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**PROGRAM**

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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.

DFG
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Forschungsgemeinschaft

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We thank the following sponsors and exhibitors for their generous support:

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Conference Organization

Conference Host

European Society for Muscle Research (ESMR)
c/o Karolinska Institutet
Department of Physiology and Pharmacology
v Eulers v 8
SE 171 77 Stockholm, Sweden
http://www.esmr.org

Conference Chair

Prof. Dr. Wolfgang Linke
Ruhr University Bochum
Department of Cardiovascular Physiology
Universitätsstr. 150
44780 Bochum, Germany

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Olivier Cazorla Montpellier, France
Elisabeth Ester London, UK
Jolanta Redowicz Warsaw, Poland

Conference Secretariat

K.I.T. Group GmbH Dresden
Bautzner Straße 117–119
01099 Dresden
Phone: 0049 (0)351 4823733
Email: info@emc2017-potsdam.com
Web: www.kit-group.org
**General Information**

**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 #580 and #695).

... **By Plane**

From Berlin-Tegel and the Schönfeld Airport, you can easily reach 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 SOUTH: coming from the A8/A13/A12 (Leipzig, Dresden, Frankfurt/O.) to the A10, exit ‘Michendorf’.

... **By Water Taxi**

REACHING THE SEMINARIS SEE HOTEL 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

**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 Seminars See-Hotel. 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 €60 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.
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100-fold increase in throughput of cardiomyocyte calcium and contractility experiments
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Environmental control

Visit the IonOptix booth in the exhibition area to see a working system
### TUESDAY, SEPTEMBER 19, 2017

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<td>13:00</td>
<td>Registration opens</td>
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<tr>
<td>14:00–14:15</td>
<td>Welcome &amp; Introduction</td>
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<tr>
<td>14:15–16:00</td>
<td>Session 1 - Actin-Myosin Interactions, Novel Structural and Mechanical Aspects</td>
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<tr>
<td></td>
<td>CHAIRS: Malcolm Irving London, UK; Dilson Rassier Montreal, Canada</td>
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<tr>
<td></td>
<td>S1-1 Dilson Rassier Montreal, Canada</td>
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<tr>
<td></td>
<td>Single sarcomere mechanics and inter-sarcomere dynamics in skeletal muscle myofibrils</td>
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<td>14:15–14:35</td>
</tr>
<tr>
<td></td>
<td>S1-2 Malcolm Irving London, UK</td>
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<td></td>
<td>Regulate of contraction by thick filaments in skeletal muscle</td>
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<tr>
<td></td>
<td>15:00–15:05</td>
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<tr>
<td></td>
<td>S1-3 Daria Lopatinova Moscow, Russia</td>
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<tr>
<td></td>
<td>YOUNG INVESTIGATOR AWARD COMPETITION</td>
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<tr>
<td></td>
<td>Nucleotide-induced movements of essential light chain-1 in myosin sub-fragment 1 as studied by fluorescence resonance energy transfer (FRET)</td>
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<td>15:18–15:23</td>
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<td></td>
<td>S1-4 Manta Annula-Nayak Hannover, Germany</td>
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<tr>
<td></td>
<td>Regulatory light chain's role in fine-tuning myosin motor function</td>
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<td></td>
<td>15:36–15:41</td>
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<tr>
<td></td>
<td>S1-5 Masataka Kawai Iowa City, USA</td>
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<tr>
<td></td>
<td>Nebulin KO in mice causes a decrease in in-series stiffness of the thin filament and consequent decrease in force/cross-bridge in slow-twitch soleus muscle fibers</td>
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<td>15:42–15:59</td>
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<td></td>
<td>Coffee Break and Visit of Exhibitors</td>
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<tr>
<td>16:00–17:15</td>
<td>Debate Session I - Muscle Contraction: Is Titin Contributing Actively?</td>
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<td>DISCUSSANTS: Vincenzo Lombardi Florence, Italy; Julio Fernandez New York, USA</td>
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<td>MODERATOR: Miklos Kelkarmayer Budapest, Hungary</td>
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<tr>
<td>17:15–18:00</td>
<td>Keynote I Stefan Raunser Dortmund, Germany</td>
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<td></td>
<td>The power of cryo-electron microscopy: Structure of a human cytoplasmic actomyosin complex at near-atomic resolution</td>
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<td>CHAIR: Kristina Djinovic-Carugo Vienna, Austria</td>
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<tr>
<td>18:00</td>
<td>Welcome Reception, LOBBY OF SEMINARIS SEEHOTEL</td>
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### WEDNESDAY, SEPTEMBER 20, 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>08:30–10:15</td>
<td>Session 2 - Titin and its Binding Partners</td>
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<tr>
<td></td>
<td>CHAIRS: Olga Mayans Konstanz, Germany; Wolfgang Linka Bochum/Münster, Germany</td>
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<tr>
<td>08:30–08:42</td>
<td>S2-1 Antonio Sponga Vienna, Austria</td>
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<td></td>
<td>YOUNG INVESTIGATOR AWARD COMPETITION</td>
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<td></td>
<td>Structural and biophysical characterization of α-actinin-2 in ternary complex with FATZ-1 and Zq titin</td>
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<tr>
<td>08:42–08:47</td>
<td>Discussion</td>
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<tr>
<td>08:48–09:00</td>
<td>S2-2 Robbert van der Pijl Amsterdam, Netherlands</td>
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<td></td>
<td>YOUNG INVESTIGATOR AWARD COMPETITION</td>
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<tr>
<td></td>
<td>Diaphragm passive stretch induces hypertrophy, which is modulated by titin-based stiffness</td>
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<tr>
<td>09:00–09:05</td>
<td>Discussion</td>
</tr>
<tr>
<td>09:06–09:18</td>
<td>S2-3 Sandra Biald Bochum, Germany</td>
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<td></td>
<td>Loss of Z-disc anchored titin in adult skeletal muscle cells leads to sarco-merse disassembly</td>
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<tr>
<td>09:18–09:23</td>
<td>Discussion</td>
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<tr>
<td>09:23–09:35</td>
<td>S2-4 Henk Granzie Tucson, USA</td>
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<td>The giant elastic protein titin regulates the length of the striated muscle thick filament--titin rules</td>
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<tr>
<td>09:35–09:40</td>
<td>Discussion</td>
</tr>
<tr>
<td>09:40–09:52</td>
<td>S2-5 Zsolt Martonfalvi Budapest, Hungary</td>
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<tr>
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<td>Force generation by titin folding</td>
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<tr>
<td>09:52–09:57</td>
<td>Discussion</td>
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<tr>
<td>09:57–10:09</td>
<td>S2-6 Jorge Alegre-Cebollada Madrid, Spain</td>
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<tr>
<td></td>
<td>Native redox posttranslational modifications as regulators of titin mechanical properties</td>
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<tr>
<td>10:09–10:14</td>
<td>Discussion</td>
</tr>
</tbody>
</table>
Program

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
Discussion

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
Discussion

12:15–12:20 Discussion

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

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
Discussion

12:51–12:56 Discussion

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

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
Discussion

14:35–14:40 Discussion

Program

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

15:00–15:05

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
Discussion

15:18–15:23

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
Discussion

15:36–15:41

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

15:54–15:59

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

10:09–10:14  S5-5  Pieter de Tombe, Chicago, USA

Impact of titin strain on the cardiac slow force response

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**

**DISCUSSIONS:** 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 Hüttiemeister, 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
Program

16:51–17:03  S7-3  Gaia Ghirardi  Padova, Italy  YOUNG INVESTIGATOR AWARD COMPETITION
The physiopathological role of mitochondrial calcium uptake in skeletal muscle homeostasis
Discussion

17:03–17:08

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
Discussion

17:21–17:26

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
Discussion

17:39–17:44

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)

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.
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.

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.

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.

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).

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Andrey N. Fomin, Wolfgang Linke
K.I.T. Group

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We can be everywhere for you!
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 myofibrils through ryanodine receptors. Calcium binding transforms the myosin head into a conformation that prevents its binding to actin, blocking a filament's movement on actin filaments, and allows 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 muscle 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.

**REFERENCES**

1. Behrmann E, Müller M, Penczek PA, Mannherz HG, edented level of molecular detail. Nature 2015; Fusi et al. 2016). 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 a system with micro-nanotubes to measure contractions of isolated half-sarcomeres and sarcomeres, (ii) a 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. This confocal microscopy system allows for activation and relaxation of one sarcomere with microfluidic perfusion to point-activate/deactivate 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 mechanosensing 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)

Daria Logvinova, Alexander Shevelev, Olga Nikolaeva, Dmitriy Levitin

A. N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Laboratory of proteins structural biochemistry, Moscow, Russian Federation

We applied FRET to examine how conformational changes occurring in myosin subfragment 1 (S1) upon formation of the S1-ADP-BeF\textsubscript{x} 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 S-IAF (acceptor) or 1,5-IAEDANS (donor). At physiological ionic strength (150 mM KCl) the distances of 4.6 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-BeF\textsubscript{x}. 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-BeF\textsubscript{x} complex, thus indicating that this part of LC1 is at the S1 active site. For Cys91, Cys160, and Cys 180 located in LC1 C-terminal part, these distances decreased upon formation of the S1-ADP-BeF\textsubscript{x} complex; this effect was the most pronounced in the case of 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 dynamics of S1-ADP-BeF\textsubscript{x} complex, from 5 to 4.45 nm, (M. Plosa) slow myosin 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

Mamta Arundale-Nayak, Walter Steffen, Arnab Nayak, Bernhard Branner

Medical School Hannover, Institute of Molecular and Cell Physiology, Hannover, Germany

Myosin motors drive diverse motile processes, ranging from intracellular cargo transport, cell division to muscle contraction and the whole process is regulated by various motor regulatory proteins. The biological function of myosin motors varies with different regulatory light chains. ‘Fast’ and ‘slow’ signify 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 TIMP- 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

**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

Olson Rassaler

MVIG University, Montreal, Canada

The sarcomere is the smallest functional unit of striated muscles. Sarcomeres are connected in series in a myofilament 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 a system with micro-nanotubes to measure contractions of isolated half-sarcomeres and sarcomeres, (ii) a system with atomic force microscopy to measure the force of isolated myofibrils, and (iii) a system with microfluidic perfusion to point-activate/deactivate control and activate one half-sarcomere. This confocal microscopy system allows for activation and relaxation of one sarcomere with microfluidic perfusion to point-activate/deactivate 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 mechanosensing 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.
Nebulin KO mice causes a decrease in in-series stiffness of the thin filament and consequent decrease in force in slow-twitch soleus muscle fibers

**Session 2**

**Titin and its Binding Partners**

*Robbert van der Pijl*1, Josh Strom1, Mei Methawasin1, John Smith1, Justin Kolb1, Kristina Djinovic-Carugo1, Stefan Conijn2, Henk Granzier1, Coen Ottenheijm1,2

1University of Iowa, Anatomy and Cell Biology, Iowa City, IA, United States
2University of Arizona, Cellular and Molecular Medicine, Tucson, United States

**S2-1**

**Structural and biophysical characterization of α-actinin-2 in ternary complex with FAT1-2 and Zq titin**

*Antonio Spongola, Arianalda Chamorro-Ramírez, Géza Gulyás, Ioana Suba-Leonard, Joan L. Arolas, Kristina Djinovic-Carugo*

Max F. Perutz Laboratories, University of Vienna, Dept. Structural and Computational Biology, Vienna, Austria

The sarcomere is the minimal contractile unit in striated muscle and is delimited by Z-disks. α-Actinin isoform 2 (α-actinin-2) is a key protein in Z-disc 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-disc proteins such as titin and FAT1-2, which participate in Z-disc formation and regulation. FAT1-2, also known as myosin-1 and calsarcin-2, is a relatively small (30 kDa) intrinsically disordered protein believed to be an adapter linking α-actinin-2 to other Z-disc proteins, while titin is a giant multimeric protein (~3,800 kDa; including folded and disordered parts) that spans half the sarcomere and combines elastic, architectural and signaling function. Although both FAT1-2 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 [Akinson 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 FAT1-2 by combinatorial NMR, SAXS, and crystallography (our unpublished data). Importantly, we have managed to solve the crystal structure of a soluble FAT1-2 construct in complex with a half dimer of α-actinin-2 at 3.2 Å. Structural analysis shows that FAT1-2 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 α-actinin-2 rod. This finding is consistent with previous reports of a similar interaction between α-actinin-2 and titin [Ribeiro Ede et al. Cell 2014]. More recently, we have focused on addressing the question whether interactions of FAT1-2 and Zq titin are competitive, synergic or independent. Using SAXS, we have characterized the binding affinity and stoichiometry of binary (α-actinin-2/titin) and ternary (α-actinin-2/titin/FAT1-2) 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**

*Josh Strom1, Mei Methawasin1, John Smith1, Justin Kolb1, Kristina Djinovic-Carugo1, Stefan Conijn2, Henk Granzier1, Coen Ottenheijm1,2

1University of Iowa, Anatomy and Cell Biology, Iowa City, IA, United States
2University of Arizona, Cellular and Molecular Medicine, Tucson, United States

**BACKGROUND:** Titin has been proposed to play a key role in mechanosensing and titin-based trophicity by 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 unilaerial diaphragm denervation (UDD) in mice, a model that induces a transient hypertrophy in the denervated diaphragm. Using ultrasound imaging, we reveal that the denervated diaphragm undergoes crosstitch passive stretch (26 ± 2% muscle lengthening), corresponding to sarcomere stretch from 2.9 to 3.7 μm. 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 stretch plays a role in the hypertrophy response, we used mouse models: one with decreased (RBM20ΔRRM; RBM20) and one with increased (ThnΔΔαx; IA) titin-based passive tension. In RBM20 mice the denervated diaphragm 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 identified after UDD, in particular Ankyr-1 & 2, FHL1 and Mγ1. MurF1. Interestingly, Ankyr1 was differentially involved, with a higher induction in the RBM20 mice and a blunted induction in the IA mice. Ankyr2 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. MAR1 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**

*Sandra Swist, Andreas Unger, Wolfgang Linke, Joanne van der Pijl, Henk Granzier*

1University of Mannheim, Mannheim, Germany
2Vrije Universiteit (VUmc), Physiology, Amsterdam, Netherlands

**BACKGROUND:** Titin has been proposed to play a key role in mechanosensing and titin-based trophicity by 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.

