Budapest, 30 August-3 September 2018

47th European Muscle Conference

Program & Abstracts

www.emc2018-budapest.hu

For its 48th European Muscle Conference the European Society for Muscle Research will return to the United Kingdom to have its annual scientific meeting at the University of Kent in Canterbury.

This meeting is jointly organised by Dr Elisabeth Ehler (King's College London) and Prof Michael Geeves (University of Kent) with the help of several members of the UK, European and US muscle community.

We are looking to an exciting mix of muscle biology research that reflects the varied interests of our society from the molecular to the physiological level and from health to disease.

www.emc2019.com

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Cover photo:
Angle-shadowed thick filaments from honeybee asynchronous flight muscle.
Electron micrograph courtesy of Károly Trombitás.

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WELCOME ADDRESS

Dear Colleagues,
Dear Muscle Aficionados:

Welcome to the European Muscle Conference! Welcome to Budapest! This meeting is the annual gathering of the members of the European Society for Muscle Research (http://www.esmr.org). Everyone who registered to our meeting is automatically a member of this society, which oversees and supports muscle research in Europe and beyond. This year's meeting is the 47th consecutive conference in a series that has been started by Professor Marcus Schaub of Switzerland. The conference venue changes, from year to year, between European cities, and earlier the Society decided that this year's meeting will be organized in Budapest, capitol of Hungary. The topics of the conference usually span the entire spectrum of muscle-related research. It is no different in this year's conference either; however, a new focus will be given to the emerging field of motor protein pharmacology. Furthermore, there will be a number of workshops, given by sponsoring instrument companies, which will provide a direct, hands-on opportunity to interested researchers to become acquainted with important methods employed in the field. There are important discoveries of muscle research — actin 75 and titin 40 years ago — to be remembered and celebrated, therefore our meeting, in a way, intends to bridge the past with the future.

I sincerely hope that the host city, Budapest, will be a most welcoming environment for our conference. The venue is the Basic Medical Science Center of Semmelweis University, the oldest and most prestigious medical tranining university of Hungary which will celebrate its 250th anniversary next year. Hopefully you will find time to discover the rich historical and cultural heritage of Budapest as well. A tour, part of our social program, will will show you some of the most important sites of this grand city. The Gala Dinner of the conference will be in the cupola of the Royal Castle of Buda, in the main hall of the National Gallery of Hungary. I am delighted that Professor Andras Muhlrad accepted to give a dinner talk.

Finally, I want to express my gratitude to the numerous people who helped in organizing this meeting: Katalin Mátray and her staff at K&M Congress, and members of the Local Organizing Committee and the Scientific Committee.

I wish you an intellectually rich convention and enjoyable stay in Budapest!

Miklós Kellermayer, Professor
EMC2018 Chairman

ACKNOWLEDGEMENTS

The organizers of the conference acknowledge the generous support of the companies and organizations below

Aurora Scientific

Auro-Science Consulting Ltd.

IonOptix Ltd.

Journal of General Physiology

Journal of Muscle Research and Cell Motility

Lumicks

MDE Co. Ltd for Medical Research, Development, Manufacture

National Heart Program NKFIA; NVKP-16-1-2016-0017

Oxford Instruments – Asylum Reseach AFMs

Semmelweis University

CONFERENCE ORGANIZATION

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

Under the Auspices of the Hungarian Biophysical Society
H-1027 Budapest, Fő u 68.
www.mbft.hu

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GENERAL INFORMATION

Conference venue
Basic Medical Science Center of Semmelweis University
H-1094 Budapest, Tűzoltó utca 37-47.
http://semmelweis.hu/eok/en/

Registration desk
The registration desk, located in the lobby of the conference venue, will be at your service throughout the duration of the conference.

Opening hours

Thursday, 30 August	12:00–18:00
Friday, 31 August	08:00–18:00
Saturday, 1 September	08:00–18:00
Sunday, 2 September	08:00–18:00
Monday, 3 September	08:00–14:00

On-site registration fees

Full Participant	580.00 €
Young Investigator	450.00 €
Accompanying Person	150,00 €

Participants are entitled to the following services:
- Admission to the scientific program and the exhibition,
- Congress folder,
- Congress badge,
- Handout,
- Coffee and refreshment during the breaks,
- Lunch,
- Welcome party on 30 August 2018,
- Budapest sightseeing by bus on 1 September 2018

Accompanying persons are entitled to the following services:
Congress badge,
Coffee and refreshment during the breaks,
Welcome party on 30 August 2018,
Budapest sightseeing by bus on 1 September 2018

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

Internet access
Free wifi is provided throughout the conference venue. The corresponding password will be displayed at the registration desk.

Weather
The weather in the first week of September is usually warm in Budapest, daytime temperature ranges between 22-25 °C, however, nights are already cool (between 11-13 °C). Rain may occur.

Dress code
Dress is informal at all occasions.

Currency, exchange, bank cards
The official currency is the Hungarian Forint. Exchange facilities are available at the airport, in hotels, at banks. ATMs are available throughout the country. American Express, Visa, Diner, Eurocard, MasterCard, JCB cards are mostly accepted in hotels, restaurants and city stores, but you should ask before ordering a service, or buy.

Insurance
The Organizing Committee does not assume responsibility for injuries or losses occurring to persons or personal belongings during the conference.

Electricity supply and phone
In Hungary electricity is supplied at 230 V, 50 Hz like in most European countries. The 2-pin connecting plug is different from that used in some other countries (e.g. USA, UK, Japan etc.). Phoning and mobile servicing background is according to European standards.

Travel
For timetables please visit the websites:
https://www.bud.hu – Liszt Ferenc Budapest International Airport
http://futar.bkk.hu – Budapest Public Transportation
The Budapest Card booklet is included in your congress folder.

Toll must be paid on motorways. Annual, monthly and ten-day vignettes are available. https://www.hungary-vignette.eu/

Anti-harrasment
47th European Muscle Conference is dedicated to a harassment-free conference experience for everyone. Please visit the congress website to check our anti-harassment policy.

MEALS AND SOCIAL PROGRAM

Coffee and refreshments will be available in the lobby of the Congress venue during Coffee breaks as indicated in the program.

Lunch included in the registration fee.
Buffet lunch will be served in the lobby of the Congress venue as indicated in the program.

Welcome reception included in the registration fee
The EMC 2018 Welcome Reception will take place in the lobby of the Basic Medical Science Center of Semmelweis University (the Congress venue) from 18:00 pm on Thursday, 30 August 2018.

Budapest sightseeing by bus included in the registration fee

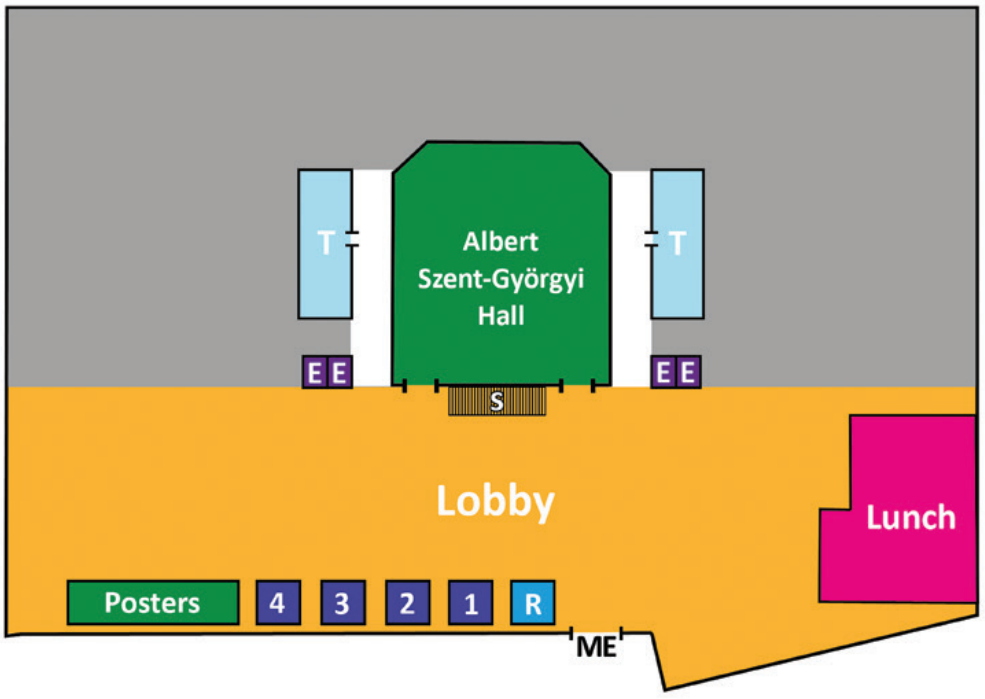
Departure from the Congress venue at 17:15 on Saturday, 1 September 2018. Length of the tour: 3 hours

Banquet dinner 65.00 EUR per person (incl. 27% VAT)
The banquet dinner will take place in the Hungarian National Gallery in the Buda Castle from 19:00 on Sunday, 2 September 2018.

Floor Plan

Venue

Basic Medical Science Center of Semmelweis University
GROUND FLOOR



Exhibitors

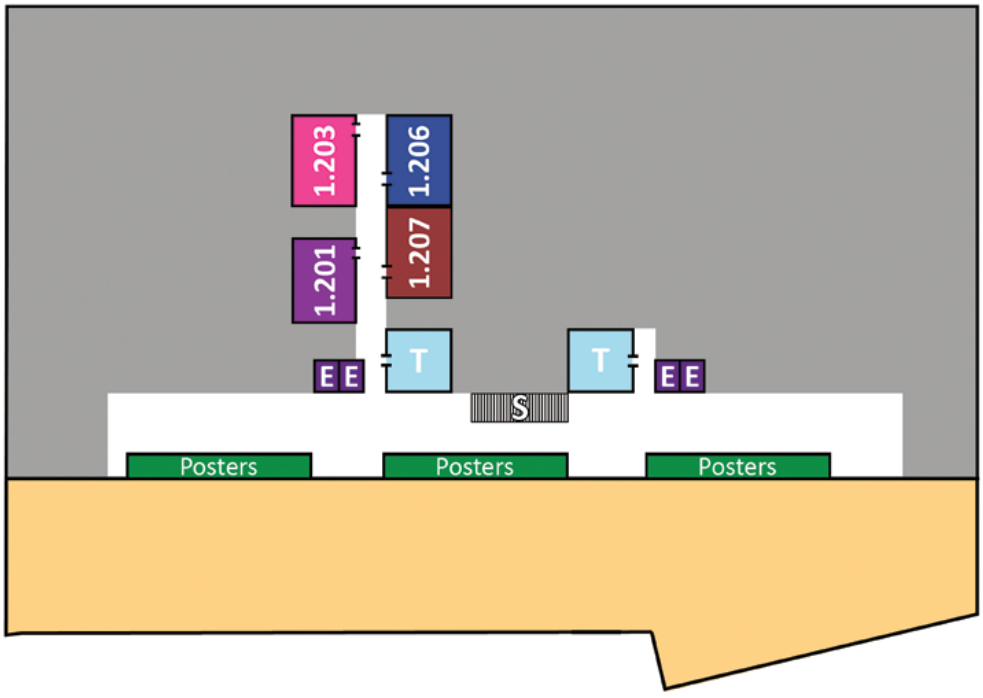
1- Aurora Scientific
2- IonOptix
3- Oxford Instruments – Asylum Research
4- MDE

E- Elevators
ME- Main entrance
R- Registration
S- Staircase
T- Toilets

Venue

Basic Medical Science Center of Semmelweis University

FIRST FLOOR



WORKSHOPS

1.201 - Aurora Scientific
1.203 - Asylum Research
1.206 - Lumicks
1.207 - IonOptix

E - Elevators
S - Staircase
T - Toilets

PROGRAM

Thursday, 30 August 2018

12:00–16:00 **REGISTRATION**

Lobby

16:00–18:00 **OPENING CEREMONY**

Albert Szent-Györgyi Hall

OC-1 *Joseph M Chalovich, Greenville, USA:* Bernhard Brenner - memorial talk
OC-2 *László Nyitray, Budapest, Hungary:* Carolyn Cohen - memorial talk
OC-3 *Stefan Galler, Salzburg, Austria:* In memoriam: Caspar Rüegg (1930-2018)

Chamber music recital

OC-4 Plenary talk
James Spudich, Stanford, USA: The myosin mesa: On the underlying molecular basis of hyper-contractility caused by hypertrophic cardiomyopathy mutations

18:00–21:00 **WELCOME RECEPTION**

Lobby
included in the registration fee

Friday, 31 August 2018

9:00–10:50 SESSION 1. SKELETAL MUSCLE MECHANICS

Albert Szent-Györgyi Hall
Chairs: *Marco Linari, Florence, Italy and Masataka Kawai, Iowa City, USA*

9:00-9:25	S1-1	Keynote presentation *Pieter P. de Tombe, Freiburg, Germany:* Myofilament length dependent activation: Molecular Mechanisms
9:25-9:38	S1-2	*Letizia Zullo, Genova, Italy:* The relevance of form and function in the octopus arm hydrostatic limb
9:38-9:58	S1-3	*Vincenzo Lombardi, Florence, Italy:* Effect of Temperature on Thick Filament-Based Regulation of Mammalian Skeletal Muscle
9:58-10:18	S1-4	*Masataka Kawai, Iowa City, USA:* Strain sensitivity of the elementary steps of the cross-bridge cycle, and the Le Chatelier principle
10:18-10:31	S1-5	YOUNG INVESTIGATOR AWARD COMPETITION *Andrea Mendoza, Montreal. Canada:* Depletion of thick filaments in individual sarcomeres affects inter-sarcomere dynamics and force production by single myofibrils
10:31-10:44	S1-6	*Matthew Gage, Lowel, USA:* Varying stability of the immunoglobulin domains in titin's N2A region

10:50-11:10 COFFEE BREAK

Lobby

11:10–13:00 SESSION 2. MUSCLE CYTOSKELETON

Albert Szent-Györgyi Hall
Chairs: *Carol Gregorio, Tucson, USA and Elisabeth Barton, Gainesville, USA*

11:10-11:35	S2-1	*Elisabeth Barton, Gainesville, USA:* Coordinators of Mechanical signal transduction through sarcoglycans and archvillin
11:35-11:50	S2-2	*H Lee Sweeney, Gainesville, USA:* Myosin X drives filopodia of mammalian myoblasts to promote cell fusion
11:50-12:05	S2-3	YOUNG INVESTIGATOR AWARD COMPETITION *Celine Bruyere, Mons, Belgium:* How does the fusion of myoblasts modulate their mechanics?
12:05-12:30	S2-4	*Mert Colpan, Tucson, USA:* Regulation of actin dynamics at thin filament pointed ends in the heart
12:30-12:45	S2-5	*Nicanor Gonzalez-Morales, Montreal, Canada:* Screen in Drosophila identifies a crucial enzyme for the incorporation of Z-disc proteins.
12:45-13:00	S2-6	*Wolfgang Linke, Münster, Germany:* Specific cleavage of the titin springs in situ uncovers titin's role in active muscle contraction

11:00–12:00 WORKSHOP 1: ASYLUM RESEARCH - VIDEO-RATE AFM OF MUSCLE PROTEINS

Room 1.203

12:00-13:00 WORKSHOP 2: IONOPTIX - ON ISOLATED CARDIAC MYOCYTES EXPERIMENTATION: HIGH THROUGHPUT CALCIUM/CONTRACTILITY MEASUREMENTS AND MECHANICS

Room 1.207

13:00–15:00 LUNCH AND POSTER VIEWING

Lobby

15:00–16:50 SESSION 3. NEUROMUSCULAR SIGNALING AND INTERACTION

Albert Szent-Györgyi Hall
Chairs: *Tomasz J. Prószyński, Warsaw, Poland and Gregorio Valdez, Roanoke, USA*

15:00-15:20 S3-1 *Gregorio Valdez, Roanoke, USA:* MicroRNAs in muscular dystrophy: Modulators of molecular mechanisms involved in repairing muscle damage

15:20-15:40 S3-2 *Said Hashemolhosseini, Erlangen, Germany:* Canonical Wnt and Hippo regulators ensure proper synaptic gene transcription of acetylcholine receptors at the neuromuscular junction

15:40-15:55 S3-3 *Rüdiger Rudolf, Mannheim, Germany:* Postnatal development of the interaction between sympathetic neurons and NMJs

15:55-16:15 S3-4 *Tomasz J. Prószyński, Warsaw, Poland:* SH3BP2 as a novel scaffold protein regulating muscle postsynaptic machinery

16:15-16:30 S3-5 YOUNG INVESTIGATOR AWARD COMPETITION
Laura Geid, Vienna, Austria: Characterizing the agrin-dependent internalisation of muscle specific kinase

16:30-16:45 S3-6 YOUNG INVESTIGATOR AWARD COMPETITION
Lara-Jane Kepser, Marburg, Germany: The role of the actin-binding protein CAP2 for mammalian skeletal muscle development and function

16:50-17:10 COFFEE BREAK

Lobby

17:10–19:00 SESSION 4. CARDIAC CONTRACTILITY AND FAILURE

Albert Szent-Györgyi Hall
Chairs: *Béla Merkely, Budapest, Hungary and Michael Regnier, Seattle, USA*

17:10-17:30 S4-1 *Béla Merkely, Budapest, Hungary:* Cardiac resynchronization in heart failure: reverse remodelling

