spots" map of titin that can support the clinical evaluation of newly recorded mSNPs.

... P4-9

Identification and functional annotation of E2-activated ERα and ALC-1 regulated genes in human cardiomyocytes based on microarray analysis data

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QUESTION: The estrogen receptor alpha (ERα) is a known ligand inducible transcription factor that regulates a large number of target genes. In a recent study, we demonstrated the atrial essential myosin light chain 4 (ALC-1) as a novel co-repressor of 17β-estradiol (E2)-activated ERα in human cardiomyocytes. In the present study, we aimed to profile the target genes, gene networks and pathways which are differentially regulated by E2-activated ERα and ALC-1 in human cardiomyocytes.
METHODS: AC16 cells, a human adult left ventricular cardiomyocyte-like cell line, were transfected with ERα vector or hALC1 vector alone or co-transfected with both vectors, and treated with 10nM E2 or vehicle for 24 h. RNA was isolated and analyzed by whole transcriptome microarray analysis on a human Clariom S array by Affymetrix. Differentially regulated genes were used for GO enrichment and pathway analysis.
RESULTS: Microarray analysis showed that 215 genes were differentially regulated in cells transfected with ERα vector or co-transfected with ERα and hALC-1 vectors in response to E2-treatment. In 135 of those genes, the interaction of ERα and hALC-1 reversed the gene expression profile caused by E2-activated ERα only. GO enrichment analysis of these 135 genes revealed that hALC-1 in combination with E2-activated ERα affects several biological processes, including positive regulation of signal transduction (GO: 0009967), regulation of protein phosphorylation (GO: 0001932), and regulation of cellular protein metabolic process (GO: 0032268). Pathway analysis of these genes showed a significant enrichment of genes involved in ECM-receptor interaction, in Notch signaling pathway, and in TGF-beta signaling pathway, among others.
CONCLUSION: This study highlights diverse gene networks and cell regulatory pathways through which the E2-induced interaction of ERα/ALC-1 operates to modulate distinct mechanisms in human cardiomyocytes.

... P4-10

Diabetes affects adaptive titin modification in response to acute myocardial ischemia/reperfusion

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Acute myocardial infarction is a leading cause of mortality worldwide, particularly in patients with diabetes mellitus. Our group recently reported that in the acute phase after myocardial ischemia/reperfusion (I/R) posttranslational modification of the sarcomeric protein titin increases cardiac myocyte stiffness in the viable non-ischemic myocardium and plays a significant role in the maintenance of ventricular stability. Here, we hypothesized that due to preexisting diabetes-induced alterations of titin properties diabetic leptin receptor deficient mice (*Lepr*^{db}) lack this important mechanism for rapid cardiac adaptation after I/R.
I/R was induced in heterozygous controls (*Lepr*$^{db/+}$) and *Lepr*^{db} mice by 60 minutes reversible ligature of the left anterior descending artery. Hearts were excised and cardiac tissue was collected 24 hours after reperfusion. Western Blot analysis was performed using phosphospecific antibodies. In non-diabetic *Lepr*$^{db/+}$ mice I/R significantly increased PKCα activity and phosphorylation of the residues S12022 and S11878 in the elastic titin PEVK region. The observed changes in titin phosphorylation status were comparable to changes observed in diabetic *Lepr*^{db} mice under baseline conditions without I/R. In line with our hypothesis I/R did not further increase PKCα activity in the diabetic animals and resulted in adverse effects on titin phosphorylation at S12022 (increased) and S11878 (decreased). In diabetic *Lepr*^{db} mice, but not in non-diabetic controls I/R caused a significant activation of ERK1/2 and phosphorylation of residue S4010 in the titin N2-B region. In summary, cardiac titin of diabetic *Lepr*^{db} mice was differentially modified after myocardial I/R compared to non-diabetic controls. We speculate that these changes alter the adaptive response of the sarcomeres to myocardial injury and may contribute to the worsened outcome of type 2 diabetes patients after I/R.

... P4-11

Characterization of a minipig model of hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is form of heart disease that is characterized clinically by increased contractility and impaired relaxation of the heart, and is associated with increased risk of heart failure and sudden cardiac death. Mutations in genes encoding sarcomere mutations are often associated with HCM, however the mechanisms by which these mutations lead to molecular, cellular, and organ-level pathophysiology remain unclear. In addition, HCM has a highly variable natural history, yet current risk stratification tools are limited in their ability to inform prognosis or guide therapy. Circulating biomarkers could provide early indicators of cardiac dysfunction and could enhance the assessment of emerging therapies. To enable better understanding of HCM pathophysiology on molecular, cellular, and organ levels, as well as to identify potential biomarkers, we generated a genetic model of HCM in the Yucatan minpig bearing the R403Q mutation in *MYH7*. We have characterized the first cohort of animals, which demonstrate a clinical course that is strikingly similar to HCM in humans. We also examined whether there were any detectable associations between the disease-associated changes in cardiac structure and function with protein biomarkers from isolated left ventricle tissue and plasma. Utilizing unbiased LC/MS/MS mass spectrometry in parallel with tissue-calibrated isobaric techniques and aptamer-based protein quantification, we examined differences between R403Q and WT animals and from isolated LV tissue and plasma and present our initial findings.

... P4-12

The role of Akt/GLUT/HK salvage pathway in the induction of a cardioprotective phenotype in SHR conplastic strains by adaptation to chronic hypoxia

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Recently we have shown that conplastic strain of spontaneously hypertensive rats (SHR-mt^{BN}) harbouring mitochondrial genome of the more ischemia-resistant BN strain exhibits a stronger protection induced by adaptation to chronic hypoxia (CNH) than that of SHR. This phenomenon was also manifested by a lower sensitivity of mitochondrial permeability transition pore opening[1]. However the mechanism beyond these effects is not fully understood.

QUESTIONS: In the present study we aimed to evaluate the possible protective role of HIF1α-dependent salvage pathway of protein kinase B (Akt)/hexokinase (HK) /glucose transporters (GLUT) in CNH-induced cardioprotection in SHR-mt^{BN}.

METHODS: Both experimental groups (SHR and SHR-mt^{BN}) were exposed for 3 weeks to CNH (FiO_2 0.1; 24 h per day) and control groups were kept in normoxic conditions for the same period of time. Expression of Akt1 and 2, GLUT1 and 4, HK1 and 2 was assessed at mRNA and protein level. Moreover, colocalizations of GLUT1 and GLUT4 with the sarcolemma, counterstained by WGA, were analyzed using quantitative immunofluorescence microscopy (Olympus IX2-UCB).

RESULTS: Our results showed that CNH induced mRNA expression of HIF1a and protein expression of Akt2 only in conplastic strain SHR-mt^{BN}. HK2, GLUT1 and GLUT4 were upregulated in both strains after CNH. Beside that the increased translocation of GLUT4, but not of GLUT1, to the sarcolemma was more pronounced in SHR-mt^{BN}.

CONCLUSION: We assume that the pleiotropic effect of Akt2 together with stimulated glucose metabolism can potentially contribute to a stronger CNH-afforded cardioprotection in SHR-mt^{BN} than in SHR.

1) Neckar et al. Clin Sci (Lond). 2017;131(9):865-881.

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... P4-13

Model of independent stochastic burst-like transcription can explain observed functional and transcriptional variability among cardiomyocytes from Hypertrophic Cardiomyopathy patients

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Studying various missense mutations in genes associated with Hypertrophic Cardiomyopathy (HCM) we found substantial variability in mRNA count and ratio of mutant/wildtype mRNA and a significantly larger variance of functional parameters of cardiomyocytes from heterozygous patients as compared to healthy controls. We proposed that this could be due to the mechanism of burst-like transcription where mutant allele and wildtype allele are activated and inactivated independently and stochastically. This concept was further fortified by our observation that 27% of nuclei of cardiomyocytes of an HCM patient with mutation R723G in β-myosin heavy chain (β-MyHC-*MYH7*) showed no active transcription sites.

To test if independent stochastic burst-like transcription could account for our experimental observations we developed a numerical model describing (i) stochastic activation/inactivation of the mutant and wildtype *MYH7*-alleles, (ii) production of pre-mRNA (measured as observed active transcription sites), (iii) splicing of pre-mRNA to mRNA and mRNA decay (measured through mutant/wildtype mRNA), and (iv) production and decay of β-MyHC protein (measured indirectly through functional experiments). The only adjustable parameters of the model were the activation/inactivation rate constants of transcription of the *MYH7*-alleles, and the splicing rate constant for generation of mRNA from pre-mRNA. All other rate constants were available from the literature.

With the best fit of the three adjustable parameters, the model predicts essentially identical cell-to-cell variability in mutant/wildtype mRNA to the experimentally observed variability and essentially identical cell-to-cell variability of functional parameters estimated from predicted mutant/wildtype protein abundance. We conclude that independent stochastic burst-like transcription of mutant and wildtype alleles could indeed be a mechanism producing cell-to-cell functional variability causing HCM-phenotype.

... P4-14

Distinct signalling pathways mediate phosphorylation of sarcomeric proteins in the right and left ventricle of the failing heart

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BACKGROUND: Treatment options for heart failure (HF) are still limited maybe because of the distinct remodelling processes and molecular mechanisms in the right (RV) and left ventricle (LV) of the failing heart.

METHODS: Post-MI rats and HF patients with reduced ejection fraction were compared to sham-operated and healthy controls, respectively. LV and RV sarcomeric protein phosphorylation and function were studied.

RESULTS: In HF rats LV myofilament calcium sensitivity (pCa_{50}) was higher compared to control, and higher in the LV *vs.* RV. This goes along with decreased TnI phosphorylation in the LV and RV, and decreased MyBP-C phosphorylation but only in the RV. Cardiomyocyte passive tension ($F_{passive}$) was increased in both ventricles, but it was higher in the RV *vs.* LV. CaMKII restored $F_{passive}$ in the RV but had no effect in the LV. PKG lowered $F_{passive}$ in both ventricles with a more pronounced effect in the RV *vs.* LV. Titin phosphorylation was increased only at S12884 in the LV. Titin phosphorylation was decreased at S3991/S4080, increased at S4043, but unaltered at S12742/S12884 in the RV, because of distinct kinase activities of LV and RV.

In biopsies from human HF pCa_{50} was higher in both ventricles compared to controls, and was higher in the LV *vs.* RV. TnI phosphorylation was decreased in the LV and RV, but MyBP-C phosphorylation was decreased only in the LV. $F_{passive}$ was increased in both ventricles and restored by PKG with a more pronounced effect in the LV *vs.* RV. Titin phosphorylation was unaltered at S4010/S4062/S12022, decreased at S4099, and increased at S11878 in the LV. In contrast, titin phosphorylation was decreased at S4010, increased at S4062/S11878/S12022, and unaltered at S4099 in the RV, because of distinct kinase activities of LV and RV.

CONCLUSIONS: Distinct kinase-mediated pathophysiological modulation of phosphorylation in the LV and RV in HF may have important therapeutic relevance when uniformly targeting cardiomyocyte signalling pathways.

Poster Session 5

Heart Muscle Structure and Function

... P5-1

Mathematical model of electromechanical coupling in human cardiomyocytes allowing for cooperative effects of the crossbridge attachment on CaTnC kinetics

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Our new mathematical model of the excitation-contraction coupling (ECC) in human cardiomyocytes combines human-specific O'Hara-Rudy (ORd) electrophysiological model (O'Hara ea, PLoS Comp Biol 2011) and a sub-model of myocardium mechanics and its calcium activation adopted with respective retuning from our developed earlier Ekaterinburg-Oxford model (EO-model) (Sulman ea, BMB 2008). EO-model describes ECC in intact cardiomyocytes for some species of laboratory animals (e.g. rabbit, rat, mouse), being parametrically adapted to each particular one. We will briefly call here the new model as Ekaterinburg-ORd model for the convenience.

The concept of cooperativity implying that affinity of TnC for calcium increases with a number of strongly attached crossbridges is a key point of the EO-model. This concept (within the EO-model) underlies a lot of mechano-electric and mechano-calcium feedbacks observed in the intact myocardium twitches. However, such cooperativity seemed to disagree with experimentally proved length-independence of the Hill coefficient of the pCa-Force relationship in the skinned heart muscle (Konhilas ea, J Physiol 2002). To overcome this disagreement we recently modified the cooperativity concept to simulate and explain all features of the mechano-dependence/independence in both intact and skinned heart muscles (Dokuchaev ea, Biophysics 2016). According to the modified concept CaTnC affinity significantly increases immediately after the crossbridge attachment but then decreases partly during a transient process towards quite moderate steady-state level.

Noteworthy, that the modified versions of cooperativity in the EO-model was necessary exclusively in the case of the skinned muscles simulation. However, this modified version proved to be also absolutely necessary for the adequate simulation of the twitches in our new Ekaterinburg-ORd model of the intact human myocardium.

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... P5-3

Cardiological involvement in idiopathic inflammatory myopathies and the diagnosis of cardiac involvement in idiopathic inflammatory myopathy by cardiac magnetic resonance tomography

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QUESTIONS: Cardiological involvement is a common extra-muscular manifestation of idiopathic inflammatory myopathies, data on long-term outcomes in these patients are lacking, the aim of the study is to observe the cardiac involvement in idiopathic inflammatory myopathies and to determine the diagnostic value of cardiac magnetic resonance (CMR) imaging in patients with idiopathic inflammatory myopathies.
METHODS: This was a retrospective study of all patients referred to the Tongji Hospital in Wuhan for the evaluation of cardiac involvement in idiopathic inflammatory myopathy by electrocardiogram (EEG)、echocardiogram and cardiac magnetic resonance (CMR) including functional imaging and late gadolinium enhancement (LGE) imaging from September 2013 to December 2015. Cardiac characteristics were recorded.
RESULTS: 56 patients (42 ± 16 years, 29% male) and 38 matched controls (41 ± 15 years, 37% male) underwent cardiac evaluations including electrocardiogram, echocardiogram, CMR (comprising cine- and late-gadolinium-enhancement (LGE) imaging). Compared to controls, cardiac involvemen rate of IIM is higher (37 vs. 7, p=0.005). Abnormal rate of the electrocardiogram for the IIM is 16.1%, higher than that of the healthy control which the rate is 7.9%. Abnormal rate of the echocardiogram for the IIM is 12.5%, higher than that of the healthy control which the rate is 5.3%. Among the 56 IIM patients, 20 (35.7%) had at least one abnormal CMR finding: 18 (28%) demonstrated an impaired left ventricular ejection-fraction (LV-EF)
CONCLUSION: Our data suggest that cardiac involvement is a common feature of IIMs. For the CMR, the positive rate of the heart involvement is higher than other traditional tests include electrocardiogram and echocardiogram, seems to offer a measurable diagnostic tool for cardiac involvement of idiopathic inflammatory myopathies.

... P5-4

Effect of phosphorylation of tropomyosin on the calcium regulation of the actin-myosin interaction in myocardium

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INTRODUCTION: Phosphorylation of proteins of the cardiomyocyte contractile apparatus is one of the mechanisms of the regulation of their function. Phosphorylation of tropomyosin (Tpm) changes in ontogenesis, as well as in pathologies of myocardium (Rao *et al. Am J Physiol Heart Circ Physiol.* 2007). Phosphorylation of Tpm disrupts the ability of the mouse heart to withstand mechanical stress (Schulz *et al. J Biol Chem.* 2012).
AIM: To study effect of Tpm phosphorylation on the calcium regulation of the actin-myosin interaction in myocardium using an *in vitro* motility assay.
METHODS: With the *in vitro* motility assay, we analyzed calcium dependence of the sliding velocity of regulated thin filaments containing F-actin, troponin and WT Tpm or S283D Tpm over atrial and ventricular myosin of pig. With an optical trap, we directly measured a bending stiffness of thin filament with WT Tpm or S283D Tpm (Nabiev et al. Biophys J. 2015). Tropomyosin with Ser-283 pseudo-phosphorylation was used as phosphorylated form of Tpm.
RESULTS: S283D Tpm did not affect the maximal sliding velocity of thin filaments and pCa_{50} of the *p*Ca-velocity relation but did not inhibit the movement of the filaments at low Ca^{2+}. S283D Tpm increased the bending stiffness of thin filament ($7.0 \pm 0.4\ 10^{-26}$ N×m^2 and $5.9 \pm 0.6\ 10^{-26}$ N×m^2).
CONCLUSIONS: Disturbance of relaxation due to phosphorylation of tropomyosin may have a different effect on the function of the heart muscle in physiological and pathological states.
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... P5-5

Hypobaric hypoxia enhances expression of Connexin 43 in the rat left ventricular myocardium

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BACKGROUND: Ventricular arrhythmias are the major cause of death in worldwide. Adaptation to intermittent hypobaric hypoxia (IHH) potentiates endogenous protective pathways reducing the incidence of ventricular arrhythmias[1], however the molecular principle has not been fully elucidated. The increased incidence of arrhythmias in the mammalian heart is supposed to be accompanied by remodelling of the cellular distribution of gap junctions formed mainly by connexins 43 (Cx43).
AIM: We aimed to determine Cx43 expression and phosphorylation in normoxic left ventricle and hypoxic one, manifesting antiarrhythmic phenotype, together with Cx43 distribution during brief ischemia/reperfusion injury (I/R) in both groups.
METHODS: Male Wistar rats were adapted to IHH (7000 m, 8-h per day, 25 exposures) and subsequently hearts were exposed to brief ischemia (15 min) and reperfusion (10 min) ex vivo. The expression and phosphorylation of Cx43 (p-Cx43) were assessed by Western blotting and of distribution of Cx43 located in intercalated discs ("end to end" junction) and in longitudinal sarcolemma ("site to site" junction) was assessed as area of fluorescence by quantitative immunofluorescence microscopy using WGA as a counterstaining of sarcolemmal region.
RESULTS: Adaptation to IHH increased expression of Cx43 and p-Cx43 at Ser368 in the LV compared to the normoxic control. These findings were corroborated by immunofluorescence staining which revealed substantially higher fluorescence signals of Cx43 and p-Cx43 at Ser368 after IHH. Additionally IHH tended to increase the ratio of "end to end"/"site to site" junctions which subsequently has not changed during I/R.
CONCLUSION: Our results suggest that the antiarhythmic phenotype of heart adapted to IHH is accompanied with higher expression and phosphorylation of Cx43 Ser368 and moderate change in Cx43 distribution.
KEY WORDS: Heart, Hypoxia, Connexin43, Arrhythmia

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... P5-6

Structural and biochemical characterisation of human cardiac troponin C mutations associated with genetic cardiomyopathies

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Troponin C mutations have been linked to genetic hypertrophic and dilated cardiomyopathies. We aimed to understand, at the molecular level, how several cardiomyopathies associated mutations in troponin C (Y5H, A8V, A31S, E59D, D75Y, C84Y, D145E and I148V) affect troponin structure and its cooperative-allosteric regulation of thin filament activity. Circular dichroism, and ATPase assays demonstrated that these mutations had little or no effect on the folding or the thermal stability of the troponin complex. ATPase assays, fluorescence spectroscopy and transient kinetics were used to assess the effect of these mutations on the Ca2+ dependent inhibition and activation of the acto-myosin ATPase, the size of the cooperative unit, the transition between the blocked and closed state and the affinity and kinetics of Ca2+ interaction with troponin and thin filaments. We found that several cardiac TnC mutations affect both inhibition and activation of the actomyosin ATPase. Kinetics measurement show that several troponin C mutations affect the rate of Ca2+ dissociation and/or the proportion of thin filaments in the blocked state. Isothermal titration microcalorimetry (ITC) was used to evaluate the effect of cardiac TnC mutations on the interactions of TnC with its binding partners. The results obtained show that most TnC mutations affect the binding of TnC to TnI. In addition, TnC A31S and C84Y decrease the binding affinity of Tn complex to tropomyosin while TnC D145E and I148V substantially decrease the ability of troponin complex to bind to the thin filament. We are currently using NMR structural methods to study the effect of the mutations on the structure of troponin C. Preliminary NMR data indicate that most TnC mutations have an impact on the structure of troponin C. Overall these results suggest that HCM linked mutations in TnC affect allosteric transitions in the troponin complex and provide insight into the mechanism by which troponin C mutations affect contractility in hypertrophic and dilated cardiomyopathy.

... P5-7

Cold acclimation induces prolonged changes in β-adrenergic response of rat myocardium

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Cold acclimation or hardening is a well-known intervention increasing organismal resistance to different pathological stimuli[1]. It has been shown that cold acclimation in man may affect functional parameters of the cardiovascular system mediated by adrenergic β-receptors (β-ARs)[2], but the molecular mechanism underlying this phenomena is not fully understood. The aim of the study was to assess rat organismal metabolic response to adrenergic stimulation and to analyze hardening-induced changes in β-adrenergic signaling in the left ventricle after cold acclimation and their durability.

Male Wistar rats were divided into 3 groups: i) control (C, 5 weeks at 26°C,); ii) cold exposure (CL, 5 weeks at 8°C); iii) recovery phase (CLR, 5 weeks at 8°C and 2 weeks at 26°C). Metabolic rate (MR) in all experimental groups was assessed by respirometry after infusion of noradrenaline (1.8μg/ml/100g). The levels of β-ARs in the left ventricle (LV) were assessed by RT-PCR, Western blotting, as well as by radioligand binding assays.

Our results showed that CL increased basal MR and it returned back after CLR. NA infusion increased MR in both C and CL groups, but this response was attenuated in CLR group. In parallel, we observed increased expression of β1AR in CL and its decline in CLR group. Accordingly, competitive binding assay revealed increased β2/β1-AR ratio in CLR group. In addition, mitochondrial swelling rates induced by calcium overload was decreased after CL In conclusion the increased β2/β1 ratio could explain the observed decline in b adrenergic response after CLR, because β2-AR, in contrast to β1-AR, can couple to the inhibitory Gi proteins. Therefore, our results suggest that Gi-regulated signaling may play an important role under cold acclimation and together with reduced mitochondrial swelling may contribute to the cytoprotective effect of hardening.

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... P5-8

A human CSRP3/MLP mutation is methylated, causes mis-splicing and expression of pathological isoforms, ultimately leading to heart failure

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BACKGROUND: CSRP3 or MLP is expressed in striated muscle and localizes, among others, to the sarcomeric Z-disc and nucleus. CSRP3 is a cardiomyocyte mechanical stretch sensor, and plays important roles in the regulation of sarcomeric architecture. Several cardiomyopathies have been associated with various CSRP3 mutations. Among those, we previously demonstrated that the W4R missense mutation (CSRP3:c.10TàC;p.W4R) leads to dilated and hypertrophic cardiomyopathies in patients and knock in animals.