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**CONCLUSION:** In skeletal muscle, titin-based stiffness modulates hypertrophic remodeling. MAR1 might play an important role in titin-based hypertrophy signaling.

**S2-4**

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

*Henk Granzier*, Pasila Tanino, Balazs Kiss, birging, different technologies, such as cryo-electron microscopy (cryo-EM), Mel Meinhau, John Smith, Justin Kolb, Siegfried Labert

1University of Arizona, Cellular and Molecular Medicine, Tucson, United States
2University of Mannheim, Mannheim, Germany

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, defined by 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 develop an in vivo nanomechanical model, ThnC1-2, in which two of titin’s super-repeats (C1 and C2) were deleted. Structural studies in both cardiac and skeletal muscles of ThnC1-2 mice reveal a reduced thick filament length, in line with the conceptually shorter C1-2 DCM phenotype. Thus, thick filament length control is titin-based and is crucial for maintaining muscle health.

**S2-6**

Native redox posttranslational modifications as regulators of titin mechanical properties

Elias Herrojo-Gilad1, Cristina Sánchez-González2, Diana Vallesquez-Carrellas1, Elena Bonzón-Kulichko1, Enrique Calvo1, Jesús Vázquez1, Jorge Almagro-Cebollada1, Fundación CNIC, Molecular Mechanics Laboratory, Cell and Developmental Biology Area, Madrid, Spain

1 Fundación CNIC, Cardiovascular Proteostasis Laboratory, Vascular Cardiology Area, Madrid, Spain

2 Fundación CNIC, Proteostasis Unit, Vascular Pathophysiology Area, Madrid, Spain

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 cardiac myocytes. However, the identity, extent and specific residues targeted by these modifications in vivo remain unresolved. 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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**REFERENCES:**


**S3-1**

Cardiomyocyte dyadic structure and function in health and disease

William Louch

University of Oslo, Institute for Experimental Medical Research, Oslo, Norway

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 Ca2+ 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 Ca2+ handling proteins including the L-type Ca2+ 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 Ca2+ release. Elevated stress placed on the myocardial wall of the failing, dilated heart is a key trigger of disrupted dyad function, 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

Beatrix Diienes1, Pétter Szentesi1, Tamás Cziжkár2, Miklós Szatmáry3, László Zsolt Szabó4, Bernhard E. Flucher1, László Csonóczy5

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

2 Sapientia Hungarian University of Transylvania, Department of Electrical Engineering, Targu Mures, Romania

3 Institute of Biomedical Engineering, Budapest, Hungary

4 University of Oslo, Institute for Experimental Medical Research, Oslo, Norway

In adult mammalian skeletal muscle the primary function of the surface membrane Ca2+ channel (Dihydropyridine receptor; DHPR or Cav.1.2β) is to voltage sensing for excitation-contraction (EC) coupling. Unlike its adult isoform which does not conduct significant Ca2+ current during physiological activation, the embryonic splice variant (Cav.1.1,2β) displays normal channel function and gives rise to a considerable Ca2+ influx. The isoform shift after birth is essential for proper tissue type specification and healthy myocardial function. The consequences of the preserved Cav1.2β influx on EC-coupling and on the release of Ca2+ from the sarcoplasmic reticulum (SR) through the SR Ca2+ release channel (Ryanodine receptor; RyR) were examined in mice expressing only the embryonic isoform. Fibres were enzymatically isolated from the limb digitorum brevis muscles of Cav1.1,2β and Cav1.1,2β mice and either RyR1 or Cav1.2β transgenic mice expressing modified SR Cav1.2β release events (CRB) in intact fibres were detected. The altered channel function gave rise to otherwise not seen in vivo CRE in adult fibres with unusual characteristics. Their appearance depended on the presence of extracellular Ca2+ and required the activation of RyR1, however, typical 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 RyR1 act in concert. It was also accompanied by modified SR Cav1.2β release properties as reactivated activation resulted in greater decline in the amplitude of the CRB transients (the ratio of the second and first amplitude being 70 to 3% in control and 42 to 5% in Cav1.1,2β mice).

Our results imply that the Cav1.2β influx through the DHPR gives rise not only to mitochondrial damage and fibre type disorganization, but most likely it modifies the structure of the cardiac myocytes, giving rise to a considerable Ca2+ influx. The isoform shift after birth is essential for proper tissue type specification and healthy myocardial function.

**S3-3**

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

Dániála Filipová1, Anna Brunn2, Martina Deckert2, Agapis Bakalidou1, Gabrielle Pfeifer1, Symonn Pepelapodou1

1 University Hospital of Cologne, Institute of Vegetative Physiology, Cologne, Germany

2 University Hospital of Cologne, Department of Neuropathology, Cologne, Germany

3 University Hospital of Cologne, Institute of Neuropathology, Cologne, Germany

On a molecular level excitation-contraction (EC) coupling in skeletal muscle can be described as the coordinated functions of two Ca2+ 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 Ca2+ 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−/−) mice displaying normal channel function and giving rise to a considerable Ca2+ influx. The isoform shift after birth is essential for proper tissue type specification and healthy myocardial function. The consequences of the preserved Ca2+ influx on EC-coupling and on the release of Ca2+ from the sarcoplasmic reticulum (SR) through the SR Ca2+ release channel (Ryanodine receptor; RyR) were examined in mice expressing only the embryonic isoform. Fibres were enzymatically isolated from the limb digitorum brevis muscles of Cav1.1,2β and Cav1.1,2β mice and either RyR1 or Cav1.2β transgenic mice expressing modified SR Cav1.2β release events (CRB) in intact fibres were detected. The altered channel function gave rise to otherwise not seen in vivo CRE in adult fibres with unusual characteristics. Their appearance depended on the presence of extracellular Ca2+ and required the activation of RyR1, however, typical 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 RyR1 act in concert. It was also accompanied by modified SR Cav1.2β release properties as reactivated activation resulted in greater decline in the amplitude of the CRB transients (the ratio of the second and first amplitude being 70 to 3% in control and 42 to 5% in Cav1.1,2β mice).

Our results imply that the Cav1.2β influx through the DHPR gives rise not only to mitochondrial damage and fibre type disorganization, but most likely it modifies the structure of the cardiac myocytes, giving rise to a considerable Ca2+ influx. The isoform shift after birth is essential for proper tissue type specification and healthy myocardial function.
Abstracts

Eighty-five DEGs were found in RYRY1-/- and 1047 in RYR1-/- samples. Taken together, these findings strongly suggest important roles of RyR1 and DHPR and in E18.5 RYR1-/- samples. Taken together, these findings strongly suggest important roles of RyR1 and DHPR and the EC coupling-related Ca2+ signaling in the late steps of muscle development.

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

Maria Fjeldstad1, Filip Margetiny1, William Miller1, Gregory Lnenicka2, *

1 University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom
2 Cytokinetics Inc., Research and Early Development, South San Francisco, CA, United States

*Correspondence: Gregory Lnenicka, g.lnenicka1@ed.ac.uk

Membrane excitability and excitation-contraction coupling at neuromuscular junctions in larval Drosophila were investigated. Our results show that Drosophila muscle fibers produce synthetically-driven regenerative responses, with a threshold at about -25 mV resembling cardiac action potentials recorded from the vertebral atriocentric valve (PMID: 19843444). These observations suggest that activation of voltage-sensitive Ca channels in vertebral Drosophila muscle membranes may not always be mitigated by high resting K permeability (PMID:23990639, 766192, 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. 

S3-5

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

Arthur Cheng1, Darren Hewett1, Leo Kim1, Nickie Durham1, Aaron Hinken1, Adam Kennedy1, Ronald Terjung2, Jeffrey Jaspers1, Fadi Malik1, Hakan Westerblad1

Karolinska Institutet, Physiology and Pharmacology, Stockholm, Sweden

Cytekinesis Inc., Research and Early Development, South San Francisco, CA, United States

*Correspondence: Arthur Cheng, a.cheng@ki.se

A fast skeletal muscle troponin activator (FSTα): CK-2066260 mitigates the fatigue-induced decline in skeletal muscle contractile force by lowering the metabolic cost. FSTα, a calcium-sensing small molecule, potently activates the sarcomeric Ca2+-release channel RyR1. Here, we investigated whether fatigue could be ameliorated by the FSTα, CK-2066260, a calcium-sensing small molecule that activates the RyR1 channel. We show that CK-2066260 reduces fatigue in both in-vitro and in-vivo preparations. CK-2066260 mitigates fatigue in in-vitro preparations by lowering the metabolic cost and in-vivo preparations by lowering the metabolic cost and increasing the threshold for fatigue. These findings have potential therapeutic implications for treating fatigue in skeletal muscle.

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

Session 4

S4-1

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

*Lucie Carrier

University Medical Center Hamburg-Eppendorf, Department of Experimental Pathology and Toxicology, Hamburg, Germany

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) and dilated cardiomyopathy (DCM) or with diastolic dysfunction (DDCM) and reduced ejection fraction. One of the main goals of my lab is to evaluate whether induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) can be used to model disease mechanisms of inherited cardiomyopathies and to test molecular therapies. We used fibroblasts from healthy individuals (control) and patients with hypertrophic cardiomyopathy (HCM) carrying sarcomere gene mutations. Fibroblasts were reprogrammed into iPSCs and further differentiated into CMs and EHTs. Additionally, we used CRISPR/Cas9 gene editing to correct the mutation in iPSCs (iC0M-CMs) and to differentiate into EHTs. The iPSC-CMs were cultured in 2D for 7-30 days and cell area and contractility were measured using 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.

The giant sarcomere protein titin has a numerous important roles in cardiomyocytes, including providing passive tension and facilitating myofibril formation. An internal pro-moter has recently been identified in the titin gene (TNNT2) that is predicted to regulate expression of a previously unstudied isoform, Crontos. To study the role of Crontos titin in human hearts, we genetically engineered human induced pluripotent stem cells (iPSCs) using the CRISPR/Cas9 system to introduce homozygous frameshift mutations in the TNNT2 gene. TNNT2 was mutated upstream of the Crontos titin gene, and the resulting constructs were used to generate human induced pluripotent stem cells (iPSC-CMs). The iPSC-CMs were differentiated into cardiomyocytes (iPSC-CMs) visibly contract, though weakly, and immunofluorescence studies indicate the formation of short, dispersed myofilaments. The myofilaments are positively stained for the to the cardiac myofilament marker α-actinin isovatal form, with the C-terminal MIR and MI-M10 regions of titin but not the N-terminal Z-disc domain, consistent with Crontos 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, Ex2 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 predomi-nantly Crontos titin, which is sufficient for rudimentary sarcomere formation but not normal function. Ongoing studies will investigate the role of Crontos titin during normal human heart development and maturation.

S4-2

Cronotos titin in developing human cardiomyocytes: degraded no more

Becky Zaubrencher1, Nathan Sniadecki2, Wolfgang Linke2, Charles Murry1, *Michael Regnier1

1 University of Washington, Bioengineering, Seattle, United States
2 Ruhr University Bochum, Cardiovascular Physiology, Bochum, Germany

*Correspondence: Michael Regnier, michael.regnier@rub.de

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 (TNNT2) that is predicted to regulate expression of a previously unstudied isoform, Crontos. To study the role of Crontos titin in human hearts, we genetically engineered human induced pluripotent stem cells (iPSCs) using the CRISPR/Cas9 system to introduce homozygous frameshift mutations in the TNNT2 gene. TNNT2 was mutated upstream of the Crontos titin gene, and the resulting constructs were used to generate human induced pluripotent stem cells (iPSC-CMs). The iPSC-CMs were differentiated into cardiomyocytes (iPSC-CMs) visibly contract, though weakly, and immunofluorescence studies indicate the formation of short, dispersed myofilaments. The myofilaments are positively stained for the to the cardiac myofilament marker α-actinin isovatal form, with the C-terminal MIR and MI-M10 regions of titin but not the N-terminal Z-disc domain, consistent with Crontos 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, Ex2 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 predomin-antly Crontos titin, which is sufficient for rudimentary sarcomere formation but not normal function. Ongoing studies will investigate the role of Crontos titin during normal human heart development and maturation.
(ii) to investigate how MyHC composition of the individual human stem-cell derived cardiomyocytes (hSC-CMs) and no correlation between twitch contraction kinetics and from 0% to 100% (n=45-63). Preliminary data so far show average 527±529 16±24% and stem cell derived cardiomyocyte to twitch kinetics of the same human embryonic protein expression of cardiac myosin heavy chain Surprisingly, twitch contraction kinetics seem to be dom - in individuals of both mRNAs suggests that transcrip - tion of the two genes occurs in a burst-like fashion. Surprisingly, twitch contraction kinetics seem to be dom - inated by parameters other than the HMyC-composition.
Abstracts

(LDA). The high frequency contractions of IFM that power the rapid wing beats are produced by periodically stretch- ing and relaxing myofibrils (stretch oscillation). 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, troponins bound to thin filaments and thick filaments may transmit force between the filaments on stretch (Peretz-Edwards et al., 2011). We have investigated the interaction between tropomyosin-troponin (TnT-Tn) and thick filaments. Unlike cardiac muscle, IFM has a TnT-Tn complex with two iso- forms of TnC (TnC1F and TnC2F) and two isoforms of Tm (Tm1 and Tm2). TnC1F regulates stretch activation and there is no homologue in cardiac muscle. Force produc- tion in IFM is the same calcium sensitivity and cooperativity as cardiac trabecule, suggesting simi- lar regulation by TnC2F and cardiac TnC. In pulldown experiments with IFM thick filaments or filaments assem- bled from pure myosin, we found the Tm-Tn complex with both IFM isoforms binds to thick filaments, and the interaction is not calcium sensitive. Unexpectedly, Tm1 allows force generation in IFM 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. Tm2 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 spe- cific to Tm1, not to IFM myosin, and that the interaction is in the N-terminus. These results suggest tropomyosin-bridges activate the thin filament by pulling directly on Tm.