17:30-17:45 S4-2 YOUNG INVESTIGATOR AWARD COMPETITION
Anastasia Knyazeva, Sankt-Peterburg, Russia: Time-depending changes in expression patterns of genes, encoding Z-disk proteins, in aortic coarctation model of cardiac hypertrophy

17:45-18:00 S4-3 *Jorge Alegre-Cebollada, Madrid, Spain:* Nanomechanical phenotypes in cardiac myosin-binding protein C mutants that cause hypertrophic cardiomyopathy

18:00-18:20 S4-4 *Michael Regnier, Seattle, USA:* Structural studies of cardiac muscle contraction with dATP

18:20-18:40 S4-5 *Zoltán Papp, Debrecen, Hungary:* Cellular mechanisms leading to cardiomyocyte diastolic dysfunction

18:40-18:55 S4-6 YOUNG INVESTIGATOR AWARD COMPETITION
Roksana Nikoopour, London, UK: Structural and biophysical characterisation of titin missense variants in genetic myopathies / cardiomyopathies

15:00–16:00 WORKSHOP 3: LUMICKS - MECHANICAL MANIPULATION OF SINGLE MOLECULES WITH OPTICAL TWEEZERS

Room 1.206

16:00–17:00 WORKSHOP 4: AURORA SCIENTIFIC - MUSCLE FIBER AND MYOFIBRIL MECHANICS

Room 1.201

Saturday, 1 September 2018

9:00–10:50 **SESSION 5. MOLECULAR MOTORS**

Albert Szent-Györgyi Hall
Chairs: *Mihály Kovács, Budapest, Hungary and Anne Houdusse, Paris, France*

9:00-9:25	S5-1	Keynote presentation *Anne Houdusse, Paris, France:* Allosteric tuning of myosin force generation: new avenues towards therapeutical treatment
9:25-9:40	S5-2	*Pasquale Bianco, Florance, Iitaly:* A synthetic nanomachine based on the fast myosin isoform of skeletal muscle
9:40-9:55	S5-3	*Maria Jolanta Redowicz, Warsaw, Poland:* Role of myosin VI in myoblast function and differentiation into myotubes
9:55-10:15	S5-4	*Michael Geeves, Canterbury, UK:* Myosin: isoforms and cardiomyopathies
10:15-10:30	S5-5	*László Nyitray, Budapest, Hungary:* Paralog selective regulation of non-muscle myosin 2 filaments by S100 protein binding and C-terminal phosphorylation
10:30-10:45	S5-6	*Alf Mansson, Kalmar, Sweden:* Blebbistatin reveals otherwise hidden state in ATP turnover and force-generation by actomyosin

10:50-11:10 **COFFEE BREAK**

Lobby

11:10–13:00 **SESSION 6. SMOOTH MUSCLE CONTRACTION AND PATHOLOGY**

Albert Szent-Györgyi Hall
Chairs: *Avril Somlyo, Charlottesville, USA and Christian Aalkjaer, Aarhus, Denmark*

11:10-11:30	S6-1	*Avril Somlyo, Charlottesville, USA:* A role for RSK2 in the contraction of pressurized arteries through activation of smooth muscle myosin and the Na+/H+ exchanger
11:30-11:55	S6-2	*Steffen-Sebastian Bolz, Toronto, Canada:* Integrating the circadian dimension - how the molecular clock regulates mechano-sensitive signalling in resistance artery smooth muscle cells
11:55-12:08	S6-3	*Gabriele Pfitzer, Cologne, Germany:* Role of telokin in the vasoregulation of murine portal vein
12:08-12:32	S6-4	*Christian Aalkjaer, Aarhus, Denmark:* The remarkable cardiovascular system of giraffes
12:32-12:46	S6-5	*Stefan Galler, Salzburg, Austria:* Is "catch" force present during active contraction of mollusk catch muscles?
12:46-13:00	S6-6	*Jacques Gilloteaux, Namur, Belgium:* The catch contraction: biophysical and ultrastructural evidences of paramyosin interactions

11:00–12:00 **WORKSHOP 1: ASYLUM RESEARCH - VIDEO-RATE AFM OF MUSCLE PROTEINS**

Room 1.203

12:00–13:00 **WORKSHOP 2: IONOPTIX - ON ISOLATED CARDIAC MYOCYTES EXPERIMENTATION: HIGH THROUGHPUT CALCIUM/CONTRACTILITY MEASUREMENTS AND MECHANICS**

Room 1.207

13:00-15:00 **LUNCH AND POSTER VIEWING**

Lobby

15:00–17:00 SESSION 7. THIN FILAMENT AND ACTIN-BINDING PROTEINS

Albert Szent-Györgyi Hall
Chairs: William Lehman, Boston, USA and Yuichiro Maeda, Nagoya, Japan

15:00-15:25 S7-1 *William Lehman, Boston, USA:* A new twist on tropomyosin assembly and binding onto actin-based thin filaments.
15:25-15:40 S7-2 *Neil Kad, Kent, UK:* Single molecule imaging reveals how Cardiac Myosin Binding Protein-C sensitizes thin filaments to calcium
15:40-15:55 S7-3 *Balázs Kiss, Tucson, USA:* Nebulin stiffens the thin filament and augments cross-bridge interaction in skeletal muscle
15:55-16:20 S7-4 *Yuichiro Maeda, Nagoya, Japan:* Atomic resolution structures of F-form actin: mutual switching between G/F transition and ATPase
16:20-16:45 S7-5 *Joanna Moraczewska, Bydgoszcz, Poland:* Myopathy causing mutations A4V and R91C in tropomyosin Tpm3.12 affect actin polymerization at the pointed end
16:45-17:00 S7-6 *Kiisa Nishikawa, Flagstaff, USA:* Measuring the biochemistry and biophysics of calcium-dependent interactions between titin and actin

15:00–16:00 WORKSHOP 3: LUMICKS - MECHANICAL MANIPULATION OF SINGLE MOLECULES WITH OPTICAL TWEEZERS

Room 1.206

16:00–17:00 WORKSHOP 4: AURORA SCIENTIFIC - MUSCLE FIBER AND MYOFIBRIL MECHANICS

Room 1.201

17:15-20:00 SIGHTSEEING TOUR BY BUS

included in the registration fee
meeting point: Congress registration desk in the lobby

Sunday, 2 September 2018

9:00–10:50 **SESSION 8. MOTOR PROTEIN PHARMACOLOGY**

Albert Szent-Györgyi Hall
Chairs: *András Málnási-Csizmadia, Budapest, Hungary and Steven Marston, London, UK*

9:00-9:25	S8-1	Keynote presentation *András Málnási-Csizmadia, Budapest, Hungary:* Motor Pharmacology: novel inhibitors for different myosin-2 isoforms
9:25-9:40	S8-2	*Alfredo J Lopez -Davila, Hannover, Germany:* Quantifying calcium and myosin contributions to thin filament activation in slow twitch human muscle fibres
9:40- 10:00	S8-3	*Georgios Tsiavaliaris, Hannover, Germany:* Selective inhibition of myosins by halogenated carbazoles and arylindole derivatives
10:00-10:20	S8-4	*Roman Bart, Ghent, Belgium:* Medicinal chemistry and use of myosin II inhibitor (S)-blebbistatin and its derivatives
10:20-10:35	S8-5	*Steven Marston, London, UK:* Investigation of small molecules that reverse the uncoupling caused by HCM and DCM mutations in contractile proteins
10:35-10:50	S8-6	*Mate Gyimesi, Budapest, Hungary:* Development of a highly specific skeletal muscle relaxant directly acting on the myosin motor domain

10:50-11:10 **COFFEE BREAK**

Lobby

11:10–13:00 **SESSION 9. MUSCLE ENERGETICS**

Albert Szent-Györgyi Hall
Chairs: *Brian R MacIntosh, Calgary, Canada and Corrado Poggesi, Florence, Italy*

11:10-11:35	S9-1	*Brian R MacIntosh, Calgary, Canada:* Myoplasmic free [Ca^{2+}] and force during intermittent submaximal contractions of intact mouse single fibres
11:35-11:47	S9-2	*Malek Kammoun, Compiegne, France:* In vivo and in vitro muscle metabolic profiles of TIEG1 KO muscle mice using spectroscopy techniques (MRS / NMR)
11:47-11:59	S9-3	*Shokoufeh Mahmoodzadeh, Berlin, Germany:* Ahnak1 expression declines the cardiac function in the aged hearts
11:59-12:24	S9-4	*Corrado Poggesi, Florence, Italy:* The missense E258K-MyBP-C mutation increases the energy cost of tension generation in both ventricular and atrial tissue from HCM patients
12:24-12:36	S9-5	YOUNG INVESTIGATOR AWARD COMPETITION *Miljenko Panajatovic, Basel, Switzerland:* Role of PGC-1α associated mitochondrial biogenesis in statin-induced myotoxicity
12:36-12:48	S9-6	*Stefano Cagnin, Padova, Italy:* Single cell analysis reveals the involvement of the long non-coding RNA Pvt1 in myofiber metabolism modulation
12:48-13:00	S9-7	YOUNG INVESTIGATOR AWARD COMPETITION *Klemen Dolinar, Ljubljana, Slovenia:* Nucleosides block AICAR-stimulated activation of AMPK in skeletal muscle and cancer cells

11:00–12:00 **WORKSHOP 1: ASYLUM RESEARCH - VIDEO-RATE AFM OF MUSCLE PROTEINS**

Room 1.203

12:00-13:00 **WORKSHOP 2: IONOPTIX - ON ISOLATED CARDIAC MYOCYTES EXPERIMENTATION: HIGH THROUGHPUT CALCIUM/CONTRACTILITY MEASUREMENTS AND MECHANICS**

Room 1.207

13:00-15:00 **LUNCH AND POSTER VIEWING**

Lobby

15:00–16:50 **SESSION 10. CONTRACTION REGULATION, EC COUPLING**

Albert Szent-Györgyi Hall
Chairs: László Csernoch, Debrecen, Hungary and Daniela Rossi, Sienna, Italy

15:00-15:25 S10-1 *László Csernoch, Debrecen, Hungary:* The role of septins in skeletal muscle
15:25-15:45 S10-2 *Vincent Jacquemond, Lyon, France:* Defective Ca^{2+} signaling in centronuclear myopathies
15:45-16:00 S10-3 *Feliciano Protasi, Chieti, Italy:* Transverse tubule plasticity drives the assembly of calcium entry units in muscle during exercise
16:00-16:20 S10-4 *Daniela Rossi, Sienna, Italy:* Role of triadin mutations in inherited arrhythmia syndromes
16:20-16:35 S10-5 *Laurence Stevens, Lille, France:* Phosphorylation/glycosylation states of MLC2 regulatory protein in skeletal muscle in disuse conditions
16:35-16:50 S10-6 *Shin'ichi Takeda, Tokyo, Japan:* New insights of intracellular calcium regulation mechanism in dystrophin-deficiency

14:00–15:00 **WORKSHOP 3: LUMICKS - MECHANICAL MANIPULATION OF SINGLE MOLECULES WITH OPTICAL TWEEZERS**

Room 1.206

15:00–16:00 **WORKSHOP 4: AURORA SCIENTIFIC - MUSCLE FIBER AND MYOFIBRIL MECHANICS**

Room 1.201

17:00–18:00 **EMC BUSINESS MEETING**

Albert Szent-Györgyi Hall

19:00–23:00 **GALA DINNER IN THE HUNGARIAN NATIONAL GALLERY**

optional (65,00 € per person); transfer not included
H-1014 Budapest, Szent György tér 2.

Concert of the Failoni Chamber Ensemble
A. Vivaldi: The Four Seasons - The Summer
F. Schubert: Trout Quintet - Theme and Variations
P. de Sarasate: Gypsy Airs
S. Joplin: Ragtime
J. Brahms: Hungarian Dances

Dinner lecture
Andras Muhlrad: Pioneering Hungarian Muscle Researchers

Monday, 3 September 2018

9:00–10:50 **SESSION 11. MUSCLE DEVELOPMENT, REGENERATION AND DISEASE**

Albert Szent-Györgyi Hall
Chairs: *Aikaterini Kontrogianni-Konstantopoulos, Baltimore, USA and Mark Sussman, San Diego, USA*

9:00-9:25 S11-1 *Aikaterini Kontrogianni-Konstantopoulos, Baltimore, USA:* Expression of truncated obscurins leads to maladaptive responses in the heart
9:25-9:40 S11-2 *Chiara Tesi, Florence, Italy:* Myocardial overexpression of ANKRD1 affects developmental cardiac remodeling and leads to adult diastolic dysfunction
9:40-9:55 S11-3 *Andreas Unger, Münster, Germany:* Compartmentalization of titin & Novex 3 during sarcomere assembly in regenerating skeletal muscle
9:55-10:20 S11-4 *Mark Sussman, San Diego, USA:* Myocardial regeneration: Uncommon sense for common problems
10:20-10:35 S11-5 YOUNG INVESTIGATOR AWARD COMPETITION
Frank Li, Tucson, USA: Nebulin's C-terminus is necessary for proper sarcomeric structure and function
10:35-10:50 S11-6 *Jose Renato Pinto, Tallahassee, USA:* Pathogenic troponin T mutations with opposite effects on myofilament Ca^{2+} sensitivity attenuate each other's cardiomyopathy phenotypes in mice

10:50–11:10 **COFFEE BREAK**

Lobby

11:10–13:00 **SESSION 12. INTEGRATIVE MUSCLE BIOLOGY**

Albert Szent-Györgyi Hall
Chairs: *Else Marie Bartels, Copenhagen, Denmark and Jessica Pingel, Copenhagen, Denmark*

11:10-11:35 S12-1 Keynote presentation
Else Marie Bartels, Copenhagen, Denmark: From basic muscle research to applications in the clinic
11:35-11:55 S12-2 *Frank Suhr, Leuven, Belgium:* Striated muscle tissue mechanosensors in health and disease
11:55-12:10 S13-3 *Anastassios Philippou, Athens, Greece:* Hormonal responses following resistance exercise performed at maximum movement velocity
12:10-12:30 S12-4 *Jessica Pingel, Copenhagen, Denmark:* Disturbances of the homeostasis of the neuro-muscular-tendon tissue-complex in contractures of individuals with Cerebral Palsy
12:30-12:45 S12-5 YOUNG INVESTIGATOR AWARD COMPETITION
Lance A Riley, Gainesville, USA: The muscle clock regulates titin splicing and sarcomere length
12:45-13:00 S12-6 *Humberto Santo Neto, Sao Paolo, Brazil:* Denervation-related muscle atrophy is mitigated by photobiomodulation with no changes in autophagy

13:00–13:30 **YOUNG INVESTIGATOR AWARDS CEREMONY**
CLOSING CEREMONY

Albert Szent-Györgyi Hall

13:30-14:30 **LUNCH**

Lobby

WORKSHOPS

Workshop 1: Asylum Research - Video-rate AFM of muscle proteins

Room 1.203
11:00–12:00 on Friday, 31 August, Saturday, 1 September and Sunday, 2 September

The advent of „fast-scanning" AFMs almost a decade ago improved imaging speeds but still only allowed processes to be captured with a temporal resolution of approximately ten seconds. The recent introduction of a practical video-rate AFM has improved imaging speeds by yet another order of magnitude and now makes it possible to capture movies with a temporal resolution better than a second with simple operation. This free workshop will give an introduction to video-rate imaging and biophysical applications with lectures and live hands-on demonstrations with the Asylum Research Cypher™ VRS Video-Rate AFM.

Workshop 2: IonOptix - Workshop on isolated cardiac myocytes experimentation: high throughput calcium/contractility measurements and mechanics

Room 1.207
12:00–13:00 on Friday, 31 August, Saturday, 1 September and Sunday, 2 September

In the first half of this workshop we will demonstrate the high throughput system for calcium and contractility measurements on intact, isolated myocytes, our protocols for these experiments, plus the analysis and statistical treatment of the resulting data sets. In the second half of the workshop we will address mechanical experiments on intact and permeabilized cardiac myocytes and muscle strips.

Workshop 3: Lumicks - Mechanical manipulation of single molecules with optical tweezers

Room 1.206
Time: 15:00-16:00 on Friday, 31 August, Saturday, 1 September and Sunday, 2 September

The dynamic single molecule workshop will showcase Lumicks C-trap instrument, a unique combination of optical tweezers and confocal microscopy. You will see the instrument, basic operation and be able to manipulate and visualise a single DNA molecule as well as ask questions from our single molecule experts.

Workshop 4: Aurora Scientific - Muscle fiber and myofibril mechanics

Room 1.201
Time: 16:00-17:00 on Friday, 31 August, Saturday, 1 September and Sunday, 2 September

Instrumentation and methodology for contractile measurements of muscle fibers
Please join us for a workshop focused on the experimental methodology for making contractile measurements in single permeabilized fibers. A brief didactic session will focus on an overview of the operating principles behind the instrumentation as well as capabilities. This will be followed by a live demonstration of the system with time for discussion and Q&A.

POSTER PRESENTATIONS

Poster should be on display during the whole conference.