AIM: To investigate the molecular interplay between the CSRP3:c.10TàC;p.W4R mutation and the development of heart failure

RESULTS: RT-PCR analysis performed on Csrp3W4R/W4R knock in mice revealed the presence of different CSRP3 splice variants. Notably, a CSRP3 mRNA missing exon 2 (Δ2mRNA) is highly prevalent. Minigene experiments confirmed alternative splicing and skipping of exon 2 only in the presence of 10T à C, while other nucleotide substitutions, even those leaving the arginine in place, did not result in mis-splicing. Alternative splicing impairs translation of the full length CSRP3 protein and induces translation of a 7KD carboxyterminal isoform. In addition, another novel 17KD isoform of Csrp3 with an intron inclusion has been identified. The expression of this isoform might be linked to the change in methylation state due to a methylated CpG site formed by the 10T à C. Moreover, animals overexpressing Δ2mRNA develop heart failure reminiscent of what can be observed in human mutation carriers.

CONCLUSIONS: CSRP3:c.10TàC;p.W4R causes cardiomyopathy and associated heart failure via multiple molecular mechanisms, including a splicing defect leading to the expression of novel, pathological carboxyterminal CSRP3 proteins, loss of CSRP3 protein, defects in protein / protein interaction and mislocalization of the mature protein. Therefore, a single mutation may cause disease via multiple mechanisms and hence explain pleiotropy observed in patients.

... P5-9

Dobutamine increases mechanical efficiency in isolated rat papillary muscle by increasing external work without affecting oxygen consumption

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Dobutamine is clinically used in end-stage heart-failure. However, the energetic consequences of catecholamines on isolated muscle are not clear. We developed a method to investigate the effect of dobutamine on external work, oxygen consumption and mechanical efficiency in healthy rat right ventricular papillary muscle.

Papillary muscles were mounted in a glass oxygen chamber filled with Tyrode"s solution, in which PO_2 was measured. The muscle was stimulated at 0.5 Hz and stepwise stretched to the length were isometric force was maximal (L_{max}). Then, muscle length was set to 92.5% of L_{max} and sinusoidal length changes of ±7.5% of L_{max} were imposed. We determined the stimulus phase for which work output was maximal at 5 Hz stimulation. A single work loop-run consisted of 3 minutes sampling the decrease in PO_2 in the chamber, 4 minutes stimulation of the muscle at 5 Hz followed by 3 minutes sampling of the recovery. For each muscle, three consecutive runs were performed. Dobutamine was added (3 μM) only in the second run and washed away before the start of the third run. Efficiency was calculated from the oxygen consumption and amount of work delivered in each run.

Relative to the first run, dobutamine increased the efficiency by a factor of 1.7 ± 0.26 whereas in control it decreased by a factor of 0.7 ± 0.037 of the initial value (p<0.001, n=5). The increase in efficiency is concomitant with an increase in external work, which increased by a factor 1.8 ± 0.39 compared to the first run. Oxygen consumption remained constant throughout the runs. However, after washout of dobutamine, efficiency dropped to control values. One muscle showed extremely high, blebbistatin-resistant oxygen consumption, possibly indicating mitochondrial damage induced by dobutamine.

We conclude that dobutamine can increase efficiency in isolated papillary muscles by increasing work rather than by reducing oxygen consumption.

... P5-10

Novex-3 titin as a potential novel signalling node in cardiac and skeletal muscle development

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QUESTION: The Novex-3 titin isoform is much smaller than conventional titin isoforms and contains a large, unique 2,150 amino acid region as a truncated C-terminus. Because of the short length of the protein, it is unlikely to bridge the elastic I-band region of the sarcomere and therefore must have a different function than the full-length titin isoforms.

METHODS: Yeast-two-hybrid screens were performed to identify potential interaction partners of Novex-3 titin. Putative interactions were validated in co-immunoprecipitation experiments using recombinantly expressed proteins. The sarcomeric localization of the Novex-3 region and binding partners in cardiac myocytes was examined using confocal laser scanning microscopy and immunoelectron microscopy, employing a custom-made Novex-3 antibody. Developmental changes in the Novex-3 titin expression level were detected by Western blot and subsequent densitometric analysis of embryonic, neonatal and adult murine heart tissue.

RESULTS: In yeast-two-hybrid screens, using the three C-terminal immunoglobulin-like domains of the Novex-3 region as bait and a human cardiac cDNA library as prey, the small peptidyl-prolyl-cis/trans-isomerase Pin1 was identified as a Novex-3 interactor. Pin1 is a post phosphorylation control element, isomerizing phospho-Ser/Thr-Pro motifs. The interaction between Novex-3 titin and Pin1 was confirmed in co-immunoprecipitation experiments; using anti-HA agarose beads, the recombinant Novex-3 C-terminus was precipitated from HEK cell lysates together with HA-tagged Pin1. Both proteins co-localized at the Z-disk periphery of mouse heart and skeletal muscle sarcomeres. The expression level of Novex-3 relative to full-length titin was found to be altered during development, with higher proportions of Novex-3 expressed in embryonic and neonatal heart and skeletal muscles than in adult tissues.

CONCLUSIONS: The interaction between Novex-3 titin and Pin1 may play an important role in myocyte differentiation and sarcomere development. Via the cis/trans isomerization, Pin1 could control the binding of Novex-3 titin to other signalling proteins involved in the regulation of cardiac development.

P5-11

The impact of titin oxidation and unfolding on cardiac and skeletal muscle function

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BACKGROUND: Titin elasticity in striated muscle is modulated by oxidative stress through reversible weakening of Ig-domain stability via S-glutathionylation of buried cysteines. The titin springs contain up to 100 immunoglobulin-like (Ig) domains, which are centrally involved in the mechanism of titin elasticity. However, it is unknown whether titin S-glutathionylation occurs *in vivo* and whether it mediates titin misfolding and aggregation, which may represent new concepts in the pathophysiology of cardiac muscle.

METHODS AND RESULTS: To promote the oxidation of titin, we exposed perfused mouse hearts to oxidative stress (0.1 mM H_2O_2) or stretched skeletal muscles in the presence of 2 mM GSH and 0.5 mM diamide *ex vivo*. We also used aortocaval shunt mouse hearts (a volume overload model). Titin oxidation was quantified by mass spectrometry following *Isotope-coded affinity tag (*ICAT) labelling. Our results demonstrate that hundreds of cysteines in titin become oxidized upon stretch of muscles and that the elastic titin I-band region shows a higher level of cysteine oxidation than the functionally inelastic A-band region. We then generated several recombinant human Ig domains from the elastic titin region, which were found to be oxidized in muscle or heart, and studied the effect of *in vitro* oxidation of these domains on their misfolding and aggregation. Unfolding of Ig domains at a temperature of 57°C, followed by oxidation, resulted in increased aggregation, which was inhibited upon prior incubation with the small heat shock protein, αB-crystallin.

CONCLUSION: Our study shows that titin oxidation occurs *in vivo*. *In vitro*, oxidized titin is prone to aggregation, which can be prevented by αB-crystallin. The mechanisms of oxidative stress-induced titin aggregation may provide useful information for mechanistic studies targeting striated muscle diseases with high oxidative stress.

P5-12

Impact of cGMP-PKG pathway modulation on titin phosphorylation and titin-based myocardial passive stiffness

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QUESTION: Titin phosphorylation by cyclic guanosine monophosphate (cGMP)-dependent protein kinase G (PKG) lowers cardiomyocyte passive stiffness (Fpassive), thus mediating a mechanical signaling process that is impaired in heart failure (HF). Here, we elucidate which elements of the nitric oxide (NO) cGMP-PKG signaling network are critical for titin phosphorylation and stiffness *in vivo*.

METHODS: We used genetic knockout (KO) mouse models with cardiomyocyte-specific deletion of the guanylyl cyclase (GC)-A receptor and cGMP-dependent PKG (cGKI), as well as global deletion of soluble GC (sGC), as well as wildtype controls. We assessed titin phosphorylation in the heart by immunoblotting and quantitative mass spectrometry (MS) using stable isotope labeling of amino acids (SILAC). We measured Fpassive before and after PKG administration and explored oxidative stress effects on cGMP-PKG signaling in myocardial biopsies from HF with preserved ejection fraction (pEF) patients and the regulatory action of PKG on CaMKII activity.

RESULTS: In all KO models, the important PKG-dependent phosphosite S4080 within the N2-Bus region of titin was hypophosphorylated compared to their matched wild-type (WT). Unexpectedly, MS analysis revealed that most titin phosphosites within the molecular spring segment of titin, including those present in the Ig-domain regions, were hyperphosphorylated in the cGKI KO hearts compared to WT hearts. Only a few sites showed a phosphorylation deficit or were unchanged, perhaps because of compensatory processes following PKG loss. This was associated with upregulation of CaMKII and a clear rise of Fpassive in KO vs. WT cardiomyocytes. While PKG administration lowered Fpassive of KO cardiomyocytes in all models, this effect was more pronounced in the cGKI KO. Increased oxidative stress in HFpEF biopsies and cGKI KO correlated with increased CaMKII and reduced PKG activities.

CONCLUSIONS: Our findings suggest that a network formed by cGMP/PKG/oxidative stress/CaMKII plays an important role in the regulation of cardiomyocyte and diastolic stiffness.

Poster Session 6

Muscle Development, Turnover and Repair

P6-1

The actin-binding properties of *Drosophila* Zasp52 contribute to myofibril assembly

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In sarcomeres, α-actinin crosslinks thin filaments and anchors them at the Z-disc. *Drosophila* Zasp52 also localizes at Z-discs, and interacts with α-actinin via its extended PDZ domain, thereby contributing to myofibril assembly and maintenance, yet the detailed mechanism of Zasp52 function is unknown. Here we show by *in vitro* biochemistry that Zasp52 contains two actin-binding sites, the extended PDZ domain and the ZM area. Furthermore, site-directed mutagenesis identifies amino acids in the PDZ domain and immediately preceding the ZM motif, which are crucial for the interaction with actin. A co-sedimentation assay shows that Zasp52 binds directly to F-actin. We also observe that Zasp52 contributes to F-actin bundling, possibly enabled by Zasp52 dimerization, which we show by a crosslinking assay. Finally, we observe a strong genetic interaction between actin and Zasp52, as well as Zasp66, during indirect flight muscle assembly, indicating that the interaction of these proteins with actin plays a critical role during myofibril assembly.

P6-2

Physiological function of myogenic cells is impaired in piglets with low birth weight

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In the pig the number of muscle fibers is largely fixed at birth. Therefore, satellite cells (SC) are the primary mediators of hypertrophic growth of existing fibers, muscle maintenance and regeneration. Breeding for high reproductive performance has increased pig litter size up to 10% in the last decade also enhancing the number of piglets with low birth weight (LBW) characterized by a persisting musculoskeletal phenotype. Here, we investigate mechanisms underlying the observed growth retardation at the cellular level during early postnatal period which is critical for lifelong development. For this purpose, subpopulations of SC were separated to assess specific functional alterations caused by LBW.

DNA, RNA, protein, and activity of muscle enzymes (CK, ICDH, LDH) were measured in tissue extracts. SC subpopulations were isolated from muscle of 4 day old piglets via Percoll gradient centrifugation. To assess molecular and functional SC characteristics, the proliferation, differentiation, the O_2-consumption and the bioenergetic profile, and the expression of myogenic genes and proteins were measured.

During the first 4 days of life, body weight of LBW piglets and their normal birth weight litter mates increased by 30% and 41%, respectively. In addition, significantly less myogenic cells (28%) could be extracted from LBW muscle referred to muscle weight. The proliferative capacity of cells did not depend on birth weight. CK activity in muscle tissue was reduced and together with the analyses of differentiation and expression of myogenic genes and proteins in isolated cells points to a reduced differentiation potential of myogenic cells from LBW piglets.

In conclusion, subpopulations of SC are present in LBW piglets and their separation enables detailed investigation of mechanisms leading to growth retardation. Results point to a disturbed myogenic differentiation which will be analyzed further to open up new possibilities to influence muscle growth and maintenance.

P6-3

Expression and localisation of the 3-hydroxylacyl-coA dehydratase (HACD) enzymes in developing zebrafish embryos

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Centronuclear myopathy in Labrador retrievers is the most common inherited neuromuscular disorder in dogs: the causative mutation in HACD1 is carried by 15–20% of the breed worldwide. A congenital myopathy with fibre type disproportion due to a HACD1 mutation has also been documented in a human family. There are four HACD enzymes in mammals, each with a distinct tissue distribution: HACD1 and HACD4 are specific to muscle and leukocytes respectively; HACD2 and HACD3 are ubiquitous. These enzymes are required for the elongation of very long chain fatty acids however their tissue specific functions are incompletely understood. To evaluate the suitability of zebrafish for studying HACD enzymes functions we aimed to identify and characterise the expression of HACD enzymes during embryonic development and in adult tissues.

Using conventional and quantitative RT-PCR, cloning and *in situ* hybridisation we have demonstrated that, as in mammals, zebrafish express two main isoforms of HACD1: a full length, active isoform that is specifically expressed in developing and mature striated muscles and a short isoform that does not contain the essential amino acids for HACD activity. Full length HACD2 was ubiquitously expressed in adult zebrafish tissues and upregulated early in embryonic development with strong expression

in the eyes and brain. We identified two shorter HACD2 isoforms of uncertain significance. HACD3 was also widely expressed, however both HACD2 and HACD3 transcripts have relatively low abundance in skeletal muscle. We documented putative zebrafish HACD4 at low levels in a number of tissues.
In conclusion, the zebrafish homologues of the four HACD enzymes have expression and tissue distribution similar to that seen in mammals; in particular HACD1 shows strong, specific expression in developing and mature striated muscles. This suggests that zebrafish are ideal for studying HACD enzyme functions including the pathogenesis of HACD1-deficient myopathies.

P6-4

Glycerol-induced injury activates fibrosis in rat muscle through upregulation of transforming growth factor-β1

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INTRODUCTION: Skeletal muscles regenerate efficiently in response to various injuries. However, disturbance of the regeneration process results in fibrous and fat tissue accumulation. Glycerol-induced injury is a novel method to induce muscle adipogenesis. Our previous studies showed that glycerol-induced injury activates both fibrous and adipogenic tissue accumulation during regeneration in mouse muscles[1,2]. However, there is no information about the outcome of regeneration following glycerol-induced injury in rat. Therefore, the aim of the current study was to investigate skeletal muscle regeneration in rats after glycerol-induced injury.
METHODS: Tibialis anterior (TA) muscles of adult male Wistar rats were injected with glycerol 50%, (*n*=20). Muscle regeneration was evaluated at different time points (4, 7 and 14 days) following glycerol-induced injury.
RESULTS: Our results showed impaired muscle regeneration with extensive fibrous tissue deposition with lack of adipocytes infiltration. On the other hand, an increased level of transforming growth factor-β1 (TGF-β1) protein production was observed at day 4 by immunohistochemistry. Next, TGF-β1 activity was blocked by a neutralizing antibody. Neutralization of TGF-β1 activity significantly improved muscle regeneration and decreased fibrosis. These findings suggest that TGF-β1 is a key factor in the development of fibrosis and impaired regeneration in rats.
CONCLUSION: Targeting TGF-β1 ameliorated fibrosis and enhanced regeneration in glycerol-injured rat muscle.
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P6-5

Effects of heat treatment on force recovery after fatiguing contraction in rat fast-twitch muscle

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The aim of this study was to examine whether heat treatment can facilitate recovery of force production after fatiguing contraction (FC). Both lower legs of the rats were immersed to hot water of 42°C (20 min day-1 for 5 days). Thereafter, gastrocnemius muscles were electrically stimulated *in situ* until the force was reduced to ~50% of the initial force. After 0 min or 60 min of recovery, the superficial gastrocnemius muscles were excised and used for biochemical and skinned fiber analyses. After 0 min of recovery, the effect of heat treatment was not found in all parameters examined. After 60 min of recovery, heat treatment but not FC elicited increases in the amounts of heat shock protein 70 and αB-crystallin. Skinned fiber analyses showed that, after 60 min of recovery, *1)* the ratio of force at 1 Hz to that at 50 Hz remained depressed in fatigued muscles from both non- and heat-treated rats, but the extent of the depressions was smaller in heat-treated than in non-treated rats; *2)* FC decreased the ratio of depolarization-induced force to the maximum Ca^{2+}-activated force in non-treated but not in heat-treated rats; and *3)* neither FC nor heat treatment affected the rise of caffeine-induced force responses. These results suggest that heat treatment may facilitate force recovery after FC by influencing sarcoplasmic reticulum Ca^{2+} release.

P6-6

Bone marrow derived cells contribute to the satellite cell niche during skeletal muscle regeneration

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Skeletal muscle regeneration is regulated by cellular and extracellular components of the satellite cell niche. Cells located in this unique microenvironment including myogenic cells at various developmental stages, cells of the immune system, the connective tissue and the vascular system have been described. However, origin of these cells has not been unambiguously determined and its identification could contribute to development of cell based therapy of the skeletal muscle disorders. In our work we intravenously transplanted mouse GFP+ freshly isolated unseparated bone marrow cells into whole-body lethally irradiated immunocompetent mice 4 weeks before the cardiotoxin-induced injury of the recipients" skeletal muscles. Seven and 28 days after the toxin injection, the injured muscles were examined for presence of GFP+ cells by direct fluorescence, protein immunohistochemistry and immunogold transmission electron microscopy. Using immunohistochemistry and cell ultrastructure observation we identified cell types of the bone marrow origin located in the satellite cell niche. In the injured muscles GFP positivity was determined in numerous immune cells (7 days after toxin injection) and rarely in endothelial cells of the blood vessels and in myogenic cells. Interestingly, many GFP+ fibrogenic cells indicated increased renewal of the connective tissue probably due to the irradiation and revealed the bone marrow as a significant source of the fibroblasts besides the resident fibro-adipogenic progenitors. To summarize, the bone marrow derived cells contributed to the cellular component of the satellite cell niche during the skeletal muscle regeneration. These cells originated not only from the hematopoietic stem cells, but obviously, also from other stem/progenitor cells residing in the bone marrow, e.g. the mesenchymal stem cells and the endothelial progenitors.
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P6-7

Myogenic differentiation of *Pax7-/-* pluripotent stem cells in teratomas

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In muscular dystrophies endogenous pool of myogenic precursors, i.e. satellite cells, declines and is insufficient to effectively repair damaged tissue. Transplantation of stem cells, like embryonic stem cells (ESCs), that could replenish satellite cells population and support muscle regeneration could be considered as possible therapy of such diseases. Understanding the molecular mechanisms that drive the differentiation of ESCs is crucial to make foundation and strengthen the basis for the therapeutic use of these cells in the future. Pax7 is the key factor that drives the specification of skeletal muscle precursor cells, controls embryonic and fetal muscle differentiation in the developing embryo, and is responsible for maintaining satellite cells in adult muscle.
The aim of our research was to determine the role of Pax7 at the early and advanced stages of *in vivo* myogenic differentiation of ESCs. Two types of ESCs: control (*Pax7+/+)* and lacking functional Pax7 (*Pax7-/-)* were transplanted under the skin of mice to generate teratomas. Such model allowed us to analyze terminal myogenic differentiation, including the formation of myoblasts, myotubes, and innervated mature muscle fibers. Thus we were able to investigate the such stages of myogenic ESC differentiation that cannot be achieved in *in vitro* culture.
RESULTS: Skeletal muscle tissue was formed in all teratomas. However, teratomas arising from *Pax7-/-* ESCs differed from the control ones in the expression of certain mesodermal and myogenic markers. Interestingly, in *Pax7-/-* teratomas smaller area was occupied by cells/fibers expressing skeletal myosin.
CONCLUSION: In the absence of functional Pax7 initiation of myogenic differentiation of ESCs is modulated. In addition, Pax7 role in the advanced myogenesis stages was also revealed by us using tratoma model of differentiation.

Poster Session 7

Muscle Exercise, Metabolism, Energetics and Plasticity

P7-1

Does the Hfe gene mutation have an effect on physical performance in a hereditary hemochromatosis mouse model?

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QUESTIONS: Hereditary hemochromatosis (HH) is an autosomal recessive disorder characterized by iron overload with a prevalence of 1/300 in Caucasians of North European origin. Hepcidin is the main regulator of systemic iron homeostasis and its expression is partially controlled by HFE (hemochromatosis protein). The mutation of the Hfe gene is the main cause of this HH disease and iron overload. A recent study has shown that 80% of French athletes who access international podiums carry a heterozygous mutation for Hfe gene. The aim of our work is to evaluate the different parameters of physical performance in knockout (KO) and heterozygous (HT) for Hfe gene, and in wild-type (WT) mice.
METHODS: 104 sedentary (27 HT, 36 KO, 41 WT) male mice (7 months) were used in this study. Their body composition were measured by nuclear magnetic resonance (LF90, Bruker, Germany). The evaluation of the physical performance of each mouse was carried out on a one way treadmill equipped with an indirect calorimetry system (Phenomaster, TSE, Germany). The first protocol consisted of increasing the speed by 1 $cm.s^{-1}$ every 15 s. Oxygen consumption (VO_2) and Respiratory Exchange Ratio (RER) peaks were determined as the highest VO_2 and RER values achieved during the test. The second protocol was performed at 75% of the best speed achieved in the first test (Smax). This second protocol allows us to determine

the VO_2 and RER mean as well as the running time and distance.
RESULTS: We observed a higher Smax ($m.s^{-1}$) in HT mice than in KO and WT (HT = 0.64±0.1 vs KO = 0.53±0.1, WT=0.48±0.1, p<0.001) as well as for VO_2 peak ($ml.h^{-1}$) (HT=197.69±25.55 vs KO=181.25±25.74, WT=165.72±27.90, p<0.001). During the second protocol, the VO_2 mean observed for HT mice was significantly higher than those of KO and WT.
CONCLUSIONS: Our results show that higher oxygen consumption combined with higher speed in HT mice may confer a selective advantage for a better exercise performance.

... P7-2
Absence of gonad-related factors alters exercise performance in mice

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QUESTIONS: In order to better define the role of male and female gonad-related factors (MGRF, presumably testosterone, and FGRF, presumably estradiol, respectively) on mouse performance gain during postnatal development, we analysed the effect of castration initiated before puberty in male and female mice.
METHODS: Male and female wild type C57BL/6 mice were analyzed at the age of 3 months. Male and female mice were both castrated (ablation of gonads) at 1 month of age, before the onset of puberty. Lean and fat masses (g) of each mice were measured by nuclear magnetic resonance (LF90, Bruker, Stuttgart, Germany). Exercise performance of each mouse was evaluated on a one-way treadmill, equipped with a calorimetric system (TSE, Frankfurt, Germany). Mice performed an incremental treadmill speed test until exhaustion (0.01 m.s-1 increment every 15s). Oxygen consumption (VO2) was measured during running. Absolute VO_2 peak ($ml.h^{-1}$) was determined as the highest value of VO2 achieved over 15s and expressed relatively to body lean mass ($ml.g^{-1}.h^{-1}$). Maximal speed (Smax, $m.s^{-1}$) was the highest speed until exhaustion.
RESULTS: In male, castration induced a decrease in lean mass (21.6±0.8 vs 17.5±0.3; p<0.001) and an increase in fat mass (2.7±0.8 vs 3.6±0.8; p<0.09) and in female, we observed the same result both in lean mass (16.9±0.9 vs 15.6±0.6; p<0.01) and in fat mass (5.6±1.7 vs 2.7±1.0; p<0.01). We found that both absolute VO2peak (182±12 vs 145±7; p<0.001), VO2peak relative to lean mass and Smax (0.56±0.03 vs 0.49±0.03; p<0.01) were reduced by castration in male but not in female: there was no effect on absolute VO2peak and Smax, and VO2peak relative to lean mass was increased (9.1±0.7 vs 10.3±0.6; p<0.01).
CONCLUSIONS: In female and male mice, castration alters body composition and, only in male, performance. It seems that both MGRF and FGRF play a beneficial role in body composition and, only MGRF, in exercise performance.