SS6-5 Approach and mechanotransduction of titin/o-actinin bond

Juan Carlos Ribeiro 1, Marco Grisoni 1, Nikos Pisani 1, Andrea Ghisleni 1, Georg Ramm 1,2,3, *

1Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria
2Department of Physics, Technische Universität München, Garching, Germany
3Division of Biochemistry, Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria

RATIONALE: 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). The cross-linking of actin and myosin at the boundaries of their filamentous structures is essential for myocardial integrity and function. In the Z-discs – the lateral bound- aries of the sarcomere machinery – o-actinin cross-links actin filaments from adjacent sarcomeres, and therefore essential for cardiac function. Using the Z-disc as an example, we investigated the role of titin isoform on the SFR. Right ven- tricular trabecule were isolated and mounted in an exper- imental 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 concentra- tion reflecting ~50% of maximum twitch force develop- ment. Presence of the giant titin isoform (HM) was associ- ated 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 slower as the SL strain was identical in both muscle groups. Therefore, our data suggest that cytoskeletal strain may underlie direct the cellular mechanisms that lead to the increased intra- cellular (Ca2+) that causes the SFR, possibly by involving cardiac myocyte integrin signaling pathways.

SS6-6 The Z-disc is between differentially spliced titin Z-repeats and the calmodulin-like domain o-actinin.

Using o-actinin as a model of structural, biochemical and cell biophysics approaches we provided insight into molecular architecture of o-actinin and into phosphoswitch-based mechanism controlling titin Z-repeats-o-actinin interaction (Ribeiro et al., 2014). Currently, the mechanisms that underlie the 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 individual interaction between o-actinin and titin is weak if force is applied, but depending on the direction of force application, the unbinding forces can be much larger. Our results suggest a model where multiple titin Z-repeat-o-actinin interactions cooperate to ensure long-term stable titin anchoring while allowing the individual components to exchange dynamically (Grisoni et al., 2017, PNAS).

SS6-7 Impact of titin strain on the CARICARD slow force response

Younis Al-Mou, Mengjie Zhang1, Jody Martin1, Marion Geaer1, Pieter de Tombe1,2

1 Loyola University Chicago, Cell and Molecular Physiology, Maywood, United States
2University of Wisconsin, Animal Sciences, Madison, United States
3University of Illinois Chicago, Physiology and Biophysics, Chicago, United States

The cross-linking of actin and myosin at the boundaries of their filamentous structures is essential for myocardial integrity and function. In the Z-discs – the lateral boundaries of the sarcomere machinery – o-actinin cross-links actin filaments from adjacent sarcomeres, and therefore essential for cardiac function. Using the Z-disc as an example, we investigated the role of titin isoform on the SFR. Right ven- tricular trabecule were isolated and mounted in an exper- imental 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 concentra- tion reflecting ~50% of maximum twitch force develop- ment. Presence of the giant titin isoform (HM) was associ- ated 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 slower as the SL strain was identical in both muscle groups. Therefore, our data suggest that cytoskeletal strain may underlie direct the cellular mechanisms that lead to the increased intra- cellular (Ca2+) that causes the SFR, possibly by involving cardiac myocyte integrin signaling pathways.

SS6-8 Autotherapy as a therapy for myofibrillar myopathy

Avnika A. Ruparelia1, Caitlin Williams1, Emily A. Mc Kabul1, Vitalia Corschitz1, Emily C. Baxter1, Keith Schubert1, Gary Ramqvist1, Robert Bryson-Richardson1

1Monash University, School of Biological Sciences, Melbourne, Australia
2Monash University, The Clive and Vera Ramaciotti Centre for Structural Cryo-Electron Microscopy, Melbourne, Australia
3Monash University, Department of Biochemistry and Molecular Biology, Melbourne, Australia

In order to better understand the mechanism of disease in myofibrillar myopathy we generated zebrafish models for two types 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 mus- cle maintenance and address the question which form of Klotho (secreted or membrane-bound) is responsible for the muscle-specific phenotype. We found that mice demonstrate several characteristics of sarcopenia in- cluding reduced myofiber diameters and a depletion of the muscle stem cell (satellite cell) pool. To investigate the regeneration process, we injured the myofibrillar muscles of Delta-Klotho mice with cardiotomy. Delta-Klotho mice showed regeneration deficits, which were more pronounced with increasing age. Satellite cells contribute essentially to the regeneration process. Therefore, we analysed the functionality of satellite cells on isolated myofibers where the satellite cells are still in their endoge- nous niche, but are cultured independently of systemic in- fluences. This ex vivo analysis demonstrated a perturbed function of satellite cells from delta-Klotho mice whereas in vitro assays with isolated myoblasts showed no influ- ence 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.
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 tendons, which allows for the generation of mechanical tension across the developing fibers. This tension is essential for myofibrillogenesis and the formation of periodic sarcomeres. However, so far, ten- sion 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 func- tional. 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 mechanically at muscle attachment sites to form myofibrils and provide insights into the molecular mechanism of how tension build-up is function- ally linked to myofibrillogenesis during muscle morpho- genesis.

S6-4

Measuring molecular tension at muscle attachment sites during myofibril assembly

Judith Hüttemeister*
S6-3

The role of titin in sarcomere assembly and dynamics

Judith Hüttemeister, Franziska Rudolph, Michael Radke, Katharina da Silva Lopes, Michael Gotthardt
Max Delbrück Center for Molecular Medicine, Berlin, Germany

The giant striated muscle protein titin determines sarco- meric structure and elasticity. It spans the half-sarcomere from the N-terminus integrated into the Z-disc and the C-terminus into the M-band. To visualize sarcomere assembly and dynamics we gen- erated titin knockin mice with GFP fused to the M-band region of titin and ditagged to the Z-disc region, respective- ly. Cardiomyocytes and myotubes derived from these ani- mals provide an opportunity to follow titin mobility and turnover in living cells.

During the de novo assembly of sarcomeres in cells de- rived from double-heterozygous GFP/dsRed mice titins N- and C-terminus integrate synchronously and not stepwise into the sarcomere. Photoactivating experiments reveal that sarcomeric titin is not a rigid backbone, but is active- ly 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.

S6-5

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

Lucas Smith*, Hui Jean Kok*, Ray Spradlin*

Graham Lamb
La Trobe University, Dept of Physiology, Bundoora Campus, Melbourne, Australia

Reactive oxygen and nitrogen species (ROS/RNS) are pro- duced in skeletal muscle in normal exercise, and at exces- sive levels in various disease states. They may potentially affect muscle function by perturbing many of the excita- tion-contraction coupling steps, though the predominant sites affected appear to be the contractile apparatus and the myonuclear receptors (RyR3) in the sarcolemmal retic- ulum. Acute exposure to moderate levels of ROS, such as hydrogen peroxide and superoxide, increase the Ca2+-sen- sitivity of the contractile apparatus in fast-twitch mamma- lian fibres via S-glutathionylation of Cys134 on fast TnI. In contrast, RNS, such as GSNO, reduce Ca2+-sensitivity by competitive action at the same site, preventing the effects of S-glutathionylation. Both reactions protect that labe|
Mouse with a line expressing MCU, by crossing a skeletal muscle specific gene to mice, and this is likely an important factor in muscle dysfunction in a range of diseases and abnormal states. ROS/RNS seem not to greatly affect the action potential-induced Ca^2+ release mechanism, but neither is mitochondrial protein leakage through the RyRs. This can potentiate force responses by raising resting cytoplasmic Ca^2+, resulting in increased Ca^2+ occupancy of TRC. 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.

**S7-4**

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

Hafsa Hamdan, Fae Vogel, Sandra Van der Linden, Carla Offringa, Gerard de Wit, Mohammad Haroon, Willem van der Laarne, Richard Jaspers

Laboratory for Myology, IRIS Universiteit Amsterdam, Dept. Human Movement Sciences, Amsterdam, Netherlands

Mitochondria are molecular machines that are central to protein synthesis, cellular energy metabolism and cell death. One of the paradigms of aging proposes that autophagy, mitophagy, mitochondrial degradation and reduced rRNA expression are hallmarks of aging. We found that mitochondrial Ca^2+ accumulation is required to guarantee skeletal muscle performance. Finally, a clear metabolic route regulating mitochondrial Ca^2+ accumulation is required to guarantee skeletal muscle performance. A novel negative regulator of mitochondrial Ca^2+ accumulation is 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

Imperial College London, Molecular Medicine, London, United Kingdom

The loss of skeletal muscle mass is a common co-morbidity 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 hypertrophy, 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.

**S8-1**

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

Eva Patera

Ruhr University Bochum, Cardiovascular Physiology, Bochum, Germany

The development of effective treatments for heart failure remains challenging. In left ventricular (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 hypertrophy, 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.
Abstracts

respiration and mechano-elastic properties of cardiomyocytes in vivo, coinciding with increased titin phosphorylation and providing mechanosensory input to the myofiber. Mitochondrial dysfunction. Mitochondria-based therapeutic approaches, the effects of overexpression of mitochondrial complex I on cardiac tissue health. Methods: Cardiac fibroblasts isolated from wildtype, 1095 knockout (KO) and 1095 heterozygous (Het) mice were subjected to 6 hours ischaemia and 24 hours reperfusion. Mitochondrial complex I activity was assessed by luciferase reporter assay and results were compared to wildtype. Results and Conclusions: Mitochondrial complex I activity was significantly lower in KO fibroblasts compared to wildtype and Het fibroblasts. This suggests that mitochondrial complex I is a key component of cardiac tissue health and that targeting this complex may be a promising therapeutic approach. Abstracts

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S8-4 The Spy-C method for in situ replacement of cardiac myosin binding protein-C in sarcomeres

S8-5 The molecular defects in Ca2+-regulation due to mutations that cause hypertrophic cardiomyopathy can be reversed by small molecules that bind to troponin

S8-6 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 (AAV) 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, our presence resulted in improvement of the histological features and in the contractile efficiency 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 to cardiac toxicity. Our observation led us to develop new vectors with skeletal muscle restricted expression. Results with this new generation of vectors will be presented.

S8-7 Initial therapeutic results of gene therapy in canine models of muscular dystrophies

The primary defect in LGMD2A, secondary deficiency of calpain 3 has been observed in a number of muscular diseases, including muscular dystrophies due to mutations in the giant titin, a known calpain 3 partner. In order to establish a new generation of vectors, we have developed an efficient and stable transgene expression in the skeletal muscle after intramuscular and loco-regional administration. Moreover, our presence resulted in improvement of the histological features and in the contractile efficiency 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 to cardiac toxicity. Our observation led us to develop new vectors with skeletal muscle restricted expression. Results with this new generation of vectors will be presented.

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

Catherine Wingate1, David Pingree2, Peter Arthur3, Anthony Blakkar4, Kristin Nowak2

1 The University of Western Australia, School of Human Sciences, Perth, Australia
2 The University of Western Australia, School of Molecular Sciences, Perth, Australia
3 The University of Western Australia, Harry Perkins Institute of Medical Research, School of Biomedical Sciences, Perth, Australia

Duchenne 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 mouse) 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 were frozen for histology and serra taken. EDL and soleus muscles from mdx/CC mice showed a significant increase in resistance to exocytosis-contraction induced damage (P<0.05) along with a decrease in muscle necrosis (P<0.05) compared to mdx controls. Serra was analysed for creative 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.
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 and 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 (10^10 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). 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 and 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 (10^10 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.

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REFERENCES
4. Walter Herzog. 2010. Work in Physiology Monitors the Folding Trajectory of a Single Protein. Nature Communications 1:1-5. 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 key 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 inducing 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_T 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_T by [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 re-binding 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_T respectively. Separation of P_i re-binding/releasing from f and f_T results in a flat k_T([P_i]) dependence in contrast to the observed steep k_T([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 P_i release results in strong loss of [P_i]-dependent rate-modulation of k_T. Strongest rate modulation of k_T by [P_i] is obtained when P_i release and P_i re-binding are directly coupled the rate-limiting transitions f and f_T in the least energetic cross-bridge cycle. Experimental force and k_T([P_i]) data from 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_T. 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 and 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 (10^10 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.

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

*Dawn Low, Gangyuan Le, Lien Phung, Sira Karvinen, David Thomas

University of Minnesota, Minneapolis, United States

Estrogen deficiency impairs skeletal muscle force generation in aged women and ovarioctomized rodents. Myosin has been implicated and here we test the hypothesis that in vivo potentiation of force and the super relaxed state (SRS) of myosin contribute to the erectile functions. METHODS: To test post-tetanic potentiation (PTP) of force, a stimulating nerve cuff was surgically implanted on the common peroneal nerve of ovari-intact (Control) or ovarioctomized (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 (G2). RESULTS: Sag increased (P<0.05) as the inter-twitch interval was increased from 1.0xCT (L: 7.0 ± 0.2% peak force (P) vs S: 2.0 ± 0.5% (P) vs 3.0xCT (L: 15.6 ± 2.6% P vs S: 22.4 ± 2.3% (P)). Sag was significantly greater (P<0.05) at S than in L all unfused tetani. Summation, a non-linear process, was assessed by the active tension generated than L in all unfused tetani. Summation, a non-linear process, was assessed by the active tension generated than L in all unfused tetani. CONCLUSIONS: Estrogenic compounds rescue Ovx-induced low PTP. Estrogen deficiency in female mice via ovariectomy but not aging impairs the number of 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.

P1-6 On the nature of unloaded-inducing postural muscle stiffness decline

*Boris Shenkanen, Irena Petrova, Sergey Tyaganov, Timur Mirzoev

SSC IF Institute of Biomedical Problems, RAS, Myology Laboratory, Moscow, Russian Federation

At the end of Xth century it was shown that stiffness of isolated muscle and 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; Tourtell et al., 2002; Ogneva, Shenkanen et al, 2011). However it is unclear, which molecular machinery deter- mines this stiffness loss. We demonstrated that the pas- sive 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 con- trol 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 ana- lyze 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-treat- ed animals did not differ significantly from the cage con- trol level. Thus, the calpain activation breakdown of cy- toskeletal 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 Founda- tion of Basic Research #16-04-00529.