Poster Viewing/Discussions are scheduled during lunch time, from 12:30-14:00 on Friday, 31 August, Saturday, 1 September and Sunday, 2 September

POSTER SESSION 1. SKELETAL MUSCLE MECHANICS

P1-1 *György Ferenczy, Budapest, Hungary:* Hydrophobic surface unraveling in force-induced titin-domain unfolding

P1-2 *Kolos Turtóczki, Budapest, Hungary:* Monitoring sarcomeric titin unfolding by ANS-binding and two-photon microscopy

P1-3 *Christina Karatzaferi, Devon, UK:* Skeletal muscle in renal insufficiency: is calcium sensitivity affected?

P1-4 *Malin Persson, Stockholm, Sweden:* Nitrosative stress generates an impaired function of myosin to form force-generating cross-bridges

P1-5 *Lorenzo Marcucci, Padova, Italy:* Experimental testing and numerical modelling of passive behavior in muscle fibers and bundles

P1-6 YOUNG INVESTIGATOR AWARD COMPETITION
Yaeren Hernandez, Tucson, USA: Titin's role in Skeletal Muscle Function; Sarcogenesis and Passive Tension

P1-7 *Ian C. Smith, Calgary, Canada:* Contractile function of vastus intermedius fibres from young rats on a high-fat, high-sucrose diet

P1-8 *Venus Joumaa, Calgary, Canada:* Equatorial and meridional x-ray reflections after active stretch and shortening in skeletal muscle

P1-9 YOUNG INVESTIGATOR AWARD COMPETITIO
Daiki Watanabe, Tokyo, Japan: Effects of S-glutathionylation on passive force in human and rat skeletal muscle fibres

POSTER SESSION 2. MUSCLE CYTOSKELETON

P2-1 *Julius Kostan, Vienna, Austria:* Structural insight into the myotilin-actin interaction

P2-2 *Adam Istvan Horvath, Budapest, Hungary:* Subcellular spatial control of non-muscle myosin 2 redistribution and stress fiber strain by Molecular Tattoo

P2-3 YOUNG INVESTIGATOR AWARD COMPETITION
Yakupova Elmira, Pushino, Russia: Possible functional role of titin amyloid aggregation

P2-4 YOUNG INVESTIGATOR AWARD COMPETITION
Verena Kanoldt, Planegg, Germany: Elucidating the role of vinculin and its splice isoform metavinculin in cells and mice

P2-5 *Zsolt Mártonfalvi, Budapest, Hungary:* Calcium dependent elasticity of native titin filaments

P2-6 *Miklós Kellermayer, Budapest, Hungary:* The topology of interactions between titin and the thick filament

P2-7 YOUNG INVESTIGATOR AWARD COMPETITION
Janelle Geist, Baltimore, USA: Myosin binding protein-C slow function, regulation, and disease implications

P2-8 *Caroline Cieniewski-Bernard, Lille, France:* Impact of O-GlcNAcylation changes on desmin behavior in differentiated myotubes

POSTER SESSION 3. NEUROMUSCULAR SIGNALING AND INTERACTION

P3-1 *Tatjana Straka, Mannheim, Germany:* Distribution of Sympathetic Innervation in Skeletal Muscles

P3-2 YOUNG INVESTIGATOR AWARD COMPETITION
Maria Shalagina, Izhevsk, Russia: Role of ceramide in lipid raft disturbance in short-term hindlimb suspension

P3-3 YOUNG INVESTIGATOR AWARD COMPETITION
Vladimir A. Protopopov, Izhevsk, Russia: Clomipramine prevents GLUT4 and NADPH oxidase alterations in rat soleus muscle during 4 days of hindlimb suspension

P3-4 *Artur Fedianin, Kazan, Russia:* Synaptic transmission in rat's soleus muscle during microgravity

P3-5 *Alena Militskova, Kazan, Russia:* Modulation of multisegmental responses in leg muscles during postural tasks

P3-6 *Sergey Tyganov, Moscow, Russia:* Effects of plantar stimulation on anabolic signalling in rat soleus muscle during gravitational unloading

P3-7 YOUNG INVESTIGATOR AWARD COMPETITION
Vid Jan, Ljubljana, Slovenia: Innervation of cultured human myotubes leads to isoform-specific upregulation of Na+,K+-ATPase subunits

POSTER SESSION 4. CARDIAC CONTRACTILITY AND FAILURE

P4-1 *Anna Kostareva, Saint Petersburg, Russia:* FLNC missense variants associate with early-onset restrictive cardiomyopathy combined with congenital myopathy

P4-2 *Árpád Kovács, Bochum, Germany:* Chronic stimulation of the NO/sGC/cGMP/PKG signalling pathway improves diastolic function in a rat model of HFpEF

P4-3 *Nicoletta Piroddi, Florence, Italy:* Design of muscle contraction assist devices by liquid crystalline elastomers

P4-4 YOUNG INVESTIGATOR AWARD COMPETITION
Melissa Herwig, Bochum, Germany: Crucial role of protein kinase G in regulating Ca2(+)/calmodulin-dependent protein kinase-II phosphorylation and oxidation and thereby diastolic function

P4-5 *P. Bryant Chase, Tallahassee, USA:* HCM mutation cardiac troponin C A8V alters cardiomyocyte nucleus structure in a knock in mouse model

P4-6 *Wang Yang, Hajkou, China:* SOCS1 box expression in mechanical stretching cultured mice cardiac ventricle

P4-7 *Tünde Berecz, Budapest, Hungary:* Cardiomyocytes derived from induced pluripotent stem cells of patient with DiGeorge syndrome show altered beating frequency and irregularity

P4-8 *Mihály Ruppert, Budapest, Hungary:* Myofilament Ca^{2+} sensitivity correlates with alterations in cardiac contractility during the progression of pressure overload-induced left ventricular myocardial hypertrophy

P4-9 *Yu-Ting Yan, Taipei, Taiwan:* Multiple functional roles of HSPB7 in heart

P4-10 YOUNG INVESTIGATOR AWARD COMPETITION
Daniil Shchepkin, Yekaterinburg, Russia: Effects of phosphorylation of myosin regulatory light chain on the actin-myosin interaction in ventricle and atria

P4-11 *Suman Nag, South San Francisco, USA:* Role of human cardiac RLC in modulating the super-relaxed state of myosin: A cardiomyopathy perspective

P4-12 *Petr Vikhorev, London, UK:* Contractility of ventricular myofibrils from patients with dilated cardiomyopathy associated mutations

P4-13 YOUNG INVESTIGATOR AWARD COMPETITION
Detmar Kolijn, Bochum, Germany: Structural and functional changes in HFpEF patients primarily associated with women and inflammation

P4-14 YOUNG INVESTIGATOR AWARD COMPETITION
Attila Oláh, Budapest, Hungary: Exercise-induced alterations of myocardial sarcomerdynamics are associated with hypophosphorilation of cardiac troponin I

P4-15 *Jamal Bouitbir, Basel, Switzerland:* Mechanisms of cardiotoxicity associated with tyrosine kinase inhibitors in H9c2 cells and mice

P4-16 *Samantha Harris, Tucson, USA:* Loss of cMyBP-C N'-terminal domains induces spontaneous oscillatory contractions (SPOC) in permeabilized myocytes from Spy-C mice

POSTER SESSION 5. MOLECULAR MOTORS

P5-1 *András Kengyel, Pécs, Hungary:* Autoregulatory functions of myosin 16 domains

P5-2 YOUNG INVESTIGATOR AWARD COMPETITION
Aseem Salhotra, Kalmar, Sweden: Presence of ATP during heavy meromyosin incubation reduces actin velocity in vitro

P5-3 YOUNG INVESTIGATOR AWARD COMPETITION
Salavat Nabiev, Yekaterinburg, Russia: Phosphorylation of essential light chain of skeletal myosin is an on/off switch of the actin-myosin interaction

P5-4 *Marlene Norrby, Växjö, Sweden:* Controlled surface silanization for actin-myosin based nanodevices

POSTER SESSION 6. SMOOTH MUSCLE CONTRACTION AND PATHOLOGY

P6-1 *Péter József Molnár, Budapest, Hungary:* Signal transduction pathways of the thromboxane prostanoid receptor in urinary bladder smooth muscle

P6-2 *Jacques Gilloteaux, Namur, Belgium:* The 'catch' smooth muscle contains small fusiform cells: stem cells, sensors or else?

P6-3 *Krisztina Vén, Budapest, Hungary:* Vasoconstrictor effect of lysophosphatidic acid (LPA) depends on fatty acid chain saturation and age

POSTER SESSION 7. THIN FILAMENT AND ACTIN-BINDING PROTEINS

P7-1 *Dmitrii I. Levitsky, Moscow, Russia:* Comparison of structural and functional properties of different isoforms of skeletal muscle tropomyosin

P7-2 *Joseph M Chalovich, Greenville, USA:* Novel regulatory elements within the COOH-terminus of human cardiac troponin T

P7-3 *Małgorzata Śliwinska, Bydgoszcz, Poland:* Effects of myopathy-related mutations A4V and R91C on regulatory functions of tropomyosin Tpm3.12

P7-4 YOUNG INVESTIGATOR AWARD COMPETITION
Zofia Ostrowska-Podhorodecka, Bydgoszcz, Poland: Tropomyosin isoforms regulate cofilin 1 activity by modulating the conformation of actin filament

P7-5 *Frieder Schöck, Montreal, Canada:* How actin-binding and α-actinin-binding of Zasp52 contribute to myofibril assembly in Drosophila

P7-6 *Balázs Kiss, Tucson, USA:* Experimentally varying the number of super-repeats in the Neb gene of the mouse: assessing the role of nebulin in thin filament length regulation

P7-7 YOUNG INVESTIGATOR AWARD COMPETITION
Michaela Yuen, Amsterdam, the Netherlands: Leiomodin3 - more than just another thin filament pointed end protein?

P7-8 YOUNG INVESTIGATOR AWARD COMPETITION
Yusuke Kanematsu, Hiroshima, Japan: The ATP hydrolysis mechanism of fibrous actin

P7-9 *Yurii Borovikov, St Petersburg, Russia:* The effect of the Gly126Arg mutation in Tpm1.1 on the interaction between myosin and actin in ATP hydrolysis cycle

P7-10 *Sergey Bershitsky, Yekaterinburg, Russia:* Effects of stabilization of flexible sites in the alpha-tropomyosin molecule

P7-11 *Natalia Koubassova, Moscow, Russia:* Effect of point substitutions in tropomyosin on its bending stiffness probed by molecular dynamics

P7-12 YOUNG INVESTIGATOR AWARD COMPETITION
Armen Simonyan, Saint Petersburg, Russia: The E173A substitution in γ-tropomyosin disturbs the transition of contractile system to relaxation

P7-13 YOUNG INVESTIGATOR AWARD COMPETITION
Venukumar Vemula, Kalmar, Sweden: Actin filament multiplication for biocomputation

P7-14 *Belinda Bullard, York, UK:* The function of two tropomyosin isoforms in regulating the contraction of insect flight muscle

P7-15 YOUNG INVESTIGATOR AWARD COMPETITION
Josine De Winter, Amsterdam, the Netherlands: Thin filament-based impaired muscle relaxation kinetics in KBTBD13-related NEM (NEM6)

P7-16 YOUNG INVESTIGATOR AWARD COMPETITION
Stefan Conijn, Amsterdam, the Netherlands: Mutations in slow skeletal troponin I (TNNI1) cause contractile dysfunction

P7-17 YOUNG INVESTIGATOR AWARD COMPETITION
Martijn van de Locht, Amsterdam, the Netherlands: Mutations in Fast Skeletal Troponin C (TNNC2) cause contractile dysfunction

P7-18 *Sylvia Bogaards, Amsterdam, the Netherlands:* A nebulin-dendra2 mouse model to localize individual nebulin molecules in sarcomeres

P7-19 *William J. Lehman:* A new twist on tropomyosin assembly and binding onto actin-based thin filaments

POSTER SESSION 8. MOTOR PROTEIN PHARMACOLOGY

P8-1 YOUNG INVESTIGATOR AWARD COMPETITION
Demeter Turos, Budapest, Hungary: In vivo neural regeneration induced by non-muscle myosin-2 inhibition

P8-2 *Galina Kopylova, Yekaterinburg, Russia:* Influence of omecamtiv mecarbil on the actin-myosin interaction in ventricle and atria

P8-3 YOUNG INVESTIGATOR AWARD COMPETITION
Shaima Hashem, London, UK: Modulation of cardiac myosin dynamics by Omecamtiv Mecarbil

P8-4 YOUNG INVESTIGATOR AWARD COMPETITION
Darshan Trivedi, Stanford, USA: Mavacamten Stabilizes the Super-Relaxed State of β-Cardiac Myosin: Deciphering the Mode of Action from Myosin Molecules to Muscle Fibers

P8-5 *Alan Fappi, Sao Paulo, Brazil:* EPA/DHA potentiates muscle autophagy and UPS during glucocorticoid atrophy process

P8-6 *Josine De Winter, Amsterdam, the Netherlands:* Fast skeletal muscle troponin activator tirasemtiv improves in vitro muscle function in the Tg.ACTA1D286G nemaline myopathy mouse model

P8-7 *Alla Kostyukova, Seattle, USA:* Piperine binding destabilizes the myosin neck via interactions with the regulatory light chain

POSTER SESSION 9. MUSCLE ENERGETICS

P9-1 *Flávia Guarnier, Chieti, Italy:* Basal metabolism is increased in mice susceptible to malignant hyperthermia and heat stroke

P9-2 *Takashi Migita, Kurume, Japan:* Effect of prior knowledge of acceleration increase on oxygen uptake and oxygenation during running

P9-3 *Maria Julia Marques, Sao Paulo, Brazil:* Physical exercise combined to corticoid/omega-3 therapy improved muscle function and respiratory performance in old mdx mouse

P9-4 *Natalia Dachanidze, Tbilisi, Georgia:* Dynamic changes of energy metabolism in rat heart muscle cells

P9-5 *Kohji Hirakoba, Kyushu, Japan:* Contribution of muscle activity in leg muscles to metabolic rate during uphill slope running in middle-aged men

POSTER SESSION 10. CONTRACTION REGULATION, EC COUPLING

P10-1 YOUNG INVESTIGATOR AWARD COMPETITION
Jana Kohútová, Prague, Czech Republic: Cardioprotective Regimen of Intermittent Hypobaric Hypoxia Affects Phosphorylated Status of Connexin 43 and Expression of Its Upstream Kinases in The Rat Heart

P10-2 *Reggiani Carlo, Padova, Italy:* A A 3D diffusional model of the [Ca^{2+}] in cytosol, sarcoplasmic reticulum and mitochondria of murine skeletal muscle

P10-3 *Werner Melzer, Ulm, Germany:* Halothane-modulation of voltage-dependent ca^{2+} release in malignant hyperthermia muscle fibres

P10-4 *Nadège Zanou, Lausanne, Switzerland:* High intensity interval training (HIIT)-induced Ca^{2+} leak through RyR1 channel is involved in mitochondrial plasticity in skeletal muscle

P10-5 *Peter Szentesi, Debrecen, Hungary:* Elementary calcium release events and calcium waves in skeletal muscle fibers of the honey bee apis mellifera

P10-6 *Marco Linari, Florence, Italy:* Thick filament mechanosensing is a downstream mechanism in dual filament regulation of cardiac muscle performance

P10-7 *Sergej Pirkmajer, Ljubljana, Slovenia:* Early vertebrate origins and diversification of FXYDs and other small transmembrane regulators of ion transport

P10-8 YOUNG INVESTIGATOR AWARD COMPETITION
Quinton Banks, Baltimore, USA: Optical recordings of action potential initiation and propagation in mouse skeletal muscle fibers

POSTER SESSION 11. INTEGRATIVE MUSCLE BIOLOGY

P11-1 YOUNG INVESTIGATOR AWARD COMPETITION
Timur Mirzoev, Moscow, Russia: Muscle protein synthesis during early recovery from disuse atrophy: a role of stretch-activated channels in the activation of anabolic signalling

P11-2 *Rungrudee Srisawat, Nakhon Ratchasima, Thailand:* Protective Effects of Thai Pomegranate Juice on Oxidative Stress Induced by Ischemia-Reperfusion in Rat Skeletal Muscle

P11-3 *Julia von Maltzahn, Jena, Germany:* Wnt7a protects skeletal muscle from cancer cachexia

P11-4 *Valeria Stefania, Vienna, Austria:* Molecular architecture of Muscle Z-disc assemblies: ZASP:α-actinin-2-FATZ-1 interactome

P11-5 *Apostolos Papandreou, Athens, Greece:* Physiological and molecular responses to high intensity interval training in flatwater kayak athletes

P11-6 *Boris Shenkman, Moscow, Russia:* Signaling pathways involved in the slow-to-fast myosin transition during unloading

P11-7 *Takayuki Akimoto, Tokyo, Japan:* Role of microRNAs in endurance-exercise-induced skeletal muscle adaptation

P11-8 *Maria Dourida, Athens, Greece:* Endocrine responses after a single bout of moderate aerobic exercise in healthy adult humans

POSTER SESSION 12. MUSCLE DEVELOPMENT, REGENERATION AND DISEASE

P12-1 *Charlotte Gineste, Marseille, France:* In vivo characterization of skeletal muscle function in the Tfam KO mouse model

P12-2 *Natalia Smolina, Saint Petersburg, Russia:* Self-made electrical stimulator as a tool to govern muscle cell differentiation

P12-3 *Lucile Hoch, Corbeil-Essonnes, France:* Identification of a new alpha-sarcoglycan degradation inhibitor using high content screening to treat LGMD2D