... P7-3
Eccentric training prevents skeletal muscle wasting in colon 26 tumor-bearing mice

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Cancer cachexia is a multifactorial syndrome characterized by severe skeletal muscle wasting. Previous studies showed that isometric training by neuromuscular electrical stimulation (NMES) failed to prevent muscle atrophy in tumor-bearing mice. Here we investigated the effects of eccentric (ECC)-NMES training, which allows greater torque production than isometric-NMES training, on muscle wasting in colon 26 (C-26) tumor-bearing mice. CD2F1 mice were divided into 4 groups: control (CNT), CNT+ECC, C-26, and C-26+ECC. Cancer cachexia was induced by a subcutaneous injection of C-26 cells. ECC-NMES (45 V, 100 Hz, 2 s on/4 s off, 20°/s, 4 sets of 5 contractions) was performed unilaterally to the left triceps surae muscles every other day starting one day after injection of C-26. After four weeks, the weight of gastrocnemius muscles was decreased in C-26 group. This change was accompanied by a marked increase in the expression levels of glutamine synthetase (GS) protein, the ratio of autophagy marker microtubule-associated protein 1 light chain 3 (LC3) B-II/ LC3B-I, and muscle-specific E3 ubiquitin ligase muscle ring finger 1 (MuRF-1) mRNA. ECC training inhibited the loss of muscle weight and increase in GS protein and MuRF-1 mRNA in C-26 mice. In contrast, augmentation of the LC3B-II/LC3B-I ratio in C-26 mice was not attenuated by ECC training. ECC training prevents skeletal muscle atrophy in C-26 mice, which presumably results from the inhibition of ubiquitin-proteasome pathway.

... P7-4
Lipid peroxidation and antioxidant system activity changes of rat blood and cardiac muscle cells under chronic stress

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The functional states of pro- and antioxidant systems in blood and heart muscle cells in rats with long-term emotional stress have been studied. It has been shown that daily rhythm disorders produce psycho-emotional stress in animals and that, this is accompanied by quantitative changes in physiological parameters and hormones in the blood. In the present study, it was observed that such stress increased lipid peroxidation in blood and heart muscle cells. Also, activities of antioxidant enzymes, superoxide dismutase, and catalase were diminished, indicating deterioration of the antioxidant system. In addition, there were decreased activities of mitochondrial enzymes participating in energy metabolism, indicating decreased energy levels in heart muscle cells. These results suggest the likelihood that emotional stress is a key factor that can cause a whole range of diseases of the cardiovascular system.

... P7-5
Acute and long-term effects of reduced capillary perfusion on skeletal muscle function and adaptive remodelling

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QUESTION: Microvascular rarefaction (loss of functional capillaries) is suspected of contributing to skeletal muscle dysfunction, but the relative contribution to performance decline of rarefaction is unknown because concurrent declines in, e.g. muscle fibre size, oxidative capacity occur in pathologies such as chronic heart failure. To investigate the effect of microvascular rarefaction on muscle performance in otherwise healthy tissue, capillary perfusion was reduced in the rat extensor digitorum longus (EDL) by arteriolar blockade using microsphere injections.
METHODS: Bilateral EDL twitch force and fatigue-resistance were determined by stimulating at 10Hz to elicit isometric contractions for 180s. Carotid pressure and bilateral femoral artery blood flow were monitored simultaneously. To assess capacity for adaptive remodelling during chronic ischaemia, functional overload of EDL was performed via extirpation of a muscle synergist coupled with injections of microspheres, followed by 2-week recovery period.
RESULTS: Fatigue index (maximum force at end /beginning of stimulation) in control EDL was 49.40 ± 1.81%, and decreased in proportion to microsphere injection (r2 0.795; P = 0.016). Contralateral EDL had unchanged fatigue resistance (r2 0.015; P = 0.563). Histological sampling of EDL indicated that impaired muscle performance correlated with a reduction in perfused capillaries (r2 0.462, P = 0.031). Interestingly, chronically reduced capillary perfusion did not influence adaptive remodelling of EDL and mechanical performance did not differ from control (F = 0.017; P = 0.899).
CONCLUSION: These experimental data highlight the sensitivity of muscle endurance to acute changes in microvascular perfusion. Conversely, muscle function is not deleteriously affected by arteriolar blockade in the long-term. Our methodology offers a convenient model with which to determine the short and long-term effects of constrained microcirculation upon active muscle.

Poster Session 8

Regulation of Muscle Contraction

... P8-1
The chaperone co-inducer BGP-15 alleviates ventilation induced diaphragm dysfunction

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QUESTIONS:
– Does the chaperone co-inducer BGP-15 increase the diaphragm muscle function during exposure to controlled mechanical ventilation?
– Can BGP-15 reverse the posttranslation modification that are induced by CMV on myosin? and does it have an impact on mitochondria structure and function during exposure to CMV?
METHODS: In this study we have used a novel experimental intensive care unit (ICU) model, allowing time-resolved studies of diaphragm structure and function in response to long-term mechanical ventilation and the effects of a pharmacological intervention strategy (the chaperone co-inducer BGP-15).
RESULTS: The dramatic loss in diaphragm muscle fiber function in response to mechanical ventilation was due to post-translational protein modifications (PTMs) of myosin, but 10 days BGP-15 treatment improved diaphragm muscle fiber function dramatically (~100%), without improving diaphragm atrophy, in parallel with protection from myosin PTMs mediated via HSP72 induction, PARP-1 inhibition, and improvement of mitochondrial function and content.
CONCLUSION: BGP-15 offers an efficient intervention strategy in reducing ventilation induced diaphragm dysfunction (VIDD) in mechanically ventilated ICU patients.

P8-2

Study of the effects of tropomyosin dimers on actin-myosin interaction at molecular level

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BACKGROUND: In striated muscle actin-myosin interaction is controlled by Ca^{2+} via thin filament associated proteins: troponin and tropomyosin (Tpm). Genes TPM1 and TPM2 encode corresponding α- and β-chains of Tpm forming αα- and ββ-homodimers and αβ-heterodimer. Most mammalian striated muscles contain αα- and αβ-Tpm (Boussouf *ea,* 2007). Expression of α- and β-Tpm in muscle depends on the animal species and age and changes in pathologies.

AIM: Comparison of the mechanical and kinetic characteristics of the myosin interaction with thin filaments containing αα- and ββ-homodimers and αβ-heterodimer of Tpm at molecular level.

METHODS: Using an optical trap we measured characteristics (step size, force and their durations) of single interactions of myosin molecule with thin filament with different Tpm dimers and the bending stiffness of these filaments (Nabiev *ea,* 2015). The maximal sliding velocity of the filaments was measured in an *in vitro* motility assay.

RESULTS: The maximal sliding velocities of the thin filaments containing αα- αβ- and ββ-Tpm were 7.2±0.4, 5.4±0.4, 5.6±0.4 $\mu m \cdot s^{-1}$, respectively. The corresponding bending stiffness of the same filaments was 6.1±0.8, 5.0±0.7, 4.1±0.4×10^{-26} $N \cdot m^2$. Average step size and force of myosin interaction with all thin filaments did not significantly depend on the Tpm dimers. Displacement lifetimes of myosin interaction with thin filaments with αα- αβ- and ββ-Tpm were 43±1, 33±1, 33±1 ms, respectively.

CONCLUSION: The decrease in stiffness of thin filaments with αβ- and ββ-Tpm compared to those with αα-Tpm, which presumably depends on change in the Tpm stiffness, may decrease the size of cooperative unit (Shchepkin ea, 2017) which in the presence of drag force in the motility assay reduces the sliding velocity as we observe. The decrease in the lifetime of myosin interaction with the same thin filaments acts in the same way by reduction in the involvement of other myosin molecules. *Supported by RFBR grants 16-04-00688.*

P8-3

Effects of N202K and R133W mutations in β-chains of tropomyosin on structural and functional properties of its αβ-heterodimers

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Tropomyosin (Tpm) is an actin-binding protein that plays a key role in the regulation of muscle contraction. Fast skeletal muscles express two Tpm isoforms, α (Tpm 1.1) and β (Tpm 2.2). As a result, dimeric Tpm molecules can form αα-homodimers and αβ-heterodimers (ββ-homodimers are unstable and occur very rarely). Besides, in many muscles αβ-heterodimers are the dominant form of Tpm. It seems possible that the properties of αβ-heterodimers can be essentially different from those of αα- and ββ-homodimers. However, by now the works on recombinant Tpms were performed almost exclusively with αα- or ββ-homodimers. We applied different methods to investigate the effects of myopathic mutations R133W and N202K in β-chains of Tpm on the properties of αβ-heterodimers and to compare them with the properties of ββ-homodimers with the same mutations in both β-chains. We showed that both these mutations had no effect on the Tpm structure. These myopathic mutations decreased the Tpm affinity to actin for Tpm ββ-homodimers, but not for αβ-heterodimers. In the case of αβ-heterodimers, we observed interesting effects of these mutations on the thermal stability of Tpm–F-actin complexes: both mutations decreased the stability of these complexes to a level observed for ββ-homodimers (N202K) or even lower (R133W). The results of the *in vitro* motility experiments have shown that these mutations strongly decrease the sliding velocity of thin filaments containing either Tpm ββ-homodimers or αβ-heterodimers. Moreover, the effects of R133W mutation were much more pronounced in the case of αβ-heterodimers. Thus, the results showed that the properties of Tpm αβ-heterodimers with mutations in the β-chain can substantially differ from those of Tpm ββ-homodimers with the same mutations in both chains. These results clearly indicate that the effects of myopathic mutations in the Tpm β-chain should be studied only on the αβ-heterodimers. Supported by RFBR grant 16-34-00654.

P8-4

Study of the effect of cardiomyopathic mutations of Tpm on the stiffness of thin filaments using the optical trap

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BACKGROUND: One of the reasons of pathogenesis of most cardiomyopathies (CM) is hereditary changes in the genes coding muscle proteins, particularly tropomyosin (Tpm). Part of Tpm mutations in the region of Tpm binding to troponin T near Cys-190 (L185R, I172T, E180V, E180G) and in the overlap junction between N- and C-terminal ends of Tpm (M281R and I284V) leads to HCM. Part of the Tpm mutations in this overlap junction (M8R, K15N, A277V) leads to DCM [Redwood & Robinson, 2013].

AIM: To study of the effect of CM-related mutations of Tpm on the bending stiffness (BS) of thin filaments.

METHOD: Using an optical trap we measured BS of reconstructed thin filaments containing mentioned above CM-related mutations (Nabiev *ea,* 2015).

RESULTS: BS of thin filaments with WT Tpm used as a reference was 5.9±0.6 (mean±SEM, in 10^{-26} $N \cdot m^2$ here and below). We found that BS of the filament with I284V Tpm (3.5±0.47) was significantly lower and that of thin filament with K15N, M8R, E180V Tpm (~7.6) was significantly higher than that of the filaments with WT Tpm. BS of the filaments with the other studied mutations did not significantly differ from that with WT Tpm.

CONCLUSION: The CM-related mutations in Tpm affect BS of thin filaments with corresponding Tpm in different way. There is no direct correlation between BS of all thin filaments and thin filament activation in the *in vitro* motility assay (Matyushenko ea, 2017). BS of the filament is not the only parameter explaining effect of Tpm mutations on functional characteristics of actin-myosin interaction in cardiac muscles. Specific molecular mechanisms by which Tpm mutations participate in the pathogenesis of CM are complicated and show themselves in different degree at different levels of organization. *Supported by RFBR grants16-34-00493.*

P8-5

Titin-mediated thick filament activation, through a mechanosensing mechanism, introduces sarcomere-length dependencies in mathematical models of rat trabecula and whole ventricle

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Recent experimental evidence in skeletal muscle demonstrated the existence of a thick-filament mechanosensing mechanism, acting as a second regulatory system for muscle contraction, in addition to calcium-mediated thin filament regulation. These two systems cooperate to generate force, but the extent to which their interaction is relevant in physiologically contracting muscle was not yet assessed experimentally. Therefore, we included both regulatory mechanisms in a mathematical model of rat trabecula and whole ventricle. No additional regulatory mechanisms were considered in our model. Our simulations suggested that mechanosensing regulation is not limited to the initial phases of contraction but, instead, is crucial during physiological contraction. An important consequence of this finding is that titin mediated thick filament activation can account for several sarcomere length dependencies observed in contracting muscle. Under the hypothesis that a similar mechanism is acting on cardiac muscle, and within the limits of a finite element left ventricle model, we predict that these two regulatory mechanisms are crucial for the molecular basis of the Frank-Starling law of the heart

P8-6

Myocyte Ca2+ cycling is impaired in the non-ischemic remote parts of the heart early after myocardial infarction

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We tested the hypothesis that myocyte Ca2+ cycling is depressed in the nonischemic remote myocardium (RM) in the early phase after acute myocardial infarction and thereby contributes to the impaired contractile function of the heart.

Myocardial ischemia was induced for 1 h by reversible ligation of the left anterior descending artery (LAD) of male C57BL/6J mice. 24 h later, single myocytes were isolated from the RM for measurements of Ca2+ cycling and sarcomere length at baseline and after isoproterenol stimulation. Biochemical analyses comprised measurements of expression and phosphorylation of Ca2+ regulatory proteins and the assessment of PKA activity. We found the peak height of myocyte Ca2+ transients

to be reduced by 19.1±3.5% compared to healthy control cells (P<0.01). Further, the speed of cytosolic Ca2+ increase and decrease were reduced by 17.3±5.6% and 24.3±8.6%, respectively (P<0.05). Sarcomere shortening was also depressed. Interestingly, all of these parameters showed a strong increase upon stimulation with the beta-adrenoceptor agonist isoproterenol, although overall PKA activity was not different from control tissue. Protein expression of the intracellular Ca2+ transporters RyR2 and SERCA2a was indistinguishable from sham-operated hearts. Expression of the SERCA2a regulator phospholamban (PLN) was also unchanged; however, we found 2.9±0.4-fold more unphosphorylated PLN monomers, the PLN species that inhibits SERCA2a, in RM than in sham-controls (P<0.001); PLN monomers that carried both one (P<0.05) and two phosphate residues (P<0.01) were reduced in RM. Phospho-specific antibodies revealed normal phosphorylation of PLN at Thr17, but markedly reduced phosphorylation at its PKA-dependent phosphorylation site Ser16.
We conclude that myocyte Ca2+ cycling and sarcomere function are depressed in RM 24 h after myocardial infarction. The underlying causes involve decreased PLN phosphorylation at its PKA-dependent phosphorylation site.

... **P8-7** .....

Molecular markers associated with different time-periods of muscle disuse: role of regulatory proteins

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In vertebrate striated muscle, troponins and tropomyosin are responsible, in part, not only for transducing the effect of calcium on contractile protein activation, but also for inhibiting actin and myosin interaction when calcium is absent. The regulatory troponin (Tn) and tropomyosin (Tm) complex, characterized by the existence of multiple slow and fast isoforms, undergoes many changes in various muscle inactivity models. In disuse conditions, such as real or simulated microgravity, the changes consist in a general slow-to-fast transition in troponins C, T and I isoforms. After 15 days of hindlimb suspension in rats, TnT and TnC appear as sensitive markers of unloading, troponin I and tropomyosin being less affected. The regulation of TnT expression appears as a very fast and complete process and is focused on the rearrangement in the pattern of the fast isoforms. These changes agree with the notion that the expression of fast TnT and myosin heavy chain (MHC) isoforms occurs in a coordinated manner. This was not the case for TnC that encompasses many changes but partial exchanges of slow isoforms with their fast counterparts, and a distinct regulation from MHC isoform expressions occurred as it is shown in skeletal muscles after bed rest. Three days of muscle disuse on human subjects after 3 day-dry immersion are sufficient to significantly induce changes in regulatory protein expressions, but troponins C and I appear as sensitive markers of short-term unloading, while troponin T and tropomyosin are less affected. This suggests that the slow and fast counterparts of the Tn subunit isoforms are regulated independently in response to unloading. Time courses and degrees of these transitions differ between the three subunits and according to the type and duration of disuse.
This work was funded by the French spatial agency "Centre National d'Etudes Spatiales" (CNES).

... **P8-8** .....

Loss of the endoplasmic reticulum resident antioxidant selenoprotein S (SEPS1) impairs fast twitch contractile function in mouse hindlimb muscles

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Emerging interest surrounds endoplasmic reticulum (ER) resident selenoproteins due to their potential regulatory capacity to maintain redox homeostasis and intracellular Ca2+ signalling crucial for cellular function. Selenoproteins are important for skeletal muscle growth and development, and their role in regulating contractile function is increasingly recognised. Selenoprotein S (SEPS1) is one of seven ER resident antioxidant selenoproteins implicated in ER stress reduction and cellular stress responses. Given the growing importance of selenoproteins to proper ER function and skeletal muscle contractile function, the role of SEPS1 in muscle metabolism and contractile function was investigated *ex vivo.* Adult male SEPS1 global homozygous (SEPS1-/-), heterozygous (SEPS1+/-) and wildtype (SEPS1+/+) littermates were generated by PGK-Cre. Here, we identify SEPS1 as a highly expressed skeletal muscle protein, with fibre type specific localisation and expression. In SEPS1+/- or SEPS1-/- mice, the reduction and or deletion of SEPS1 reduced physical activity, compared to SEPS1+/+. While energy expenditure, body composition and whole mouse and muscle anthropometrics and morphology remained unchanged. In the fast twitch EDL, a downward shift in the force frequency curve was observed in SEPS1-/- compared to SEPS1+/+, suggestive of reduced strength. During 4 minutes of intermittent, submaximal stimulation the reduction and or deletion of SEPS1 reduced force production, which remained evident following 10 minutes of recovery, as SEPS1+/- and SEPS1-/- produced ~20% less force compared to SEPS1+/+. This impairment was associated with reduced mRNA levels of the thioredoxin antioxidant system and ER stress markers. While, in slow twitch *soleus* muscles, SEPS1 deletion did not compromise contractile function, and gene markers of thioredoxin antioxidant system increased. Thus, SEPS1 appears a novel regulator of contractile function and cellular stress responses in fast twitch muscle.

... **P8-9** .....

Thin filament regulation in insect flight muscle and how it differs in cardiac muscle

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The indirect flight muscle of insects (IFM) and cardiac muscle have mechanical properties in common. Both contract rhythmically and both show length-dependent activation (LDA). The high frequency contractions of IFM that power the rapid wing beats are produced by periodically stretching opposing muscles (stretch activation). Cardiac muscle is also activated by a rapid stretch at each beat. Both LDA and stretch activation are more pronounced in IFM than in cardiac muscle. In Lethocerus (water bug) IFM, troponin bridges between thick and thin filaments may transmit force between the filaments on stretch (Perz-Edwards et al, 2011). We have investigated the interaction between tropomyosin-troponin (Tm-Tn) and thick filaments. Unlike cardiac muscle, IFM has a Tm-Tn complex with two isoforms of TnC (TnCF1 and TnCF2) and two isoforms of Tm (Tm1 and Tm2). TnCF1 regulates stretch activation and there is no homologue in cardiac muscle. Force production in IFM with TnCF2 has the same calcium sensitivity and cooperativity as cardiac trabeculae, suggesting similar regulation by TnCF2 and cardiac TnC. In pulldown experiments with IFM thick filaments or filaments assembled from pure myosin, we found that the Tm-Tn complex with both Tm isoforms binds to thick filaments, and the interaction is not calcium sensitive. Unexpectedly, Tm1 alone binds to thick filaments but Tm2 alone does not. Two regions of sequence differ in the isoforms: one some way from the N-terminus and one at the C-terminus. Tm1 is predicted to have less stable end-to-end association that Tm2. The two isoforms isolated from IFM do not form heterodimers. Tm1 binds to skeletal myosin S1, showing that the interaction between Tm and thick filaments is specific to Tm1, not to IFM myosin, and that the interaction is in the myosin head region. These results suggest troponin bridges activate the thin filament by pulling directly on Tm.

Poster Session 9

Signalling Mechanisms in Muscle

... **P9-1** .....

Role of mitoKATP in skeletal muscle

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$MitoK_{ATP}$ is a mitochondrial ATP-sensitive potassium channel, located in the inner mitochondrial membrane, involved in the control of mitochondrial structure and function. Similarly to its plasma membrane counterpart, $MitoK_{ATP}$ is a hetero-oligomer composed by a tetramer of mitochondrial K^+ channel forming subunits (**mitoK**) associated with four ATP-sensitive regulatory subunits (**mitoSUR**) arranged in an octameric complex. In HeLa cells, MitoK overexpression causes a drastic reduction of mitochondria membrane potential, fragmentation of the mitochondrial network and a collapse of cristae. Here we are investigating the patho-physiological role of the mitoKATP in skeletal muscle. Hind limb muscles injected with AAV9 (Adeno Associated Virus Serotype 9) particles expressing mitoK (AAV9-mitoK) showed impaired mitochondrial function, reduced muscle mass and fibre size accompanied by fibre degeneration and marked impairment of motor ability. Taken together our preliminary results indicate that mitoK plays a role in the control of muscle mitochondria function and on muscle trophism. Future experiments will elucidate the mechanism underlying these effects.

... **P9-2** .....

Muscle *igf1* deletion leads to impaired glucose homeostasis

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Skeletal muscle comprises 40% of total body weight and is critical for glucose (Glc) clearance. The mobilization of Glc transporters such as GLUT4 to the plasma membrane is mediated by insulin activation of its receptor. Because insulin-like growth factor 1 (IGF1) shares high homology with Insulin and mediates its actions via the same family of receptors, it also can contribute to Glc clearance. To investigate how muscle IGF1 controls Glc metabolism, we developed a Muscle IGF1 Deficient (MID) mouse model in which skeletal muscle IGF1 production can be eliminated via tissue specific inducible Cre deletion. Quadriceps muscles (QUAD) and serum were taken from 16 week-old male MID (N=5) and age-matched wild-type (WT) mice (N=5). ELISA was used to determine IGF1 and Insulin levels. Reductions in muscle IGF1 protein content (50%, p<0.05), IGF1 serum levels (25%, p<0.05), and In-

sulin levels (60%, p<0.05), were observed in MID mice, compared to their age-matched WT. Despite lower muscle IGF1 content, MID mice exhibited no reduction in muscle mass, but body weight increased due to hyperlipidemia. Fed and fasted Glc levels did not differ between groups. Still, MID mice displayed impaired Glc tolerance with unchanged Insulin tolerance. To determine the fate of Glc, we performed *in vivo* Glc uptake under insulin-stimulated conditions; Glc levels did not change between MID and WT mice. To determine the contribution of IGF1 deletion on Glc intolerance, we quantified GLUT4 protein levels in QUADs by WB at basal state, and found a reduction in GLUT4 levels in MID muscles. Using RNA-seq analysis to investigate significant *Igf1* deletion dependent changes in expression, we found a large number of genes implicated in Glc transport, actin cytoskeleton regulation, and actin nucleation by ARP-WASP complex altered in MID muscles. Collectively this data indicates that skeletal muscle IGF1 plays an important role in Glc metabolism through multiple pathways.