P1-7 Direct isometric muscle strain analyses using speckle tracking technology. A validation study

*Laura Kaczynski, Prich, Andrea Holshard Larsen, John Hjortbak, Jordi Sanchez Dalf, Katya Lykke Lambertsen

University Hospital Odense, Department of Orthopaedics, Odense, Denmark

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

P1-8 Dysfunction of titin in aged mice and its implication for muscle stiffness decline

Nicanor Gonzalez-Morales, *Frieder Schock

McGill University, Biological, Montreal, Canada

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 ovalarily distributed at the Z-discs. In humans, mutations in the actin-binding protein Filamin C result in myopathies, but the underlying molecular func- tion is not well understood. Here we show using Drosoph- ila indirect flight muscle that the Filamin ortholog Cheesio 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 responsible for interaction with the titin ortholog Sallimus, and we demon- strate a genetic interaction of Filamin with titin and actin. Filamin mutants disrupting the actin- and the titin-binding domain display distinct phenotypes, with myofibrils aligning up in parallel or perpendicularly to the myofilibr, re- spectively. Thus, Z-discs require Filamin to withstand the strong contractile forces acting on them.
Complexes in which myosin molecules were associated to titin filaments could not be identified. Occasionally we observed myosin filaments with thin filaments attached to their surface. Thus, the electrostatic driven self-association is stronger in either myosin and titin than their binding to each other. Conceivably, associated protein C, and additional mechanisms are required to modulate and regulate the situ interactions between titin and the myosin thick filament.

Extraocular muscles (EOM) in desmin knockout and R349P desmin mutant mice

Extraocular muscles (EOM) are classified as a separate muscle group given their distinct gene and protein expressions and morphology compared to other skeletal muscles. EOM show different physiological properties and are selectively spared in several muscular dystrophies. We have investigated the functional changes in the peripheral structures of the EOM in case of impaired motor activity, primarily related to the restriction of signaling 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 Ηmax/Мmax ratio indicates the rising reflex excitability of the motoneurones of the corresponding motor center and the increasing in the number of motoneurones, responsible for different stimulation. It was found that the threshold of the M-response of MG decreased and its amplitude increased which induced the functional changes in the peripheral structures of the neuromuscular apparatus. It is known that the support affaternation play in important role in motor control [Griгорieva 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 generators of the support.

This work was supported by RFBR, research project no. 15-04-06911a, partially supported by RSF, research project no. 15-15-20336.

Motor evoked potentials induced by epidural spinal cord stimulation can be used for evaluation of the spinal cord functional state after spinal cord injury (Iafarova et al. 2014). Previously it was shown that local hypothermia after spinal cord contusion delay the functional recovery in m. gastrocnemius and m. soleus, and m. tibialis anterior on 3, 7, 14, and 30 days after SCI. All procedures were made in accordance to bio ethics 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 threshold of ER and MR significantly increased on 14 day after SCI and on 3 day 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 group of hypothermia. The threshold of ER and MR significantly increased on 3 day after hypothermia treatment. Previously it was shown that local hypothermia after spinal cord contusion delay the functional recovery in m. gastrocnemius and m. soleus, and m. tibialis anterior on 3, 7, 14, and 30 days after SCI. All procedures were made in accordance to bio ethics 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 threshold of ER and MR significantly increased on 14 day after SCI and on 3 day in group of hypothermia treatment. 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the work was a developmental methodology for conditioning of spinal evoked responses by using Jendrassik maneuver and skull stimulation at 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 ± 3 years in control and with Jendrassik maneuver with increasing maneuver duration of 1 to 10s. The stimulation was carried out by Neurosoft Neuro M4V-4 stimulus (Russia). Registration of the responses was performed by bipolar self-adhesive electrodes. Statistical data was processed with using of Winocoxt-test. Differences were considered as significant at p < 0.05. Changes in the responses parameters to TSCS with 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. Soleus by 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 Jendrassik maneuver in rehabilitation cardiology processes. 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  *Artur Abovyan, Irina Lusva, Nafis Abovyan, Anton Eremeev, Igor Lavrov  Kazan Federal University, Institute of Fundamental Medicine and Biology, Kazan, Russian Federation  

The main goal of this study was evaluation of reaction exittance 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 vertical position. The stimulation was carried out using six different values of stimulation strength: 10, 20, 30, 40, 50, and 60 volts. The intensity of GM responses was adjusted based on threshold for the appearance of noticeable muscle twitch. GM responses were recorded using needle electrodes. The GM reactivity was measured by the amplitude of the evoked action potential. The experiments were conducted on male Wistar rats. The gravitational unloading was modeled by hanging animals in the vertical position. The stimulation was carried out using six different values of stimulation strength: 10, 20, 30, 40, 50, and 60 volts. The intensity of GM responses was adjusted based on threshold for the appearance of noticeable muscle twitch. GM responses were recorded using needle electrodes. The GM reactivity was measured by the amplitude of the evoked action potential. The work was supported by the Russian Science Foundation (Project № 15-15-20036).

P3-5 Theoretical analysis of Ca2+-handling in skeletal muscle fibers of Calsequestrin-null mice  *Lorenzo Marucchi1,2, Carlo Reggiani3, Marta Canato1, Gar Stienen4  1University of Padova, Biological Sciences, Padova, Italy  2University of Padova, CMBM, Padova, Italy  3KU University, Laboratory of Physiology, Institute for cardiovascular research, Amsterdam, Netherlands  

Muscle contraction and relaxation is a complex phenomenon finely regulated by the Ca2+ ions movements between muscle cells compartments and Ca2+ binding proteins. Calsequestrin (CSEQ) is the most abundant Ca2+ binding protein in the sarcomeric reticulum, and allows for storing of 20 mM or more of Ca2+, still maintaining the free calcium concentration to 1 nM or less. Surprisingly, control of Ca2+ movements in muscle fibers of mice carrying null mutation of the gene coding for CSQ (CSQ-KO), the CSQ predominant isoform in fast skeletal muscles, are close to those of wild type mice (WT), both inwitch and tetanic contraction, as well as free [Ca2+]i 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 Ca2+ channels (SOC), a modified permeability of RyR at which CSEQ 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 role of Ca2+ handling, through a mathematical model which include diffusional term in Ca2+ movements within compartments, to exploit the crucial position of mitochondria close to calcium release units and the presence of microdomains. We simulate the predicted [Ca2+]i in the main compartments (SR, cytosol and mitochondria) and buffers (CSQ, Troponin and Parvalbumin) fitting model parameters on WT from our experimental data on (PDE) 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 analysis of the model simulate underline the relevance of the changes in RyR permeability and of the contribution of SOCE to maintain the contractile performance.
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; zymography) 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 percentage of the total area of histological section, decreased 36% in pifithrin-α-treated (9.2 ± 1.022% 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 zymography (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-β, 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-α ameliorates dystrophic cardiomyopathy in the mdx mice model of Duchenne muscular dystrophy.

P4-3
Nahumto Santo Neto, Isabel C. Chagas Barbin, Juliano Alves Pereira, Maria Julia Marques
University of Campinas, Structural and Functional Biology, Campinas, Brazil

P4-4
Intrinsic MYH7 expression regulation may contribute to tissue level allelic imbalance in hypertrophic cardiomyopathy
Julith Montag, Mandy Byrington, Julia Rose, Anna-Lena Weber, Pia Ernstberger, Anne Mayer, Edgar Becker, Britta Keyser, Jolanda van der Venlen, Carolyin Y. Ho, Antoni Francisco, Bernhard Brenner, Theresa Kraft
Hannover Medical School, Hannover, Germany
University Medical Center, Amsterdam, Netherlands
Boston Children’s Hospital, Boston, United States
Hospital Clinic Barcelona, Barcelona, Spain

QUESTIONS: How does the level of cardiac CK and fibrosis area relate to the severity of HCM in the different mutations of MYH7? Does the level of cardiac CK and fibrosis area differ between non-HCM donors and in HCM-patients? Are the differences of cardiac CK and fibrosis area caused by mutations of MYH7 or other mechanisms? What is the effect of mutations of MYH7 on the level of cardiac CK and fibrosis area in HCM-patients? How do the differences of cardiac CK and fibrosis area between controls and HCM-patients change with different MYH7-missense mutations?

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 extent 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
Galina Kopytova, Danil Shchepekin, Alexander Matyushenkov, Sergey Bershitsky
Institute of Immunology and Physiology, Russian Academy of Sciences, Biological Mophology, Yekaterinburg, Russian Federation
A.N. Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russian Federation

INTRODUCTION: In cardiomyopathies, pathological changes in the heart include atrial enlargement and fibrillation (Pahibarak 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⁺⁺ 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 of Tpm molecules.

METHOD: We analyzed Ca⁺⁺ 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, M85K, K155N) 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 pCa₅₀ of pCa-velocity relation. Mutation E180G increased Ca⁺⁺ sensitivity of pCa-velocity relation. Mutations I284V and M281T increased the maximal velocity and the Ca⁺⁺ sensitivity of the velocity. Ca⁺⁺ sensitivity estimated by the ratio pCa₅₀ of pCa-velocity relation was higher for thin filaments containing Tpm with mutations E40K, E54K, E180V, E180G, I172T, I284V, and M281T as compared to those with WT Tpm. Mutations L185R, M85K, and K155N did not affect significantly pCa-velocity relation.

CONCLUSIONS: In general, these mutations have less pronounced effect on the Ca⁺⁺ regulation of the actin-myosin interaction in atria. The overlap junction between N- and C-terminal of Tpm molecules has a pronounced effect on the Ca⁺⁺ regulation of the actin-myosin interaction in atria. Ca⁺⁺ regulation of the actin-myosin interaction in atria.

P4-6
Altered myofibrillar structure and function in dogs with Duchenne muscular dystrophy cardiomyopathy
Younss Alt Mou, Alain Lacampagne, Thomas Irving, Valerie Scheuermann, Stephanie Blott, Bijan Ghalai, Pietier de Tombe, *Olivier Cancio
Insam 1046 - INRS UMR 2014 – Université de Montréal, Montréal, France

AIMS: Duchenne Muscular Dystrophy (DMD) is associated with progressive depressed left ventricular (LV) function. However, DMD effects on myofibril 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 myofibrillar structure and function alterations in this dog model with spontaneous cardiac failure.

METHODS AND RESULTS: We employed synchrotron x-rays diffraction to evaluate myofibrillar lattice spacing at various sarcomere lengths (SL) on permeabilized LV myocardium. We found a negative correlation between SL and lattice spacing in both sub-epicardial (EP) and sub-endocardial (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 nm). Furthermore, cross bridge cycling indexed by the kinetics of tension redevelopment (ktr) was faster in ENDO GRMD myofibrillar at short SL. We measured post-translation modifications of key regulatory contractile proteins. S-glutathionylation of cardiac Myosin Binding Protein-C (cMyBP-C) was unchanged and PKA dependent phosphorylation of cMyBP-C was significantly reduced in tients, we identified imbalanced as well as balanced 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 extent 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.
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-MHC) 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 sarcomeric protein, (β-MHC). 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-MHC-mutation R723G and of a donor heart. We used fluorescence in situ hybridization on 16 µm thick sections of cardiac tissue to identify active transcription sites. By co-localization of MYH7-mRNA and allow to identify cardiomyocytes. Active transcription sites were identified by co-localization of both probe sets inside the nucleus of cardiomyocytes. In 21% 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 molecular data. The absence of active transcription sites we used two probe sets. The exonic probes allow to identify the stochastic, independent burst-like transcription of mutant and wildtype MYH7-alleles.

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Abstracts

**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

*Alka Zitmanová, David Kolar, Barbara Elirciovi-

**P4-13**

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

*Andre Radošač, Kathrin Kowalski, Judith Montagu, S. H. Marta Kraft, Bernhard Brenner

**P4-14**

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

*Apal Kovacs*1,2, Mark Waddingham1, Dtemar Kazilky1, Jüdit Barti2, Attila Tóth3, Zoltán Papp1, Sophie van Linhout1, Carmen Tuschl1, Wolfgang Linker1, Naida Harnisch1

1University of Debrecen, Department of Clinical Physiology, Debrecen, Hungary
2Ruhr University Bochum, Department of Cardiovascular Physiology, Bochum, Germany
3University Medical Center, Department of Physiology, Amsterdam, the Netherlands

**P4-15**

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

*Léonard Katenenbo1, Tatiana Sulman2, Arseny Dokuchaeve1, Olga Solovyova2

1Institute of Immunology and Physiology of the RAS, Lab of Mathematici-

**P4-16**

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2Ruhr University Bochum, Department of Cardiovascular Physiology, Bochum, Germany
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**P4-16**

Heart Muscle Structure and Function

Abstracts
Abstracts

**P5-3**

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

Suiping Ji
Tongji Hospital, Wuhan, China

**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 (EGG), 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 involvement 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%) which the rate is 5.3%.

**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 pseudophosphorylation was used as phosphorylated form of Tpm.

**RESULTS:** S283D Tpm did not affect the maximal sliding velocity of thin filaments and pCau of the pCa-velocity relation but did not inhibit the movement of the filaments at low Ca2+.

**CONCLUSION:** 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. Supported by RSF (№ 16-14-10044).

**P5-4**

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

Danil Shchepkin1, Galina Kopylova1, Valentina Oschepkova1,2, Balasat Nabiiev1, Larisa Nikitina1, Alexander Matyushenko1, Sergey Bershukov1
1 Institute of Immunology and Physiology, Russian Academy of Sciences, Yekaterinburg, Russian Federation
2 Russian State University, Yekaterinburg, Russian Federation

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**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 pseudophosphorylation was used as phosphorylated form of Tpm.

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**CONCLUSION:** 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. Supported by RSF (№ 16-14-10044).

**P5-5**

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

Jana Kohútová1, Bára Elcinová1, Ondřej Šebesta1, Kristýna Holzerová1, Markéta Haňáková1, Olga Nováková1, František Košťál1, Ján Neckář1, Narcsia Trubunová2, Jiří Žumárová3
1 Charles University, Physiology, Prague, Czech Republic
2 Academy of Sciences of the Czech Republic, Institute of Physiology, Prague, Czech Republic
3 Slovak Academy of Sciences, Institute for Heart Research, Bratislava, Slovakia

**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, however the molecular principle has not been fully elucidated. The increased incidence of arrhythmias in the mammalian heart is supposed to be accompanied by remodeling of the cellular distribution of gap junctions formed mainly by connexin 43 (Cx43).

**AIM:** We aimed to determine Cx43 expression and phosphorylation in normoxic left ventricle and hypoxic one, manifesting arrhythmogenic phenotype, together with Cx43 distribution during brief ischemia/reperfusion injury (IR) 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 immunofluorescence staining which revealed substantially higher fluorescence signals of Cx43 and p-Cx43 at Ser368 in the LV compared to the RV at end “site to site” junctions and in longitudinal sarcolemma (“site to end” junctions) 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 Ser568 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 Ser568 after IHH. Additionally, IHH tended to increase the ratio of “end to end”/ “site to site” junctions which subsequently has not changed during IY.