P12-4 *Simona Boncompagni, Chieti, Italy:* Exercise prevents formation of Tubular Aggregates in ageing skeletal muscle fibers

P12-5 *Yundong Peng, Bad Nauheim, Germany:* Gα12/13 signaling plays a critical role in satellite cell quiescent maintenance and skeletal muscle regeneration

P12-6 *Iwona Grabowska, Warsaw, Poland:* Adipose tissue derived mesenchymal stem cells in regeneration of large damages of skeletal muscles

P12-7 YOUNG INVESTIGATOR AWARD COMPETITION
Evangelos Zevolis, Athens, Greece: Cardiomyoblast (H9c2) molecular responses during differentiation

P12-8 *Fangyuan Qian, Nanjing, China:* A novel DNAJB6 mutation in distal myopathy with rimmed vacuoles

P12-9 *Anabelle Silva Cornachione, Sao Paulo, Brazil:* Eccentric exercise training improves soleus muscle morphology and function of mdx mice

P12-10 *Szilvia Benkő, Debrecen, Hungary:* Nod-like receptors in C2C12 myoblasts and myotubes during differentiation and regeneration

P12-11 *Sandra Swist, Bochum, Germany:* Isolation of specific titin RNA-binding proteins using the streptomycin-binding RNA aptamer

P12-12 YOUNG INVESTIGATOR AWARD COMPETITION
Mate Penzes, Budapest, Hungary: The role of non-muscle myosin II in the angiogenesis and neuronal regeneration after stroke

P12-13 *Paula Tavares, Coimbra, Portugal:* BCAA's may prevent muscle atrophy induced by immobilization. Influence of physical exercise

P12-14 *Daniel Ribeiro, Sao Paulo, Brazil:* Paradoxical sleep deprivation induces differential biological response in rat masticatory muscles: inflammation and myogenesis

P12-15 YOUNG INVESTIGATOR AWARD COMPETITION
Gerda Mawududzi Sanvee, Basel, Switzerland: Effects of insulin on statin-induced myopathy and insulin resistance in c2c12 myotubes

P12-16 YOUNG INVESTIGATOR AWARD COMPETITION
Julia Kreutzberg /Schuld, Bonn, Germany: Absence of properly dimerizing FLNC leads to Z-disc destabilization and lesion formation in skeletal muscle fibers

P12-17 *Stanislava Avrova, St Petersburg, Russia:* Molecular mechanisms of muscle dysfunction resulting from the myopathy-causing E41K mutation in the TPM2 gene

P12-18 YOUNG INVESTIGATOR AWARD COMPETITION
Olga Karpicheva, St Petersburg, Russia: Destabilization of blocked functional state of thin filaments by cap myopathy-causing mutation Glu150Ala in TPM3 gene

P12-19 YOUNG INVESTIGATOR AWARD COMPETITION
Elena Rogozovets, St Petersburg, Russia: Improper thin filament activation by γ-tropomyosin with the Arg90Pro mutation associated with congenital fibre type disproportion

P12-20 *Rositsa Milcheva, Sofia, Bulgaria:* Quantitative analysis of sialyltransferase expressions in mouse skeletal muscle by real time RT-PCR

P12-21 *Katerina Todorova, Sofia, Bulgaria:* The muscle phase of trichinellosis is associated with up-regulation of the enzyme ST6GalNAc1

P12-22 *Dinara Silantyeva, Kazan, Russia:* The effect of hypothermia on muscle spasticity in a chronic spinal cord injury

P12-23 YOUNG INVESTIGATOR AWARD COMPETITION
Martin Dahl-Halvarsson, Gothenburg, Sweden: Drosophila model of myosin myopathy rescued by overexpression of a TRIM-protein family member

P12-24 YOUNG INVESTIGATOR AWARD COMPETITION
Kathleen Broughton, San Diego, USA: Engineering evolution: Tetraploidization of human cardiac stem cells to enhance functional activity

P12-25 *Maxim Baltin, Kazan, Russia:* Evaluation of recovery of motor functions spinal cord in the local delivery of methylprednisolone

P12-26 YOUNG INVESTIGATOR AWARD COMPETITION
Jiao Liu, Lund, Sweden: A pilot physiological study on a novel muscle myopathy in broiler pectoralis major muscle

P12-27 *Renata Dmitrieva, Saint-Petersburg, Russia:* LMNA G232E and R482Q mutations prevent progression from fetal developmental program to adult skeletal muscle differentiation in vitro

P12-28 YOUNG INVESTIGATOR AWARD COMPETITION
Argyro Papadopetraki, Athens, Greece: Effect of cardiomyoblast secretome, with and without mechanical preconditioning, on hypoxia reoxygenation injury

P12-29 YOUNG INVESTIGATOR AWARD COMPETITION
Athanasios Moustogiannis, Athens, Greece: The effects of muscle cell aging on myogenesis

P12-30 *So-ichiro Fukada, Osaka, Japan:* Molecular mechanisms underlying maintenance of the quiescent state of muscle satellite cell

P12-31 YOUNG INVESTIGATOR AWARD COMPETITION
Jan Spaas, Diepenbeek, Belgium: Skeletal muscle contractile properties in experimental autoimmune encephalomyelitis mice

P12-32 YOUNG INVESTIGATOR AWARD COMPETITION
Britta Eggers, Bochum, Germany: An optimized protocol for the differentiation and analysis of fiber types from murine muscles by laser microdissection and mass spectrometry

P12-33 *Jon Tinsley, Abingdon, UK:* Quantification of developmental myosin fibres in muscle biopsies as a clinical biomarker for muscle disease

P12-34 YOUNG INVESTIGATOR AWARD COMPETITION
Maria Angels Rodriguez Garcia, Umeĺ, Sweden: Extraocular muscles in desmin knockout mice

P12-35 *Luis Cea, Providencia, Chile:* Connexin-based hemichannels mediate the skeletal muscle damage induced by dysferlin deficiency

P12-36 YOUNG INVESTIGATOR AWARD COMPETITION
Robbert van der Pijl, Tucson, USA: MARP1 is a negative regulator of passive stretch-induced hypertrophy

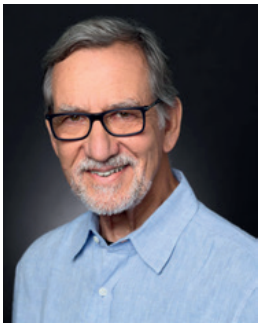
P12-37 *Jessica Pingel, Copenhagen, Denmark:* Pathological changes in muscle signaling mechanisms in muscle contractures of children with cerebral palsy

P12-38 *Minttu Marttila, Helsinki, Finland/Boston, USA:* Zebrafish models of ACTA1-related myopathies recapitulate human disease

P12-39 YOUNG INVESTIGATOR AWARD COMPETITION
Marloes van den Berg, Amsterdam, the Netherlands: Diaphragm weakness in critically ill patients is associated with upregulation of the titin-binding protein MARP1

P12-40 YOUNG INVESTIGATOR AWARD COMPETITION
Tomasz Domoradzki, Warsaw, Poland: IGF-I modifies microRNA expression and release in rat skeletal myoblasts during differentiation

BIOGRAPHIES OF PLENARY AND KEYNOTE SPEAKERS



James Spudich

James Spudich, Douglass M. and Nola Leishman Professor of Cardiovascular Disease, is in the Department of Biochemistry at Stanford University School of Medicine. He received his B.S. in chemistry from the University of Illinois in 1963 and his Ph.D. in biochemistry from Stanford in 1968. He did postdoctoral work in genetics at Stanford and in structural biology at the MRC Laboratory in Cambridge, England. From 1971 to 1977 he was Assistant, Associate, and Full Professor in the Department of Biochemistry and Biophysics, University of California, San Francisco. In 1977, he was appointed Professor in the Department of Structural Biology at Stanford University. Spudich served as Chairman of the Department of Structural Biology from 1979-1984. Since 1992 he has been Professor in the Department of Biochemistry, and served as Chairman from 1994-1998. From 1998 to 2002, he was Co-Founder and first Director of the Stanford Interdisciplinary Program in Bioengineering, Biomedicine and Biosciences called Bio-X. He is also an Adjunct Professor at the National Center for Biological Sciences, Tata Institute of Fundamental Research and InStem in Bangalore, India. In 1998 he co-founded Cytokinetics, focused on treatments for diseases characterized by compromised muscle function like amyotrophic lateral sclerosis and heart failure. In 2012 he co-founded MyoKardia, focused on developing targeted therapies for the treatment of rare genetically-based cardiovascular diseases such as hypertrophic and dilated cardiomyopathy.

Over the last several decades the Spudich laboratory studied the structure and function of the myosin family of molecular motors in vitro and in vivo, and they developed multiple new tools, including in vitro motility assays taken to the single molecule level using laser traps. That work led them to their current focus on the human cardiac sarcomere and the molecular basis of hypertrophic and dilated cardiomyopathy. They postulated in 2015 that a majority of hypertrophic cardiomyopathy mutations are likely to be shifting beta-cardiac myosin heads from a sequestered off-state to an active on-state for interaction with actin, resulting in the hyper-contractility seen clinically in HCM patients. This hypothesis is different from earlier prevailing views, and this *viewing an old disease in a new light* is the basis of their current research.

Pieter de Tombe, Ph.D., FAHA, FAPS

Dr. Pieter de Tombe received his undergraduate training in Chemistry in the Netherlands. He received his Ph.D. graduate training in Physiology at the University of Calgary, Canada (1985-1989) studying cardiac sarcomere dynamics in Dr. Henk ter Keur's laboratory, followed by Post-Doctoral training in bioengineering at the Johns Hopkins University, Maryland (1989-191) studying cardiac pump function and energetics in Dr. Kiichi Sagawa's laboratory. He was appointed to his first independent faculty position at Wake-Forest University, North Carolina (1991-1996), in the division of Cardiology as Assistant Professor (1991), and Associate Professor (1996). In 1996 he joined the department of Physiology and Biophysics at the University of Illinois at Chicago (UIC) rising to the rank of Professor in 2002. In 2008, he was appointed the James DePauw Professor of Physiology and Chair of the department of Cell and Molecular Physiology at the Loyola University Chicago Stritch School of Medicine. May 1, 2017, Pieter relocated back to UIC (part-time) as well as the Yacoub Institute/Imperial College at Harefield, UK, and the University of Freiburg, Germany (part-time). Pieter's research program focuses on the cellular mechanisms underlying depressed cardiac function in heart failure, and the regulation of sarcomere dynamics underlying the Frank-Starling mechanism. He has trained numerous graduate students and Post-Doctoral fellows, published >150 journal articles, and has been funded by the Whitaker foundation for bioengineering, the American Diabetes Association, the American Heart Association, and the National Institutes of Health (the latter continuously since 1992). He is an Associate editor for Pflugers Archiv European Journal of Physiology, Reviews of Physiology Biochemistry Pharmacolo-

gy, and the Journal of Cellular and Molecular Cardiology, and member of the editorial boards several other journals. He regularly serves on national and international grant review panels, including the American Heart Association and the National Institutes of Health. Pieter was council officer of the International Society of Heart Research, the Association of Chairs of Physiology, and the Science Policy Committee of the American Physiological Society.

Anne Houdusse

Keywords: *myosin, force production, unconventional myosin, molecular motors*

Anne Houdusse is a CNRS research director in the Cell Biology department (UMR 144) at the Institut Curie, Paris, France. With undergraduate and graduate degrees from Ecole Normale Superieure and Pasteur Institute Paris, Anne Houdusse was trained as a structural biologist and a chemist, particularly X-ray crystallography. With a HFSPO fellowship, Anne studied myosin motors as a post-doctoral fellow at the Brandeis University where, with Carolyn Cohen and Andrew Szent Gyorgyi, she laid the foundation for her challenging work on structures of conventional myosins. In 1999, she was given an ATIP award and came back to Paris to establish her own independent laboratory. The focus of her group's research at the Institut Curie has been the understanding of how motors produce force and how they are recruited and regulated in the cell. Studies of myosin V and myosin VI have established the essential elements of how all molecular motors produce directional force. This was in part funded via a Human Frontier grant in 2005-2008. This contribution has been recognized in 2009, with the FEBS/EMBO Women in Science Award. She was also awarded in 2013 with the CNRS Silver Medal and was elected member of EMBO.

András Málnási-Csizmadia

Keywords: *myosin, molecular motors, rapid kinetics, drug development*

András Málnási-Csizmadia is the leader of the Motor Pharmacology Research Group of the Hungarian Academy of Sciences at the Eötvös University, Budapest. He graduated in the Structural Biochemistry Doctoral program at the Eötvös University supervised by László Nyitray and co-supervised by Andrew G. Szent-Györgyi at the Brandeis University, Boston. As a post-doctoral fellow in Clive R. Bagshaw laboratory in Leicester, U.K. he investigated the structure-function relationship by combining the rapid enzyme kinetics methods with site directed intrinsic fluorescence labeling of myosin motor domain. In 2001 he returned to the Department of Biochemistry of Eötvös University as a Wellcome Trust fellow to establish his own research group. He studied molecular communication pathways between the functional domains of myosin motor domain. He was also supported by HHMI and received the EMBO Young Investigator Award. In the first announcement he received an ERC grant and few years later an ERC PoC grant as well. He has developed the Molecular Tattooing techniques and created series of novel myosin inhibitors. In 2017 he received the Hungarian Academy of Sciences Award shared with Mihály Kovács and László Nyitray.

Else Marie Bartels

Degrees:
PhD 1974 in Biophysics, University of Copenhagen, Denmark
DSc 1990 in Physiology The Open University, UK
Research Librarian 2001, The Royal School of Library and Information Science, Denmark

Postdoctoral Training:
Courses in Computing, Biophysics and Molecular Biology, Environmental Chemistry, Databases and Search Techniques, Project-Work Methods and Projects Management, Evaluation and Work Environment
Teacher Training
Management training

Administrative and Management Experience
Since 1971 participation in the organization of teaching at various university departments
Since 1974 writing of Grant proposals and Research Reports
Over the years member of organizing committees for scientific meetings and congresses
Since 1980 project coordinator for various research projects and laboratories
1998-2008 Course Co-ordinator responsible for the daily running of The Danish National Library of Science and Medicine's Course Centre
2006-2008 Coordinator of Advisory Services for the Sciences, Copenhagen University Library
2008-2018 Head of Laboratory of Biochemistry and Physiology, the Parker Institute

Teaching Experience
Since 1969 tutor in Biophysics, Physiology and Biochemistry at Copenhagen University(1969-76), Oxford University (1978-1995) and UK Open University (1977-1995).
Since 1974 lecturing at all levels, and supervision of BScs, MScs and PhDs at the above mentioned universities
1996-2008 Course Coordinator and teacher at courses at Copenhagen University Library
Since 1976 course designer and writer of textbook chapters

Positions Held Since PhD Graduation (24.1.74):
1974-1995 Research Fellow and later Senior Research Fellow, first at Copenhagen University, later at The Open University Oxford Research Unit, and at Laboratory of Physiology and Nuffield Orthopaedic Hospital, Oxford University, supported by Danish and British Research Councils and research foundations as ARC, Welcome and the SRC
1995-2008 Senior Researcher and Academic Adviser at Copenhagen University Library
2008-2018 Senior Researcher and Head of the Biochemistry and Physiology Laboratory at the Parker Institute, Bispebjerg and Frederiksberg Hospital
From June 2018 Emerita researcher at department of Neurology, Bispebjerg and Frederiksberg Hospital

Professional Societies memberships:
The Scandinavian Physiological Society
The Physiological Society

Publications
More than 190 publications in peer-reviewed journals, as well as several public outreach publications and textbook chapters.

ABSTRACTS

ORAL

Opening Ceremony

OC-1

Bernhard Brenner - memorial talk

Joseph M Chalovich

The Brody School of Medicine at East Carolina University
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Bernhard Brenner, M.D., professor and chairman of physiology at the Medical School of Hannover, Germany, died on June 26th, 2017 after a courageous fight against cancer. He was 66. Bernhard was a scientist with a razor sharp intellect and an unyielding zeal to elucidate the mechanism of muscle contraction. He will be fondly remembered as an inspiring colleague and mentor to many.

Bernhard was born in Stuttgart, Germany. He studied medicine at the University of Tübingen from 1969 to 1975 and received his doctoral degree in 1979. For his thesis he studied strain-induced calcium release from striated muscle sarcoplasmic reticulum with Professor Rudhard Jacob. He demonstrated independence and a talent for innovative experimental approaches early on in his career. Bernhard designed and built his own devices for measuring fast mechanical kinetics of single muscle fibers. He perfected the preparation of permeabilized single mammalian (rabbit) skeletal muscle fibers with control over the bathing medium. He discovered that repeated cycles of stretches and quick releases of a muscle fiber under fully activating conditions could keep the striation pattern stable for hours without deterioration. Later such cycles were dubbed by some colleagues as the "Brenner Cycles" and are still a standard method in muscle labs.

From 1980 to 1985 Bernhard was a visiting research associate in the laboratory of Richard Podolsky at the National Institutes of Health (NIH), USA. While at NIH, a rather close-knit and long lasting collaborative group was formed, which included Richard Podolsky, Evan Eisenberg, Joseph Chalovich, Lois Greene, Mark Schoenberg, Leepo and Bernhard. The group took advantage of the fact that, for the first time, biochemical, mechanical and structural results could be obtained under the same experimental conditions. Biochemical results were previously obtained from rabbit muscle whereas mechanical and structural (X-ray diffraction) results were measured in frog muscle.