... **P9-3** .....

Garlic-derived S-allylmercaptocysteine and chronic aerobic exercise improve insulin sensitivity and modulate Nrf2 and NF-κB/IκBα pathways in the skeletal muscle of a non-alcoholic fatty liver disease animal model

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QUESTIONS:

1. Do garlic-derived S-allylmercaptocystine (SAMC) and aerobic exercise improve insulin sensitivity in NAFLD rat skeletal muscle?
2. Is the amelioration of insulin resistance related to the reduction of oxidative stress and inflammation in NAFLD rat skeletal muscle?

METHODS: 63 healthy female Sprague-Dawley (SD) rats were divided into 7 groups, namely: (1) rats fed with normal chow; (2) SAMC rats fed with normal chow (200mg/kg SAMC fed by oral gavage from 9-12th week); (3) exercise rats fed with normal chow (run on a rotarod wheel from 9-12th week, 30min/day); (4) NAFLD rats (rats fed with a high fat diet consisting of 30% fat from fish oil); (5) NAFLD rats with co-administration of SAMC; (6) NAFLD rats with exercise; (7) NAFLD rats with exercise and co-treatment of SAMC. After 12 weeks, rats were sacrificed. Body weight, fat composition, GTT, ALT and liver histology were obtained to confirm NAFLD in the rats. Skeletal muscle samples were collected and analysed for malondialdehyde (MDA; an oxidative marker), insulin sensitivity (Trim72, PI3K, Akt, mTOR), oxidative stress (Nrf2, Keap1, SOD1) and inflammation (NF-κB/IκBα, TNF-α, IL-6) markers. These were investigated by western blot and ELISA.

RESULTS: 1) In the skeletal muscle of NAFLD rats, Trim72 was increased while PI3K, p-Akt and p-mTOR were diminished. Oxidative stress marker MDA was higher with increased expression level of NF-κB, IL-6 and TNF-α and corresponding decreasing level of IκBα expression; 2) SAMC ameliorated high-fat diet-induced Trim72, improved PI3K, p-Akt and p-mTOR, decreased MDA, activated Nrf2 with upregulation of SOD1 expression and corresponding decreasing level of Keap1 protein expression. SAMC also reduced NF-κB expression followed by reduction of IL-6 and TNF-α as verified by IκBα expression; and 3) Chronic aerobic exercise training has the same effects on skeletal muscle as SAMC, except that it failed to reduce the oxidative stress marker MDA in the skeletal muscle of NAFLD rats.

CONCLUSIONS:

1. SAMC and chronic aerobic exercise may exert their beneficial effects on NAFLD through increased in insulin sensitivity in the skeletal muscle by reducing the level of Trim72 expression and restoring PI3K/Akt/mTOR pathway;
2. Because oxidative stress and inflammation are key components of insulin resistance, the attenuation of insulin resistance by SAMC and exercise may be partly through the reduction of inflammation and increased antioxidant enzymes.

... **P9-4** .....

Exogenous application of La^{3+} upregulates myosin heavy chain type I mRNA through activation of calcineurin in C2C12 cells

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Although there are many studies about skeletal muscle regulatory factors, such as IGF-1, FGF, HGF, and IL-6, roles of Ca^{2+}-signaling on muscle cell hypertrophy were still unclear. Our previous study using rat soleus muscle indicated that myosin heavy chain type I (MyHC I) proteins were significantly increased by the exercise after disuse atrophy. It is well known that the mechanical stress by exercise raises intracellular Ca^{2+} level in the muscle cell, therefore we examined Ca^{2+}-calcineurin signaling on synthesis of MyHC I in C2C12 cells.

C2C12 cells were induced to differentiate to myotubes by medium exchange to D-MEM containing 2%FBS. The cells were incubated in D-MEM containing 2%FBS with chemical compounds at the beginning of differentiation and removed after 24hr, and were maintained in differentiation medium for 3 days. MyHC I and IL-6 mRNA expression level in C2C12 cells were measured by the real-time PCR method.

We tried to examine the contribution of Ca^{2+} influx through Ca^{2+}-permeable channels on MyHC I and IL-6 mRNA levels in the C2C12 cells. The MyHC I and IL-6 mRNA levels were not affected by the administration of nifedipine, a L-type Ca^{2+} channel inhibitor. However, these RNA levels were increased by the administration of La^{3+} which was known as a TRP channel inhibitor. The increased mRNA levels of MyHC I and IL-6 induced by the administration of La^{3+} were attenuated by the co-administration of cyclosporine A, indicating that the effects of La^{3+} on these mRNA levels were mediated by the calcineurin activation. Furthermore, the effects of La^{3+} on MyHC I mRNA levels were attenuated by the co-administration of anti-IL-6 receptor antibody.

These results indicate that Ca^{2+} influx through the L-type Ca^{2+} channel does not affect the expression levels of MyHC I and IL-6 mRNA, but La^{3+} influx through the some kind of ion channels, other than TRP channels, upregulates these mRNA levels by the activation of calcineurin-NFAT-IL-6 pathway in a Ca^{2+}-independent manner.

... **P9-5** .....

Effect of calcineurin activation by organic acids on expression of interleukin-6 and myosin heavy chain class II_b mRNA levels in mouse myocytes

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BACKGROUD AND QUESTIONS: Caffeoylquinic acid (CQA), oleic acid (OA) and linoleic acid (LA) are organic acids and presents in several kinds of foods: CQA is a kind of plant polyphenols in coffee beans and many vegetables, and OA and LA are unsaturated fatty acids and includes in seed, fish, and many other plants.It has been reported that CQA, OA and LA had act as a calcineurin activator, but the effects on skeletal muscle still remains unclear. Our recent study indicated that augmentation of myosin heavy chain class I (MyHC I), IL-6 and heat shock protein (HSP) 70 mRNA levels in C2C12 myocytes in a calcineurin activation-dependent way.

In the present study, we examined the effects of calcineurin activators on expression levels of MyHC II_b and IL-6 mRNAs in C2C12 cells.

METHODS: C2C12 cells were cultured until semi-confluence in the medium containing 15% serum. Then, induction from C2C12 myoblasts to myotubes was initiated by medium exchange to the medium containing 2% serum. C2C12 cells were incubated in the medium containing 2% serum with or without agents, which are calcineurin activators such as CQA, OA and LA, and calcineurin inhibitor such as cyclosporine A, or IL-6 and anti-IL-6 receptor antibody. The mRNA expression levels were measured by quantitative RT-PCR method using Taqman probes.

RESULTS: First, we tested the effect of calcineurin activators on IL-6 mRNA level in C2C12 cells. Then, the expression level of IL-6 mRNA were significantly increased by CQA, OA and LA. Second, we examined the effect of IL-6 on MyHC IIb mRNA expression. The mRNA was significantly augmented by addition of IL-6 and was decreased by anti-IL-6 receptor antibody. Third, we observed the effect of calcineurin activators on MyHC IIb mRNA expression. The MyHC IIb mRNA level was increased by CQA, OA and LA.

CONCLUSION: These results indicated that secretion of IL-6 in C2C12 myocytes induced by calcineurin activation might augment MyHC IIb mRNA in the cells.

... **P9-6** .....

Cav1.1 is involved in eEF2k activity increase in rat m.soleus during hindlimb suspension. NH125 activates eEF2k

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Decrease of protein synthesis rate was observed in m.soleus as early as 3 days of hindlimb suspension (HS). It was shown that phosphorylation of eukaryotic elongation factor 2 (eEF2) by its specific Ca2+/CaM-dependent kinase (eEF2k) decreased eEF2 affinity to a ribosome, which led to protein synthesis rate drop. We supposed that eEF2k is activated in m.soleus under HS in Ca2+-dependent manner with Cav1.1 involvement, which reasons to eEF2 activity decrease and consequently to drop of protein synthesis rate. We divided rats into 5 groups: control (C), HS group during 3 days, 3-day HS groups with Cav1.1 blocker nifedipine (HS-Nif), Ca2+ chelator BAPTA-AM (HS-B), and eEF2k inhibitor NH125 (HS-N). We didn"t find decrease of m.soleus mass in all HS groups. eEF2 mRNA expression and its protein content didn"t change in all HS groups as compared to C. We observed significant increase in eEF2k mRNA expression and its protein content in all HS groups as compared to C. P-eEF2 (T56) level was significantly greater in HS group than in C. Nifedipine decreased P-eEF2 level partially after 3 days of HS, BAPTA-AM completely prevented the rise in P-eEF2 content, which did not differ from that in C. Surprisingly NH125 increased 9 fold the rise in the level during HS. We observed reduction in protein synthesis rate after 3 days of HS. Despite the reduction of P-eEF2 level in HS-B group, the protein synthesis rate was as low as in HS. NH125, in turn, preserved the protein synthesis rate during HS at the C level. We showed that phosphorylation level of p70S6k (T389) didn"t change after 3 days of HS, but it increased 3 fold in HS-N group as compared to C. Thus, eEF2k is activated in m.soleus under HS which is Ca2+-dependent process with involvement of Cav1.1. NH125, in contrast, activates eEF2k, which leads to rise in P-eEF2 level. Increase in the phosphorylation level of p70S6k is likely to prevent the protein synthesis rate in HS-N. The work was supported by grant RFBR 15-04-05729-a

... P9-7

The effects of two different stretching protocols on skeletal myotubes

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QUESTIONS: Mechanical loading of differentiated myotubes mimics the loading pattern of mature skeletal muscle. Alterations in signaling and gene expression responses have been reported upon various protocols of mechanical loading applied on myoblasts. The purpose of this study was to investigate the effects of two mechanical loading protocols on signaling and gene expression responses of myotubes.

METHODS: C2C12 myoblasts were cultured on elastic membranes up to their 7th day of differentiation and then underwent two different stretching protocols [i.e., 2% elongation, 0.25Hz frequency, 24h (Long Term, LT) vs. 18% elongation, 1Hz frequency, 15min (Short term, ST)]. Cells were harvested and lysed 24h after the completion of each protocol. P-Akt and P-ERK1/2, as well as NFkB expression was determined by immunoblotting of cell lysates in stretched and non-stretched myotubes. RT-PCR was used to measure loading-induced changes in the expression of Myf5, MyoD, Myogenin, MRF4, IGF-1Ea, IGF-1Eb, Foxo, Fuca, p53, Murf1, Atrogin, Myostatin Il-1b, Il-6 and INF-γ.

RESULTS: Passive stretching of myotubes resulted in no significant decreases of P-Akt and P-ERK1/2 in both protocols, while NFkB expression increased significantly in LT compared to the decreased expression in ST. Expression levels of Myod, MyoG and IGF-1 isoforms, increased while the apoptotic factors FOXO and p53 decreased significantly in the LT ($p<0.05$). In contrast, ST resulted in the upregulation of MyoD, Atrogin, Myostatin Il-1b, Il-6, INF-γ, while it downregulated Murf1, FOXO, and p53 ($p<0.05$).

CONCLUSION: It was demonstrated that myotubes responded differentially to the different loading protocols used. Although both protocols resulted in downregulation of apoptotic factors, LT upregulated myogenic and anabolic factors while ST increased the expression of atrophy and inflammatory factors. These findings suggest that LT may enhance myogenesis, while ST may induce inflammation and atrophy in skeletal myotubes.

... P9-8

Transcription factors regulating E3-ligases MuRF-1 and MAFbx expression at the early stage of muscle disuse

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Postural muscle disuse is followed by the progressive atrophy development. Essential role in this process play MuRF-1 and MAFbx E3-ligases. It is believed that these events are triggered at the early stage of unloading. We investigated the transcription factors which can trigger their expression. Using the rat hindlimb unloading model we didnt find the decreasing in soleus mass but Akt (Ser 473) and FOXO3a (S253) phosphorylation levels (factors that can regulate E3-ligases expression) significantly decreased (60% and 45% respectively) while the content of MuRF-1 and MAFbx mRNA significantly increased (1.4- and 1.9 fold, $p<0,06$) in soleus muscle after 24 hrs unloading compared to control. Also HDAC4 accumulation (which can stimulate myogenine expression and E3-ligases activation) was found in the nuclear fraction. Thus the proteolytic signaling pathway was found to be activated as early as after 24 hrs of unloading. After three days of unloading, soleus mass was significantly decreased, MuRF-1 and MAFbx mRNA expression was upregulated (3.8 and 6.1 fold respectively, ($P \leq 0.05$)) and pAkt(S473) and pFOXO3(S253) levels significantly decreased (60% and 45% respectively) compared to control. The rate of Myogenine mRNA expression was increased after 3 days of unloading only.

CONCLUSION: MuRF-1 and MAFbx mRNA expression is regulated by FoxO3 after 1day of unloading. Myogenin may join to this process in addition to FoxO3 on 3rd day of unloading. This work was supported by RFBR (grant № 17-04-0183).

... P9-9

A possible role of stretch-activated ion channels in the activation of anabolic signalling in rat soleus muscle during an acute recovery from disuse atrophy

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A better understanding of molecular mechanisms by which postural muscle is recovered after a period of disuse-induced atrophy is of great importance for both space physiology and rehabilitation medicine. However, triggering mechanisms responsible for the activation of anabolic signalling pathways in skeletal muscle during an acute recovery from mechanical unloading are vaguely defined. The purpose of the study was to evaluate a possible role of stretch-activated channels (SAC) as mechanosensors which could be involved in the regulation of mRNA translation initiation in rat soleus muscle during early recovery from disuse atrophy (hindlimb unloading). Wistar male rats were subjected to 14-day hindlimb unloading (HU) followed by 12 hours of recovery (reloading). *In vivo* blockade of SAC during recovery was performed by injection of 10 mg/kg gadolinium (Gd3+). The phosphorylation status of the key mTORC1-dependent anabolic markers was assessed by WB. HU induced a significant decrease in p70s6k, S6 protein and 4E-BP1 phosphorylation ($p<0.05$). Following 12-h reloading phosphorylation level of these anabolic markers significantly increased above control values ($p<0.05$). Treatment of rats with Gd3+ during a period of reloading prevented a full phosphorylation of p70s6k, S6 protein and 4E-BP1 so that their phosphorylation level did not differ from the control group. Thus, this study has identified that SAC may play an important role in skeletal muscle mechanotransduction involved in reloading-induced activation of protein synthesis. The obtained data suggest that functional stretch-activated channels are necessary for complete activation of mTORC1 signalling in rat soleus muscle during acute recovery from disuse-induced atrophy. The study was supported by RFBR grant # 16-34-00530

... P9-10

Impact of stretch-activated ion channels inhibition on the transduction of mechanical signal to mTORC1 in rat soleus muscle under hindlimb unloading

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Mechanical forces play a crucial role in the regulation of skeletal muscle mass, however, molecular force transducers, which convert mechanical stimuli into biochemical events, remain poorly studied. The aim of this study was to evaluate the impact of stretch activated ion channels (SAC) on the transduction of a mechanical signal in rat soleus muscle after 7-day hindlimb suspension (HS). Twenty-four adult male Wistar rats weighing 220±5 g were randomly divided into 3 groups: 1) Control (vivarium cage control days followed by *ex vivo* bout of eccentric contractions (EC)); 2) HS (HS for 7 days followed by *ex vivo* bout of EC) in a medium without GdCl3); 3) GD (HS for 7 days followed by EC in a medium with SAC blocker, 20 μM GdCl3). Upon completion of the EC, muscles were subjected to Western blot analyses in order to determine the content of phosphorylated forms of the key anabolic markers. We found that phosphorylation of ribosomal S6 kinase (p70s6k), 4E-binding protein 1 (4E-BP1) and S6 ribosomal protein significantly decreased after 7-day unloading compared to control animals. However, we also found a similar decrease in phosphorylation of p70s6k, 4E-BP1 and S6 protein in the GD group, and this decrease did not differ from that of the HS group. The results of the study suggest that there is a functional inactivation of the SAC during hindlimb suspension, since the inhibition of these channels did not lead to further decline in EC-induced phosphorylation of the key mTORC1-dependent markers. The study was supported by RFBR grant # 16-34-60055 and the Program of the Presidium of the RAS.

... P9-11

Signaling pathways regulating contractility in smooth muscle from the rat aorta and pulmonary artery

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To investigate the signaling pathways regulating contractility in the aorta and the pulmonary artery, we measured muscle force and phosphorylation of the regulatory light chains (RLC) of both smooth- and non-muscle (NM) myosin. Small rings of aorta and pulmonary artery were stimulated to contract with phenylephrine, KCl and the phorbol ester PDBu. For phenylephrine and KCl activation, the Rho kinase inhibitor (Y-27632) decreased force in both the aorta and the pulmonary artery, however the decrease in force was greater for the aorta. Two-dimensional electrophoresis was used to resolve the phosphorylated and nonphosphorylated RLCs of non-muscle and smooth muscle myosin. For the aorta, inhibition of Rho kinase significantly reduced phosphorylation of both smooth muscle and NM myosin; for the pulmonary artery, only the decrease in phosphorylation of the smooth muscle RLC was significant. For PDBu, inhibition of Rho kinase did not decrease force or either smooth muscle or NM RLC phosphorylation. Further, the expression of NM myosin was significantly higher in the aorta than in the pulmonary artery. These data suggest that for both KCl depolarization and α–agonist activation, Rho kinase significantly contributes to the mechanism for force regulation in these types of smooth muscle. The greater Y-27632 induced decrease in force in the aorta, compared to the pulmonary artery, may be due to the higher expression of NM myosin and the larger reduction in NM myosin phosphorylation in this tissue. These data suggest that a Rho kinase dependent pathway regulates NM myosin phosphorylation, and the activation of NM myosin contributes to the regulation of force in vascular smooth muscle.

... P9-12

Enhanced capacity for CaMKII signaling lowers contraction-induced calcium release and slows contraction of fast-twitch muscle and fatigued slow-oxidative muscle

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BACKGROUND: The multi-meric phosphotransferase CaMKII is implicated in the facilitation of calcium release and mitochondrial biogenesis through a contraction-induced activation via threonine 287 phosphorylation, an increases its expression after a high training volume of contractions. Here we tested whether overexpression of α/β CaMKII enhances excitation-induced calcium release and modifies contractile characteristics in dependence of the aerobic muscle type.

METHODS: *Gastrocnemius medialis* and *soleus* muscle, was transfected with pCMV-driven expression plasmids for native rat α and β CaMKII or an empty control (n=11). Contractile effects were characterized during electrically paced isometric tetanic contractions in intact muscle-tendon preparations. Alterations in intracellular calcium during tetanic contractions were measured using a fluo-4 FF-based detection system in α/β CaMKII-, or control-transfected, single fibers of mouse *m. flexor digitorum brevis*. Effects were verified at a 5% significance level.

RESULTS: Transfection increased α/β CaMKII protein levels 4-fold in muscle fibers and enhanced contraction-induced Thr287 phosphorylation of beta CaMKII in slow-twitch *soleus* and fast-twitch *gastrocnemius* muscle. In *m. gastrocnemius*, α/β CaMKII overexpression extended the time until maximal contractile velocity was reached (+ 3%) and reduced the maximal rate of force development at the start (-6%) and end (-14%) of 50 repeated contractions. Fatigue, and the content of mitochondrial proteins, was not affected by α/β CaMKII overexpression. In *m. soleus* α/β CaMKII overexpression shortened the time until maximal contractile velocity was reached and this reverted to an increase when fatigue was established. Sarcoplasmic calcium concentration was 23% less increased in α/β CaMKII transfected fibers of *m. flexor digitorum brevis*.

CONCLUSIONS: Enhanced capacity for α/β CaMKII signaling diminishes calcium release in fast type muscle and shifts contractile properties towards a slow type. The slowdown of muscle contraction in fatigued, α/β CaMKII overexpressing slow-oxidative muscle, suggests that contractile effects of enhanced CaMKII signaling capacity are modified by a mitochondria-related mismatch in calcium or energy buffering.

... P9-13

The effect of myofillar myopathy assocated desmin mutants on protein turnover

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Myofibrillar myopathies (MFM) are genetic muscle disorders with z-disc abnormalities and progressively disorganized intermyofibrillar networks. Additionally, it often presents with uncharacteristic inclusions and vacuoles. Thus far, genes implicated in MFM include: DES, CRYAB, MYOT, ZASP, FLNC, BAG3, FHL, and DNAJB6. Over 70 unique mutations have been described in desmin alone, with most being missense mutations leading to disorganized desmin networks. In order to explore underlying mechanisms and potential treatments we generated inducible myoblastic cell models (C2C12) expressing modest levels of either wild-type desmin or mutant desmin. Forty-eight hours after inducing the transgene we examined basal and stress induced proteasomal response with a destabilized gfp reporter. We focused on one mutant D399Y, in the central rod domain of desmin which is associated with increased aggregation. Both naïve C2C12 cells and two lines expressing wild-type desmin showed ~6 fold increase in GFP intensity after 6 hours of the proteasomal inhibitor MG132 (5 uM) treatment. Two clonal lines expressing D399Y produced only a 2-fold increase under the same conditions. All MG132 treated cell lines showed a marked increase in HSP27 protein levels. Additionally, after oxidative stress, 200uM H2O2 for 2 hours, mutant desmin cells had approximately 50% reduction in GFP signal increase (from 4 fold to 2 fold) compared to control cells. Likewise utilizing a fluorescently-tagged LC3 sensor we see an increase in autophagy in the D399Y cell line compared to control lines. These data suggest enhanced protein clearance in response to the expression of MFM associated desmin mutants, at least at this early stage of pathology. Perhaps this is an early compensation for the expression of the mutant desmin as well as the low level aggregates (<4%). Given that these are generally slowly developing diseases it will be interesting to follow these pathways over time.