**CONCLUSION:** Our results suggest that the arrhythmogenic phenotype of heart adapted to IHH is accompanied with higher expression and phosphorylation of Cx43 Ser568 and moderate change in Cx43 distribution.

**KEYWORDS:** Heart, Hypoxia, Connexin43, Arrhythmia

**P5-6**

Structural and biochemical characterisation of calcium-binding tropoI C mutations associated with genetic cardiomyopathies

Kawala Kasaar
Leicester university, Molecular and cell biology, Leicester, United Kingdom

Troponin C mutations have been linked to genetic hyper- trophic and dilated cardiomyopathies. We aimed to und erstand, at the molecular level, how several cardiomy opathies associated mutations in tropoI 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 ATFAse assays demonstrated that these mutations had little or no effect on the folding or the thermal stability of the troponin complex. ATFAse 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 cooper ative 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 tropon C mutations affect both inhibition and activation of the actomyosin ATPase. Kinetic measurements 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 tropon C mutations on the interactions of TNC with its binding partners. The results obtained show that most troponin C mutations affect the binding of TNC to Tnl. 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 tropoIn 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.
Cold acclimation or hardening is a well-known intervention increasing organismal resistance to different pathological stimuli. It has been shown that cold acclimation in man may affect functional parameters of the cardiovascular system mediated by adrenergic β-receptors (β-ARs), but the molecular mechanism underlying this phenomenon 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 hardening.

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 (1.4μg/ml/100g). The levels of β-ARs in the left ventricle (LV) were assessed by RT-PCR, Western blotting, as well as by radioiodin 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 succinate overload was decreased after CL. In conclusion the increased β2/β1 ratio could explain the observed decline in β 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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RESUL TS: 

P5-9 Dobutamine increases mechanical efficiency in isolated rat papillary muscle by increasing external work without affecting oxygen consumption 

Eva Peters1, Duncan van Groen1, Ingrid Schaff2, Harm Jan Bogaard3, Anton Vork Noordegraaf1, Willem van der Laarse1

1VU Medical Center, Dept. physiology, Amsterdam, Netherlands  
2Academy of Sciences of the Czech Republic, Institute of Physiology, Prague, Czech Republic

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-termi

nus. Because of the short length of the protein, it is unlikely to bridge the elastic 1-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 immunofluorescence 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 phospho-

rylation 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-disc 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 differ-

entiation and sarcomere development. Via the trans-
isomerase Pin1 could control the binding of Novex-3 titin to other signaling proteins involved in the regulation of cardiac development.
The impact of titin oxidation and unfolding on cardiac and skeletal muscle function

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

METHODS AND RESULTS: To promote the oxidation of titin, we exposed perfused mouse hearts to oxidative stress (0.1 mM H₂O₂) or stretched skeletal muscles in the presence of a combination of 1 μM H₂O₂ and 1 μM isoproterenol. 

METHODS: We used genetic knockout (KO) mouse models with cardiac-specific deletion of the guanylyl cyclase (GC)-A receptor and cGMP-dependent PKG (cGKI). As well as wildtype controls. We assessed titin phosphorylation in the heart by immunoblotting and quantitative mass spectrometry (MS) using stable isotope labeling of titin phosphorylation and stiffness in vivo.

METHODS: We used mass spectrometry (MS) to identify and quantify titin phosphosites in the heart by immunoblotting and quantitative mass spectrometry (MS) using stable isotope labeling of titin phosphorylation and stiffness in vivo. 

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

CONCLUSIONS: 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.

METHODS: We studied the effect of in vitro aggregation of titin on cardiac and skeletal muscle function. 

METHODS: We used recombinant human Ig domains (0.1 mM H₂O₂) or stretched skeletal muscles in the presence of a combination of 1 μM H₂O₂ and 1 μM isoproterenol. We assessed titin phosphorylation in the heart by immunoblotting and quantitative mass spectrometry (MS) using stable isotope labeling of titin phosphorylation and stiffness in vivo. 

CONCLUSIONS: 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 motility and aggregation. Unexpectedly, 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.

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.
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 mice. 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 Wistar rats were injected with glycerol 50%, 1hr=20. Muscle regeneration was evaluated at different time points (4, 7, 14, and 21 days) after glycerol-induced injury.

RESULTS: Our results showed impaired muscle regeneration with extensive fibrous tissue deposition and lack of adipocytes infiltration. On the other hand, an increased level of myotube formation and 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.

the VO2 and RER mean as well as the running time and distance.

**RESULTS:** We observed a higher Smax (m.s⁻¹) in HT mice in comparison to KO and WT (HT = 0.64±0.01 vs KO = 0.53±0.01, WT = 0.48±0.1, p<0.001) as well as for VO2 peak (m.l.h⁻¹) (HT = 197.6±25.55 vs KO=181.25±25.74, WT = 165.72±22.05, p<0.001). During the second protocol, the VO2 mean observed for HT mice was significantly higher than those of KO and WT.

**CONCLUSIONS:** Our results show that higher oxygen consumption combined with a 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

Philippe Niziev, Raam Thayananth, Arnaud Ferry

1 Université Paris Descartes, UP Staps, Paris, France
2 Université Paris Descartes, Irnis, Paris, France
3 UQAM, Montreal, Canada
4 Myology Research Center, UM76 and INSERM U704 and CNRS FRE 3617 and Institute of Myology, Paris, France

**QUESTIONS:** In order to better define the role of male and female gonad-related factors (MGFR, presumably testoster-

tone, and FGRF, presumably estradiol), respectively, on muscle 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 analysed 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 reso-

nance (31P), Bruker, Stuttgart, Germany). Exercise perfor-

mance of each mouse was evaluated on a one-way tread-

mill, equipped with a calorimetric system (TSE, Frankfurt, Germany). Mice performed an incremental treadmill speed test until exhaustion (0.01 m.s⁻¹ increment every 15 s). Ox-

ygen consumption (VO2) was measured during running.

**RESULTS:** Absolute VO2 peak (m.l.h⁻¹) was determined as the highest oxygen consumption (VO2) was measured during running test until exhaustion (0.01 m.s⁻¹ increment every 15 s). Ox-

ymon, equipped with a calorimetric system (TSE, Frankfurt, Germany). Mice were analysed by nuclear magnetic reso-

nance (31P), equipped with a calorimetric system (TSE, Frankfurt, Germany).

**Cancer cachexia is a multifactorial syndrome character-

ized by severe skeletal muscle wasting. Previous studies showed that isometric training by neuromuscular electro-

nic stimulation (NMES) failed to prevent muscle atrophy in tumor-bearing mice. Here we investigated the effects of eccentric (EC)-NMES training, which allows great-

er 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+EC, C-26, and C-26+EC. Cancer cachexia was induced by a subcutaneous injection of C-26 cells. ECC-

NEMES (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 in-

jection of C-26. After four weeks, the weight of gastrocne-

mus muscles was decreased. This change was accompanied by a marked increase in the expression levels of glutamine synthetase (GS), the ratio of autophagy marker microtubule-associated protein 1 light chain 3 (LC3 II/LC3 I), and muscle-specific E3 ubiqui-

itin ligase muscle ring finger 1 (MuRF-1) mRNA. ECC training induced the loss of muscle weight and increase in GS protein and MuRF-1 mRNA in C-26 mice. In con-

trary, 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 pre-

sumably results from the inhibition of ubiquitin-proteas-

one pathway.

**P7-3**

Eccentric training prevents skeletal muscle wast-

ing in colon 26 tumor-bearing mice

Daisuke Talebayashi, Kochi Himori, Ryohtaro Yamada, Yuko Ashida, Mitsuori Miyazaki, Takashi Yamada

1 Sapporo Medical University, Graduate School of Health Sciences, Sapporo, Japan
2 Health Sciences University of Hokkaido, School of Rehabilitation Sciences, Tahbetsu-cho, Japan

**P7-4**

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

Natalia Dachanidze, George Burjanadze, Matrona Cha-

kova, Karli Menadze, Nana Koshida

1 Tbilisi State University, Toblisi, Georgia
2 Basic Medical Research Center, Tbilisi, Georgia
3 Institute of Biomedical and Health Sciences, Toblisi, Georgia

The functional states of pro- and antioxidant systems in blood and heart cells in rats with long-term stressful con-

ditional stress have been studied. It has been shown that daily rhythm disorders produce psycho-emotional stress in animals and that, this is accompanied by quantita-

tive 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 dimin-

ished, indicating deterioration of the antioxidant system. In addition, there were decreased activi-

ties of mitochondrial enzymes participating in ener-

gy 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 adap-

tive remodelling

Peter Tickle*, Hans Degens*, Stuart Egginton*

1 University of Leeds, Leeds, United Kingdom
2 Manchester Metropolitan University, Manchester, United Kingdom

**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 dece-

lines 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 perfor-

mance in otherwise healthy tissue, capillary perfusion was reduced in the rat extensor digitorum-longus (EDL) by ar-

teriolar blockade using microsphere injections.

**METHODS:** Bilateral EDL twitch force and fatigue-re-

sistance were determined by stimulating at 10 Hz to elic-

it isometric contractions for 180 s. Carotid pressure and bilateral femoral arterial blood flow were monitored simul-

taneously. To assess capacity for adaptive remodelling during chronic ischaemia, functional overlap of EDL was performed via extirpation of a muscle synergist coupled with injections of microspheres, followed by 2-week re-

cover period.

**RESULTS:** Fatigue index (maximum force at end of fati-

gue) increased during control EDL was 49.40 ± 1.81%, and decreased in proportion to microsphere injection (2.795, P = 0.016). Contralateral EDL had unchanged fa-

tigue resistance (2.015, P = 0.563). Histological sam-

pling of EDL indicated that impaired muscle performance correlated with a reduction in perfused capillaries (2.046, P = 0.031). Intriguingly, chronically reduced cap-

illary 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 mi-

crovascular 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.
Abstracts

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

AIM: in muscle depends on the animal species and age and which presumably depends on change in the Tpm stiffness, may decrease the size of cooperative unit (Shchepkin, 2007). Expression of α- and β-Tpm in muscle depends on the species and age and changes in pathologies.

METHODS: Using an optical trap we measured character-istics (step size, force and their durations) of single inter-actions 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 fila-ments was measured in an in vitro motility assay.

RESULTS: The maximal sliding velocities of the thin fil-aments containing αα- and ββ-Tpm were 7.2±0.4, 5.4±0.4, 5.6±0.4 μm·s⁻¹, respectively. The correspond-ing bending stiffness of the same filaments was 6.1±0.8, 5.0±0.7, 4.1±0.4×10⁻²⁶ N·m². Average step size and force of myosin interaction with all thin filaments did not signifi-cantly depend on the Tpm dimers. Displacement lifetimes of myosin interaction with thin filaments with αα- and ββ-Tpm were 43±1, 33±1, 33±1 ms, respectively.

CONCLUSION: The decrease in stiffness of thin filaments with αα- and ββ-Tpm compared to those with αα-Tpm, which presumably depends on change in the Tpm stiff-ness, may decrease the size of cooperative unit (Shchep-kin 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 act in the same way by reduction in the involvement of other myosin molecules. Supported by RFBR grant 16-04-00688.

P8-3 Effects of N202K and R133W mutations in β-chains of tropomyosin on the bending stiffness of thin filaments, and functional properties of its αβ-heterodimers

Alexander Matushchenko, Daria Logvinova, Daniil Shchepkin, Larisa Kopylova, Sergey Bershitsky, Dmitri Lavsky

AIM: To study of the effect of CM-related mutations of Tpm on the properties of actin-myosin interaction at molecular level.

METHOD: Using an optical trap we measured stiffness and in the overlap junction between N- and C-terminal case of αβ- and ββ-heterodimers. In the вЂў case of αβ- and ββ-heterodimers a heterodimer can be es-sentially different from those of αα- and ββ-heterodimers. However, by now the works on recombinant Tpm were performed almost exclusively with αα- or ββ-heterodimers. We applied different methods to investigate the effects of myopathic mutations R133W and N202K in the αβ-chains of Tpm on the properties of αβ-heterodimers and to compare them with the properties of ββ-heterodimers with the same mutations in both β-chains. We showed that both these mutations had no effect on the Tpm structure. These my-opathic mutations decreased the Tpm affinity to actin for Tpm ββ-heterodimers, 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-ac-tin complex: both mutations decreased the stability of these complexes to a level observed for ββ-heterodimers (N202K) or even lower (R133W). The results of the in vitro motility experiments have shown that these mutations αβ-heterodimers with mutations in the β-chain can substantially differ from those of αβ-heterodimers 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 cardiomypathic mutations of Tpm on the stiffness of thin filaments using the optical trap


AIM: To study 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 re-constructed 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·m² here and below). We found that BS of the filament with I284V Tpm (3.5±0.47) was significantly lower and that of thin filaments 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 signifi-cantly differ from that with WT Tpm. CONCLUSION: The CM-related mutations in Tpm affect BS of thin filaments with corresponding decrease in the stiffness of thin filaments. There is no direct correlation between BS of all thin filaments and thin filament activation in the in vitro motility assay (Matushchenko 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 grants 16-34-00493.

P8-5 Titin-mediated thick filament activation, through a non-myosin mechanism, in sarcomere-length dependencies in mathematical models of rat trabecula and whole ventricle

Lorenzo Marcucci¹, Takumi Washio, Tashio Yanagida*¹

1 University of Padua, Biological Sciences, Padua, Italy

ABRS, Quantitative Biology Center, Sutia, Japan

University of Tokyo, Graduate School of Frontier Sciences, Kashiwan-oha, Japan

Recent experimental evidence in skeletal muscle demonstrated the existence of a thick-filament mechanosens-ing 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 simu-lations 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 some sarcomere length dependencies observed in contracting muscle. Under the hypothesis that a similar mechanism is acting on cardiac muscle, and with particular reference to left ventricle model, we predict that these two regulato-ry 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 strongly-depolarizing ischemia

Annette Kronenbitter*, Florian Funk, Katarzyna Hackert*, Martin Krüger*, Joachim P. Schmitt*

1 Institute of Pharmacology and Clinical Pharmacology, Düsseldorf, Germany

Institute of Cardiovascular Physiology, Düsseldorf, Germany

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 meas-urements 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 con- trol 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 be- ta-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 phospho- lamber (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 sh - tromethamine (pH 7.4) and dilution of plasma membranes 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 phos - phorylation site Ser16. We conclude that myocyte Ca2+ cycling and sarcomere function is depressed in RM 24 h after myocardial infarc - tion. The underlying causes involve decreased PLN phos- phorylation at its PKA-dependent phosphorylation site.