At the time the Eisenberg lab proposed the existence of weak binding states within the actomyosin ATP hydrolysis cycle. Bernhard's high-speed mechanical and X-ray diffraction measurements provided the first evidence of these weak binding states in muscle fibers. The results indicated that tropomyosin-troponin does not block cross-bridge binding to actin in relaxed muscle, contrary to the widely accepted "steric blocking" model of muscle regulation. Further experiments showed that the specific attachment of cross-bridges to actin in the weak binding states is essential in the path-way to contraction. He showed that, rather than controlling the binding of myosin to actin, tropomyosin-troponin regulates contraction by altering the rate of cross-bridge cycling kinetics. Calcium shifts the equilibrium between the inactive and active forms of actin-tropomyosin-troponin to the active form that has rapid cycling kinetics.

The conclusion that calcium regulates cross-bridge kinetics in muscle fibers came from the experimental protocol that Bernhard used. Muscle fibers in a medium with a defined calcium concentration were subjected to a short period of unloaded shortening followed by re-stretch to isometric conditions. Bernhard showed that the rate constant of force redevelopment, kTR, represents cross-bridge turnover kinetics. His observation that kTR increased as the calcium-concentration (and force) were increased supported the earlier suggestion by F.J. Julian that calcium changes cross-bridge turnover kinetics in a graded way, while the number of cross-bridges involved in active cycling remains essentially constant. This "rate modulation" concept was in contrast to the "recruitment" model, where tropomyosin was thought to control the number of cycling cross-bridges without changing the turnover kinetics. Many scientists that work on skeletal or cardiac muscle still use the kTR measurement to investigate effects on rate modulation of cross-bridge cycling.

The PNAS article that described the measurement of kTR is only one example of an important article in which Bernhard was the sole author; he was undeniably creative. He made lasting contributions to our understanding of the regulation of striated muscle contraction and of the changes in structure and kinetics associated with force generation of cross-bridges. His findings are reflected in modern physiology text books.

Bernhard's ideas were sometimes counter to the prevailing thought of the muscle field but he was always ready for objective and spirited discussions. He studied the work of his peers and was able to offer insightful comments about their work. Yet, Bernhard was equally critical of the work of his students and associates. He was open to criticism and suggestions for alternative ways of thinking. Bernhard's first priority was the truth wherever it could be found.

After returning to German, Bernhard completed his habilitation in 1987 at the University of Tübingen on the molecular mechanism of muscle contraction. Bernhard's first academic appointment was in 1988 at the University of Ulm in the Physiology department of Professor Reinhard Rüdel. In 1993 he became the Director of the Institute for Molecular and Cell Physiology, Hannover Medical School (MHH), Hannover, Germany where he served with passion even during his lengthy illness.

He continued to study mechanisms of force generation fusing fiber studies and in addition, in vitro single motor molecule analyses. Studies on skeletal and non-muscle myosin revealed two active site conformations of a single myosin molecule. In one conformation the myosin could complete the ATPase cycle. In the other conformation, the intact ATP dissociated. This observation should be important for head–head coordination of processive myosins. Bern-

hard also applied his expertise to dynein, kinesin and Tau protein. With his life-long interest in cardiac physiology, Bernhard focused the later part of his career on studies of mutations in cardiac myosin that are related to Hypertrophic Cardiomyopathy. He and his group in Hannover made major contributions in defining the structure–function relationship of human cardiac and slow skeletal myosin. They revealed that a variable region of the myosin converter domain is a major element for tuning cross-bridge compliance. His group recently proposed a new hypothesis for the marked heterogeneity in expression of both, the fraction of mutated β-myosin heavy chain and contractile performance among individual cardiomyocytes in the myocardium of patients with Hypertrophic Cardiomyopathy. They found burst-like, stochastic on/off-switching of myosin transcription, which most likely is independent for the mutant and the wildtype fMYH7-allele. The transition that Bernhard made from muscle mechanics to gene expression is emblematic of Bernhard's drive to learn. When faced with a difficult research problem he acquired whatever skills were necessary to solve that problem.\

For more than 30 fears Bernhard was a passionate and popular teacher at medical school for which he received several awards. In his last years he was committed to implement fa new way of teaching basic physics. He integrated physics directly into the physiology course, giving undergraduate students a much more concrete and practical understanding of the subject. Bernhard was also a highly valued and respected colleague, serving for several years as elected member of the Senate of Hannover Medical School where he fought to promote excellence in both research and academics in the preclinical institutes of the medical school. Bernhard was often critical but fair and his input was always valued.

Bernhard will be remembered as one who made his way quickly to the microphone at conferences to ask a question or to provide an insightful interpretation of data. Many students, postdoctoral fellows and colleagues were inspired by his wisdom, his critical thinking and scientific objectiveness. Bernhard Brenner will be greatly missed by his family, his students, his colleagues and his friends.

OC-3

In memoriam: Caspar Rüegg (1930-2018)

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The scientific community mourns Caspar Rüegg, one of the most important muscle researcher who died on 18 January 2018. In grateful memory, we would like to pay tribute to the works and personality of our mentor.

Johann Caspar Rüegg was born on January 28, 1930, in Zürich, Switzerland. After studying medicine in Zürich (MD, 1955) and biochemistry in Cambridge (PhD, 1960), he worked in Heidelberg ("Habilitation", equivalent to Dr. Sc., 1963), Oxford and Bochum. From 1974 until his retirement in 1998, he was a professor of Physiology at the University of Heidelberg and the Director of the Second Institute of Physiology. Caspar Rüegg's mentors and early collaborators included neurophysiologist W.R. Hess (Nobel laureate in 1949) and muscle researchers K. Bailey, H.H. Weber, J.W.S. Pringle and W. Hasselbach. It was this early interaction that defined Rüegg's lifelong dedication to muscle research.

Calcium and new muscle cell preparations

Rüegg's early experimental work confirmed Ca_{ϵ}^{+} as an intracellular messenger. Together with colleagues, he injected Ca_{ϵ}^{+} buffer solutions into giant muscle fibers of crabs while measuring force. These experiments showed how much the Ca_{ϵ}^{+} concentration increases inside muscle cells when stimulated for contraction (Portzehl et al. 1964).

Furthermore, Rüegg contributed in developing "skinned" or "demembranated" muscle cell preparations with the aid of glycerol or detergents (Rüegg and Weber 1963; Jewell et al. 1964). Here he built on earlier work by Nobel laureate Albert Szent-Györgyi. The new preparation allowed investigating the contractile apparatus of muscle cells, in which ATP-driven rowing motions of the lateral heads of myosin filaments exert force on actin filaments and cause filament sliding.

In pioneer experiments, Rüegg and colleagues were able to measure the force of skinned skeletal and smooth muscle preparations as a function of Ca_{ϵ}^{+} concentration (Filo et al. 1965). Subsequently, Rüegg and his team succeeded in measuring ATP consumption during contraction at high time resolution (Griffiths et al. 1980). The measurement was based on an ingenious technique, in which the ATP hydrolysis was enzymatically coupled to the oxidation of NADH, which was monitored by fluorescence.

Modulation of contraction

Over the decades, it became apparent that the effect of Ca_c^+ on the contractile apparatus was not always the same, but was modulated by changes in the chemical surroundings of the contractile apparatus. Rüegg and his co-workers found that intracellular inorganic phosphate reduced not only overall muscle strength, but also the Ca_c^+ sensitivity of the contractile apparatus (Herzig and Rüegg 1977). These effects presumably contribute to fatigue when the muscle produces more inorganic phosphate through excessive ATP hydrolysis.

Likewise, Rüegg and co-workers could show that the Ca_c^+ sensitivity of the contractile apparatus depends on posttranslational modification of proteins. They found a reduction in Ca_c^+ sensitivity by phosphorylation of the troponin I subunit in the heart (Herzig et al. 1981b). The troponin complex, together with tropomyosin, constitutes the molecular switch that controls the interaction of rowing myosin heads with actin filaments in a Ca_c^+-dependent manner. Rüegg and his co-workers further observed an increase in Ca_c^+ sensitivity upon phosphorylation of the myosin regulatory light chain, which is bound to the neck region of the myosin head (Morano et al. 1985). These findings demonstrated that heart muscle cells are able to adapt their Ca_c^+ sensitivity to changing physiological requirements.

New modulators of heart and smooth muscle

In experiments with skinned muscle preparations, Rüegg and co-workers found synthetic drugs that made the contractile apparatus of heart muscle cells more sensitive to Ca_c^+ (Herzig et al. 1981a, Rüegg et al. 1984). These so-called Ca_c^+ sensitizers opened a new possibility of increasing the power of failing hearts without having to raise the intracellular Ca_c^+ concentration, which may be fatal. The Ca_c^+ sensitizer, levosimendan, which was later developed by others, has been in clinical use in many countries for ~15 years.

In similar experiments on smooth muscles, Rüegg and his colleagues found the first inhibitor of protein phosphatases, okadaic acid, which is a toxin of marine dinoflagellates. This substance enhanced smooth muscle contraction like a Ca_c^+ sensitizer by inhibiting the dephosphorylation of the myosin heads (Takai et al. 1987). This finding supported the hypothesis that Ca_c^+ regulates smooth muscle contraction via phosphorylation/dephosphorylation of myosin heads.

Further work on smooth muscle cells contributed significantly in elucidating the mechanisms of action of the intracellular messengers cAMP and cGMP (e.g. Herzig et al. 1981b; Pfitzer et al. 1982).

"Catch" muscles of mollusks

In addition to his groundbreaking studies on mammalian skeletal, cardiac and smooth muscle cells, Rüegg kept a keen interest in the contractile mechanisms of invertebrate muscles. Using "catch" muscles of mussels, he examined their ability to maintain high force without significant energy consumption. For decades, the catch state was thought to be due to a slowdown of the ATP-driven rowing motions of myosin heads. However, as early as the 1960's, Rüegg inhibited myosin heads with thiourea and observed that the catch state was unaffected (Rüegg 1963). He concluded that the catch mechanism was based on a separate holding structure. This hypothesis was proven to be true more than four decades later, when specific inhibitors of the myosin heads became available (Galler et al. 2005). These studies were of general interest because the smooth muscles of blood vessels also enter into a state of high energy efficiency ("latch" state) when maintaining a contracted state for hours to regulate the blood flow.

Asynchronous flight muscles of insects

Rüegg also investigated asynchronous flight muscles of insects to study their extremely fast oscillatory contractions, which in some cases can produce up to 1000 wing beats per second. Together with colleagues, he was able to demonstrate that skinned fibers of these muscles oscillate spontaneously when they are activated by constant Ca_c^+ concentration (Jewell et al. 1964; Jewell and Rüegg 1966). Consequently, they concluded that the fast contraction-relaxation cycles were not caused by oscillations of the intracellular Ca_c^+ concentration, but were generated by the contractile apparatus itself.

Psychosomatic medicine

In addition to his contribution to muscle physiology which he published in about 200 articles, Rüegg wrote excellent books on psychosomatic medicine (Rüegg, 2011, 2012, 2016) after his retirement. Here he discussed the questions of how the brain affects our health and whether our thinking alters neuronal circuits of the brain.

Appreciation

Rüegg was awarded with the prestigious Adolf Fick Prize of the German Physiological Society, of which he was an honorary member. Furthermore, he was an honorary editor of the Journal of Muscle Research and Cell Motility and an associate member of the Swiss Academy of Sciences. In addition, Rüegg was in the appreciation of countless colleagues worldwide with whom he was in personal contact. Everyone enjoyed his extremely profound knowledge of the complex muscle field which he also summarized in remarkable review articles and books (e.g. Rüegg 1971; Rüegg 1992).

Rüegg campaigned vehemently for the promotion of young talents and courageously supported colleagues in difficult situations of their careers. Rüegg was certainly not only one of the most excellent of scientists, but also one of the most popular and friendly of muscle researchers.

Every encounter with Caspar was engaging and stimulating. Also at a time when he had long turned to the theoretical work of psychosomatic medicine, he demonstrated large enthusiasm for muscle research. Unforgettable is his presumably last visit of a muscle laboratory, in which he attended a catch muscle experiment in Salzburg: He followed the recording of the force trace with almost childlike excitement and then repeatedly he moved between the experimental setup and the tea table while initiating a lively discussion arising from his immense knowledge. When he left, beaming all over his face, he said "Thank you for allowing me to breathe the spirit of muscle research again!"

Thank you, Caspar, for allowing us to breathe your fascinating spirits! Your intellectual heritage lives on in the worldwide muscle community, which you have shaped and enriched hugely.

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OC-4

Plenary talk

The myosin mesa: On the underlying molecular basis of hyper-contractility caused by hypertrophic cardiomyopathy mutations

James Spudich

Stanford University, Stanford, CA, USA

After 40 years of developing and utilizing assays to understand the molecular basis of energy transduction by the myosin family of molecular motors, all members of my laboratory are now focused on understanding the underlying biochemical and biophysical bases of human hypertrophic (HCM) and dilated (DCM) cardiomyopathies. Our primary focus is on HCM since these mutations cause the heart to be hypercontractile, and we hope to understand the molecular basis of this increased power output. HCM is most often a result of single missense mutations in one of several sarcomeric proteins, the sarcomere being the fundamental contractile unit of the cardiomyocyte. More than 40% of all HCM mutations occur in the motor domain of human ß-cardiac myosin, while another ~40% occur in myosin binding protein-C. Associated with HCM worldwide are heart failure, arrhythmias, and sudden cardiac death at any age. We are using in vitro molecular studies of biochemically reconstituted human sarcomeric protein complexes to lay the foundation for understanding the effects of HCM-causing mutations on power generation by the contractile apparatus of the sarcomere. With a detailed molecular understanding of the resultant increase in power output caused by HCM mutations, one should be able to exquisitely design appropriate small molecule therapies, which are desperately needed for treatment of these diseases.

Session 1. Skeletal Muscle Mechanics

S1-1

Keynote presentation

Myofilament length dependent activation: Molecular mechanisms

Pieter de Tombe

Magdi Yacoub Institute, London, UK

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

S1-2

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

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

Introduction

The Octopus vulgaris arm is a muscle hydrostat with extraordinary motor capabilities. The arm 'bulk' is composed mainly of transverse (T) and longitudinal (L) muscles acting synergistically. T and L are made by uninucleated striated cells sharing similar physiological properties and embedded in a dense connective matrix.

Objective

We aim at deciphering T and L structure, mechanics, mode of activation and their contribution to whole arm movements.

Methods

Confocal Microscopy was used to perform High-resolution morphometric study of muscles, connective tissues, and elastic fibers organization. Muscle biomechanics was investigated with a Dual-Mode Lever arm system on in-vitro preparations.

Results

L show a higher rate of elastic fibers organized in parallel to the main muscle force vector compared to T. T and L have different activation properties; T have a higher twitch to tetanus ratio than L and a force-frequency curve shifted to the right, hence they behave as slow muscles.

T and L have similar concentric force/velocity curve, suggesting an equivalent molecular motors dynamics.

Conclusion

T and L activation system and global mechanical output strongly depend on their architectural organization within the connective matrix. This significantly contributes to muscle performance and can serve the functional needs of body muscles during various movements.

S1-3

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

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

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

S1-4

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

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

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2 University of Arizona, USA
3 Soochow University, China

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

S1-5

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

Andrea C Mendoza, Professor/Dean Dr. Dilson E Rassier

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Background

The force produced by a myofibril should depend on its cross-sectional area, but not the number of active sarcomeres since they are arranged in series. However, a previous study performed in our laboratory (de Souza Leite et al., PNAS, 114:8794-8799, 2017) showed that depleting the thick filament of one sarcomere within an activated myofibril decreased the force production.

Purpose

In this study we examined how depletion of thick filaments in individual sarcomeres within a myofibril affects force production.

Methods

Myofibrils isolated from rabbit psoas were activated/relaxed using a perfusion system. An extra micro-perfusion needle filled with high ionic strength solution was used to erase thick filaments in real time before myofibril activation. Force sarcomere length non-uniformities were measured upon activation.

Results

The force produced by myofibrils with intact sarcomeres was higher than the force produced by myofibrils with one sarcomere lacking thick filaments ($p = 0.01$). Depleting the thick filaments of two sarcomeres decreased the force further ($p = 0.01$). The mechanism of the force decrease was associated with length adjustments developed by sarcomeres upon activation.

Conclusion

Our results suggest that the myofibril force is affected by intra-sarcomeres dynamics and the number of active sarcomeres in series.

S1-6

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

Colleen Kelly, Matthew J. Gage

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

Session 2. Muscle Cytoskeleton

S2-1

Coordinators of mechanical signal transduction through sarcoglycans and archvillin

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

S2-2

Myosin X drives filopodia of mammalian myoblasts to promote cell fusion

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

S2-3

How does the fusion of myoblasts modulate their mechanics ?

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

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

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

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

References

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S2-4

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

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

S2-5

Screen in Drosophila identifies a crucial enzyme for the incorporation of Z-disc proteins.