... P9-14

Mechanically unfolded titin immunoglobulin domains refold more accurately when assisted by chaperone alpha-B-crystallin

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The spring region of the giant muscle protein titin contains many immunoglobulin-like (Ig) domains, which unfold and refold under low (physiological) stretch forces. Under potentially proteotoxic stress, the Ig segments tend to unfold and form aggregates, affecting myocyte passive stiffness. Small heat shock proteins such as alphaB-crystallin (αBC) translocate under physiological or pathological stress to the titin springs. The translocated small heat shock proteins (sHSPs) could suppress the abnormal stiffening by interacting with denatured Ig domains. To better understand this protective function, we studied the unfolding-refolding behavior of an 8-Ig-domain titin construct $(I91)_8$ by single-molecule AFM force spectroscopy, in the absence/presence of recombinant αBC (at pH7; pH6; or pH5) or 'control' protein of similar size. Titin Ig domains were unfolded at 175 pN constant force applied for a variable "denature" time (t_D), then the force was set to zero for a variable "quench" time (t_Q) to allow for domain refolding, and finally a "probe" pulse (175 pN; t_P = 5 s) was applied to test how many domains had refolded. Interestingly, Ig domain unfolding kinetics were little affected by αBC. However, upon lowering pH from 7 to 6, the refolded fraction (number of refolded Ig domains during t_Q indexed to number of unfolded Ig domains during t_D) decreased slightly, indicating domain misfolding. At pH5, the refolded fraction dropped by half and ~50% of titin Ig domains showed misfolding events, an effect independent of t_D (variation, 2 - 40 s). Importantly, αBC (10 μM or 20 μM) normalized the refolded fraction to values observed at pH7, whereas 2 μM αBC caused partial protection; control protein had no protective effect. The refolded fraction depended strongly on t_Q (variation, 0.5 - 10 s), under all experimental conditions. Ig domain refolding kinetics were greatly slowed by lowering pH from 7 to 5, as quantified on refolded fraction vs. t_Q plots, on which means were fit by simple exponentials. Again, αBC normalized the refolding kinetics to those observed at pH7 in the absence of αBC. We conclude that αBC promotes the folding efficiency of titin Ig domains and protects them from misfolding, especially under acidic stress, which is frequently encountered in muscle cells.

... P9-15

Ultrastructural and biochemical characterization of the interaction between activator of Hsp90 ATPase protein 1 (Aha1) and titin in cardiac muscle cells

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Aha1 is a ubiquitously expressed co-chaperone that associates with heat-shock protein 90 (Hsp90), thereby accelerating its chaperone cycle. Aha1 also shows Hsp90-independent chaperone function, implicating it into cellular protein quality control mechanisms. The function of Aha1 in striated muscle cells is unknown. Aha1 was localized in striated muscle cells by immunogold electron microscopy (EM) and correlative immunofluorescence microscopy. Preferential binding of AHA1 to the elastic titin region was observed. Analysis of the epitope – Z-disc distance on immunoelectron micrographs of cardiac myocytes stained for AHA and titin epitopes revealed a preferential association of Aha1 with the titin N2-B domain. In addition, ultrastructural and histological analysis of Aha1-KO mouse hearts demonstrated reduced myofibrillar integrity, increased mitophagy, and fibrosis, accompanied by the massive presence of intracellular aggregates. Immuno-EM studies on ultrathin sections from Aha1-KO hearts showed Hsp90 to be translocated to the elastic titin region (N2-B domain), which was not observed in normal cardiac myocytes. Biochemical interactions tests, including gel filtration and fractionation, pulldown-assays and co-immunoprecipitation, using recombinant Aha1 and various elastic titin constructs, confirmed the Aha1-titin N2-B domain interaction. The association of the co-chaperone with titin springs implicates a protective effect on I-band structure and possibly, the involvement of Aha1 in Hsp90-mediated protein quality control of cardiac myocytes.

... P9-16

Ceramide in skeletal muscle during hindlimb unloadin

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QUESTIONS: It is known that the physical inactivity is accompanied by dysfunction of skeletal muscles, including decline of contractile properties, fatigue, atrophy with reduced protein synthesis and increased protein degradation, changes in the profile and characteristics of contractile proteins, etc. Such alterations follow immobilization of patients due to severe somatic and neurological diseases or traumas, as well as weightlessness especially in long duration space flight. Microgravity effects in skeletal muscles involve a number of intracellular signaling pathways and some of them have been studied in detail. However, the role of sphingolipid messengers in the development

of disuse muscle atrophy and dysfunction is not completely understood. We have shown the accumulation of ceramide in muscles (m.soleus) subjected to hindlimb unloading (HU) during 4 and 30 days, and the similar phenomenon we previously observed in rats subjected to immobilization stress of different duration.

METHODS: In the present work, we studied the lipid profile of rat skeletal muscles (using HTLC), the expression of the main enzymes of sphingolipid metabolism and the localization of ceramide and GLUT4 transporter in muscle fibers (immunohistochemically) during HU (6-12 hours, 4 days and 14 days). For HU we used generally accepted tail suspension model.

RESULTS: It has been shown that by the early stage of unloading (6–12 hours) the levels of acid sphingomyelinase and ceramide in m. soleus enhance with simultaneous decrease of sphingomyelin. This effect persists during subsequent periods of the experiment. Along with the increase in ceramide formation during early stages of unloading, the amounts of cholesterol and phosphatidylcholine in muscle decrease with parallel decline of GLUT4 immune fluorescence in muscle fibers. Inhibitor of ceramide formation partially or completely eliminates effects of HU in m. soleus.

CONCLUSION: Our results evidence the possible role of sphingolipids in muscle dysfunction caused by mechanical unloading.

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Poster Session 10

Skeletal Muscle Diseases

P10-1

Comparative transcriptome analysis of skeletal muscle in ADSSL1 myopathy

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QUESTIONS:
ADSSL1 myopathy is a recently identified myopathy characterized by includes adolescent symptom onset, predominant distal muscle involvement, mild facial weakness and mild serum CK elevation. ADSSL1 protein encoded by the *ADSSL1* gene is a key modulator for purine nucleotide interconversion by catalysis of the initial step in conversion of inosine monophosphate to adenosine monophosphate. To understand the pathomechanism of this novel myopathy, we generated transcriptome profiles of muscles from control subjects and patients with ADSSL1 myopathy.

METHODS: We analyzed RNA expression in vastus lateralis muscle samples from three control subjects and four patients with ADSSL1 myopathy In order to construct cDNA libraries with the TruSeq RNA library kit, 1ug of total RNA was used. The protocol consisted of polyA-selected RNA extraction, RNA fragmentation, random hexamer primed reverse transcription and 100nt paired-end sequencing by Illumina HiSeq2500.

RESULTS: A comparison of the gene expression demonstrated 740 up-regulated genes and 521 down-regulated genes in myopathy group. We analyzed the unigenes and pathways related to the purine metabolism pathway. We found that *ADSSL1, PGM1, ADSL, AMPD1, GMPD1, GMPR, NT5C2, ADCY2, PDE4D, ENPP4, ADCY9* and *NT5C1A* genes were significantly down-regulated, and *GUK1, APRT, NME1, ITPA, HDDC3, ADA, NUDT5* and *IMPDH1* genes were significantly up-regulated in myopathy group.

CONCLUSIONS: The present study demonstrated that the pathogenic mechanism of ADSSL1 myopathy might be caused by the altered expression of these genes. Additionally, our findings provide new insights for future studies in the ADSSL1 myopathy.

P10-2

Myofibrillar dysfunction in a rat model of critical illness myopathy is prevented by neuromuscular electrical stimulation

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Critical illness myopathy (CIM) is the most frequent cause of weakness in patients in the intensive care unit. Here we investigated whether neuromuscular electrical stimulation (NMES) training inhibits skeletal muscle dysfunction in steroid-denervation (SD) rats, a widely used animal model for CIM. SD rat was induced by cutting the sciatic nerve and subsequent daily injection of dexamethasone (5 mg/kg) for 7 days. For NMES training, plantarflexor muscles were stimulated supramaximally via a surface electrode (50 Hz, 2 s on/4 s off). NMES training was performed every day and consisted of four sets of five isometric contractions produced at five min intervals. After the intervention period, plantarflexor muscles were excised and medial gastrocnemius (MG) muscles were used for physiological and biochemical analyses. There was a significant reduction in the maximum Ca^{2+}-activated force production of chemically skinned fibers in MG muscles from SD rats. These changes were associated with severe myosin loss and aggregation of hypernitrated actin in SD MG muscles. Moreover, the protein expressions of the redox enzymes NADPH oxidase (NOX) 2/gp91phox, NOX4, neuronal nitric oxide synthase (nNOS), and catalase were increased in SD MG muscles. NMES training prevented all these SD-induced alterations except upregulation of nNOS. These data show that NMES training prevents the myofibrillar dysfunction in SD rats presumably by counteracting the loss of myosin and redox modifications in actin molecules. These data imply that NMES training can be an effective adjuvant therapy for muscle weakness in CIM patients.

P10-3

Eccentric exercise prevents impaired contractility and autophagy flux in skeletal muscle from adjuvant-induced arthritis rat

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We have previously demonstrated that skeletal muscles in adjuvant-induced arthritis (AIA) rats show intrinsic contractile dysfunction, which is presumably caused by aggregation of actin molecules. The purpose of this study was to determine whether eccentric (ECC) exercise prevents muscle weakness and actin aggregates in AIA rat. AIA was induced in the knees of Wistar rats by an injection of complete Freund's adjuvant. To induce ECC contractions, planter flexors were electrically stimulated via surface electrodes (45 V, 30 Hz, and 2 s /4 s duty cycle) while the ankle was forcibly dorsiflexed by servomotor (20°/s). ECC exercise was applied every other day for 3 weeks and consisted of 4 sets of 5 contractions. After the intervention period, gastrocnemius (GAS) muscles were excised for mechanical and biochemical analysis. Maximum Ca^{2+}-activated force per cross-sectional area was reduced in skinned fibers from AIA GAS muscles. These contractile dysfunctions were accompanied by formation of actin aggregates, which were associated with 3-nitrotyorsine, ubiquitin, and p62. Moreover, the expression levels of NADPH oxidase 2, neuronal nitric oxide synthase, and p62 were increased, whereas the ratio of LC3B-II to LC3B-I was decreased in AIA GAS muscles. ECC exercise prevented all these AIA-induced alterations and increased the expression of αB-crystallin, which may protect against protein aggregation. These data show that ECC exercise prevents contractile dysfunction and actin aggregates in skeletal muscle of AIA rats. The combined effects of reduced ROS production, restoration of autophagic flux, and improved chaperone function would account for the ECC exercise-induced inhibition of actin aggregates in AIA muscles.

P10-4

miR-424-5p: a novel negative regulator of ribosomal biogenesis which contributes to muscle wasting

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The loss of skeletal muscle mass is a common co-morbidity in a number of chronic diseases and in older individuals which worsens quality of life and increases mortality. The loss of muscle mass is linked to a shift in the balance of protein turnover in favour of catabolism. Ribosomes are molecular machines that are central to protein synthesis, so defective ribosomal biogenesis is likely to impact this balance. We found that miR-424, a microRNA located in a cluster on the X-chromosome, was significantly upregulated and associated with disease severity in the quadriceps of patients with chronic obstructive pulmonary disease (COPD), intensive care unit-acquired weakness, sarcopenia and those requiring aortic surgery. Furthermore, pre-surgery levels of miR-424 in the quadriceps of patients undergoing aortic surgery was proportional to quadriceps muscle loss over the following 7 days. In silico studies predicted that miR-424 targeted components of the pre-initiation complex (PIC) required to synthesise ribosomal RNA (rRNA), including RNA polymerase I (POLR1A), upstream-binding transcription factor (UBTF) and RRN3. Transfection of the miRNA into a myoblast cell line reduced the expression of these mRNAs as well as rRNA expression and protein synthesis consistent with the predictions. Over-expression in mice tibialis anterior muscles caused rapid fibre atrophy, with 21% muscle mass loss, and reduced rRNA and UBTF expression. In conclusion,

we put forward miR-424 as a novel negative regulator of ribosomal biogenesis and propose that it is likely to contribute the inhibition of protein synthesis in muscle wasting patients.

... **P10-5**

The inflammatory profile of fast twitch skeletal muscle is amplified by reduced Selenoprotein S (SEPS1) expression

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A hallmark of muscle myopathies, including Duchenne Muscular Dystrophy (DMD) is excessive inflammation. As such, there is great interest in characterising novel genes that regulate inflammation, due to their potential to modify disease severity and progression. One such candidate is Selenoprotein S (Selenos: *Seps1*). Polymorphisms in *Seps1* are associated with elevated pro-inflammatory cytokines, and *in vitro* SEPS1 is protective against inflammatory stress. SEPS1 is an endoplasmic reticulum protein highly expressed, but poorly characterised in skeletal muscle. Given these features, we investigated whether the genetic reduction *of Seps1* impacted the inflammatory profile and disease progression in the *mdx* mouse. Male *mdx* mice with a heterozygous *SEPS1* deletion (*mdx*:*Seps1*-/+) were generated and these mice had an approximate 50% reduced SEPS1 protein expression in hind limb muscle. *mdx:Seps1*-/+ mice gained less total and lean body mass, and had a higher oxygen consumption during the first few hours of their active period. In the *EDL*, the inflammatory profile of *mdx*:*Seps1*-/+ mice was exacerbated with mRNA expression of *monocyte chemoattractant protein 1* (*Mcp-1*) (P=0.034), macrophage marker *F4/80* (P=0.030) and *transforming growth factor-β1* (*Tgf-β1*) (P=0.056) elevated. This was associated with a shift in muscle fibre distribution whereby *mdx:SEPS1*-/+ mice had smaller muscle fibres. Despite these changes, the strength and endurance of the EDL were unaltered when stimulated *ex vivo*. In the soleus muscle the reduced SEPS1 expression had no effect on inflammation, muscle size or function. In conclusion, reduced SEPS1 appears to specifically exacerbate the inflammatory profile of fast twitch muscle fibres that could lead to a higher metabolic cost and lower mass. A longer duration study may reveal that the exacerbated inflammation caused by a reduced SEPS1 negatively impacts disease progression.

... **P10-6**

25(OH) Vitamin D protects from atrophy *in vitro*

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Cachexia is a common complication occuring in up to 60% of cancer patients characterized by several metabolic alterations leading to severe loss of skeletal muscle mass. Besides increasing morbidity and mortality indirectly by worsening the side effects of chemotherapy, cachexia can be a direct cause of death of a large proportion of cancer patients. However, to date, there are no effective and established therapeutic options to treat cachexia. 25-hydroxyvitamin D (25(OH)D) blood levels have been correlated with the incidence and evolution of some cancers, suggesting that vitamin D might constitute a useful prognostic marker in cancer patients. In addition, the vitamin D system has also been shown to play a key role in the maintenance of muscle homeostasis and functionality. This has led to the hypothesis that vitamin D might also be used as anti-cachexia treatment. However, the therapeutic efficacy of vitamin D supplementation on muscle function and its underlying mechanism of action are still largely uncharacterized. Here, we show that 25(OH)D protects C2C12 myotubes from cytokine-induced atrophy through activation of the Akt-FOXO3 axis. In addition, we find that the intracellular conversion of 25(OH)D to 1,25-dihydroxyvitamin D (1,25(OH)2D) is essential for its anti-atrophic function. Altogether, our data indicate that 25(OH)D can sustain skeletal muscle cell homeostatic functions *in vitro*, an action that could potentially be exploited to treat cancer-induced cachexia *in vivo*.

... **P10-7**

A selective androgen receptor modulator, TEI-SARM2, improves muscle function in rat model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD), which is caused by the mutation of dystrophin gene, is a severe muscle wasting disease. Dystrophin-deficient mdx mice is widely used as DMD animal model, however, mdx mice only partially mimic degeneration and regeneration phenotypes. To overcome this problem, the DMD model of rats (DMD rats) was previously generated by CRISPR/Cas. DMD rats exhibit age-dependent progressive muscle degeneration and pathophysiological features, similar to DMD patients and more severe than mdx mice. Thus, DMD rats enable us to evaluate chemical compounds as a drug candidate for DMD patients. TEI-SARM2, an oral non-steroidal selective androgen receptor modulator (SARM), is selected as a pharmaceutical candidate for the treatment of muscle wasting diseases by its potent muscle anabolic activity. Here, we sought to evaluate the possibility of TEI-SARM2 as a therapeutic agent for DMD using DMD rats. During 8-month treatment of daily oral administration, TEI-SARM2 significantly preserved muscle strength, measured by grip test and isometric tetanic force. Interestingly, TEI-SARM2 did not increase skeletal muscle weights of their limbs. In addition, treatment of weekly oral administration of TEI-SARM2 also improved muscle function measured by grip test and isometric tetanic torque with minimum side effects on testes and prostates. Altogether, our results suggest that TEI-SARM2 has a strong potential for improving the function of dystrophic muscle and especially, weekly treatment of TEI-SARM2 will be a promising therapeutic option for DMD.

... **P10-8**

Oleic acid rescues palmitic acid-induced damage in human skeletal muscle fibroblasts undergoing transdifferentiation into adipocytes

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BACKGROUND: Fatty degeneration in skeletal muscle is a hallmark of many myopathies, sarcopenia, obesity and type-2 diabetes, but the precise source of this fatty infiltration remains elusive. Our research group has shown that primary human skeletal muscle fibroblasts (but not myoblasts) have the potential for transdifferentiation into adipocytes *in vitro*, suggesting that these cells may be the cause of adipocyte accumulation in muscle[1]. This transdifferentiation of skeletal muscle fibroblasts can be triggered in culture with the addition of fatty acids (oleic and palmitic acid). To better understand the effect of these fatty acids on muscle fibroblasts, in this study oleic and palmitic acid were applied separately.
METHODS: Primary skeletal muscle derived fibroblasts were obtained from human muscle biopsy samples taken from the *vastus lateralis* muscle of healthy volunteers. Following isolation and expansion, cells were purified by immuno-magnetic cell-sorting (MACS) to separate CD56+ (enriched for myoblasts) and CD56- (enriched for fibroblasts) fractions, and subsequently treated with a physiological concentration of fatty acids for 72 hours (oleic and palmitic acid at 300-600μM each, combined or separately) to induce the transdifferentiation into adipocytes. After 72h, cells were fixed and analysed by immunohistochemistry using antibodies against the adipogenic transcription factors C/EBPα and PPARγ, and Oil Red O which stains lipids.
RESULTS: We observed that palmitic acid had a toxic effect on muscle fibroblasts as cells died after exposure to 300μM or 600μM palmitic acid. Interestingly, this toxic effect was rescued when palmitic acid was combined with oleic acid at the same concentration, whilst the most adipogenic response of the fibroblasts was seen with treatment with oleic acid alone.
CONCLUSION: Oleic acid seems to protect muscle cells against palmitic-induced damage.
This work was funded by the BBSRC.
[1]Agley CC *et al.* (2013). *J Cell Sci* 126, 5610-25.

... **P10-9**

Effect of AHK2, a novel modulator of ryanodine receptors, in Duchenne muscular dystrophy

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In the mdx mice, model of Duchenne muscular dystrophy (DMD), the sarcoplasmic reticulum ryanodine receptor (RyR) is abnormally nitrosylated and this leads to calstabin depletion from the protein complex and subsequent calcium leak through the channel. RyR modulators enhance RyR-calstabin binding preventing calcium leak, reducing biochemical and histological evidence of muscle damage and improving muscle function. In the present work we show that treatment of 1 month-old mdx mice during 5 weeks with AHK2, a novel RyR stabilizer, reduces histopathological and biochemical evidence of muscle damage and ameliorates overall muscle weakness. Likewise, in dystrophin-deficient human myotubes, AHK2 significantly increases RyR1-Calst1 binding and normalizes intracellular calcium levels. Moreover, treatment of 4 month-old mdx mice with AHK2 during 5 weeks also ameliorates cardiac function and cognitive deficits. Indeed, cardiomyocyte sarcolemmal integrity, determined by Evans blue dye (EBD) uptake, is impaired in mdx mice after beta-isoproterenol challenge, while trieatment with AHK2 protects sarcolemmal integrity. Finally, treatment with AHK2 normalizes the abnormal defensive behaviour of mdx mice, after an acute stress. Overall, our results demonstrate that AHK2 is effective in improving not only skeletal muscle dystrophic phenotype but also the impairments observed in cardiac muscle and CNS in the mdx mouse model of DMD. Thus, they support AHK2 as a potential therapeutic drug to prevent the progression of muscular, cardiac and neurological defects in DMD patients.

P10-10

The effects of m. trapezius latent trigger point vibration on postural stability

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Recently, it has been putting greater emphasis on cervical disturbances as a pathophysiological component of disequilibrium. This study investigates how myogenic trigger points vibration of cervical muscles can affect postural stability. 38 relevantly healthy volunteers (10M/28F, aged 20-25 years) participated. Of these 29 with latent myogenic trigger points of trapezoid muscle and 9 without revealed triggers (control group). Both groups were evaluated once and on equal terms. To evaluate postural stability we used force plate standard test. Muscle vibration was performed once within 1 minute with vibration frequency 100Hz. Findings were assessed previous to muscle vibration and 30, 60, 180 seconds after it. Results getting previous to vibration is taken as 100%; according that all findings were evaluated. The most significant difference between individuals with myogenic trigger points and control group was detected in 30 sec after vibration. So, statistical analysis of balance quality rate discrepancy showed growth on 3,65%±1,16($p<0,05$), in study population and insignificantly decrease in control. Both control group and individuals with myogenic trigger points have a tendency to increase in 60 and 180 sec after vibration. However, only individuals with triggers showed significantly growth on 6,96%±2,05 and 8,69%±2,28 ($p<0,05$) correspondingly. Shifting linear velocity represents significant decrease in 30 sec in population with trigger points. Meanwhile, in control group this rate slightly increase. Interestingly, that in individuals with triggers points showed significant reduction in frontal plane comparing with sagittal plane on 8,2%±4,0 and 4,8%±2,6 concordantly. It was shown that force plate indexes were improved significantly after trapezoid muscle trigger points vibration. This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities № 17.9783.2017/BC

P10-11

Positive end-expiratory pressure ventilation causes diaphragm fiber shortening in critically ill patients

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Rationale: Critically ill patients develop diaphragm weakness which leads to prolonged ventilator dependency. Weakness is not fully understood, but may be caused by diaphragm fiber adaptations to long term flattening of the diaphragm dome caused by an increased end-expiratory lung volume when positive-end expiratory pressure (PEEP) is applied during mechanical ventilation (MV).
Aim: To study whether (1) PEEP ventilation leads to shorter diaphragm fibers by reduction of the number of sarcomeres in series and/or decreasing myosin and/or actin filament length to maintain optimal filament overlap, and (2) the involvement of giant mechanosensing protein titin in this process.
METHODS: We measured diaphragm displacement by ultrasound, filament length and force-sarcomere relations in individual diaphragm fibers, and the number of sarcomeres in series and intact mechanics in diaphragm strips. Experiments were performed on (diaphragm biopsies) of critically ill patients and on 18h-MV rats.
RESULTS: PEEP ventilation leads to 10mm caudal diaphragm displacement in critically ill patients (at 10cmH2O), and 0.7mm in MV rats (at 2.5cmH2O). Force-sarcomere length relation, and thin and thick filament length in diaphragm fibers was comparable between critically ill and control patients. Similarly, in MV rats the force-sarcomere length relation and filament length was unaltered. However, the optimum diaphragm length for force production was 12% reduced in MV compared to control rats, caused by a 12% reduction of the number of sarcomeres in series. In rats with a more compliant titin isoform, the optimum diaphragm length for force production was less reduced during PEEP ventilation than in wild type rats.
CONCLUSION: PEEP ventilation shortens diaphragm fibers due to sarcomere absorption, which may be modulated by titin. These adaptations might contribute to diaphragm weakness in critically ill patients when the original diaphragm length is restored during weaning from the ventilator.