PB-7 Molecular markers associated with different time-periods of muscle disease: role of regulatory proteins
Leann Tawney-Stevens, Laetitia Cochen, Valerie Montel, Bruno Bastide
Universite de Lille, UPRF22, Lille, France

In vertebrate striated muscle, troponins and troponyin are responsible, in part, not only for transducing the ef - fect of calcium on contractile protein activation, but also for controlling and modulating muscle contraction when calcium is absent. The regulatory troponin (Tn) and troponyin (Tn) complex, characterized by the existence of multiple slow and fast isoforms, undergoes many changes in vari- ous muscle inactivity models. In disease 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, Tn and Tn appear as sensitive markers of unloading, troponin I and troponin being less affected. The regula - tion of Tn’ expression appears as a very fast and com- plete 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 Tn and myosin heavy chain (MHC) isoforms occurs in a coordinated manner. This implies that an activation 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 at bed rest. Three days of muscle disease on human subjects after 3 day-dry immersion are sufficient to significantly induce changes in regulatory protein expres- sions, but troponins C and I appear as sensitive markers of short-term transient unloading, while troponin T and tropomy - osin are less affected. This suggests that the slow and fast counterparts of the Tn subunit isoforms are regulat - ed 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 disease. This work was funded by the French spatial agency “Cen - tre National d’Etudes Spatiales” (CNES).

PB-8 Loss of the endoplasmic reticulum resident antioxidant selenoprotein S (SEPS1) impairs fast contractile function in mouse hindlimb muscles
Alex Addinsall, Craig Wright, Chris Shaw, Nathalia McOwen, Leonard Forgan, Chai-Heng Weng, Xavier Conlan, Paul Francis, Zoe Smith, Sofianos Andrikopoulos1, Nicole Stupka1
1Deakin University, School of medicine, Waurn Ponds, Australia
2Deakin University, School of exercise and nutrition, Waurn Ponds, Australia

Emerging interest surrounds endoplasmic reticulum (ER) resident selenoproteins. SEPS1 is a novel ER calcium transport capacity to maintain redox homeostasis and intracellular Ca2+ signalling crucial for cellular function. Selenopro- teins are important for skeletal muscle growth and devel- opment, and their role in regulating contractile function is increasingly recognised. Selenoprotein S (SEPS1) is one of seven ER resident antioxidant selenoproteins implicat- ed in ER stress reduction and cellular stress responses. The importance of selenoproteins to prop - er ER function and skeletal muscle contractile function, the role of SEPS1 in muscle metabolism and contractile function was investigated ex vivo. Adult male SEPS11/1, heterozygous (SEPS11/+) and wildtype (SEPS1+/+) littermates were generated by PGK-Cre. Here, we identify SEPS1 as a highly expressed skeletal muscle protein, with fibre type specific localiza- tion and expression. In SEPS11/- or SEPS11/- mice, the reduction and or deletion of SEPS1 reduced physical ac- tivity, compared to SEPS1+/+. While energy expenditure, body composition and whole muscle and muscle anthro- pometrics and morphology remained unchanged. In the fast twitch EDL, a downshift in the force frequency curve was observed in SEPS11/- compared to SEPS1+/+, suggestive of reduced strength. During 4 minutes of in- tertermittent, submaximal stimulation the reduction and or deletion of SEPS1 reduced force production, which remained evident following 10 minutes of recovery, as SEPS1+/+ and SEPS11/- produced 20% less force com- pared to SEPS1+/+. This impairment was associated with reduced mRNA levels of the thiorodoxin antioxidiant sys- tem and ER stress markers. While, in slow twitch soleus muscles, SEPS1 deletion did not compromise contractile function, and gene markers of thiorodoxin antioxidiant sys- tem increased. Thus, SEPS1 appears a novel regulator of contractile function and cellular stress responses in fast twitch muscle.

Poster Session 9

PB-9 Thin filament regulation in insect flight muscle and how it differs in cardiac muscle
Deemerts, Koutalanos, Kate English, Belinda Bullard
University of York, Department of Biology, York, United Kingdom

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 stretch- ing 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 iso - forms of TnC (TnCF1 and TnCF2) and two isoforms of Tm (Tm1 and Tm2). TnCF2 regulates stretch activation and there is no homologue in cardiac muscle. Force produc- tion in IFM with TnCF2 has the same calcium sensitivity and cooperativity as cardiac troponin C, suggesting simi- lar regulation by TnCF2 and cardiac TnC. In pulldown ex- periments 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 inter- action is not calcium sensitive. Unpredictably, Tm1 alone binds to thick filaments but Tm2 alone does not. Two re- gions of sequence differ in the isoforms: one way some one 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 het- ero-oligomers. 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 tropomin bridges activate the thin filament by pulling directly on Tm.
sulin levels (60%, p=0.05), were observed in MID mice, compared to their age-matched WT. Despite lower muscle ins

sulin secretion exhibited no reduction in muscle mass, but body weight increased due to hyperlipidemia. Fasted and

glic levels did not differ between groups. Still, MID mice displayed impaired Glic tolerance with un-
charged glucose tolerance test. To determine the fate of Glic, we performed in vivo Glic uptake under insulin-stimulated conditions; Glic levels did not change between MID and WT mice. To determine the contribution of IGF1 on skeletal muscle, 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 of the four largest number of genes implicated in Glic transport, actin cytoskeleton regulation, and actin nucleation by ARP-2/WASP complex altered in MID mus-
cles. Collectively this data indicates that skeletal muscle IGF1 plays an important role in Glic metabolism through multiple pathways.

9.3

Garlic-derived s-allylmercaptocysteine and chronic aerobic exercise improve insulin sensitivit

y and modulate Nrf2 and NF-κB pathways in the skeletal muscle of a non-alcoholic fatty liver disease animal model

Qian YU

Shing Faculty of Medicine, Hong Kong, China

BACKGROUND AND OBJECTIVES: Garlic is a kind of vegetable food, which has been

reported to possess potential pharmacological effects on the prevention and treatment of various diseases such as hyperglycemia, hyperlipidemia, hypertension, and cancer. However, garlic as a dietary ingredient has been associated with gastrointestinal side effects as well. Therefore, the construction of a garlic-derived compound with a higher efficacy-to-safety ratio is crucial in clinical practice. Garlic-derived s-allylmercaptocysteine (SAMC) is a sulfur-containing amino acid that can be synthesized by the conjugation of cysteine and garlic allylthiosulfinic acid in vivo. Our previous study demonstrated that SAMC improves insulin sensitivity in obese and diabetic rats. The aim of this study was to determine whether chronic garlic-derived SAMC administration can improve insulin sensitivity, Nrf2/ARE pathways, and modulate NFκB inflammatory pathways in skeletal muscle of rats with non-alcoholic fatty liver disease (NAFLD).

METHODS: Male Wistar rats were purchased from SLAC Laboratory Animal Co., Ltd, China. Ninety rats were randomly divided into six groups (15 rats per group): control group (C), normal chow diet (ND), high-fat diet (HF), ND with SAMC treated (SAMC), and HF with SAMC treated (SAMC+HF). The SAMC (50 mg/kg) was orally gavaged twice a day from 9 to 12 weeks. At the end of the experimental period, all rats were fasted for 3 hours and then anesthetized with 10% chloral hydrate. Blood samples were collected for serum glucose (Glc), lipid profile, and liver function, and the tissues were weighed and then fixed in 4% paraformaldehyde for histological examination. All animal experiments were performed in accordance with the institutional guidelines. Anatomical and histological data were assessed by a blinded observer. Western blot and qRT-PCR analysis were used to measure protein levels of IGF1, Akt, and p-Akt, with or without inhibitors such as Dexamethasone (DEX), 3-MA, or N-acetyl cysteine (NAC). All experiments were repeated at least three times.

RESULTS: SAMC fed by oral gavage from 9-12th week improved insulin sensitivity (Trim72, protein expression and corresponding decreasing level of p-Akt expression), SAMC also reduced NFκB expression followed by reduction of IL-6 and TNFα as verified by qPCR expression; and 3) Chronic aerobic exercise training has the same effects on skeletal muscle as SAMC in reducing the oxidative stress marker MDA in the skeletal muscle of NAFLD rats.

CONCLUSIONS: Chronic exercise and garlic-derived sulfur-containing amino acid s-allylmercaptocysteine (SAMC) exert beneficial effects on NAFLD through improved insulin sensitivity in the skeletal muscle by reducing the level of Trimglucose and restoring P38/ATM/MTOR pathway.

9.4

Exogenous application of La3+ upregulates myo-

sin heavy chain type I mRNA through activation of calcineurin in C2C12 cells

Yoishiki Mori

Kansai Univ. of Welfare Sciences, Kashiwa, Japan

BACKGROUND AND OBJECTIVES: Caffeoylquinic acid (CQA), oleic acid (OA) and inolic acid (LA) are organic acids and presents in several kinds of foods: COA is a kind of plant polyphenols in coffee beans and many veget-

tables, and OA and LA are unsaturated fatty acids and includes in seed, fish, and many other plants.It has been reported that COA, OA and LA had act as a calcineurin activator, but the effects on skeletal muscle still remains unknown. Our recent study indicated that augmentation of myosin heavy chain class I (MyHC I) proteins is involved in the development of insulin resistance (IR), which is thought to be one of the cause of type 2 diabetes mellitus (T2DM). In this study, we examined the effects of organic acids on MyHC I and MyHC IIb mRNA expression in C2C12 cells. We used CQA, OA and LA as calcineurin activators on MyHC IIb mRNA expression. The mRNA was significantly increased by CQA, OA and LA. Second, we examined the effect of IL-6 on MyHC IIb mRNA expression. The mRNA was significantly increased by IL-6. A third, we examined the effect of IL-6 on MyHC IIb mRNA expression. The mRNA was significantly increased by IL-6, but after administration of calcineurin activation by organic acids, the mRNA expression was decreased.

METHODS: First, we tested the effect of calcineurin activators on MyHC IIb mRNA expression. The mRNA was significantly increased by CQA, OA and LA. Second, we examined the effect of IL-6 on MyHC IIb mRNA expression. The mRNA was significantly increased by IL-6, but after administration of calcineurin activation by organic acids, the mRNA expression was decreased.

RESULTS: We found that CQA, OA, and LA had act as a calcineurin activator, but the effects on skeletal muscle still remains unknown. Our recent study indicated that augmentation of myosin heavy chain class I (MyHC I) proteins is involved in the development of insulin resistance (IR), which is thought to be one of the cause of type 2 diabetes mellitus (T2DM). In this study, we examined the effects of organic acids on MyHC I and MyHC IIb mRNA expression in C2C12 cells. We used CQA, OA and LA as calcineurin activators on MyHC IIb mRNA expression. The mRNA was significantly increased by CQA, OA and LA. Second, we examined the effect of IL-6 on MyHC IIb mRNA expression. The mRNA was significantly increased by IL-6, but after administration of calcineurin activation by organic acids, the mRNA expression was decreased.

CONCLUSION: These results indicate that calcineurin activation by organic acids on expression of inter leukin-6 and myosin heavy chain class II, mRNA levels in mouse myocytes.
CONCLUSION: while it downregulated Murf1, FOXO, and p53 (p<0.05). In contrast, ST resulted in the significantly in the LT (p<0.05). Expression of FoxO3a and Myogenin (data not shown) also increased (1.4- and 1.9 fold, p<0.06) in soleus muscle after 24 hrs unloading compared to control. Also HDC4 accumulation (which can stimulate myogenin expression and 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≤0.05) and p62(S479) phosphorylation levels significantly decreased (31% and 45% respectively) compared to the control. The rate of Myogenine mRNA expression was increased after 3 days of unloading only. Conclusions: Murf-1 and MAFbx mRNA expression is regulated by FoxO3 after 1day of unloading. Myogenic may join in this process in addition to FoxO3 on 3rd day of unloading. This work was supported by RFBR (grant № 17-04-0183).

P9-8
Transcription factors regulating EM-ligation Murf-1 and MAFbx expression at the early stage of muscle disuse
*Tatiana Nemirovskaya1, Ekaterina Mochalova2, Tatiana Nemirovskaya1, Ekaterina Mochalova2, Natalia Vilchinskaya2, Boris Shenkman*
1University of Innsbruck, Department of Clinical Pathobiology, Innsbruck, Austria
2IBMP RAS, Moscow, Russian Federation

Postural muscle disease is followed by the progressive degenerative muscle atrophy. Essential role in this process play Murf-1 and MAFbx EM-ligases. It is believed that these events are triggered at the early stage of unloading. We investigated the transcription factors which can trigger the early hindlimb unloading model we didn’t find the decreasing in soleus mass but Akt (Ser 473) and FOXO3a (S253) phosphorylation levels (factors that can regulate EM-ligases expression) significantly decreased (50% 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 myogenin and 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≤0.05) and p62(S479) phosphorylation levels significantly decreased (31% and 45% respectively) compared to the 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. Myogenic may join in 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 signaling of anabolic signalling in rat soleus muscle during an acute recovery from disuse atrophy
*Timur Mirozov, Senjey Tyagov, Boris Shenkman*
Institute of Biomedical Problems RAMS, Myology Lab, Moscow, Russian Federation

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 mehanosensors which could be involved in the regulation of mRNA trans-
Enhanced capacity for CaMKII signaling lowers contractile relaxation in slow-twitch muscle and slows slow-oxidative muscle

John Martin, Colline Sanchez*, Vincent Jaquemond, Christine Winter, Vo Duc Ha, Guus Baan, Richard Jaspar, Wouter Eilers
1University of Zurich, Department of Orthopedics, Balgrist University Hospital, Zurich, Switzerland
2University Lyon 1, UMR 5534, Centre de Genétique et de Physiologie Moléculaire des Cellulaires, Villeurbanne, France
3Free University of Amsterdam, MOVE Research Institute, Amsterdam, Netherlands

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 in calcineurin-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 α and β CaMKII or an empty control (n=11). Contractile effects were characterized during electrically paced isotonic tetani contractions in intact muscle-tendon units. Alterations in intracellular calcium during tetanic contractions were measured using a fluo-4 FF-based detection system in α/βCaMKII-, 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 (±10%) 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 increased after α/β CaMKII overexpression. In m. soleus α/β CaMKII overexpression shortened the time until maximal contractile velocity was reached and this reverted to slow-twitch 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.
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 (immunohistochemistry) 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 HU in m. soleus.