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Introduction/Background

Sarcomeres, the smallest contractile unit of muscles, are formed by antiparallel cables of thin and thick filaments. The actin thin filaments are anchored to a structure called the Z-disc, which develops from small growing Z-bodies that stably grow to reach their final size. In flies, the earliest steps of Z-disc formation are governed by the physical interaction between of actinin, actin and Zasp proteins. An extended PDZ domain located at the N-terminal region of Zasp mediates actinin binding. Zasp also bears 4 LIM domains at its C-terminal region, speculated to mediate protein-protein interactions. This conformation makes Zasp an ideal candidate for recruiting Z-disc proteins. We then screened for proteins that would be recruited by Zasp to the Z-disc.

Objective/Purpose

The goal of this work is to find novel players involved in Zasp-actinin Z-disc assembly process.

Method. First, we used an evolutionary covariation screening approach to select protein with a similar evolutionary pattern as Zasp and actinin. Then, we used GFP tagged alleles to select proteins located at the Z-disc. Finally, we characterize the phenotype of dOGDH and its interaction with Zasp.

Results

From our screen, we identified the fly homolog of the oxoglutarate dehydrogenase (dOGDH) as a component of the Z-disc. We show that the depletion of dOGDH impede protein recruitment to the Z-discs, leading to very small Z-discs and huge protein aggregates in the outside of myofibrils. The aggregation phenotype is specific to Z-disc proteins, and depends on the presence of Zasp and actinin. We show that Zasp recruits dOGDH to the Z-disc, through its C-terminal LIM domains. As OGDH is a member of the tricarboxylic acid cycle, we also tested other components of the cycle.

Conclusion

We identify a novel Z-disc protein that is required downstream of Zasp and actinin to ensure the correct recruitment of Z-disc proteins, and we propose a model in which the enzymatic activity of dOGDH regulates Z-disc growth.

S2-6

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

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Background

The giant protein titin contributes to muscle force generation. However, no tool has been available to specifically cleave the titin springs in sarcomeres.

Methods

A HaloTag-TEV-protease cassette was cloned into elastic titin of a mouse model, allowing for in-situ imaging of titin, specific proteolysis during myofiber mechanics and visualization of successful cleavage on protein gels. Using permeabilized myofiber bundles, we measured passive force over the sarcomere-length (SL) range 2.2-3.4 μm and maximum Ca^{2+}-triggered force (pCa5) at 2.6 μm SL, in the absence or presence of TEV-enzyme.

Results

TEV-protease cleaved HaloTag-TEV titin in myofibers within <1/2 hour, but had no effect on wildtype titin or other proteins. Titin cleavage caused myosin-filament disarray and lowered passive tension by 50-70%, the remainder being attributable to extracellular-matrix proteins. Mean active force was reduced by ~50%, with large inter-sample variability in the proportion of active-force reduction.

Conclusions

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

Session 3. Neuromuscular Signaling and Interaction

S3-1

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

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

S3-2

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

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

S3-3

Postnatal development of the interaction between sympathetic neurons and NMJs

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

S3-4

SH3BP2 as a novel scaffold protein regulating muscle postsynaptic machinery

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Neuromuscular junctions (NMJs) are synapses formed between motor neurons and skeletal muscle fibers. Despite their crucial role, the mechanisms that orchestrate NMJ development are still poorly understood. The Dystrophin-associated Glycoprotein Complex (DGC) is a major laminin receptor in skeletal muscles required for maintenance of muscle integrity and proper development of the postsynaptic machinery. We have identified SH3BP2 scaffold as a novel cytoplasmic protein that associates with DGC.

We demonstrated that SH3BP2 is concentrated at the NMJ postsynaptic machinery and also at the muscle contractile machinery. Protein complex purification experiments combined with mass spectrometry analysis revealed that SH3BP2 interacts with several postsynaptic proteins including alpha and gamma subunits of acetylcholine receptors (AChRs), agrin co-receptor Lrp4, as well as several components of the DGC.

Cultured myotubes depleted of SH3BP2 had impaired ability to cluster AChRs. Similarly, muscle-specific deletion of SH3BP2 affected organization of the NMJ postsynaptic machinery in vivo. Thus, SH3BP2 is a novel scaffold protein that organizes postsynaptic specialization by linking its crucial components to the DGC.

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S3-5

Characterizing the agrin-dependent internalisation of muscle specific kinase

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

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

S3-6

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

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

Session 4. Cardiac Contractility and Failure

S4-1

Cardiac resynchronization in heart failure: reverse remodelling

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Cardiac resynchronization therapy has been shown to reduce all-cause mortality, heart failure (HF) events and symptoms in those patients, where subsequent left ventricular reverse remodelling can be occurred with releating left ventricular volume reduction and stroke volume increase. Despite the clear evidences from large number of multicenter randomized trials, there is still a higher amount of patients who fail to develop reverse remodeling.

Evaluation of symptomes, QRS morphology, QRS width and left ventricular ejection fraction (LVEF) are crucial during the optimal patient selection. In the literature, baseline LVEF or left ventricular volumes and diameters predict the subsequent response to CRT implantation. However, in the actual guidelines the implantation criteria refer LVEF Ł35%, patients over this value might also benefit from the therapy. At the same time significantly decreased LVEF (≤ 25%) might lead to higher risk for HF events or all-cause mortality.

The response depends on several parameters, such as left ventricular lead position or right to left ventricular activation delay (RV-LV AD). First data has been shown that in LBBB patients lateral or posterior left ventricular lead position and also a longer RV-LV AD predicts a lower risk of HF events or all-cause mortality and confirmed the previous empiric clinical experience.

In parallel with the development of reverse remodelling the questions and decision between CRT-P vs. CRT-D implantation or the CRT upgrade from a previous device are waiting for further clarifications. For the latter question BUDAPEST CRT upgrade study might provide clear evidences (NCT02270840).

S4-2

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

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

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

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

Results Cardiac hypertrophy progression was validated by echocardiographic measurement. Significant increase of left ventricular mass along with no increase of left ventricular internal dimension at end-diastole confirmed the development of concentric hypertrophy by week 10 of aortic banding. Estimation of cell diameter in LV revealed cardiomyocytes enlargement after 8 weeks of pressure overload. Additionally, we observed gradual increase of Nppa expression at each time point in LV and IVS. Expression of Actn2, Cmya5 and Ldb3 was downregulated in LV after 1 week with subsequent increase to normal level and further decline to 10 weeks of aortic constriction. In RV we observed upregulation of the same gene expression after 2, 8 and 10 weeks of pressure overload. Expression pattern of above genes in IVS also reflected upregulation: mRNA level of Actn2 increased after 1 week, Cmya5 – after 10 weeks and Ldb3 after 1, 8 and 10 weeks. Other genes, selected for

the study (Fhl1, Fhl2, Synpo2, Ilk, Myoz2, Csrp3), did not demonstrate clear change in expression pattern, which did not differ between time points and parts of myocardium. In contrast with mRNA level changes, immunoblotting analysis identified significant increase of Actn protein level after 10 weeks of aortic constriction in LV myocardium, whereas no changes were detected in RV and IVS.

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

S4-3

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

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

S4-4

Structural studies of cardiac muscle contraction with dATP

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We have demonstrated that nucleotide 2 deoxy-ATP (dATP) enhances cardiac muscle contraction and ventricular function in both normal and diseased hearts. Our molecular dynamics simulations suggest that when dADP.Pi is in the nucleotide binding pocket of myosin, it results in exposure of more positively charged residues on the actin binding surface compared with ADP.Pi. We hypothesized that this results in increased electrostatic interactions between myosin and the actin filament, even in resting muscle, and results in increased strong crossbridge formation and contraction during cardiac systole. To test this hypothesis, we used small angle X-ray diffraction to investigate sarcomere structure under relaxed and activation conditions in physiological (170mM) and low ionic strength (IS) (100mM) solutions, at short (2.0 μm) and long (2.3 μm) sarcomere lengths (SL). We also performed Brownian Dynamic simulations of myosin bindnng to actin for several trajectories distances. From the equatorial fibers diffraction pattern analysis, lattice spacing (d1,0) was not significantly different with ATP vs dATP in skinned rat trabeculae in either IS conditions. The intensity ratio (I1,1/I1,0) significantly increased with dATP in relaxed (pCa9) muscle at physiological IS at both SL, indicating that more myosin heads were positioned closer to actin filaments. This same result was observed during activation (pCa5.2). When IS was reduced, the intensity ratio difference between muscle with dATP vs. ATP was significantly reduced or eliminated. Furthermore the intensity ratio with dATP at SL 2.0 μm was deceased when IS was decreased from 170 and 100mM, suggesting reduced electrostatic interaction between myosin and actin. Analysis of the meridian fiber diffraction pattern revealed that the distance of the M3 reflection increased with dATP during relaxation (pCa9). This increased axial distance (M3) among neighboring crowns of myosin heads suggests activation of thick filaments with dATP. The M6 reflections did not significantly differ between ATP and dATP. Preliminary data from BrownDye simulation suggest that myosin heads with dATP may have faster binding rates at 6.19, 8, 10 and 15 Ĺ distance to actin filaments. Taken together, our data suggest dATP triggers repositioning of the myosin S1 domain closer to actin filaments and activates thick filaments, facilitating increased and faster strong binding to actin and force generation. Our combined structure and simulation studies support our hypothesis that this occurs via enhanced initial myosin-actin electrostatic interactions with dATP to improve cardiac systolic function. AG055594, HL128368.

S4-5

Cellular mechanisms leading to cardiomyocyte diastolic dysfunction

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

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

S4-6

Structural and biophysical characterisation of titin missense variants in genetic myopathies / cardiomyopathies

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Myopathies and cardiomyopathies are genetic conditions affecting skeletal and cardiac muscle. They are often caused by mutations in sarcomeric genes, such as TTN which encodes the giant protein titin. Due to its size, TTN gene variants are also found in unaffected individuals (as shown by the 1000 Genomes Project) and it is difficult to assess their impact. In this project, biophysical techniques such as X-ray crystallography, differential scanning fluorimetry (DSF) and 1D Nuclear magnetic resonance (NMR) were applied to determine the structure of single and multiple titin A-band domains, in order to assess the impact of suspected and proven pathogenic variants on their stability and structure. Fibronectin type-3 domains from the titin A-band harbouring rare missense mutations were expressed in E. coli, both in wild-type (wt) and variant forms. AII wt's were confirmed folded by NMR studies, whilst some variants had structural changes induced by the missense mutation. Their DSF melting temperatures were lower by around 10°C, suggesting a reduction in stability caused by the mutation may be a common feature of genetically proven pathogenic TTN variants. X-ray structural data elucidated the structural basis of the destabilization, allowing visualization of impact of the missense mutation on the surrounding residues and tertiary structure of the protein.

Session 5. Molecular Motors

S5-1

Keynote presentation

Allosteric Tuning of Myosin Force Generation : new avenues towards therapeutical treatment

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

S5-2

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

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

S5-3

Role of myosin VI in myoblast function and differentiation into myotubes

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

In skeletal muscle, MVI is present in the neuromuscular junction, sarcoplasmic reticulum and myofiber nuclei thus implying that it is important in proper functioning of muscle (Karolczak et al. 2013, 2014). Moreover, we have also postulated that MVI could be involved in myoblast differentiation and maturation (Karolczak et al. 2015).

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

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S5-4

Myosin: isoforms and cardiomyopathies

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

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

S5-5

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

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

S5-6

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

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

Session 6. Smooth Muscle Contraction and Pathology

S6-1

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

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

S6-2

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

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

S6-3

Role of telokin in the vasoregulation of murine portal vein

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Background

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

Methods

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

Results

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

Conclusions

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

S6-4

The remarkable cardiovascular system of giraffes

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

The mean blood pressure of giraffes is about 200 mmHg.

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

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

S6-5

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

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In skinned catch muscle preparations of mollusks, the catch releasing factor cAMP causes not only quick relaxation during the catch phase but also a decay of force during active contraction at submaximal Ca_c^+ activations. Therefore, it was assumed that a certain component of catch force exists during submaximal active contractions (Butler et al. Biophys J, 90:3193–3202, 2006). To test this hypothesis, we investigated the cAMP effect at different pHs, because catch is pronounced at acidic pH and absent at alkaline pH. We found that the cAMP induced force decay at submaximal Ca_c^+ activation was largest at pH 6.8 and smaller or absent at pH 6.2 and pH 7.4. No effect of cAMP on force was observed at maximal Ca_c^+ activation of any pH. These observations are not compatible with the assumption that catch force is established during the phase of Ca_c^+ activation. The linkages causing catch may already exist during active contraction; however, they are not under tension at this condition. The cAMP induced force depression at submaximal Ca_c^+ activation is probably caused by an effect on the force-generating myosin heads.

S6-6

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

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Introduction

The anterior byssus retractor muscle under active, catch, relaxation and contracture states with biophysical recordings demonstrated each a specific ultrastructure of the contractile filaments [1-3].

Objective

During the years, uncertainty remained about the catch because of diverse techniques used instead of synchronized ones.

Method: Ultrastructure of ABRM undergoing catch state were revisited using strain gauge recordings while other assays were also treated with α-amylase.

Results

Close associations between paramyosin filaments with interconnections formed by some electron dense material, undigested by α-amylase, are shown.

Conclusion

Synchronized tension recording along with electron microscopy processing shows that paramyosin interactions could also accompanied by another intervening protein (twitchin? [4]) detected in the catch state contraction. This mechanism allows the muscle to maintain long lasting contraction with a very low energetic expenditure during long interval periods thus survival of the species along seashores.

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Session 7. Thin Filament and Actin-binding Proteins

S7-1

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

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

S7-2

Single molecule imaging reveals how Cardiac Myosin Binding Protein-C sensitizes thin filaments to calcium

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

S7-3

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

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

S7-4

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

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

S7-5

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

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

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

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

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

The project was supported by NCN, grant 2014/15/NZ1/01017.

S7-6

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

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

Session 8. Motor Protein Pharmacology

S8-1

Keynote presentation

Motor Pharmacology: novel inhibitors for different myosin-2 isoforms

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

S8-2

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

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

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

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

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

S8-3

Selective inhibition of myosins by halogenated carbazoles and arylindole derivatives

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

S8-4

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

Bart Roman

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

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

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

S8-5

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

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

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

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

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

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

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

S8-6

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

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

Session 9. Muscle Energetics

S9-1

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

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Introduction

Submaximal repeated stimulation of whole mammalian muscle in situ results in activity dependent force potentiation followed by a fatigue-induced decline in active force. The potentiation is thought to be due to increased sensitivity to Ca^{2+} whereas the decrease in active force is thought to be due to both decreased myoplasmic free [Ca^{2+}] ([Ca^{2+}]m) and decreased Ca^{2+} sensitivity.

Method

We measured [Ca^{2+}]m with indo-1 fluorescence during intermittent submaximal (40-50 Hz) stimulation of single mouse flexor digitorum brevis fibres at 32C and evaluated the change in the relationship between force and [Ca^{2+}]m with respect to the control (prefatigue) relationship.

Result

Active force began at 194 75 kPa (mean SD) and [Ca^{2+}] was 0.319 0.012 M. Initially, active force decreased to 182 48 kPa while [Ca^{2+}]m increased to 0.372 0.015 M, representing a decrease in Ca^{2+} sensitivity. Subsequently force potentiated to 221 34 kPa then fell to 69.3 27.6 kPa while [Ca^{2+}]m decreased, reaching 0.318 0.014 M at the final target force of 40% of initial.

Conclusion

At no time did the Ca^{2+} sensitivity increase beyond that seen in the control situation. The final [Ca^{2+}]m was not significantly different from the initial [Ca^{2+}]m. Calcium sensitivity recovered to the control value within 5 min and low-frequency fatigue persisted due to a post-fatigue development of persistent low [Ca^{2+}]m.

S9-2

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

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Introduction

TGFbeta inducible early gene-1 (TIEG1) is a member of the Krüppel-like family of transcription factors (KLF10). Deletion of TIEG1 results in muscle fiber hypertrophy, texture profile changes, dysfunction of mitochondrial biogenesis and defects in functional properties.

Aim

To further analyze the effect of TIEG1 gene on muscle metabolism.

Methods and Results

12 WT and 12 TIEG1 KO mice were used for in vivo spectroscopy acquisitions 9.4T (Bruker). A home built coil was developed. Resonance frequencies were 400 MHz for the proton and 162 MHz for the phosphorus. Localized 1H and 31P spectroscopy were performed with PRESS sequence providing quantification of different metabolites. While 1H-NMR spectra showed no significant difference for choline, creatine, taurine and extramyocellular lipids between WT and TIEG1 KO. 31P spectra revealed a significant difference for phosphocreatine and ATP.

For metabolomics analysis 1H-NMR spectra were obtained from soleus (N=18) and EDL (N=18) muscles isolated from WT and TIEG1 KO with a 600MHz spectrometer (Bruker, 14T). Heatmaps were generated to visually depict changes in metabolites ($p < 0.05$) as a function of mouse genotype. For both TIEG1 KO soleus and EDL muscles, there were more down regulated metabolites compared to WT muscles.

Conclusion

The present study has demonstrated a new role for TIEG1 in the homeostasis of the muscle metabolome and specifically in energetic metabolism.