P10-12

Characterisation of *MYO9A* as a pre-synaptic CMS gene

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BACKGROUND: Congenital myasthenic syndromes (CMS) are a group of rare, inherited disorders characterised by compromised function of the NMJ manifesting with fatigable muscle weakness. We identified mutations in *MYO9A* as causative for CMS but the precise pathomechanism remained to be characterised.
AIMS: To unravel the pathophysiology underlying CMS caused by mutations in *MYO9A*. We hypothesised that defects in MYO9A affect the neuronal cytoskeleton, thus leading to impaired vesicular transport.
METHODS: MYO9A-depleted NSC-34 cells (mouse motor neuron-derived cells) were used to assess the effect on the cytoskeleton using immunofluorescent and immunoblotting techniques. Vesicular transport was analysed using three main assays; a secretome study to observe effects on secreted proteins from NSC-34 cells, a biotinylation assay to assess the expression of surface proteins and a recycling assay to look at receptor recycling. In addition, an unbiased approach utilising proteomic profiling of wild-type and MYO9A-depleted NSC-34 cells was performed to identify key players of the pathophysiology.
RESULTS: Disruption of the cytoskeleton has been identified in cells depleted for MYO9A, with an upregulation of actin and a downregulation of other structural proteins including periaxin. Accordingly, defects in receptor recycling and regular transport of proteins to the cell surface were also observed in cells depleted for MYO9A. Proteomic data support a role for defective vesicular transport in NSC-34 cells and identified affected proteins which are also involved in the manifestation of other neuromuscular disorders.
CONCLUSION: Our combined data allow new insights into the pathophysiology of CMS and show that loss of MYO9A affects the neuronal cytoskeleton, leading to impaired transport and vesicular recycling of proteins. This could cause a CMS phenotype by affecting the surface expression and secretion of important NMJ proteins, as well as the structure of the nerve terminal.

P10-13

Brief prednisolone treatment improves critical illness myopathy in rats

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Glucocorticoids (GCs) are intensively used anti-inflammatory immune-suppressive drugs with diverse therapeutic and adverse effects. In immobilized patients in particular, the use of GCs is controversial. Chronic high-dose GCs exacerbate muscle atrophy and weakness, a condition termed critical illness myopathy (CIM), while brief GC treatment was shown to improve CIM by an unknown mechanism. Here, we investigated the effects of transient prednisolone treatment on CIM by using an established rat model that largely mimics the CIM geno/phenotype. The rats were divided into one control sham-operated group and two experimental groups that underwent deep sedation, neuro-muscular blockade and mechanical ventilation for five days. Experimental groups received either no drug (5D group) or prednisolone, (Pred group). Soleus myofibers were subsequently assessed for size, force and myosin:actin ratio, atrogene expression, and systemic cytokines were measured in the plasma. Our results showed that prednisolone reduced fiber atrophy, and improved maximal force and myosin:actin ratio compared with the 5D group despite activation of MuRF1 expression. Both anti-inflammatory IL-10 and leptin levels increased markedly in Pred group vs. 5D group, along with pro-inflammatory IP-10, IL-18 and the chemokine, fractalkine. The latter three cytokines indicate increased IFN-γ levels. Notably, at five days, regardless of group, soleus muscle fiber specific force correlated with circulating IL-10 levels, and soleus fiber size with circulating leptin levels. These findings suggest that transient use of prednisolone in CIM rats induces changes in both pro- and anti-inflammatory cytokines, modulating the inflammatory and immune status, thereby protecting skeletal muscles from a severe inflammatory response.

P10-14

Expanding the phenotype of *BICD2* mutations towards skeletal muscle involvement

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The cargo-vesicle adaptor protein BICD2 is homologous to *Drosophila* bicaudal D (*BicD*), which is evolutionarily conserved from flies to humans. BICD2 contains five predicted coiled-coil binding domains grouped into three binding regions. The first N-terminal binding region (CC1) binds to the dynein-dynactin complex via direct interaction with the p50 subunit of the dynactin complex, the

second (CC2) binds the kinesin motor complex, and the third (CC3) interacts with the cargo-associated RAB6 GTPase. It has been suggested that BICD2 links a variety of RAB6-positive cellular cargos to the dynein motor complex and thus initiates directional cargo movement.
Mutations in *BICD2* cause autosomal dominant spinal muscular atrophy with lower extremity predominance 2 (SMALED2) and hereditary spastic paraplegia (HSP). The interaction of mutated BICD2 with the dynein-dynactin complex and/or with RAB6A leads to fragmentation of the golgi apparatus, suggesting perturbations of BICD2-dynein-dynactin mediated trafficking with possible impairments in the development/maintenance of motor neurons.
We analyzed two independent German families with clinical, genetic and muscle MRI studies and identified the mutations p.Ser107Leu and p.Thr703Met in the *BICD2* gene, respectively. Immunofluorescence studies and immuno-electron microscopy showed striking impairment of dictyosome integrity, vesicle pathology and abnormal BICD2 accumulation either within the nuclei (p.Ser107Leu) or in the perinuclear regions (p.Thr703Met). Transfection studies confirmed BICD2 aggregation of different subcellular distribution.
Our findings extend the phenotypic spectrum of *BICD2*-associated disorders, by features of a chronic myopathy and show a novel pathomechanism of BICD2 defects in skeletal muscle. Therefore, BICD2 as a key adaptor protein in trafficking of cellular cargos, seems to be important not only in neurons, but also crucial for skeletal muscle integrity and maintenance.

P10-15
The clinical and pathologic findings of Korean patients with *RYR1*-related congenital myopathy

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INTRODUCTION: This study was designed to investigate the characteristics of five Korean patients with *RYR1*-related congenital myopathy.
METHODS: Five patients from unrelated families were diagnosed with *RYR1*-related congenital myopathy via direct or targeted sequencing of the *RYR1* gene. Clinical, mutational, and pathological findings were then analyzed.
RESULTS: Seven different mutations, including two novel mutations (c.5915A>T, c.12250C>T) were identified. All patients presented at infancy with proximal dominant weakness and delayed motor milestones. Other clinical findings were scoliosis in three, winged scapula in two, hip dislocation in one, and pectus excavatum in one. Respiratory distress and ophthalmoplegia were observed in one patient with novel recessive mutation. Two of three muscle specimens revealed myopathic pattern with core.
Discussion: We identified a novel compound heterozygous *RYR1* mutation and demonstrated clinical and pathological findings in five Korean patients with *RYR1*-related congenital myopathy.

P10-16
***Drosophila* models of Laing distal myopathy**

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BACKGROUND: Mutations in *MYH7* encoding slow/ß-cardiac myosin heavy chain are associated with cardiomyopathy or skeletal muscle disease with or without cardiac involvement. A vast majority of mutations located at the elongated a-helical rod domain of *MYH7* have been associated with Laing distal myopathy (MPD1) or myosin storage myopathy, the two skeletal myopathies with distinct morphological and clinical phenotype. Patients with MPD1 show variable clinical and muscle pathological changes.
METHODS: We developed the first fruit fly *Drosophila melanogaster* model system through the use of CRISPR/Cas9 gene editing system to investigate the *in vivo* consequences of a recurrent MPD1 mutation (K1729Δ). Homozygous and heterozygous lines, as well as hemizygous flies with exclusive expression of the K1728Δ *Mhc* allele in indirect flight and jump muscles (IFM) were established.
RESULTS: The mutants were flightless and had significant movement disability with a marked effect on crawling behavior and jumping ability. Heterozygous flies had a significantly reduced life expectancy while homozygous flies did not survive pupation. Immunofluorescence analyses of larval muscles and adult IFM of mutant flies exhibited severe disruptions and disorganization of sarcomere. Mutated myosin revealed a propensity to aggregate during early development. The impacts of the mutation at the larval stage of cardiac development indicated significant structural and functional impairments of homozygous allele on the heart while heterozygotes" heart muscles seem to remain unchanged.
DISCUSSION: The intra- and extra-familial variation of clinical and pathologic phenotypes of this mutation is intriguing. Material obtained from patients is limited, making it difficult to assess the mechanism of mutation pathogenicity and follow the course of the disease development. We created a CRISPR/Cas9 *Drosophila* model to determine the pathogenic mechanism of the recurrent K1729Δ *MYH7* mutation in patients with MPD1.

P10-17
Sarcomere dysfunction in nemaline myopathy caused by a mutation in slow skeletal troponin T (TNNT1)

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INTRODUCTION: Nemaline myopathy (NEM) is rare muscle disease caused by mutations in genes encoding proteins of the sarcomeric thin filament. Here, we investigated a muscle biopsy of a patient with a homozygous mutation in the TNNT1 gene (NEM5), resulting in a truncated slow skeletal troponin T isoform. Troponin T binds to troponin C and I to form the troponin complex, which regulates muscle contraction through interaction with calcium and tropomyosin. NEM5 patients suffer from early-onset muscle weakness and respiratory insufficiency.
AIMS: To understand the underlying mechanism of muscle weakness in NEM5.
METHODS: Patient and control biopsies were taken from the quadriceps. We performed mechanical measurements on single muscle fibers isolated from the patient"s biopsy. Fibers were activated by exposure to calcium-containing solution. Myofibril mechanics will be studied as well. Myofibril mechanics reflect the functioning of the thin and thick filaments, without confounding effects of damaged myofibrillar area. Furthermore, we will attempt troponin complex exchange experiments in control and patient myofibrils. Results of these experiments should clarify whether muscle weakness in NEM5 patients occurs solely due to the mutation or whether other defects/damage are present as well.
RESULTS: Currently, single fiber measurements have been performed on the NEM5 biopsy. The results show that the maximum force production, normalized to cross-sectional area, is severely reduced. This is in line with the severe phenotype of the patient. Furthermore, the slow-type fibers are atrophied and their calcium sensitivity of force is increased.
CONCLUSION: Based on the single fiber data we propose that sarcomere dysfunction contributes to muscle weakness in NEM5. Our planned studies should confirm this proposition.

P10-18
Effects of a chaperone co-inducer (BGP-15) on contractile properties of single fibres from soleus muscle of rats exposed to Intensive Care Unit (ICU) conditions

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We have studied the effects of a chaperone co-inducer, multi-target drug (BGP-15) on muscle structural and functional impairment associated with deep sedation, neuromuscular blockade and mechanical ventilation. We hypothesized a positive effect of BGP-15 on fibre structure and function in our model of intensive care intervention and Critical Illness Myopathy (CIM).
Female Sprague-Dawley rats were used. Intravenous administration of BGP-15 was given to the rats for the whole duration of the experiments (5 or 10 days). The experimental groups were extensively monitored 24 hours per day. The soleus muscles were dissected from euthanized experimental and controls animals. Muscle bundles were then chemically skinned and prepared for contractile measurements. CSA, absolute force (P0) and specific force (SF) i.e. absolute force/CSA, were measured at the single muscle fibre level. Transmission electron microscopy was used to study intermyofibrillar (IMF) mitochondrial structure.
After 5 days of treatment (deep sedation + neuromuscular blockade + mechanical ventilation) the SF decreased significantly compared to the controls. The administration of BGP-15 maintained the SF at the control level after 5 days, however after 10 days it showed no effect on SF. On the contrary CSA values were not significantly affected by BGP-15 administration, therefore the improvement in SF at 5 days is essentially caused by the improvement of P0, which is significant. Furthermore we observed that the proportion of abnormal IMF mitochondria was dramatically increased after both 5 and 10 days, of which BGP-15 treatment was seen to alleviate, significantly reducing the abnormal mitochondrial structure.
We conclude that the restoration of SF is associated with BGP-15 administration, which might be transient and partially dependent on the structural integrity of the IMF mitochondria. This is compatible with the pharmacodynamics of BGP-15 which involves also the mitochondrial lipid structures.

P10-19

Biochemical investigations to unravel myopathological perturbations caused by the Caveolin-3 p.P104L mutation

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Caveolin-3 is a muscle specific protein localized to the sarcolemma where it interacts with the dystroglycan complex (DGC) and is thus involved in the connection between the extracellular matrix (ECM) and the cytoskeleton. Muscle diseases caused by mutations in the *CAV3* gene are called Caveolinopathies. So far, more than 40 dominant pathogenic mutations have been described leading to different phenotypes molecularly associated with a mis-localization of the mutant protein to the Golgi. Hereby, associated ER-stress has been demonstrated for the p.P104L mutation. However, the further pathophysiological consequences of mutant CAV3 mis-localization and ER-stress remained elusive. Utilizing a transgenic (p.P104L) mouse model of Caveolinopathy and performing proteomic profiling along with immunoblot and morphological studies (including electron and CARS microscopy) we systematically addressed these consequences. Our morphological studies revealed Golgi and ER proliferations as well as the build-up of protein aggregates. These observations were confirmed via immunological studies and are in accordance with our proteomic data showing altered abundance of 120 proteins in diseased quadriceps muscle fibres. Proteomic findings indicated ECM remodeling and cytoskeletal vulnerability. Moreover, our proteomic findings suggested that further DGC components are affected by the perturbed protein processing machinery leading to the formation of protein aggregates which could be confirmed via CARS microscopy. Hence, our combined data classify (p.P104L) Caveolinopathy as an acquired protein folding disease with sarcolemmal affection and thus expand the pathophysiological knowledge of this disorder, an important aspect in the therapeutic management of CAV3-patients.

P10-20

The high Ca^{2+}-sensitivity associated with the Glu139del and Arg91Gly mutations in tropomyosin is caused by freezing of tropomyosin near the closed position

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Deletion of glutamic acid residue at position 139 (Glu-139del) and substitution of arginine 91 for glycine (Arg-91Gly) in β-tropomyosin are caused by point mutations in TPM2 gene. The latter are associated with cap myopathy and distal arthrogryposis, respectively, and both are characterized by high Ca^{2+} sensitivity of myofilaments. To understand the mechanisms of these defects we studied multistep changes in mobility and spatial arrangement of tropomyosin, actin and myosin heads during the ATPase cycle in reconstituted ghost fibers using the fluorescent probes associated with respective proteins and polarized fluorescence microscopy. The Glu139del and Arg91Gly mutations was shown to increase strongly the flexibility of tropomyosin and freeze the tropomyosin strands near the closed position. Both mutations inhibited the movement of the tropomyosin strands towards the blocked position at low Ca^{2+}, thus causing higher Ca^{2+} sensitivity. The Glu-139del mutation decreased while the Arg91Gly mutation increased the amount of the myosin heads strongly bound to F-actin at high Ca^{2+}, but both increased the number of such heads at relaxation; this may contribute to contractures and muscle weakness. The relative number of actin monomers in the «OFF» state at low Ca^{2+} was increased in the presence of both tropomyosins. Therefore, the ability of troponin to switch actin monomers off at low Ca^{2+} was retained. It was suggested that the high Ca^{2+} sensitivity in the presence of these mutations is not associated with a failure in troponin action, but rather arises from the abnormal position of the mutant tropomyosins on the actin filaments and hence an increase in the population of the strongly bound myosin heads at low Ca^{2+}. The use of reagents that decrease the Ca^{2+} sensitivity of the troponin complex may not be appropriate to restore muscle function in patients with the Glu139del and Arg91Gly mutations. This work was supported by the Russian Science Foundation (grant 17-14-01224).

P10-21

Turnover studies on DNAJB6 and the CASA pathway proteins

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Limb-girdle muscular dystrophy type 1D (LGMD1D) results from dominant mutations in the co-chaperone DNAJB6. The mutations confer toxic properties to the short cytoplasmic isoform DNAJB6b, and slow down the turnover of mutant DNAJB6, as well as that of the co-expressed wild-type protein. We have previously shown that DNAJB6 interacts with the chaperone-assisted selective autophagy (CASA) system. This macroautophagy pathway, important for muscle maintenance, depends on the co-chaperone BAG3, together with HSPA8 (Hsc70) and HSPB8 (Hsp22). Interestingly, overexpression of BAG3 increases DNAJB6b toxicity in zebrafish — an effect not seen with the BAG3 myopathy mutant P209L — suggesting an active role for BAG3 in LGMD1D pathogenesis.

The role of altered DNAJB6 turnover in the pathogenesis of LGMD1D, and its relationship with the CASA proteins is unknown. BAG3 could increase the toxicity of mutant DNAJB6b by aggravating its turnover block. Alternatively, if BAG3 and HSPB8 lie downstream of DNAJB6b mutations, also their turnover rates could be affected. To explore these possibilities, we have carried out cell-culture based protein turnover studies. In our setup, proteins of interest are expressed in a tetracycline-inducible system, and their turnover is followed after tetracycline removal. Coexpression or knockdown allows identification of modulating effects of other proteins.

Unexpectedly, while wild-type BAG3 had little effect, BAG3 P209L blocked the turnover of both wild-type and mutant DNAJB6b. This correlated with a shift of DNAJB6b to the insoluble fraction, suggesting that mutant BAG3 may sequester DNAJB6b into an insoluble pool that is resistant to normal degradation. Our results reinforce a functional interaction between DNAJB6 and BAG3, provide a possible explanation for the different effects of wild-type and P209L BAG3 on DNAJB6 toxicity, and place DNAJB6 downstream of the BAG3 P209L mutation, potentially linking the pathomechanisms of LGMD1D and BAG3 myopathy.

P10-22

Fast skeletal muscle troponin activator *tirasemtiv* improves *in vivo* muscle performance in a nemaline myopathy mouse model harboring the *Acta1*^{H40Y} mutation

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BACKGROUND: Nemaline myopathy (NM) is among the most common non-dystrophic congenital neuromuscular disorders. Muscle weakness results in a poor quality of life. Here, we evaluated the acute effect of *tirasemtiv* – a fast skeletal muscle troponin activator currently under clinical investigation in ALS – on *in vivo* muscle function, respiratory function and muscle trophicity in an *Acta1*^{H40Y}-based NM mouse model.

METHODS: The acute effect of *tirasemtiv* (3mg/kg; i.p.) on muscle performance was evaluated using a cross-over design in 15 *Acta1*^{H40Y} mice. *In vivo* performance of gastrocnemius muscle was studied using transcutaneous electrical stimulation during a fatiguing protocol. High-energy phosphate metabolites and intracellular pH were investigated using ^{31}P-magnetic resonance spectroscopy. *In vivo* respiratory function was studied during stress (5% CO_2) using whole body plethysmography. Endurance capacity was studied using a forced incremental running protocol. Myosin Heavy Chain (MHC) isoform composition was determined using SDS-PAGE and fiber cross-sectional area (CSA) by histology on diaphragm, soleus, EDL, gastrocnemius and tibialis muscle.

RESULTS: Submaximal force production was significantly higher upon administration of *tirasemtiv* (84±5 mN) compared to vehicle (66±6 mN). Intracellular pH was significantly lower in mice receiving *tirasemtiv* (6.98±0.02 *vs.* 6.87±0.02). No difference was observed for PCr consumption and Pi production. Tidal volume was higher (0.037±0.04 ml *vs.* 0.033±0.04 ml) and breathing frequency was lower (200±4 breaths per minute (BPM) *vs.* 209±3 BPM) during stress. No changes in endurance capacity were observed. No effect of *tirasemtiv* on MHC isoforms composition and on CSA was found.

INTERPRETATION: Our multimodal approach revealed that *tirasemtiv* improves i*n vivo* submaximal force production and respiratory function in an *Acta1*^{H40Y}-based NM mouse model. These findings are pivotal steps towards a therapeutic strategy to combat muscle weakness in NM.

... P10-23

Injection of botulinum toxin A leads to impaired muscle function, hyperreflexia, increased passive stiffness and damage of the fibrilar and non-fibrilar structures of rat skeletal muscles

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Botoxulinum A (Btx) is used for a wide range of conditions ranging from spasticity to wrinkles. Recent studies have given new insights into the clinical consequenses related to the use of Btx towards spasticity. The aim of the present study was to investigate the effect of intramuscular Btx injections on muscle structure-, metabolism, and function in skeletal muscle tissue of rats. Electrophysiological measurements have shown that the passive stiffnes of the muscle was increased after Btx injection. At the same time the reflex activity was incresed, indicating that Btx injection increased spasticity. Furthermore the gait pattern of the rats was significantly affected 3 weeks after Btx injection. The ankle joint rotated externally, the rats became flat footed, and the stride length decreased in the Btx injected leg. The changes in gait pattern were accompanied by clear evidence of microstructural changes on the tissue level by as evidenced by 3D imaging of the muscles by means of Synchrotron Radiation X-ray Tomographic Microscopy (SRXTM). The imaged showed that both the fibrillar and the non-fibrillar tissues were affected. The volume fraction of fibrillary tissue was reduced significantly and the non-fibrillar tissue increased. Furthermore, gene expression analysis showed an upregulated connective tissue turnover and an upregulation of the inflammatory marker IL-6. In conclusion: The present study reveals that intramuscular Btx injections cause essential damage of the microstructure-, metabolism, and function of the muscle, which leads to functional deficits.

... P10-24

A patient-mimicking filaminopathy mouse model reveals increased myofibrillar lesion formation as a major pathomechanism

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Mutations of the human filamin C-encoding gene (*FLNC*) cause autosomal dominant forms of progressive and often devastating myopathies and cardiomyopathies. The first identified and most frequently occurring mutation (p.W2710X) leads to the deletion of a C-terminal 16 amino acids fragment of filamin C (FLNc) causing myofibrillar myopathy. This disease is characterized by the formation of pathological protein aggregates containing FLNc, desmin and Xin in skeletal muscle fibres. We generated the first patient-mimicking knock-in mouse model harboring the ortholog of this mutation. The knock-in mice show no gross morphological abnormalities, but develop signs of muscle weakness at higher age. Most importantly, immunofluorescence analyses revealed the formation of FLNc- and Xin-positive lesions originating at the level of Z-discs as a sign of increased myofibrillar instability. These lesions, which differed from “classical” amorphous protein aggregates on the basis of their more filamentous morphology, were more abundant after physical exercise. This finding urged reassessment of filaminopathy patient biopsies, and indeed we found similar, previously unreported lesions in addition to protein aggregates. Our data indicate that FLNc is involved in the mechanical stabilization of Z-discs. We hypothesize that these lesions define a preclinical stage of *FLNC*-associated myopathies and contribute to muscle weakness prior to the formation of protein aggregates.

... P10-25

Characterization of the stromal cell population that interplay in skeletal muscle degeneration

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Chronic muscle degeneration is the end point of chronic skeletal muscle diseases. Therapy approaches towards the cure of genetic disorders that provide promising results on animal models fail to succeed in clinical trials. Fibrosis, described as the widespread increase in connective tissue content is the foremost important fact among these changes that needs to be reversed since, it blocks the access of regenerative growth factors to their targets, it exerts a restrictive action on the kinetics, disrupts the microenvironment and restricts the activation of the somatic stem cells for the regeneration of the muscle tissue. For a success towards the augmentation of muscle function, the cellular components and pathways that lead to fibrosis need to be elucidated.