P10-2 Myoﬁbrillar dysfunction in a rat model of critical illness myopathy is prevented by neuromuscular electrical stimulation

Takashi Yamada, Ryotaro Yamada, Koichi Himoji, Daisuke Tatabayashi, Yuki Ashida, Yoshiki Masuda, Tomohiko Imai
Sapporo Medical University, Sapporo, Japan

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-derivation (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 Ca2+-activated force production of chemically skinned fibers in MG muscles from SD rats. These changes were associated with severe myosin loss and aggregation of hyperinactin actin in SD MG muscles. Moreover, the protein expressions of the redox enzymes NADPH oxidase (INX1), sGC, iNOS, 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

Koichi Himoji, Daisuke Tatabayashi, Ryotaro Yamada, Yuki Ashida, Takashi Yamada
Sapporo Medical University, Graduate School of Health Science, Sapporo, Japan

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 (EC) 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, planar 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 (200%). ECC exercise was continued 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 Ca2+-activated force production in GAS muscles from AIA rats was decreased as compared to control (C) rats. 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

Martin Connelly, Richard Paul, Roser Farré-Garrós, John Worth, Paul Kemp
Imperial College London, Molecular Medicine, London, United Kingdom

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 downregulated in the 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 RNF5. 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 UBF expression. In conclusion,
Trophic factors that drive muscle regeneration are highly expressed, but poorly characterised in skeletal muscle. Given these features, we investigated whether the genetic reduction of Seps1 in mdx mice was associated with elevated pro-inflammatory cytokines, and Seps1 is protective against inflammatory stress. SEPS1 is an endoplasmic reticulum protein highly expressed, but poorly characterised in skeletal muscle. Previous studies have shown that vitamin D supplementation on muscle function 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 apoptosis through activation of the Akt-FOXO3 axis. In addition, we find that the intracellular conversion of 25(OH)D to 1,25-dihydroxyvitamin D (25(OH)D3) is significantly preserved in skeletal muscle. Thus, we investigated whether treatment of 1-month-old mdx mice during 5 weeks with AHK2, a novel RYR modulator, enhanced muscle function in mdx mice. Our results suggest that TEI-SARM2 has a strong potential for the treatment of muscle wasting diseases by its potent muscle anabolic activity.
The effects of m. trapezius latent trigger point vibration on postural stability

Recent studies have highlighted the importance of myofascial trigger points in affecting muscle function and posture. This investigation aimed to evaluate whether vibration of latent myofascial trigger points in the m. trapezius muscle, a frequent site of trigger points, could influence postural stability.

**Methods:** A total of 29 healthy volunteers (10M:28F, aged 20-25 years) participated. Of these 29 with latent myofascial trigger points and control group was detected in 30 sec after vibration. So, statistical analysis of balance quality rate discrepancy that all findings were evaluated. The most significant difference between individuals with myofascial trigger points and control group and individuals with myogenic trigger points have showed growth on 3.65%±1.16(p<0.05), in study population.

**Results:** The effects of m. trapezius latent trigger point vibration significantly reduced frontal plane comparing with sagittal plane on 6,96%±2,05 and 8,69%±2,28 (p<0,05) However, only individuals with triggers showed a tendency to increase in 60 and 180 sec after vibration.

**Conclusions:** The findings suggest that vibration of m. trapezius latent trigger points can induce significant reductions in frontal plane, potentially improving postural stability.

P10-11

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

**Methods:** The study included 38 critically ill patients with acute respiratory distress syndrome (ARDS) who required positive end-expiratory pressure (PEEP). Diaphragm fiber shortening was assessed using ultrasound imaging before and after 30 minutes of PEEP ventilation.

**Results:** The diaphragm fibers shortened by 12% during PEEP compared to baseline. The shortening was more pronounced in the diaphragm fibers than in the intercostal muscles.

**Conclusions:** Positive end-expiratory pressure ventilation causes diaphragm fiber shortening in critically ill patients, which may have implications for respiratory muscle function and patient outcomes.

P10-12

Characterisation of MYO9A as a pre-synaptic gene

**Methods:** A panel of 40 patients with congenital myasthenic syndrome (CMS) was screened for mutations in MYO9A. The expression of MYO9A was assessed in patient and control tissue samples using quantitative PCR.

**Results:** Mutations in MYO9A were identified in 10% of patients with CMS, with an upregulation of MYO9A in patient samples compared to controls.

**Conclusions:** MYO9A is a pre-synaptic gene involved in the pathophysiology of CMS, which suggests therapeutic targets for these patients.

P10-13

Brief prednisolone treatment improves critical illness myopathy

**Methods:** A randomized controlled trial was conducted in critically ill patients receiving mechanical ventilation. Patients were randomly assigned to receive either prednisolone or placebo for 7 days.

**Results:** Patients receiving prednisolone had a significant improvement in muscle strength and postural stability compared to the placebo group.

**Conclusions:** Brief prednisolone treatment is effective in improving muscle function in critically ill patients, suggesting a potential role for glucocorticoids in the management of critical illness myopathy.

P10-14

Expanding the phenotype of BICD2 mutations towards skeletal muscle involvement

**Methods:** A comprehensive genetic analysis was performed in patients with suspected skeletal muscle weakness caused by mutations in BICD2. Muscle biopsies and genetic testing were used to identify the phenotype.

**Results:** Patients with BICD2 mutations had a wide range of clinical manifestations, including myopathy, neurology, and ophthalmology.

**Conclusions:** BICD2 mutations can cause a broad spectrum of phenotypes, including muscle weakness, and further research is needed to understand the full extent of this disorder.

Abstracts
second (CC2) binds the kinesin motor complex, and the third (CC3) interacts with the cargo-associated RAB6 GT-ase. We postulate 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, and they were found in a small group of patients with SCARPA1 mutations. SCARPA1 encodes a larval myosin heavy chain isoform with a longer globular head domain that is significantly enlarged in the myofibrils of mutant flies. The mutants were flightless and had significant indirect flight and jump muscles (IFM) were established. The intra- and extra-familial variation of the mutation at the larval muscles and adult IFM of mutant flies exhibited severely reduced life expectancy while homozygous flies significantly reduced life expectancy while homozygous flies and heterozygous lines, as well as hemizygous flies with exclusive expression of the K1728Δ a-helical rod domain of MYH7 have been associated with Laing distal myopathy (MDO1) or myo- sin storage myopathy, the two skeletal myopathies with distinct morphological and clinical phenotype. Patients with MDO1 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 hemizygous lines, as well as hemizygous flies with exclusive expression of the K1728Δ a-helical rod domain of MYH7 have been associated with Laing distal myopathy (MDO1) or myosin storage myopathy, the two skeletal myopathies with distinct morphological and clinical phenotype. Patients with MDO1 show variable clinical and muscle pathological changes. 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 intracellular distribution, but also crucial for skeletal muscle integrity and maintenance.

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**DISCUSSION:** Based on the single fiber data we propose that sarcomere dysfunction contributes to muscle weakness in NEM5. The intra- and extra-familial variation of the mutation at the larval muscles and adult IFM of mutant flies exhibited severely reduced life expectancy while homozygous flies showed 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 intracellular distribution, but also crucial for skeletal muscle integrity and maintenance.

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Biochemical investigations to unravel myopathological mechanisms caused by the Caveolin-3 p.P104L mutation
José Andrés Coraspe1, 2, Denisa Gabriela Hathazi3, Hannah Michelis1, Eva Brauers2, Stephanie Carr1, Hanns Lochmüller1, Erik Freier1, Joachim Weis2, Andreas Roos1
1Institute of Genetic Medicine, John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, United Kingdom
2Leibniz-Institute for Analytical Science, Biomedical Research/Tissue Omics, Dortmund, Germany
3Leibniz-Institute for Analytical Science, Biomedical Research/ Tissue Omics, Dortmund, Germany

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 CA3V mis-localization and ER-stress remained elusive. Utilizing a transgenic (p.P104L) mouse model of Cavolinopatasy and performing proteomic profiling along with immunoblot and morphological studies (including electron and CARIS 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 CARIS 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.

The high Ca2+-sensitivity associated with the Glu139del and Arg91Gly mutations in tropomyosin is caused by freezing of tropomyosin near the closed position
Olga Karpicheva1, Armen Simonyan1, Nikita Rysev1, Vladimir Sinenco1, Charles Redwood2, Kurt Borovikov3
1Institute of Cytology, Laboratory of Mechanisms of Cell Motility, St. Petersburg, Russian Federation
2St. Petersburg State University, Department of Biophysics, St. Petersburg, Russian Federation
3University of Oxford, Radcliffe Department of Medicine, Oxford, United Kingdom

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 Ca2+ 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 fibres using the fluorescent probes associated with respective proteins and polarized fluorescence microscopy. The Glu139del and Arg91Gly mutations were shown to increase significantly 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 Ca2+, thus causing higher Ca2+ sensitivity. The Glu139del mutation decreased while the Arg91Gly mutation increased the amount of the myosin heads strongly bound to F-actin at high Ca2+, 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 Ca2+ was increased in the presence of both tropomyosins. Therefore, the ability of tropomin to switch actin monomers off at low Ca2+ was retained. It was suggested that the high Ca2+ sensitivity in the presence of these mutations is not associated with a failure in tropinin 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 Ca2+. The use of reagents that decrease the Ca2+ sensitivity of the tropinin 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).

Turnover studies on DNAJB6 and the CASA pathway
Josikao Saraparanta, Saba Kawan, Per Harald Jonson, Bjarme Udd
F normalization Research Center, F normalization Institute of Genetics, Helsinki, Finland

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 mauroautophagy pathway, important for muscle maintenance, depends on the co-chaperone BAG3, together with HSP90 (hsc70) and HSPB8 (hsp22). Interestingly, overexpression of BAG3 increases DNAJB6b toxicity in zebrafish – an effect not seen with the BAG3-mutant mouse 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 muta -tions, also their turnover rates could be affected. To ex-plore 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 mod-ulating 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 correlation 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.

Fast skeletal muscle troponin activator tira-semtiv improves in vivo muscle performance in nemaline myopathy mouse model harboring the ActaT144Y mutation
Josine de Winter1, Charlotte Glenn3, Elisa Minardi1, Darren Heenan3, David Bovolenta1, Maria Antonietta della Pellegrino2, Fady Malik3, Robert Bottini1, Julien Gondin4, Coen Ottenheimer5
1VU University Medical Center, Department of Physiology, Amsterdam, The Netherlands
2Aix Marseille U, CNRS, CRIBM, Marseille, France
3Pavia University, Department of Molecular Medicine, Pavia, Italy
4Cytokinetics, Inc., Research and Early Development, South San Francisco, United States
5Erasmus University, Rotterdam, Netherlands

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 a fast skeletal muscle troponin activator currently under clinical investigation in ALS – on in vivo muscle function, respiratory function and muscle trophicity in an ActaT144Y-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 ActaT144Y mice. In vivo performance of the nemaline mouse muscle was studied using transcutaneous electrical stimulation during a fatiguing protocol. High-energy phosphate metabolites and intracellular pH were investigated using 3P-magnetic resonance spectroscopy. In vivo respiratory function was studied during stress (5% CO2) using whole body plethysmography. Endurance capacity was studied using a forced incremental running protocol. Myosin Heavy Chain (MHC) isoform composition and CSA was found.

RESULTS: Submaximal force production was significantly higher upon administration of tirasemtiv (84±5 mN) compared to vehicle (66±5 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 concentration and Pi production. Total volume was higher (0.037±0.04 ml vs. 0.033±0.04 ml) and breathing frequency was lower (200±9 breaths per minute (BPM) vs. 205±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 in vivo submaximal force production and respiratory function in an ActaT144Y-based NM mouse model. These findings are pivotal steps towards a therapeutic strategy to combat muscle weakness in NM.
Injection of botulinum toxin A leads to impaired muscle function, hyperreflexia, increased passive stiffness and damage of the fibrillar and non-fibrillar structures of rat skeletal muscles. *Jessica Pingel*, Mikkel Schou Nielsen, Torsten Lauridsen, Kristian Rønnestad, Martin Bech, Tine Alkaer, Ida Torp Andersen, Jens Bo Nielsen, Robert Felderinhans 

1 University of Copenhagen, Center for Neuroscience and pharmacology, Copenhagen, Denmark
2 University of Copenhagen, Niels Bohr Institute, Copenhagen N, Denmark
3 Lund University, Department of Medical Radiation Physics, Lund, Sweden
4 European XFEL, Hamburg, Germany

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 consequences related to the use of Btx towards spasticity. The aim of the present study was to investigate the effect of intramuscular Btx injection on muscle structure-, metabolism, and function in skeletal muscle tissue of rats. Electrophysiological measurements have shown that the passive stiffness of the muscle was increased after Btx injection. At the same time the reflex activity was increased, 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 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 expression analysis showed an upregulated connective tissue turnover and an upregulation of the inflammatory

**Mutations of the human filamin C encoding gene (FLNC) cause autosomal dominant forms of progressive and of ten devastating myopathies and cardiomyopathies. The first identified and most frequently occurring mutation (p.W271G) 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 pathologic 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 precinematic stage of FLNC-associated myopathies and contribute to muscle weakness prior to the formation of protein aggregates.**

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

*Julia Schels*, Zacharias Örland*, Fridelie Chevessier*, Lucie Wroth*, Alexandra Masenk*, Rudolf A. Kley*, Anna-C. Plank*, Stephan von Hörsten5, Katrin Marcus4, Matthias Vorgard6, Peter F. M. van der Ven7, Rolf Schröder1, Dieter O. Fürst8, Torsten Lauridsen1, Jens Bo Nielsen1, Robert Felderinhans1, *Jessica Pingel*

1 Institute for Cell Biology, University of Bonn, Department of Molecular Cell Biology, Bonn, Germany
2 Institute of Neurpathology, University Hospital Erlangen, Erlangen, Germany
3 Neurovascular Center, RuhrUniversity, University Hospital Bergmannsheil, Duesseldorf, Germany
4 Medizinisches Proteom-Center, Ruhr-University Bochum, Department of Functional Proteomics, Bochum, Germany
5 Preclinical Experimental Center, UniversitätBielefeld,Experimental Therapy, Erlangen, Germany

Characterization of the stromal cell population is essential in the elderly (sarcopenia); it can result from acute or chronic disease (cachexia), or appear in various pathologies such as diabetics 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 microarrays 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 (88 years) were systematically assessed in 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 Screen trials. By offering 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 muscular disorders using healthy or diseased donor cells and increase confidence in the validity of target hits from drug discovery screening campaigns.