S9-3

Ahnak1 expression declines the cardiac function in the aged hearts

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Recent evidence has suggested that a high Ahank1 expression is associated with poor muscle fitness, although the underlying mechanism remains unclear. We therefore investigated the in vivo role of Ahnak1 on physical fitness (4 weeks daily treadmill running) of aged male and female mice (12-13 months old) using a homozygous Ahnak1 knock-out model (Ahnak-/-) and their wild-type littermates (WT). We observed that significantly more Ahnak-/- mice of both sexes (100%) could successfully complete the running training program than WT male (31%) and female (8%) mice. To investigate the cause of the improved physical fitness of Ahnak-/- mice, we compared the phenotype and the activity of mitochondria in their mouse hearts and primary adult mouse cardiomyocytes (aCM) with those in WT hearts by transmission electron microscopy (TEM) and seahorse analysis at the basal level. Our TEM study showed significantly lower mitochondrial number per area in female and male Ahnak-/- hearts in comparison to those of WT mice. However the Ahnak-/- hearts of both sexes have significantly more large (>1µm2) mitochondria and less small mitochondria (<0.5µm2) compared to age-and sex-matched WT hearts. Additionally, the mitochondrial activity (max. respiratory capacity) was significantly higher in aCM prepared from Ahnak-/- mice of box sexes compared to the respective WT groups. The higher efficiency of ATP production of remodeled mitochondria in aCM prepared from Ahnak-/- was associated with significantly improved contractile parameters (maximal contraction amplitude, maximal rate of force development and maximal relaxation rate) compared to the corresponding WT groups. Interestingly, peak-systolic and diastolic Ca^{2+} levels of aCM prepared from Ahnak-/- were significantly reduced suggesting increased calcium sensitivity of the myofibrillar apparatus of Ahnak-/- mice. Taken together, our data indicate that Ahnak1 deteriorates the mitochondrial remodeling and function, thus affects adversely the fitness of the aged heart.

S9-4

The missense E258K-MyBP-C mutation increases the energy cost of tension generation in both ventricular and atrial tissue from HCM patients

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

S9-5

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

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Background
Statins impair expression of PGC-1α in human and rat skeletal muscle, suggesting a role of PGC-1α in statin-induced myotoxicity.

Objective
This study aimed to investigate these effects in differentially expressed PGC-1α mouse models.

Methods
We used 3 mouse models: mice with muscle PGC-1α knockout (MKO), mice overexpressing PGC-1α (MCK), and wild-type (WT) mice. Mice treated for 3 weeks with water or simvastatin (5 mg/kg/d) by oral gavage, were assessed with grip test, metabolic treadmill and glucose tolerance test. We measured mitochondrial respiration and H2O2 production in fresh permeabilized muscle fibres.

Results
Simvastatin showed impairment in WT mice, manifested by decreased exercise capacity, glucose intolerance, and decreased mitochondrial respiration in the glycolytic muscle coupled with increased H2O2 production. MKO mice treated with simvastatin, showed decreased exercise capacity and mitochondrial respiration in oxidative and glycolytic muscle types. MCK mice showed no impairments of mitochondrial function and physical capacity.

Conclusion
Oxidative muscles are more resistant to simvastatin-associated toxicity than glycolytic muscles. PGC-1α seems to be a susceptibility factor and has an important role in mitigating of simvastatin induced myotoxicity.

S9-6

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

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

S9-7

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

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Introduction

AMP-activated kinase (AMPK), a major regulator of energy metabolism, is a promising target for the treatment of type 2 diabetes and cancer. AICAR, an adenosine analogue, is the most widely used pharmacological AMPK activator in cell-based assays. Some broadly used cell culture media, such as MEMα, contain high concentrations of nucleosides. We examined whether such media alter AICAR actions in skeletal muscle and cancer cells.

Methods

We evaluated the effect of AICAR and nucleosides on AMPK activation, glucose uptake and cell proliferation.

Results

In nucleoside-free media AICAR activated AMPK, increased glucose uptake and suppressed cell proliferation. These effects were reduced in MEMα with nucleosides. Addition of adenosine to nucleoside-free media also suppressed AICAR actions. MEMα with nucleosides blocked AICAR-stimulated AMPK activation even in the presence of methotrexate, which normally enhances AICAR actions by reducing its intracellular clearance.

Conclusion

Our findings show that nucleosides in cell culture media reduce effects of the most widely used pharmacological AMPK activator AICAR. Results of cell-based assays in which AICAR is used for AMPK activation therefore critically depend on media formulation. Furthermore, our findings highlight a role for extracellular nucleosides and nucleoside transporters in regulation of AMPK activation.

Session 10. Contraction Regulation, EC Coupling

S10-1

The role of septins in skeletal muscle

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

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

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

S10-2

Defective Ca^{2+} signaling in centronuclear myopathies

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

S10-3

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

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

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

S10-4

Role of triadin mutations in inherited arrhythmia syndromes

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

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

S10-5

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

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UREPSSS, APMS

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

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

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

This study was funded by the French spatial agency "Centre National d'Etudes Spatiales" (CNES).

S10-6

New insights of intracellular calcium regulation mechanism in dystrophin-deficiency

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

Session 11. Muscle Development, Regeneration and Disease

S11-1

Expression of truncated obscurins leads to maladaptive responses in the heart

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

S11-2

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

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

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

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

S11-3

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

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

S11-4

Myocardial regeneration: Uncommon sense for common problems

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Myocardial regenerative research remains an area of intensive study despite over a decade of frustratingly slow progress and modest clinical efficacy. A fundamental limitation in myocardial regeneration is inherently poor reparative capacity of adult mammalian heart which declines over lifespan. Augmentation of repair requires unnatural solutions to overcome normal adult myocardial biology using Regeneration Associated Cellular Effectors (RACE) to deliver functionally competent therapeutic interventions. The logic and rationale of four distinct RACE conceptual strategies will be presented including CardioEnhancers (genetic engineering), CardioChimeras (cell chimerism), CardioClusters (multi-cell three dimensional clustering), and CardioEvolvers (increased ploidy). Each RACE approach addresses a distinct biological limitation that impairs current cell-based treatments for myocardial damage, and different RACE approaches can be combined to promote synergism of biological potentiation. These next-generation approaches represent the future of myocardial regenerative research, ultimately translating into novel clinical treatments achieving desperately needed treatment of heart failure.

S11-5

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

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

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

S11-6

Pathogenic troponin T mutations with opposite effects on myofilament Ca^{2+} sensitivity Attenuate Each Other's Cardiomyopathy Phenotypes in Mice

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

Session 12. Integrative Muscle Biology

S12-1

Keynote presentation

From basic muscle research to applications in the clinic

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

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

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

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

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

S12-2

Striated muscle tissue mechanosensors in health and disease

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

S12-3

Hormonal responses following resistance exercise performed at maximum movement velocity

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Introduction

Mechanical overloading of skeletal muscle is a strong stimulus for eliciting acute hormonal changes potentially involved in muscle adaptation following resistance exercise.

Purpose

This study investigated the responses of thyrotropin (TSH), free thyroxine (fT4) and prolactin (PRL) in young volunteers after resistance exercise of the knee extensor muscles of both legs.

Methods

Nine healthy males (age: 22.5 ± 3.3 years, height: 181 ± 5 cm, body mass: 81.6 ± 5.6 kg) underwent a resistance exercise protocol (4 sets of squat and 4 sets of leg press, 8 repetitions/set at a load of 10-RM) with the velocity of movement during concentric contractions being maximum. Blood samples were collected before, immediately after and at 20 and 40 min post-exercise. Serum levels of TSH, fT4 and PRL were measured by ELISA.

Results

TSH showed a gradual, non-significant increase up to 43% at 40 min post exercise ($p>0.05$). Serum fT4 levels exhibited also a gradual increase reaching significance ($p<0.01$) at 40 min post exercise. PRL levels showed a slight decrease up to 19% 40 min post exercise ($p>0.05$).

Conclusion

Our findings suggest that resistance exercise induces acute increases in serum levels of thyroid hormones, particularly of fT4. Further studies are needed to characterize the mechanisms by which these hormonal responses are triggered and regulated during recovery after resistance exercise.

S12-4

Disturbances of the homeostasis of the neuro-muscular-tendon tissue-complex in contractures of individuals with Cerebral Palsy

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

Changes at tissue level

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

Systemic differences

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

Effect of treatments

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

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

S12-5

The muscle clock regulates titin splicing and sarcomere length

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Introduction

Disruption of the muscle clock leads to weakness with myofilament disruption. Titin's role as a sarcomeric scaffold and spring could potentially link myofilament disarray with muscle weakness.

Objective

We tested if titin isoforms change after loss of skeletal muscle Bmal1, a circadian factor. We also tested if loss of Bmal1 has an effect on sarcomere length.

Methods

iMSBmal1-/- and iMSBmal1+/+ mice were used. Titin isoforms were determined at the protein and RNA level. Sarcomere lengths were measured with immunohistochemistry using an α-actinin antibody to demarcate Z-lines.

Results

The ratio of short to long isoforms of titin protein changed in the tibialis anterior muscle of iMSBmal1-/- mice. RNASeq data indicated this shift is due to inclusion of exons 51-89 within titin's spring region. While average sarcomere length was not different, sarcomere length variability increased after knockout. Ongoing studies are testing if altering titin splicing can change sarcomere length.

Conclusion

Our results suggest that titin plays a role in regulating sarcomere length homogeneity downstream of the molecular clock.

S12-6

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

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Background

Photobiomodulation (PBM) mitigates muscle atrophy induced by microgravity and ischemia-reperfusion but whether it affects denervation-related atrophy is unknown. Autophagy plays a central role in muscle atrophy and is affected by PBM in non-muscle tissue.

Objective

To examine the effects of PBM on denervation-muscle atrophy and autophagy.

Methods

Right tibialis anterior muscles were denervated by sciatic nerve section, in adult mice. Denervated right tibialis anterior muscles were treated with PBM during 5 or 14 days. Some denervated muscles were not treated. Controls were not denervated and did not receive PBM. Atrophy of the right tibialis anterior muscle was estimated by muscle fiber cross sectional area (CSA). Autophagy was evaluated by immunofluorescence, western blot and electron microscopy.

Results

CSA was higher ($p < 0.001$) in PBM than in untreated group at day 5 (1661.6 ± 139.6 vs 1094.8 ± 46.8 μm2) and day 14 (1067.1 ± 46.8 vs 827.2 ± 63.5 μm2). No differences were found between PBM and untreated in autophagy markers: i) anti LC3 positive points, ii) LC3II/LC3I ratio and iii) autophagic figures at day 5 and 14.

Conclusion

Denervation-related muscle atrophy was mitigated by PBM without affecting autophagy. PBM emerges as a potential therapy to mitigate denervation-induced muscle atrophy in the clinical arena.

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ABSTRACTS

POSTER

Poster Session 1. Skeletal Muscle Mechanics

P1-1

Hydrophobic surface unraveling in force-induced titin-domain unfolding

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

P1-2

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

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

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

P1-3

Skeletal muscle in renal insufficiency: is calcium sensitivity affected?

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Introduction
Chronic renal insufficiency patients present with functional abnormalities of unknown etiology collectively described as uremic myopathy.

Objective
We investigated possible differences in calcium sensitivity of uremic (UREM) and control (CON) muscle.

Method
We used psoas muscle from an approved rabbit model of UREM and sham-operated CON. Isometric tension (P0) was assessed in 128 CON and 195 UREM skinned fibers at 10°C, 30°C and at pH7 and pH6.2, in various CaCl2 concentrations. Force data expressed as percentage of P0 at standard conditions (10°C, pH7) and free calcium expressed in pCa values, were fitted in the Hill equation ($p<0.05$).

Results
At 10 °C pH7, UREM and CON fibers presented with similar calcium sensitivity (pCa50 UREM 6.12±0.02 vs CON 6.20±0.03) and cooperativity (nH UREM 2.11±0.14 vs CON 2.36±0.3). Acidosis (pH 6.2) at 10oC caused a loss of calcium sensitivity, more so for UREM fibers (pCa50 UREM 5.32±0.06 vs CON 5.58±0.02). At 30 °C pH7, UREM fibers showed lower sensitivity than CON (pCa50 UREM 6.00±0.25 vs CON 6.42±0.19). At 30°C pH 6.2 calcium sensitivity was similar for both groups (pCa50 UREM 5.71± 0.13 vs CON 5.80± 0.05). Changes in cooperativity followed a similar pattern.

Conclusion
In uremic muscle, calcium sensitivity may be depressed, even in resting conditions.

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P1-4

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

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Introduction
Oxidative and nitrosative stress in muscle cells have been associated with muscle weakness observed in many diseases. Although there is indirect evidence to suggest that oxidation affects skeletal muscle myosin, the detailed acute effects of nitrosative modifications on myosin-actin interactions are not known.

Objective
In this study we examined the effects of peroxynitrite (ONOO)-derived nitrosative stress on the contractile properties of individual skeletal muscle myofibrils.

Method
Nitrosative stress were induced by adding the ONOO-generator SIN-1 or ONOO directly to myofibrils. To evaluate cross-bridge properties, forces and force development rates were measured by monitoring myofibril-induced displacements of an atomic force cantilever upon activation (pCa 4.5) and relaxation (pCa 9.0).

Results
The isometric force decreased by 50% in myofibrils treated with 10 mM SIN-1 while rates of force activation and redevelopment were unchanged. The rate of the slow linear phase of relaxation increased during nitrosative stress while the duration of this phase was not altered. Similar results were seen for myofibrils treated directly with ONOO.

Conclusion
Our results suggest that the decrease in force after acute nitrosative stress is linked to an impaired function of myosin to form force-generating cross-bridges and not from cross-bridge kinetics.

P1-5

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

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

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

P1-6

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

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

P1-7

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

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Introduction/Background

How diet-induced obesity affects skeletal muscle contractile function is not well established.

Objective/Purpose

We examined how a high-fat, high-sucrose (HFS) diet affects contractile properties of skinned rat vastus intermedius fibres.

Methods

Male rats aged 3 weeks began either a chow diet or a HFS diet. After 14 weeks on the diets, body composition was assessed with dual-energy x-ray absorptiometry. Muscles were then harvested, and contractile properties of skinned fibres were assessed at 19 °C.

Results

Fat comprised 27±4% and 14±2% of body mass in HFS and chow diet group animals, respectively. Maximum force, peak shortening rate, resting stiffness, and the ratio of force to stiffness due to cross-bridges did not differ between diet groups. However, the calcium-sensitivity of force production was higher in fibres of HFS-fed rats than fibres of chow-fed rats. This effect was more pronounced in type I than type IIa fibres.

Conclusion

The HSF diet used in this study caused a 2-fold increase in body fat, but aside from increasing the calcium-sensitivity of cross-bridge formation, contractile properties of skinned vastus intermedius fibres were not affected.

P1-8

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

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Background

The steady-state force achieved after a skeletal muscle is actively stretched or shortened is always greater or smaller than a pure isometric force obtained at the same final muscle length. The precise mechanism of these properties, termed residual force enhancement (RFE) and force depression (FD), is not well understood.

Objective

Our aim was to gain insight into the possible mechanism(s) of RFE and FD by examining the structural changes in the sarcomere after active stretch and shortening, using small angle x-ray diffraction.

Methods

We examined stiffness and the equatorial 1.0 and 1.1 and meridional M3 and M6 x-ray reflections for steady-state conditions after pure isometric and active stretch and shortening contractions in skinned rabbit psoas bundles.

Results and Conclusions

Active stretch did not affect stiffness or I1.1/I1.0 but it increased M3 and M6 spacings and decreased M3 peak intensity, compared to pure isometric contractions. Active shortening reduced stiffness, I1.1/I1.0, M3 and M6 spacings and M3 peak intensity. This suggests that the proportion of attached cross-bridges seems unaffected after stretch but decreases after shortening. Moreover, RFE and FD are likely accompanied by an increase in cross-bridge disorder and a change in their conformation.

P1-9

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

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

Poster Session 2. Muscle Cytoskeleton

P2-1

Structural insight into the myotilin-actin interaction

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

P2-2

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

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

P2-3

Possible functional role of titin amyloid aggregation

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Titin is a giant elastic muscle protein. In sarcomeres of cardiac and skeletal muscles, the amount of titin is third beside the number of actin and myosin filaments. Molecular simulations carried out in 2015 showed that 2 titin domains I27-I28/I27-I27 were able to form amyloid-like aggregates, called "intramolecular amyloids". Amyloids are protein aggregates with a cross-β-structures. The high ability of the formation of amyloid aggregates by full-length molecules of smooth muscle titin was demonstrated in our recently studies. Unlike most proteins forming amyloids under extreme conditions in vitro (at 37-100°C, acidic pH for 24 hours or longer), titin formed amyloid aggregates for 20-30 minutes in solutions with physiological ionic strength at a temperature of 4-24°C (pH 7,0-7,4).

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

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P2-4

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

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

P2-5

Calcium dependent elasticity of native titin filaments

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

P2-6

The topology of interactions between titin and the thick filament

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

P2-7

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

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

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

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

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

P2-8

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

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

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

Poster Session 3. Neuromuscular Signaling and Interaction

P3-1

Distribution of Sympathetic Innervation in Skeletal Muscles

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

P3-2

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

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Introduction

Atrophy and dysfunction of skeletal muscle developing during space flight are related to changes in cell signaling mechanisms. One of the signaling pathways is associated with sphingolipids, mainly ceramide (Cer). It is known that Cer can displace cholesterol (Chol) from a lipid raft and it causes the destabilization of its structure.

Purpose

To study the role of Cer in lipid raft disturbance caused by 12-hour hindlimb suspension (HS) of soleus muscle.