The fibro-adipogenic precursors (FAPs) are mesenchymal cells that reside in the stroma of the skeletal muscle that differentiate and contribute to the fibrosis and fatty infiltration in the muscle tissue. This cell population exerts supportive action towards the regeneration of an acute damage in the skeletal muscle but potentiates fibrosis and fatty degeneration under chronic progressive conditions. We previously showed evidence that FAPs might be activated following applied immobilization procedures without any inflammation.

Using a state-of-the-art cell isolation and characterization technique, here we show direct activation of FAPs following tenotomy and denervation in rodent models. These studies aiming at elucidating the processes, cells and pathways that contribute to skeletal muscle degeneration will help to understand the molecular fundamentals, and thus; the prevention of skeletal muscle fibrosis.

... P10-26

In vitro phenotypic comparison between young and aged human myotubes

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Muscle wasting can result from a wide range of dysregulations in muscle physiology. Muscle loss is present systemically in the elderly (sarcopenia); it can result from acute or chronic illness (cachexia), or appear in various pathologies such as dystrophies or diabetes. In this context, we developed MyoScreen™, a drug discovery engine that provides a physiological human *in vitro* model of skeletal muscle. The model relies on micropatterns that control the microenvironment and thus guide and orientate the differentiation of human primary myoblasts. To demonstrate that MyoScreenTM is a sensitive and predictive model with a potential for discovering new compounds, three healthy donors with a range of ages (4, 20, and 37 years old) and a diabetic donor (68 years) were systematically assessed in terms of proliferation, differentiation, calcium flux, acetylcholine receptor clustering and response to atrophy and hypertrophy inducers. Interestingly, the present study highlights a gradient of different features depending on patient age and health. On the other hand, the results of the age-stratified analysis demonstrate the capacity of the MyoScreenTM drug discovery platform to discriminate between donors and to measure the impact of compounds, based on High Content Screening. By combining a higher relevance to the *in vivo* situation with access to a rich panel of phenotypic readouts, MyoScreenTM represents a new paradigm that can improve our understanding of the molecular mechanisms driving muscle disorders using healthy or diseased donor cells and increase confidence in the validity of target hits from drug discovery screening campaigns.

... P10-27

Muscle inflammation following supraspinatus tears

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INTRODUCTION: Rotator cuff (RC) lesions are one of the most common conditions affecting the shoulder. The etiology of RC diseases is multifactorial but the supraspinatus (SS) tendon is particularly vulnerable to become lesioned. In patients with RC tears, increased numbers of inflammatory cells have been demonstrated in the inflamed synovial tissue. Recent studies have suggested that also the RC muscles become inflamed after RC tears and animal models suggest that acute inflammation plays a detrimental role in chronic muscle damage following RC tears. The aim of this study was to characterize inflammation in humans suffering from a RC tear.

METHODS: Tissue samples were taken from the RC tissues at the time of surgery in 9 patients scheduled for surgery. Mean age was 58 years (40-61) years. All patients had a MRI validated SS tear. Control biopsies were obtained from the deltoid muscle. In addition, we used a mouse model to study acute inflammation 5 days after a RC tear. We used multiplex analysis, proteomics, histological and immunohistochemical analyses to study the inflammatory profiles of SS muscle and tendon, deltoid muscle and bursa.

RESULTS: Multiplex analysis demonstrated acute inflammation in the lesioned SS mouse muscle compared to the

healthy contralateral SS muscle. In addition, in patients with chronic RC tears, we found differential expression levels of several matrix metalloproteinases (MMPs) known to be involved in the degradation of the extracellular matrix between SS tendon and muscle (MMP3 and MMP9), and between SS and deltoid muscle (MMP9). Also several inflammatory mediators (cytokines and chemokines) were differentially expressed between SS muscle and tendon and between SS and deltoid muscle. Immunohistochemical analyses of SS muscle demonstrated the presence of CD68+ macrophages, and CD3+ and CD8+ T cells. Proteomic analysis demonstrated the presence of inflammatory related proteins in the SS tendon and SS muscle.
DISCUSSION: We have shown that not only the tendon becomes inflamed following RC tendon tears but also the SS muscle shows sign of inflammation. Chronic inflammation differs between tendon and muscle and between muscles, which suggests that the pathophysiological mechanisms taking place in RC muscles may be a major contributor to RC disease.

Poster Session 11

Skeletal Muscle Structure and Function

... P11-1

The active force-length relationship of skinned skeletal muscle fibres during extensive eccentric contractions

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QUESTIONS: In contrast to experimentally observed progressive forces in eccentric contractions, cross-bridge and sliding-filament theories of muscle contraction predict that varying myofilament overlap will lead to increases and decreases in active force during eccentric contractions. Non-cross-bridge contributions potentially explain the progressive total forces.
However, it is not clear whether underlying abrupt changes in the slope of the nonlinear force-length relation are visible in long isokinetic stretches, and in which proportion cross-bridges and non-cross-bridges contribute to muscle force.
METHODS: We performed in-vitro isokinetic eccentric experiments of calcium-activated single skinned fibres from rat extensor digitorum longus muscles with extensive magnitudes of stretch. Fibres were stretched from initial fibre lengths L_i of 0.7, 0.85, 1.0 L_0 with constant stretch amplitude of 0.45 L_0. To investigate cross-bridge and non-cross-bridge forces, the actomyosin-inhibitor 2,3-butanedione monoxime (BDM) has been applied to the muscle fibres performing the same stretch protocol as described above for L_i of 0.85 and 1.0 L_0.
RESULTS: Here we show that maximally activated single-skinned muscle fibres behave (almost across the entire working range) like linear springs despite strongly changing underlying isometric forces. The BDM-experiments revealed a nonlinear progressive contribution of non-cross-bridge forces and suggest a nonlinear cross-bridge contribution similar to the active force-length relation.
CONCLUSIONS: The observed results support the idea of a cumulative mechanism that combines nonlinear cross-bridge and non-cross-bridge effects to result in a linear force response during active muscle stretch. This linear muscle behaviour might significantly reduce the control effort in biological locomotion. Moreover, the observed slight increase in slope with initial length is in accordance with current models attributing the non-cross-bridge force to titin.

... P11-2

Proteomic characterization of murine muscle fiber types via lasermicrodissection and LC MS / MS mass spectrometry

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QUESTIONS: Skeletal muscles are composed of different fiber types known as type I and type II fibers. Type II fibers can be divided into three subclasses, IIa, IIb and IIx fibers. All can be distinguished via specific myosin isoforms. The fiber type composition of various muscles is differing and can change due to aging or training. These shifts can also be observed in several neuromuscular diseases and could play a role in the progression of the disease itself. Subclass specific analysis of fibers enables a more accurate and detailed analysis of aging, gender or disease related effects.
METHODS: For a precise differentiation of fiber types and their isolation from complex muscle tissue a sophisticated method was established combining fiber type specific immunostaining, laser microdissection and mass spectrometry. Soleus muscle was cryosected and stained with myosin isoform specific antibodies. Fiber types were dissected, lysed, tryptically digested and analysed via LC-MS/MS. We initially used the established workflow in order to elucidate a general influence of gender and age on the protein composition of different muscles in wildtype mice.
RESULTS: First results on gender dependent proteome pattern in different fiber subclasses demonstrate the applicability of this method. Over 1000 proteins could be identified in type I fibers of male and female mice. In total 24 proteins could be identified as interesting candidates for being differently expressed in one of both sexes. E.g. proteins associated with cell stress could be identified as higher abundant in female murine muscle samples. Studies with larger cohorts are still ongoing to strengthen the preliminary findings.
CONCLUSIONS: Our results outlined gender differences on skeletal muscle fiber level. Further on we aim at the identification of differences in the protein pattern in a knock-in mouse strain harboring the most common mutation causing desminopathy in order to increase our knowledge about in disease underlying molecular pathomechanisms.

... P11-3

Assessment of the relation between produced force and way of recruiting muscle fibres to achieve this force in humans

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QUESTION: With the hypothesis that frequency of fibre contraction and spatial summation of fibre contraction together will describe a given obtained force, the aim of this study was to look at the frequency and the amplitude of contraction of a forearm muscle, *m. palmaris longus,* expressed by the S- and the T-score measured with acoustic myography, and relate these parameters to recorded force when pressing a hand dynamometer.
METHODS: 12 healthy subjects had their *m. palmaris longus* assessed by acoustic myography (AMG), using a Curo device, during force measurements with a hand dynamometer. Force production was varied from 10-90% of assessed maximal force. Muscle fatiguing was also followed to see, if the hypothesis would hold. AMG parameters determined were temporal and spatial summation during force production expressed by the S- and T-score of the ESTi Score. Linear regression analyses were applied to relate force production to spatial and temporal summation of muscle fibres in the involved muscle.
RESULTS: Our data does show that muscle strength is indeed sustained by either changing the frequency and/or changing the number of fibres which are active at a given time. This also is the case during fatigue.
CONCLUSION: AMG is a method which can be applied to describe how a subject uses a given muscle during a given movement, and it is useful in assessing muscle fatigue in a way which will help when considering training strategies in subjects with muscle trauma or disease, the elderly, or in athletes.
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P11-4

Structural changes in diaphragm fibers of critically ill patients

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BACKGROUND: Critically ill patients develop diaphragm weakness which leads to prolonged ventilator dependency. This weakness is, at least partly, caused by a lower maximal active force generated by individual diaphragm fibers of critically ill patients. Importantly, the force deficit remains after normalization of force to the reduced cross sectional area of diaphragm fibers in patients. This indicates that in diaphragm fibers of critically ill patients the functioning of myofilaments is altered.
Aim: To study whether structural changes in the myofilaments underlie the force deficit in diaphragm fibers of critically ill patients.
METHODS: Diaphragm biopsies were obtained from eight critically ill and seven control patients. Low angle x-ray diffraction experiments were conducted at the BioCAT beamline, Advanced Photon Source, Argonne National Laboratories. Individual muscle fibers were isolated from the biopsies and mounted in parallel between two halves of EM-grids (~28 fibers per grid). Sarcomere length was set at 2.5 µm and the grids were mounted between a force transducer and a length motor. Diffraction patterns were recorded during inactive and active conditions with 10ms x-ray exposure time. Data was collected with a Pilatus 3x M1 detector set at 3 meter distance from the specimen.
RESULTS: Preliminary results show that 1.1/1.0 intensity ratio is reduced in critically ill patients compared to controls (1,36 ± 0,08 vs. 0,73, respectively), suggesting that in patients the myosin heads are in closer vicinity to the thick filaments. The myosin-based M3 (14,51 nm vs. 14,47nm, respectively) and M6 spacings (7,263 nm vs. 7,251nm, respectively) are reduced in critically ill patients, suggesting structural changes in the thick filament.
CONCLUSION: Our pilot data suggest that structural changes in the thick filament develop in diaphragm fibers of critically ill patients. These changes might underlie diaphragm weakness in these patients and might contribute to weaning failure.

P11-5

Titin N2A behavior during passive and active stretch in mouse EDL muscle

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Investigation into titin's elastic behavior has accelerated in recent years, in part due to increasing evidence for an active (calcium dependent) role for this molecular spring in muscle. Despite this, most details of titin activation remain unclear including location, mechanism and functional relevance. A recently described mechanism offers insight into these details, by proposing that titin's N2A region is capable to binding to actin filaments with calcium. This titin-actin interaction shortens titin's proximal segment, storing elastic energy in the process.
QUESTION: Does titin's proximal segment elongate differently during passive and active stretch?
METHODS: We fluorescently labeled the N2A region of titin and observed changes in epitope movement during passive (low calcium) and active (high calcium) stretch using mouse extensor digitorum longus (EDL) myofibrils. The N2A antibody separates titin's I-band region into the proximal immunoglobulin (Ig) and distal PEVK segments, which were quantified to evaluate whether calcium introduction affected segmental elongation. The preparations were stretched passively and actively on a myofibril testing apparatus, and titin's behavior with stretch was visualized.
RESULTS: Myofibril proximal segments only achieve 60% of their segment length during active stretch (n = 1), when compared to passive stretch (n = 4). The active myofibril showed consistent shortening of titin's proximal segments for all six active sarcomeres, which implies that little straightening of proximal Ig domains occurred with calcium addition.
CONCLUSIONS: The limited N2A mobility with calcium supports a titin-actin binding mechanism and has the potential to explain active force enhancement known to be calcium and cross-bridge dependent. These investigations offer new insights into titin's dynamic role in muscle contraction, suggesting there is more than a passive role for titin in muscle contraction.

P11-6

Rigor bonds under denaturing conditions reduce isometric tension development and lattice spacing of skinned porcine skeletal muscle fibres

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QUESTIONS: Rigor cross-bridge attachment and low pH at body temperature occur initially during rigor onset in muscle and are involved in myofibrillar protein denaturation. From a meat science perspective, they affect the sarcomeric water holding and the meat quality. It has been shown that pH < 6.0 at a temperature > 37°C is critical, although the effects of the rigor attachment are unknown. One major consequence of the denaturation is reduced myofibrillar lattice spacing accompanied by relocation of water from inter- to extra-filamental space. The aim of this study was to test the hypothesis that during the denaturation conditions, rigor cross-bridge attachment can affect the sarcomeric structure.
METHODS: We established a new model using permeabilised/skinned porcine *longissimus dorsi* muscle fibres to study the effects of varied pH, temperature and the presence of rigor bonds on the myofibrillar structure. The preparations were exposed to 30 min with different conditions followed by evaluation at normal pH (7) and temperature (22°C) of: 1) the isometric contractile force, and 2) myofilament lattice spacing using small angle x-ray diffraction.
RESULTS AND CONCLUSIONS: Our data show that incubation at 38°C, pH 5.5 under rigor conditions reduce the lattice spacing by 20%, accompanied by 50% loss of active force, while treatment under relaxed conditions gave unchanged lattice spacing and force. The present study suggests that rigor bond attachment under denaturing conditions leads to a compressed interfilamental space, loss of sarcomeric water and affected contractile function.

P11-7

Immobilization during muscle development reduces muscle strength and lattice spacing

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Immobilization of skeletal muscle occurs during several physiological and clinical conditions, and can be associated with muscle weakness. The mechanisms and cellular signaling involved in the loss of force are not fully understood, although the mechanical activity of the muscle sarcomere appears to be important for developing and maintaining the structure/function of the contractile system. Larger animal models cannot easily be applied to study these processes, due to potential effects on whole animal physiology. The aim of our study was to establish an immobilization model in zebrafish larvae (<6 days old), where active swimming movements are not required for food intake. We applied a pharmacological approach (BTS) to block actin-myosin interaction in the skeletal muscle to keep the larvae fully immobilized up to 6 days post fertilization (dpf). In freely moving larvae, active force (single twitch stimulation at optimal length) increased gradually from day 4 to 6. In immobilized larvae, force (immediately after washout of BTS) was significantly lower and failed to increase during development. Removal of the BTS and active swimming for 1-2 days led to a partial recovery of active force. The mRNA for Muscle Ring Finger proteins (Murf 1-3) and Atrophy gene-1/muscle atrophy F-box (Atrogin-1/MAFbx) increased with development (days 4-6, using RT-qPCR) with Murf 1 being the most abundant. After immobilization the expression of these components was lower, although the Murf 1 tended to be increased early during the immobilization. Analysis of interfilament spacing using small angle x-ray diffraction showed that the spacing was decreased following immobilization. The results show that active muscle contraction is required for establishment of the functioning contractile system with adequate filament distances, and that Murf signaling is involved linking the mechanical state to the structure.

P11-8

Nebulin"s C-terminus regulates muscle structure, function, and growth

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The filamentous protein nebulin has been found to play a complex role within the muscle sarcomere. Studies in nebulin knockout models have revealed a vital role in thin filament length regulation, force generation, cross-bridge cycling, and the lateral alignment of the sarcomeres. To better understand how nebulin accomplishes its many functions, it is necessary to study the protein through its different domains. We generated a mouse model that deletes the serine-rich region and SH3 domain, nebulin"s C-terminal domains located within the Z-disc. This deletion produces a truncated nebulin that causes developmental defects in the mice, resulting in a smaller mouse with weight deficits in several limb muscles. Despite this, nebulin is localized properly and thin filament lengths are not changed, though lateral alignment is affected. We also observed structural abnormalities and force deficits reminiscent of a less severe nemaline myopathy phenotype. In studying binding partners of the C-terminus, we found novel binding partners to the serine-rich region that may contribute to the phenotype observed. Additional studies into muscle hypertrophy using an IGF-1 AAV suggest that the loss of the C-terminus could prevent muscles from responding as efficiently to efforts at restoring muscle

mass. Through these studies we conclude that nebulin"s C-terminal domains are vital to several of the protein"s key functions, especially regulating an organized and functional sarcomere.

... **P11-9**

Structural studies of human ZASP in complex with α-actinin 2

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The Enigma family proteins are specific for heart and skeletal muscle and are located at Z-discs. It is one of the prevailing protein families involved in dilated cardiomyopathies (DCM). ZASP (Z-band alternatively spliced PDZ-motif protein) belongs to this family and it is implicated in 1-4% of DCM (Avanika, 2012; Lin, 2014). ZASP is expressed in the early stage of myofibrillogenesis and it acts as a mediator between cytoskeletal elements and signaling cascades. The presence of ZASP in the Z-disc is essential for integrity of the sarcomere during contraction. In the Z-disc, ZASP interacts with various binding partners (Au, 2004; Ming Zheng, 2009), in particular with α-actinin 2 (ACTN2), which is one of the most abundant protein in the Z-disc, designed to cross-link actin filaments. Because of its direct implication in signaling pathways (Lin, 2013), ZASP could have a regulatory role on association of ACTN2 with other binding partners. In our study we wanted to explore the function and dynamics of ACTN2-ZASP complex formation *in vitro* combining different biophysical, biochemical and structural techniques such as pull-downs, cross-linking coupled with mass spectrometry (XL-MS), small angle X-ray scattering (SAXS), crystallography, microscale thermophoresis (MST), and SEC-MALLS. Previously, it has been shown that ACTN2 can bind to some of the Z-disc proteins, in particular titin, only after activation by the phospholipid PIP2 (Young, 2000; Ribeiro, 2014). The binding triggers a conformational rearrangement, generating an open conformation of ACTN2. Here, we show that ZASP can interact with both of the conformations of ACTN2. In addition, we showed by MST that ZASP binds ACTN2 with nanomolar affinity, which makes the complex amenable for crystallization and subsequent X-ray diffraction experiments. Moreover, preliminary XL-MS analysis delineated the binding sites of ZASP on ACTN2, providing constrains for modeling of ZASP-ACTN2 complex using the data obtained by SAXS. We also show that ZASP and ACTN2 can form a stable ternary complex with the Z-disc protein FATZ-1. Further studies will provide insights into formation synergies of the complex ZASP-ACTN2-FATZ-1 formation and its organization at molecular level, which might help to reveal essential implication of ZASP in the formation of the Z-disc.

... **P11-10**

Features of tension rise in fast and slow skeletal muscles of the rabbit

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The contractile activity of skeletal muscle is determined by the isoform composition of myosin heavy chains (MHC) and regulated by the level of intracellular calcium. Fibers containing the slow (I) MHC isoform have a slower rate of shortening than fibers expressed fast (IIa, IIb and IId/x) MHC isoforms.

We studied the significance of MHC isoforms for the Ca^{2+} regulation of skeletal muscle contraction in the experiments on skinned rabbit fibers with Joule temperature jump (T-jump) (Bershitsky & Tsaturyan J. Physiol., 2002) and on isolated proteins in an *in vitro* motility assay (IVMA). MHC from fast *m. psoas* were predominantly IIx and IIb isoforms, the I isoform of MHC was in fibers from slow *m. soleus*. Thin filaments were reconstructed from skeletal actin, troponin and tropomyosin. In IVMA, with myosin from *m. psoas* and *m. soleus*, regulatory proteins from *m. psoas* and *m. soleus*, respectively, were used.

At T-jump from 5°C to 30°C, tension of fast and slow fibers increased about 2.8 and 2.5 times, respectively. Surprisingly stiffness of slow fibers increased about two-fold while stiffness of fast fibers rose by only 15% as early it was shown for fast fibers (Huxley & Brown, J. Mol. Biol., 1967). At increasing of temperature the Ca^{2+} sensitivity of the fiber tension increased.

Experiments in IVMA were carried out at 21°C to 30°C, since at lower temperatures we would not be able to measure the filament sliding velocity at low Ca^{2+}. At increasing temperature, maximal filament sliding velocity over myosin from *m. psoas* and *m. soleus* increased 2.5 and 5 times, respectively. Thus, the temperature sensitivity of the contractile apparatus of slow muscle is higher than fast one, which agrees with the data obtained earlier (K. W. Ranatunga, Biophys, J., 1996).

We can assumed that difference of contractile activity and its Ca^{2+} regulation of fast and slow skeletal muscles determined by cooperative mechanisms of the actin-myosin interaction and kinetics of cross-bridge. Supported by grant of RSF No. 16-14-10044.

... **P11-11**

The rate of force generation in skeletal muscle is limited by the stress-dependent kinetics of the OFF-ON transition of the myosin-containing thick filament

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Recent studies in skeletal muscle fibres using bifunctional rhodamine (BSR) probes on the C-lobe of the regulatory light chain (RLC) of myosin showed that transition of the myosin motors from the OFF to the ON state at different $[Ca^{2+}]$ is controlled by thick filament stress (Fusi *et al., Nat Commun* 2016, doi:10.1038/ncomms13281). In this work we investigated the kinetics of the OFF-ON transition of the myosin motors and troponin during active force development triggered by a rapid ($>10^4$ s^{-1}) increase in $[Ca^{2+}]$ following UV-photolysis of caged-calcium (NP-EGTA), under conditions which preserve the physiological resting structure of the thick filament (T=25°C, 5% Dextran, 2.45 μm sarcomere length). We used polarized fluorescence to monitor changes in the orientation of BSR probes labelling the E-helix of RLC and the C-helix of troponin-C (TnC) exchanged into demembranated fibres from rabbit psoas muscle. After photolysis of NP-EGTA force increased with a sigmoidal time course and an initial lag of ~10 ms. The change in the order parameter $\langle P_2 \rangle$ of the RLC probe, associated with it becoming more perpendicular to the fibre axis, also had a sigmoidal time course. The $\langle P_2 \rangle$ change showed an initial lag of ~10 ms and was complete by ~35 ms after photolysis, when force development was only 60% complete. In contrast, changes in $\langle P_2 \rangle$ of the TnC probe were almost complete within the 10ms-force lag. After photolysis of NP-EGTA at 11°C, a temperature at which most of the myosin motors are in the ON state even at low $[Ca^{2+}]$, force rose with no lag; under these conditions the $\langle P_2 \rangle$ change of the RLC probe was smaller with slower kinetics, whereas that of the TnC probe was almost complete within 10 ms. These results suggest that the rate of force generation is limited by the kinetics of the OFF-ON transition of the thick filament and that the lag at the start of activation is associated with the positive feedback loop of thick filament mechano-sensing. Supported by MRC, UK.