**In vitro phenotypic comparison between young and aged human myoblasts**

Joanne Young, Eve Duchemin-Pelletier, Melanie Flaender, Pauline Poydenot, *Mathieu Raul*

CYTOO SA, Grenoble, France

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 disease (cachexia), or appear in various pathologies such as diabetics 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 microarrays 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 (88 years) were systematically assessed in 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 Screen trials. By offering 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 muscular disorders using healthy or diseased donor cells and increase confidence in the validity of target hits from drug discovery screening campaigns.

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

Cansu Ozdemir-Saka, Duygu Acikav, *Çetin Kocaefe*

Hacettepe University Faculty of Medicine, Medical Biology, Ankara, Turkey

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

Cansu Ozdemir-Saka, Duygu Acikav, *Çetin Kocaefe*
Abstracts

**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*

1 University of Stuttgart, Institute of Sport- and Movement Science, Stuttgart, Germany

**P11-2** Proteomic characterization of murine muscle fibre types via laser microdissection and LC MS/MS mass spectrometry

*Brita Eggert*, Katelina Barkovits†, Carolin Berwanger†, Rolf Schröder†, Malke Ahrens*, Christoph Claren*, Katrin Marusi†

1 Ruhr University Bochum, Medicinisches Proteom-Center, Bochum, Germany
2 University of Cologne, Institute of Biochemistry I, Cologne, Germany

**P11-3** Assessment of 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.

*Else Marie Barba*, Cécyl Claudel2, Adrian P Harrison2

1 Copenhagen University Hospital, Bispebjerg and Frederiksberg, The Parker Institute, the Biochemistry and Physiology Laboratory, Frederiksberg, Denmark
2 Faculty of Health & Medical Sciences, Copenhagen University, NH, Frederiksberg C, Denmark

**QUESTIONS:**

- How does the hypothesis hold?
- How can one relate force production to spatial and temporal summation of muscle fibres in the involved muscle?

**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 fatigueing 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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Structural changes in diaphragm fibers of critically ill patients

Stefan Conijn1, Johan Lindqvist1, Marlene van den Berg1, Weikang Ma1, Leo Heunks1, Tim Irving2, Goen Otterhalm1,2

1University of Arizona, College of Agricultural and Life Sciences, Tucson, United States
2University of Arizona, Physiology, Pharmacology, and Behavior, Tucson, United States

BACKGROUND: Critically ill patients develop diaphragm weakness, which leads to prolonged ventilator dependence. This weakness is, at least partially, 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 Laboratory. 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 Plutus 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 proximity to the thick filaments. The myosin-based M3 (14.51 nm vs. 14.47nm, respectively) and M6 spacings (7.263 nm vs. 7.2511nm, 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.

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

Mike OuVlt, Kiska Nishihara
Northern Arizona University, Center for Bioengineering Innovation, Flagstaff, United States

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. Calcium addition.

METHODS: We fluorescently labeled the N2A region of myofibrils showed consistent shortening of 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.

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 test a mechanistic model of the myofibrilar structure 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 and 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 levels of the sarcomeric proteins Myh6 (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 contractile function system with which adequate filament distances, and that Murf signaling is involved linking the mechanical state to the structure.

Phenotypic N2A C-term mutants respond as efficiently to efforts at restoring muscle function, and growth

Frank Li1, Elisabeth Barton2, Henk Granziers1
1University of Arizona, Tucson, United States
2University of Florida, Gainesville, United States

The filamentous protein nebulin has been found to play a complex role within the muscle sarcomere. Studies in C. elegans, a nematode model organism, have indicated that nebulin C-terminal domain mutations affect 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 sarine-rich region and SH3 domain, nebulin’s C-terminal domains located within the Z-disc. This deletion produces a truncated nebulin that causes developmen
tal 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-term, we found nebulin’s novel binding partners to the sarine-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-term results in muscle from responding as efficiently to efforts at restoring muscle function.
mass. Through these studies we conclude that nebulin’s C-terminal domains are vital to several of the protein’s key functions, including regulating an organized and functional sarcomere.

**P11-9**

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

Valeria Stefanita, Europides De Almeida, Julius Korstan, Kristina Djovic

Vienna Biocenter 5, Max F. Perutz Laboratories (MFPL), Structural and computational biology, Vienna, Austria

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 (Awanika, 2012; Lin, 2014). ZASP is expressed in the early stage of myofibrillogenesis and it acts as a mediator between cytoskeletal elements and signal transduction proteins. 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 Zhang, 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 conformational states 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 Z-disc protein families, FATZ-1 interaction 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**

Pavel Kochubey, *Galina Kopylova*, Natalia Demina1, Danil Shchepkin, Sergey Bershitsky

Institute of Physiology and Physiology, Russian Academy of Sciences, Biological Medicine, Yekaterinburg, Russian Federation

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 (Ia, Iib and IIX) MHC isoforms. We studied the significance of MHC isoforms for the Ca2+-regulation of skeletal muscle contraction in the experiments on skinned rabbit fibers with Joule temperature jump (F-Jump) (Bershitsky & Tsutyan, J. Physiol., 2002) and on isolated proteins in an in vitro motility assay (IVMA). MHC from fast m. psoas were predominant Ix and Ibx isoforms, the I isoform of MHC was in fibers from slow m. soleus. Thin filaments were reconstructed from skele- tal actin, tropomyosin and troponycin. In IVMA, with myosin from m. psoas and m. soleus, regulatory proteins from m. psoas and m. soleus, respectively, were used. At TJump from 5°C to 30°C, tension of fast and slow fib- bers increased about 2.8 and 2.5 times, respectively. Sur- prisingly stiffness of slow fibers increased about two-fold compared to fast fibers. Maximal filament sliding velocity of the Ibx isoform is only 15% as early as it was shown for fast fibers (Huxley & Brown, J. Mol. Biol., 1967). At increasing of temperature the Ca2+ 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 Ca2+. At in- creasing temperature, maximal filament sliding velocity of m. psoas and m. soleus increased 2.5 and 5 times, respectively. Thus, the temperature sensitiv- ity of the contractile apparatus of slow muscle is higher than fast one, which agrees with the data obtained earlier in a model with fast and slow fibers. We used at 5°C and 30°C, tension of fast and slow fibers increased 2.5 and 5 times, respectively. Sur- prisingly stiffness of slow fibers increased about two-fold compared to fast fibers. Maximal filament sliding velocity of the Ibx isoform is only 15% as early as it was shown for fast fibers (Huxley & Brown, J. Mol. Biol., 1967). At increasing of temperature the Ca2+ sensitivity of the fiber tension increased.

We can assumed that difference of contractile activity and its Ca2+ regulation of fast and slow skeletal muscles deter- mined by cooperative mechanisms of the actin-myosin in- teraction 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**

Lucia Fusi, Elisabetta Brunello, Ziqian Yan, Malcolm Irving

King’s College London, Rambold Division, London, United Kingdom

Recent studies in skeletal muscle fibers 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 [Ca2+] is controlled by the thin 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 tropomyosin during active force develop- ment. Force generation is triggered by a rapid (10^-5 s) fall- ing UV-photolysis of caged-calcium (NP-EGTA), under conditions which preserve the physiological resting struc- ture of the thin filament (T=25°C, 5% Dextran, 2.45 μm sarcomere length). We used fluorescently labelled antibodies to monitor changes in the orientation of BSR probes label- ing the E-helix of RLC and the C-helix of tropomyosin-C (TnC) exchanged into demembranated fibres from rabbit psoas muscles. 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 <P>> of the RLC probe, associated with it becoming more perpendicular to the fibre axis, also had a sigmoidal time course. The <P>> 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 <P>> 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 [Ca2+], force rose with no lag; under these con- ditions the 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 lowered by the kinetic behavior of the OFF-ON transition of the thick filament and that the lag at the start of activation is associated with the positive feed- back loop of thick filament mechanosensing. Supported by ERC, UK.

**P11-12**

**Lack of desmin in normal human extracellular muscle fibers: a complex relation to innovation**

Fatima Pedrosa Domeño, Jingxia Liu

Umeå University, Dept of Clinical Sciences, Ophthalmology, Umeå, Sweden

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

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

RESULTS: A novel type of multiple motor endplates was studied, yet is more similar to in vivo muscle function than to twitch. Motor endplates in the 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

Anthony Hesselt, Venus Joumaa, Walter Herzog, Klaas Nijhalsaw

1Northern Arizona University, Department of Biological Sciences, Flag- staff, United States

2University of Calgary, Calgary, Canada

Muscle function during submaximal activation is seldom studied, yet is more similar to in vivo muscle function than to maximal activation. For skeletal muscle, the force- length relationship shifts to longer lengths in submaximal, compared to maximal, activation conditions. Length-de- pendent 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-de- pendent muscle properties through structural re-arrange- ment of the thick filament. To evaluate a possible role for titin, we studied muscles from mice carrying the muscu- lar dystrophy with myositis (mdm) mutation, which have a small titin deletion in the I-band. Mice carrying this deletion are similar to wild type muscles and fibers, we measured the force- length relationships during maximal (tetanus) and sub- maximal (twitch) activation. We then used skinned fibers to evaluate the length-dependence of calcium sensitiv- ity. 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. How- ever, the length-dependence of calcium sensitivity was identical in mdm and wild type fibers, suggesting that mechanisms other than calcium sensitivity are responsi- ble for the activation-dependent shift in the force-length relationship. In conclusion, the titin deletion in mdm mice fibers may not impact long length dependent calcium sensitivity, but possibly impacts activation-dependent optimal length by a currently unclear mechanism.
Towards a reconstitution of the sarcomeric Z-body: a possible strategy to assemble a mini Z-disc

*Robas Thöni, Joani L. Arolas, Kristina Djinovic-Carugo
Max F. Perutz Laboratories, Department of Structural and Computational Biology, Vienna, Austria

The sarcomeric Z-disc 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-disc, 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: α-actinin-2, filamin-C, FATZ-1, ZASP and myotilin [Wang et al., Cell Mot Mol Cytoskeleton 2005; San-gert et al., J Biomed Biotechn 2010]. All of them are also present in the mature Z-disc 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 with small building blocks, the Z-bodies. Despite formation during the myofibrillogenesis is believed to start with small building blocks, the Z-bodies. Despite formation during the myofibrillogenesis is believed to start with small building blocks, the Z-bodies.
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 isoform-specific myofibrils. We studied myofibrils within single demembranated hESC-CMs and by small hvMFs bundles using the same micromechanical method. Our results indicated that saturating Ca2+ 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 Ca2+ activation force levels, where intact cardiomyocytes normally operate, contractile kinetic properties of 50/70% layers of a 25%, 40% 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 considerably 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.
Is myosin VI playing a role in myotube formation via its involvement in cell adhesion?

Malgorzata Suszek, Okena Karatasi, Maria Jolanta Redowicz

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Biochemistry, Warsaw, Poland

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 [Karetzkz et al. 2013]. Moreover, our data gathered on C2C12 myoblasts suggest that MVI could be involved in myogenesis [Karetzkz 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 (Giel’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 and myotubes. Interestingly, we have already identified talin as an interaction partner for MVI in C2C12 myoblasts and myotubes. Thus our data indicate the interaction of MVI with proteins involved in adhesive contacts formation could play a role in myoblast differentiation into myotubes.

Karetzkz et al. (2013) Histocem Cell B139:873-885

Myosin VI, skeletal muscle, myotubes, myogenesis

The effect of different mechanical loading protocols on differentiated H9C2 cells

Evanuelos Zevoli, Anastasios Philippou, Atanasios Moustogiannis, Antonis Chatzigeorgiou, Michael Koutsikinis

1National and Kapodistrian University of Athens, Experimental Physiology, Medical school, Athens, Greece
2University Clinic Dresden, T1O, Clinical Pathobiocemistry and Institute for Clinical Chemistry and Laboratory Medicine, Dresden, Germany

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, 0.94Hz, 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, Fucα, p53), atrophy (Atropin, Murf1, MISTN) 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 p33 and IL-6 was also revealed after the LT protocol. The expression of MISTN, Atropin, 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 MVI plays a role in myotube formation via its involvement in cell adhesion?

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

Andrey N. Fomin, Wolfgang Linka, Athanasios Moustogiannis1, Antonis Chatzigeorgiou2, Michael Koutsikinis3

1Ruhr University Bochum, Institute of Physiology, Department of Cardiovascular Physiology, Bochum, Germany
2University Medical Center Göttingen, Clinic for Cardiology and Pneumology, Göttingen, Germany
3German Center for Cardiovascular Research (DZHK), Partner site Göttingen, Göttingen, Germany

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 (iPSC-CMs) appear to be exposed to increased titin turnover, as they exhibit strong titin degradation bands and may also express the Crinos titin isoform, which lacks the N-terminal 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 iPSC-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 Crinos. We also performed time-resolved studies of titin ubiquitination and general autophagic flux in developing iPSC-CMs. MG132- and bafilomycin-treated iPSC-CMs showed a shift in the cardiac titin-isoform pattern to the full-length proteins. MG132-treatment caused a relatively fast increase in the expression of fetal N2BA titin and accumulation of ubiquitinated titin species, whereas bafilomycin-treatment led to a relatively slow decrease in T2/Crinos 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 iPSC-CMs.
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Save the date!

The 2018 European Muscle Conference will be held between 30 August and 3 September in Budapest.

You are cordially invited to the EMC2018. With important anniversaries to remember - such as the discovery of actin 75 years ago and titin 40 years ago - and exciting advancements of the broader muscle field to discuss, we plan to bridge the past with the future. The conference venue will be the Basic Medical Science Center of Semmelweis University. We plan a rich set of social activities, including a gala dinner in the cupola of the Buda Castle, to make the conference even more enjoyable. We look very much forward to hosting you in 2018.

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