... **P11-12**

Lack of desmin in normal human extraocular muscle fibers: a complex relation to innervation

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QUESTIONS: Is there a relation between motor endplates and the lack of desmin previously found in a subgroup of muscle fibers containing myosin heavy chain slow tonic (MyHCsto) in human extraocular muscles (EOMs)?

METHODS: Twenty EOMs collected from 11 healthy subjects (42-82 years old) were processed for immunohistochemistry with antibodies against desmin, MyHC isoforms and markers of motor endplates and nerve axons.

RESULTS: A novel type of multiple motor endplates was found in the human EOMs, in addition to the previously well-known single en plaque motor endplates of fast and slow myofibers and multiple en grappe endplates of myofibers containing MyHCsto. This novel type of multiple endplates consisted of several typical large en plaque motor endplates present along long segments of individual fibers lacking MyHCsto, slow and fast. Desmin was either present or absent in these myofibers with multiple en plaque endplates. Desmin was absent in a short segment in the vicinity of typical single en plaque endplates of fast and slow myofibers. Desmin was also absent in myofibers containing MyHCsto, which typically have en grappe endplates. Finally, desmin was present in myofibers receiving palisade endings, near the tendons.

CONCLUSIONS: Our data indicate that an additional type of multiply innervated myofiber lacking MyHCsto, slow and fast, exists in the human EOMs. Furthermore, the cytoskeletal organization of the endplates in the human EOMs differs fundamentally from those of limb muscles, where desmin is enriched at the neuromuscular junctions.

... **P11-13**

A role for titin in the activation-dependent shift of the force-length relationship in skeletal muscle

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Muscle function during submaximal activation is seldom studied, yet is more similar to *in vivo* muscle function than maximal activation. For skeletal muscle, the force-length relationship shifts to longer lengths in submaximal, compared to maximal, activation conditions. Length-dependent calcium effects, specifically an increase in calcium sensitivity with increasing sarcomere length, have historically been suggested as the cause of this shift in the force-length relationship. Recent evidence suggests that the titin protein may also play a role in activation-dependent muscle properties through structural re-arrangement of the thick filament. To evaluate a possible role for titin, we studied muscles from mice carrying the muscular dystrophy with myositis (*mdm*) mutation, which have a small titin deletion in the I-band region. For *mdm* and wild type muscles and fibres, we measured the force-length relationships during maximal (tetanus) and submaximal (twitch) activation. We then used skinned fibers to evaluate the length-dependence of calcium sensitivity. Our results demonstrate that in contrast to wild type muscles and fibers, which show a shift to longer optimal lengths during twitch compared to maximal contractions, the force-length relationship was the same for twitch and maximal contractions in *mdm* muscles and fibers. However, the length-dependence of calcium sensitivity was identical in *mdm* and wild type fibers, suggesting that mechanisms other than calcium sensitivity are responsible for the activation-dependent shift in the force-length relationship. In conclusion, the titin deletion in *mdm* mice does not impact length dependent calcium sensitivity, but possibly impacts activation-dependent optimal length by a currently unclear mechanism.

... P11-14

Towards a reconstitution of the sarcomeric Z-body: a possible strategy to assemble a mini Z-disk

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The sarcomeric Z-disk defines the flanking border of the sarcomere and is a highly complex macromolecular assembly composed of hundreds of different proteins. Its formation during the myofibrillogenesis is believed to start with small building blocks, the Z-bodies. Despite rigorous structural, genetic and biochemical research in the last decades on the Z-disk, the interaction and assembly properties of the Z-body is still poorly understood. So far six proteins have been found to co-localize in the early stage of myofibrillogenesis, when the Z-bodies are formed: actin, α-actinin-2, filamin-C, FATZ-1, ZASP, and myotilin [Wang et al., *Cell Motil Cytoskeleton* 2005; Sangert et al., *J Biomed Biotechnol* 2010]. All of them are also present in the mature Z-disk but no structural information on binary and ternary complexes and not to mention of a fully assembled Z-body is known to date.
In order to elucidate the structural organization of Z-bodies, as well as address the questions of stoichiometry and hierarchy of assembly, we are establishing two strategies for reconstitution of the Z-bodies containing α-actinin-2, filamin-C, FATZ-1, ZASP, and myotilin by using: (i) an *in vivo* co-expression approach in insect cells and (ii) an *in vitro* assembly with the individual full length proteins or tailored constructs produced in *Escherichia coli* and insect cells. Here we show the cloning strategy and co-expression experiments, demonstrating that the core-particle (without actin) can be expressed together in baculovirus-infected insect cells. In addition, we show for the first time the expression in insect cells of the biggest component, filamin-C. Analysis of the purified protein by SEC-MALS reveals a molecular weight of 580 kDa, which matches its dimeric state, and can be visualized by rotary shadowing electron microscopy. We are currently building the Z-bodies using α-actinin-2 as an assembly platform, capitalizing on our previous knowledge on the individual binary affinities between the components.

... P11-15

Structural insight into the myotilin-actin interaction

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Although all types of muscle cells use actin and myosin for contraction, only in skeletal and cardiac muscle these proteins are organized into sarcomeric units. These units are delimited by Z-discs, which are intricate webs of various proteins including α-actinin-2, F-actin and myotilin, the last one being at the focus of this research. Myotilin consists of two immunoglobulin-like domains (Ig1, Ig2) flanked by a unique serine-rich N-terminus and a short C-terminal tail and interacts with F- and G-actin, α-actinin-2, ZASP, FATZ and filamin C. In order to gain insight into its remarkable interaction capabilities we here investigated its actin-binding properties from the structural point of view.
First, we determined binding affinities for interaction of different myotilin constructs with G-actin using microscale thermophoresis, all showing relatively weak binding in the micromolar range. Binding to F-actin was assessed with actin co-sedimentation assays, showing that Ig2 represents the main point of interaction and that Ig1 may only play a supplementary role.
We additionally confirmed binding of myotilin to G- and F-actin using chemical cross-linking coupled with mass-spectrometry, which enabled us to identify amino acid residues flanking the interaction interface. Furthermore, 15N-HSQC titration NMR experiments were performed for Ig1, Ig2 and both domains in tandem, allowing us to further map the binding sites of F-actin on myotilin to one side of the Ig-domain β-sandwich. Subsequent mutagenesis of single residues at one or more of these binding sites diminished binding to F-actin in a dose-dependent manner. This gradual loss of binding capacity was evidently corroborated by *in vivo* FRAP experiments using C2C12 skeletal muscle cells, showing similar increased dynamics of mutant myotilin in Z-discs.
Based on these experimental data and observations published previously, we propose a model in which each Ig-like domain of myotilin specifically binds to neighboring subunits of the actin filament.

... P11-16

Mass spectrometry-based protein identification to understand the proteomic signature of human skeletal muscle

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Skeletal muscle plays a crucial role in locomotion and metabolism and constitutes approximately 40% of human body mass. It is, therefore, necessary to have a precise knowledge of its molecular signature to fully understand its role in health and disease. Proteomics is a powerful tool for cataloguing and quantifying proteins of a variety of in vitro systems and organs including skeletal muscle. However, given the high abundance of specific proteins, such as structural components and metabolic factors interfering with identification of low-abundant proteins, proteomics is not without its limitations.
In order to unravel the proteomic signature of human skeletal muscle and strengthen knowledge of its precise protein composition, we utilized human myoblasts, myotubes and skeletal muscle tissue. Different fractionation approaches were then applied at peptide (high pH fractionation) and protein (based on molecular weight) as well as organelle level (enrichment of nuclei, membrane proteins, sarcoplasmic proteins and cytoskeletal proteins). Tryptic peptides derived from all approaches were measured using state-of-the art MS workflows which led to the identification of the 10,000 most abundant proteins. We obtained relative abundances for proteins expressed in human muscle cell lines and skeletal muscle – which should serve as a valuable resource for disease diagnosis – and focused on protein signature changing during muscle cell differentiation. Additionally, we compared the protein composition in differentiated human muscle cells and complex tissue and hereby focus on the coverage of cellular processes and organelle protein composition. Our combined data represents the most comprehensive protein catalogue of human skeletal muscle described thus far and by the same token provide a deeper knowledge of its molecular composition.

... P11-17

Identification of protein interaction pathways and partners of Klf5 in myoblast differentiation program

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Klf5 is a transcription factor with a ubiquitous expression in early embryonic stem cells as well as adult somatic epithelial tissue. The function of Klf5 is diverging in a context dependent mode in cells and tissues. The diversity of the DNA binding properties and protein interaction partners of Klf5 depicts that it may exert both common and opposing actions on different tissues and cells in a context dependent way. Due to this, it is not possible to attribute a cellular function to Klf5 in a certain cell and context by it"s action on another cell or tissue type. We have identified Klf5 as an indispensable transcription factor that is required for the expression of myogenin during myogenic differentiation program.
Klf5 is a transcription factor with basal expression in skeletal muscle stem cells and fibro-adipogenic precursors. Thus, the role and function of Klf5 must be further dissected in this context to include both cell populations that are counteracting during regeneration and degeneration (fibrosis). We previously have utilized genome editing tools to generate a targeted insertion in the Klf5 gene to enable further investigation of the protein product for functional studies. We intended to use this approach to identify the protein-protein interaction partners and the post-translational modifications of Klf5. We further validated these findings using a high-throughput proximity proteomics approach which – to our knowledge - is to be employed for a transcription factor for the first time.
The functional analysis of protein interaction partners of Klf5 helped to illuminate novel pathways and partners that are novel to skeletal muscle differentiation program. We further have evaluated the interaction of Klf5 with known soluble cues and pathways shown to be important in other cell and organ systems including cytokines and Wnts.

Poster Session 12

Stem Cell Derived Myocytes and Experimental Genome Editing

... P12-1

Do β-Myosin heavy chain isoform-expressing myofibrils within human ESC-derived cardiomyocytes recapitulate the contractile features of adult human ventricular myofibrils?

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The aim of the present study was to understand whether β-myosin heavy chain isoform-expressing myofibrils within human embryonic stem cell-derived cardiomyocytes (hESC-CMs) do recapitulate the contractile function of the adult human ventricular myofibrils (hvMFs) isolated from donor hearts.

We have identified principal sarcomeric protein isoforms involved in the modulation of the force development and analyzed the steady-state and kinetic parameters of the isometric forces generated by the myofibrils within single demembranated hESC-CMs and by small hvMFs bundles using the same micromechanical method.

Our results indicated that at saturating Ca^{2+} concentration, both hvMFs and myofibrils within hESC-CMs developed force with similar kinetics, but maximum isometric force was smaller for myofibrils of hESC-CMs than for hvMFs. At submaximal Ca^{2+} activation force levels, where intact cardiomyocytes normally operate, contractile kinetic parameters of demembranated hESC-CMs and hvMFs exhibited differences, which can be attributed to a sarcomeric protein isoforms pattern of hESC-CMs specific to an early developing stage rather to the adult form of human ventricular cardiomyocytes.

Myofibrils within hESC-CMs only partially recapitulate the contractile features of adult hvMFs.

We highlight the importance of correlating sarcomeric protein isoforms content with the contractile function of myofibrils within human stem cell-derived cardiomyocytes differentiated *in vitro*, because their morphological maturation may not necessarily determine and parallel the adult ventricular-like contractile function at myofibrillar level. Human stem cell-derived cardiomyocytes at an early developing stage could provide the basis for extended studies with patient-specific cardiomyocytes carrying for instance, familiar hypertrophic cardiomyopathy-related missense mutations in sarcomeric proteins, addressing questions related to the onset of the disease during early developmental stages.

... P12-2

Subdividing porcine satellite cell subpopulations with discontinuous gradient density centrifugation

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Satellite cells (SC) and their progeny are responsible for postnatal muscle growth, regeneration and muscle plasticity. Their involvement in a wide range of myogenic processes corresponds to a high heterogeneity of SC biochemical and functional properties. As separation of SCs is challenging, functional divergent SC subpopulations are poorly analyzed and characterized. Here, we used Percoll density centrifugation to separate distinct SC/myogenic precursor cell (MPC) subpopulations.

For this aim, we isolated SC/MPCs from muscles of 4-day-old piglets by digestion with trypsin (0.25%) followed by discontinuous Percoll density gradients. Two subpopulations were isolated from the 40/50% and 50/70% layers of a 25%, 40%, 50% and 70% Percoll gradient, and compared with the total myogenic population obtained from the 40/70% interface of a 25%, 40% and 70% Percoll gradient. For analysis of growth kinetics (adhesion, proliferation) the xCELLigence system was used. In addition, myogenic marker expression was investigated by flow cytometry and differentiation assays were performed for functional characterization.

All populations showed a myogenic phenotype characterized by the ability to proliferate, to differentiate and to form myotubes. However, the isolated subpopulations exhibited distinct functional behavior, and a fast adhering and proliferating subpopulation (SPS) could be separated from a considerable slower adhering and proliferating subpopulation (SPF). Desmin expression did not differ between the subpopulations but higher Pax7 levels were found in the SPF population.

Our study illustrates that discontinuous Percoll density centrifugation is suitable for subdividing SC populations with divergent myogenic functions. With this method SC heterogeneity can be analyzed to a greater extent and this knowledge will broaden our understanding about muscle growth variations in pigs and most likely in other animals.

... P12-3

A porcine model for the ß-myosin mutation R723G suggests onset of hypertrophic cardiomyopathy during fetal development

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QUESTIONS: Familial Hypertrophic Cardiomyopathy (HCM) is the most common inherited cardiac disease. About 30% of the patients are heterozygous for mutations in the *MYH7* gene. This gene encodes the ß-myosin heavy chain (ß-MyHC), the motor protein in the sarcomeres of the human heart. Hallmarks of HCM are cardiomyocyte disarray, interstitial fibrosis and hypertrophy of the left ventricle. Etiology and pathology are largely unknown and mouse models cannot fully mimic the human disease. Therefore a large animal model for HCM has been postulated.

RESULTS: The domestic pig is a well-defined model organism for human heart diseases. Using TALEN-mediated genome editing we have successfully introduced the orthologous HCM-point mutation R723G in the *MYH7* gene of porcine fibroblasts. No selection marker or additional sequence was added, to preserve the native porcine *MYH7*-gene. These cells were used to clone pigs that are heterozygous for the HCM-mutation R723G. Most interestingly, the neonatal pigs displayed features of HCM, including mild myocardial disarray, malformed nuclei, and *MYH7* over-expression. The mutated mRNA and protein was expressed but at very low levels. In line with this, the force generation of cardiomyocytes was essentially unaffected. Surprisingly, the animals die within 24 h *post partem* despite the absence of off-target effects of TALEN-induced genome editing. Future studies on further genome edited pigs with this and other HCM-mutations will have to reveal whether this is directly linked to the mutation.

CONCLUSIONS: Here we report on the first genome edited pigs that encode for the HCM-mutation R723G. The finding of HCM specific pathology in the neonatal R723G-piglets indicates a much earlier onset of the disease than previously determined for this mutation and highlights the importance of novel large animal models for studying causative mechanisms and long-term progression of human cardiac diseases.

... P12-4

Effect of a recombinant protein derived from a 6 EGF-like domain within delta-like 1 homology (DLK1) on inhibition of myogenesis in mouse myoblast cells

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QUESTIONS: Muscle stem cells (MSCs) known to be involved in muscle regeneration are considered as cell-mediated therapeutic source for congenital or acquired muscle diseases. However, there have been lots of limitations in the application of MSCs as cell-mediated therapeutic tool because of the absence of *in vitro* culture system producing massively undifferentiated MSCs showing rapid differentiation into myogenic cell lineages *in vitro*. Recently, 6 EGF-like domains within delta-like 1 homolog (DLK1) were known to influence on inhibition of myogenesis. Accordingly, we hypothesized that 6 EGF-like domains within DLK1 might effectively stimulate self-renewal and inhibit myogenic differentiation during *in vitro* MSC culture. Therefore, we synthesized a recombinant protein including 6 EGF-like domains and their inhibition effects on myogenic differentiation was evaluated in C2C12 cells, myogenic progenitor cells, prior to application to MSCs.

METHODS: The cDNA encoding 6 EGF-like domains was derived from preadipocytes and inserted into a pET-22b (+) expression vector. Subsequently, a recombinant protein including 6 EGF-like domains was synthesized through vector expression and protein purification and lyophilization. C2C12 cells were differentiated into myogenic cell lineage in myogenic differentiation medium supplemented without or with a recombinant protein including 6 EGF-like domains, and MSC- and myogenic cell lineage-specific markers were analyzed transcriptionally.

RESULTS: During induction of differentiation, C2C12 cells stimulated by a recombinant protein with 6 EGF-like domains showed stronger expression of *Pax7* and *Myf5*, MSC-specific markers, and weaker expression of *MyoD, Myogenin, MyHC* and *Desmin,* myogenic cell lineage-specific markers than non-stimulated C2C12 cells.

CONCLUSIONS: We identified that myogenesis of mouse myoblast cells could be inhibited by a recombinant protein derived from 6 EGF-like domains within DLK1.

... P12-5

Is myosin VI playing a role in myotube formation *via* its involvement in cell adhesion?

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Myosin VI (MVI), one of unconventional myosins, is involved in numerous cellular processes associated with the actin cytoskeleton. We have previously shown that it is also expressed in the muscle fiber where it localizes to the muscle nuclei, sarcoplasmic reticulum and neuromuscular junction [Karolczak et al. 2013]. Moreover, our data gathered on C2C12 myoblasts suggest that MVI could be involved in myogenesis [Karolczak et al. 2015]. To address mechanism(s) of involvement of MVI is myoblast differentiation, we obtained a primary myoblast culture from hind limb muscle of adult *SV* mice (*Snell"s waltzer* mice; MVI-KO) that do not synthesize MVI. and of control littermates (WT mice). By means of light and confocal microscopy, we observed profound changes in the morphology and cytoskeleton organization of MVI-KO myoblasts with respect to WT cells. What is more, MVI-KO myotubes were developing in a different way than the control ones, with a fraction resembling a myosac-like morphology. Immunoblotting analysis revealed not only a change in the levels of synthesis of numerous proteins involved, among others, in cell adhesion such as talin, tensin and focal adhesion kinase but also a shift in timing of expression of Pax3 and Pax7. In line with these data is observation that organization of adhesive structures was also altered, both in the myoblasts and myotubes. Interestingly, we have already identified talin as an interaction partner for MVI in C2C12 myoblasts [Karolczak et al. 2015]. Thus our data indicate the interaction of MVI with proteins involved in adhesive contacts formation could play a role in myoblast differentiation into myotubes.

Karolczak et al. (2013) Histochem Cell Biol 139:873-885
Karolczak et al. (2015) Histochem Cell Biol 144:21-38

Myosin VI, skeletal muscle, myotubes, myogenesis

... P12-6

The effect of different mechanical loading protocols on differentiated H9C2 cells

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QUESTIONS:
Cardiomyocytes are sensitive to mechanical loading, possessing the ability to respond to mechanical stimuli by reprogramming their gene expression. In this study, signaling as well as expression responses of myogenic, anabolic, inflammatory, atrophy and apoptotic genes to two different mechanical stretching protocols were examined in differentiated cardiomyocytes.
METHODS: H9C2 cardiomyoblasts were cultured on elastic membranes and after reaching ~80% confluence they were differentiated into myotubes for 7 days. Then, they were subjected to cyclic stretch using a cell tension System (FX-5000, Flexcell International). Two stretching protocols were used: the short term (ST, 12.7% elongation, 0.5Hz, 15min) and the long term (LT, 2.7% elongation, 0.25Hz, 24h). Real-Time PCR was used to monitor the changes in mRNA expression of the IGF-1 isoforms (IGF-1Ea, IGF-1Eb), myogenic regulatory factors (MyoD, MyoG, MRF4), as well as pro-apoptotic (Foxo, Fuca, p53), atrophy (Atrogin, Murf1, MSTN) and inflammatory factors (IL-6). Western blot analysis was used to evaluate the phosphorylation of Akt and ERK1/2 signaling proteins after the stretching protocols.
RESULTS: It was documented that the LT protocol resulted in increased expression of both IGF-1 isoforms as well as MyoD and MyoG. A downregulation of Foxo ($p<0.05$) and upregulation of p53 and IL-6 was also revealed after the LT protocol. The expression of MSTN, Atrogin, Murf1, as well as the phosphorylation of Akt and ERK1/2 were not significantly affected by any of the stretching protocols used.
CONCLUSIONS: The upregulation of IGF-1 isoforms and myogenic factors combined with the downregulation of pro-apoptotic factor Foxo after the LT protocol suggest that it may be more effective compared with the ST in inducing a hypertrophic response of cardiomyotubes. Further investigation is needed to define the potential role of the increased IL-6 and p53 expression in the enhancement of the hypertrophic response of cardiomyotubes.

... P12-7

Both autophagy and the ubiquitin-proteasome system contribute to titin turnover in stem cell-derived human cardiomyocytes

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Cardiac titin exhibits dramatic changes in the isoform expression pattern during perinatal heart development. Assays for titin isoform composition provide useful information about the maturation state of cardiomyocytes. Compared to human adult heart muscle, cultured human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CMs) appear to be exposed to increased titin turnover, as they exhibit strong titin degradation bands and may also express the Cronos titin isoform, which lacks the N-terminus of the TTN molecule. We sought to investigate mechanisms of titin degradation and turnover in cardiomyocytes. We hypothesized that the ubiquitin-proteasome system (UPS) and autophagy both contribute to these processes. To test this, we cultured hiPSC-CMs until titin was detectable on protein level and treated them either with a blocker of proteolytic activity of the 26S proteasome complex (MG132) or with an inhibitor of the vacuolar-type H^+-ATPase (bafilomycin A1). We quantified the expression of full-length cardiac isoforms (fetal N2BA and N2B), titin-degradation products (T2), and Cronos. We also performed time-resolved studies of titin ubiquitination and general autophagic flux in developing hiPSC-CMs. MG132- and bafilomycin-treated hiPSC-CMs showed a shift in the cardiac titin-isoform pattern to the full-length proteins. MG132-treatment caused a relatively fast increase manly in the expression of fetal N2BA titin and accumulation of ubiquitinated titin species, whereas bafilomycin-treatment led to a relatively slow decrease in T2/Cronos products by disrupting late stages of the autophagic flux in the cells. Collectively, our results demonstrate that both UPS and autophagy participate in titin turnover at different rates and stages and likely contribute to the constantly changing cardiac titin-isoform pattern in maturing hiPSC-CMs.

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Reference: Belz et al., Fiber Optic Biofluorometer for Physiological Research on Muscle Slices (2016). Proceedings of SPIE, Vol. 9702 in Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XVI

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