Method

We used Morey's tail-suspension model to simulate the microgravity effect in muscle. In some experiments rats were pretreated with the inhibitor of acid sphingomyelinase (aSMase), clomipramine.We studied the lipid profile of rat soleus using HPTLC. Chol was detected by colorimetric enzymatic method. By confocal microscopy, we visualized lipid rafts and nAChR after the staining with cholera toxin B subunit and rhodamine-conjugated α-bungarotoxin, respectively.

Results

The amount of Cer increased and sphingomyelin and Chol decreased in suspended soleus muscle. Unloading also led to the decrease in raft labeling, indicating the disturbance in their structure. Clomipramine abolished these effects.

Conclusion

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

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

P3-3

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

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

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

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

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

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

The study was supported by RFBR (grant N 16-04-01370)

P3-4

Synaptic transmission in rat's soleus muscle during microgravity

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The mechanical unloading of skeletal muscles during space flights or in experimental or pathological, conditions limiting motility, causes morphofunctional changes of the neuromuscular system.

Here we studied the changes in neuromuscular transmission in the soleus muscle in conditions of gravitational unloading (experimental group UN) and during gravitational unloading combined with daily electrical stimulation of the spinal cord (experimental group UN + ES).

The amplitude of the 200th M-response was evaluated with respect to the 1st - decrement test at high frequency (50 Hz) sciatic nerve stimulation after 7 days of gravitational unloading.

A significant depression of the induced motor potential was recorded in the UN group with the decrement 35 ± 12%. The decrement and increment of the motor response were recorded in the UN + ES group. On average, the amplitude of the 200 M-response was 13 ± 16% in compare to the first, which corresponds to the values recorded in intact animals.

These results suggest that microgravity conditions can affect the functional state of the neuromuscular transmission of the rat's soleus muscle, at the same time, these changes prevented by stimulation of the spinal cord.

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P3-5

Modulation of multisegmental responses in leg muscles during postural tasks

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The amplitude modulation of multi-segmental responses (MMRs) can reflect the result of the integration of sensory and motor information at the spinal level during postural tonic and rhythmic motor activity. The aim of this study is to investigate effects of various postural tasks on alteration of characteristics of multi-segmental responses in healthy subjects. Six relevantly healthy individuals (23.2±2 years) were examined under three experimental conditions: eyes open on a normal floor surface; eyes closed on a normal floor surface; and eyes open on a foam surface. At all tested conditions transcutaneous electrical spinal cord stimulation was applied at the Th11-12 vertebra. The MMRs were registrated by bipolar self-adhesive electrodes from the following muscles: rectus femoris (RF), medial hamstring (MH), tibialis anterior (TA), and soleus (SOL). Our results indicate that the modulation of MMRs amplitude was mainly observed in calf muscles. Particularly, we observed suppression in amplitude of MMR in TA and SOL ($p<0,05$), but not in RF and MH ($p=0,05$) with more complex tasks (eyes closed and foam surface). Consequently, MMRs modulation under various postural tasks can be related on presynaptic inhibition under alteration of afferent input. These results can provide essential information on mechanisms of postural control.

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P3-6

Effects of plantar stimulation on anabolic signalling in rat soleus muscle during gravitational unloading

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Mechanical plantar stimulation allows to prevent muscle tone and strength impairment, fibre atrophy and increased degradation of the cytoskeletal proteins. The aim of the study was to evaluate the effects of plantar stimulation on anabolic signalling response in rat soleus during gravitational unloading.

The plantar stimulation at the early stage of the hindlimb unloading led to complete or partial prevention of the alteration of some key signaling molecules. We found that phosphorylation of GSK 3 β, IRS-1 content and overall protein synthesis was significantly decreased after 3-day hindlimb suspension (HS) compared to control animals. We also found that phosphorylation of p70S6k and eEF 2 was significantly higher in HS animals. These effects were partially or completely prevented by plantar foot stimulation. Some other key signalling factors, altered under unloading conditions, were found to be insensitive to plantar stimulation. The causes and mechanisms involved in the plantar stimulation selective effects on the signalling pathways components in the postural muscle should be analyzed in the future studies.

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P3-7

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

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Introduction

Na+,K+-ATPase (NKA), a heterodimer comprising an α (α1-3) and a β (β1-3) subunit, is fundamental to skeletal muscle ion homeostasis and contractility. NKA is regulated by small transmembrane proteins from the FXYD family (FXYD1-7). In skeletal muscle, the most prominent among these are FXYD1 (phospholemman) and FXYD5 (dysadherin).

Objective

We determined whether innervation by motor neurons alters the expression of NKA subunits, FXYD1 and FXYD5 in cultured human myotubes.

Methods

To establish innervation, human myotubes were co-cultured with explants of embryonic rat spinal cord.

Results

Once innervated by motor neurons, myotubes contracted spontaneously. Aneural myotubes were quiescent and did not contract. Using qPCR and species-specific primers, mRNA levels of the NKA α3 and β2 subunits, respectively, were 3-fold and more than 100-fold higher in innervated, contracting myotubes than in aneural myotubes. Expression of other NKA subunits, FXYD1 and FXYD5 was unaltered. In co-cultures which failed to display contractile activity expression of α3 and β2 subunits was not significantly increased, highlighting the role of contractions in regulation of NKA expression.

Conclusion

Innervation leads to isoform-specific changes in the expression of NKA subunits in human myotubes. These changes are likely induced by motor neuron-driven contractions of myotubes.

Poster Session 4. Cardiac Contractility and Failure

P4-1

FLNC missense variants associate with early-onset restrictive cardiomyopathy combined with congenital myopathy

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Introduction

FLNC gene encodes filamin C – a large actin-binding protein, which is highly expressed in heart and skeletal muscle. Mutations in FLNC are associated with various types of myopathies and inherited cardiac disorders, including hypertrophic (HCM), dilated (DCM) and restrictive (RCM) cardiomyopathies. In our study, we described new cases of filaminopathy in patients with early-onset RCM combined with congenital myopathy due to de novo mutations in FLNC.

Purpose

To evaluate the pathogenic significance of newly identified FLNC missense variants and its impact on muscle tissue morphology and development of RCM phenotype.

Method

Design of the study was approved by the Institute Ethical Review Board. The study cohort included 24 identified RCM cases and an additional RCM case not previously reported elsewhere. Targeted enrichment was performed with the SureSelect Human All Exon 60Mb Kit (Agilent Technologies), and captured DNA was sequenced on a HiSeq2000 (Illumina). Pathogenicity of identified missense variants was assessed based on the MetaSVM predictions obtained from the dbNSFP database. The identified genetic variants were classified according to ACMG guidelines. Morphological examination of skeletal and cardiac muscle biopsies was accessed using Masson, Gomori trichrome staining as well as immunohistochemical stainings of filamin C, desmin and actin. C2C12 cells were transfected with pCS2- FLNCWT, -FLNCA1186V, or -FLNCA1183L constructs, carrying wild type or mutant FLNC coding sequences respectively, and analyzed after anti-FLNC staining.

Results

We identified four unrelated cases of filaminopathy in patients, carrying FLNC (NM_001458.4) variants: c.3557C>T, p.A1186V in three probands and c.[3547G>C; 3548C>T], p.A1183L in one proband. All patients present clear restrictive cardiac phenotype combined with myopathy and arthrogryposis with early manifestation. In silico prediction revealed high pathogenicity of A1186V and A1183L substitutions, localized in the same Ig domain. Morphological analysis identified mild fibrosis in cardiac tissue, of patient, carrying A1186V mutation. In heart samples cardiac actin staining indicated the presence of intact intercalated disks, but no regular staining of filamin C in ID was detected in patient heart in comparison to control. In skeletal muscles desmin staining showed moderate sarcomere disturbance in patient myofibrils, reflected in unclear desmin localization at Z-disk area. There were no FLNC or desmin aggregates in both cardiac and skeletal muscle samples. Analysis of transfected C2C12 cells also revealed absence of FLNC aggregates in mutant variants.

Conclusion

Identified missense FLNC variants have a high pathogenicity level, confirmed by histopathological analysis of muscle tissue. Both tissue samples examination and in vitro study revealed, that progression of early-onset FLNC-related RCM combined with myopathy does not directly associate with aggregates formation.

P4-2

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

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Background

Here we investigated the role of enhancing the NO/sGC/cGMP/PKG signalling pathway in the modulation of diastolic function in a model of heart failure (HF) with preserved ejection fraction (pEF).

Purpose

To study the effect of sGC stimulation on left ventricular (LV) diastolic dysfunction, and its action on myocardial stiffness and oxidative stress.

Methods

Chronic (4 weeks) stimulation of sGC (with BAY 41-8543) was studied on 15-week-old male Dahl/SS (HFpEF) and SS-13 (CTRL) rats (n=8-12/group).

Results

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

Conclusion

Our data suggest that chronic stimulation of sGC may be a promising treatment option for HFpEF patients.

P4-3

Design of muscle contraction assist devices by liquid crystalline elastomers

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Aims

Loss of muscle contractility occurs in different, life-threatening diseases. Current treatments suggest the need for a new generation of contraction assist devices. Liquid Crystalline Elastomers (LCEs) can work as "artificial muscle", with particular focus on cardiac muscles.

Methods and Results

LCEs are biocompatible materials able to deform reversibly in response to given stimuli. Thin (20-µm) LCEs films were prepared and their light-response and mechanical properties measured from small strips (200-400 µm diameter, 3-4 mm length) isometrically mounted between a force transducer and a linear actuator. LCE film samples maximally activated and relaxed by a green light (200 mW/mm2), showed a mechanical behavior similar to force responses of isolated human cardiac myofibrils. The nature of material composition and the stimulus intensity modulated mechanical and kinetic parameters.

Conclusions

LCEs are suitable to mimic cardiac muscles. We prepared light-responsive LCEs films, highlighting how different molecular parameters affect different aspects of mechanical functions. Our results open for a new generation of LCE-based contraction assist devices.

P4-4

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

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Rationale

Myocardial diastolic stiffness depends in part on signaling pathways and phosphorylation. $Ca(^{2+})$/calmodulin-dependent protein kinase-II (CaMKII) δ and protein kinase G (PKG) are known to target titin, but it is unknown if PKG phosphorylates CaMKIIδ.

Methods and Results

CaMKIIδ phosphorylation by PKG was assessed in recombinant proteins and heart failure (HF) biopsies by autoradiography, immunoblotting and quantified in vivo by mass spectrometry (MS). Unchanged CaMKIIδ phosphorylation and increased oxidation was observed in HF biopsies. PKG-dependent phosphosites were identified within the CaMKIIδ by quantitative MS and confirmed in recombinant human CaMKIIδ. The most highly phosphorylated sites are located in the regulatory domain and the linker region. Acute intravenous injection of PKG stimulator in anaesthetized HF rats significantly improved diastolic function via increased PKG activity, reduced CaMKIIδ auto-phosphorylation and oxidation, and reduced oxidative stress and inflammation.

Conclusions

Our study shows that PKG plays a central role in regulating and maintaining the balance of CaMKIIδ activity and oxidative stress and thereby improving diastolic function.

P4-5

HCM mutation cardiac troponin C A8V alters cardiomyocyte nucleus structure in a knock in mouse model

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

P4-6

SOCS1 box expression in mechanical stretching cultured mice cardiac ventricle

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We compared investigated mice dried cardiac tissue SOCS1 expression under mechanical stretching culture. Mice anterior ventricular muscle layer were stretched to 30%, incubated in 37C for 72 hr up to dry. The medium were HyClone classical liquid medium, or with plasmid and CfTX-1 peptide. A further SDS-PAGE assay investigated the 25kDa range band, westernblot identified the SOCS1 box. The results indicated SOCS1 box were expression in wet culture and dried cardiac muscle. In SOCS1 plasmid transfection stretching cultured samples, SOCS1 box was most expressed in nuclear sediment rather than cytoplasmic supernatant, CfTX-1 peptide upregulated the SOCS1 expressions in wet cultured cardiac muscle. The conclusion of this study was SOCS1 box was a stabilized inhibitory signaling proteins in dried cardiac muscle. Mechanical stretching enhanced its expression both in cytoplasma and nucleus. Plasmid transfection increased the expressions in nucleus rather than cytomplasma. CfTX-1 peptide upregulation in wet cultured samples can be observed. It suggests that SOCS1 box may be involved in the important regulatory process during the reconstruction of myocardial mechanical load.

P4-7

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

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

P4-8

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

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

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

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

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

P4-9

Multiple functional roles of HSPB7 in heart.

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HSPB7 is a member of small heat shock protein family and specifically express at striated muscles. It also is found as an interaction protein of filamin C. Previous studies showed both HSPB7 and FLNC are expressed dynamically in cardiomyocytes from the onset of cardiogenesis to the adult. Additionally, several single-nucleotide polymorphisms (SNPs) of HSPB7 and mutations of FLNC have been identified to be associated with heart failure caused by cardiomyopathy in human patients, respectively. Using gene targeting approach, we found that conventional loss of Hspb7 causes early embryonic lethality with the fragmented myofibrils, especially the weakened Z-line structure and hexagonal thin filament array by the defects of cardiac actin and a-actinin disarrangements during cardiac myofibrillogenesis in mouse. Interestingly, the ablation of FLNC in Hspb7 KO embryo can extend its life to term and resolved the disarrangement of a–actinin but not actin, suggesting HSPB7 secures the function of FLNC at early heart development. Alternatively, the conditional loss of Hspb7 in adult heart quickly results in the disruption of the intercalated disc structure, decreasing the expression of connexin 43 and mislocalization of N-cadherin and desmoplakin, and further inducing arrhythmic sudden death with the overexpression and aggregation of FLNC in cardiomyocytes. Our study characterized HSPB7 as an intercalated disc protein and suggest it has an essential role in maintaining intercalated disc integrity and conduction function in the adult heart. Put together, our findings identify HSPB7 as a safeguard of FLNC and suggest it plays a multiple function role in conducting cardiac sarcomere assembly and maintaining intercalated disc integrity for the conduction function in heart.

P4-10

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

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

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

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

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P4-11

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

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Myosin regulatory light chain (RLC) is a major regulatory subunit of the myosin molecule, the role of which has been well-characterized in non-striated muscle. However, in striated muscle, its purpose is less well defined, and it is only in the past decade that researchers have started unraveling the function of RLC and its phosphorylation in muscle contraction. Mutations in this protein result in ~ 2% of total familial cardiomyopathy, an autosomal dominant disorder, manifested by ventricular and septal hypertrophy and myofibrillar disarray that can lead to sudden cardiac death. In this study, we report the role of human cardiac RLC in forming the super-relaxed state (SRX) of myosin in reconstituted full-length cardiac myosin thick filaments. We show that the presence of RLC fine-tunes the ability of myosin to form the SRX state and that its removal depopulates the SRX state. A similar reduction in SRX population is also achieved by phosphorylating the RLC with MLCK. The second mechanism of RLC-mediated regulation in muscle is thought to be by the binding of either Mg^{2+} or Ca^{2+} to the N-terminal EF-hand domain. Mg^{2+} did not affect myosin SRX population, but increasing Ca^{2+} enhanced the population of the myosin SRX state. Preliminary SRX studies on cardiomyopathy-causing RLC mutants R58Q (HCM), K104E (HCM) and D94A (DCM) show that none of the mutants affects the SRX population in the dephosphorylated state of the RLC; however, these mutants also do not affect the SRX population when RLC is phosphorylated, unlike the wild-type. Additionally, in micro-scale thermophoresis binding experiments, only the dephosphorylated form of the DCM-causing D94A RLC mutant exhibits weaker binding to the myosin lever arm as compared to WT RLC. Binding of the mutants resembles WT upon phosphorylation. Altogether, these observations demonstrate that either RLC phosphorylation or Ca^{2+} binding to RLC can alter the number of accessible myosin heads for contraction and cardiomyopathy-causing RLC mutants can modify this mechanism to varying extents.

P4-12

Contractility of ventricular myofibrils from patients with dilated cardiomyopathy associated mutations

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

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P4-13

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

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Background

Heart Failure with preserved Ejection Fraction (pEF) is poorly understood and predominantly present in women. Inflammation and oxidative stress levels may differ between genders resulting in distinct signalling alteration.

Objective

Here we studied how oxidative stress/inflammation affects the pEF pathophysiology by modulation of LV stiffness in distinct manners-based gender.Methods: We subdivided patients to four groups; men/women, with more (pEF+) or less (pEF-) inflammation.

Results

pEF+ women showed higher cardiomyocyte Fpassive, which was accompanied by lowest sGC, PKG activity, and global Titin hypophosphorylation compared to pEF- women and pEF+/- men. Myofilament Ca^{2+}-sensitivity and force generation capacity were lower in pEF+ compared to pEF- women and pEF+/- men, along with decreased phosphorylation of MyBP-C, Tnl and MLC2. Morphological changes were observed, pEF+ woman had higher collagen volume fraction and increased myocyte diameter.

Conclusion

Because of more inflammation and oxidative stress, women showed sever signalling pathway alterations and diastolic dysfunction. Understanding gender differences in pEF may help developing novel treatment options.

P4-14

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

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

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

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

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

P4-15

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

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Introduction

The treatment with tyrosine kinase inhibitors (TKI) shows an increase in progression-free survival in cancer. However, TKIs are susceptible to develop cardiac toxicity in patients.

Objective

The aim of our study was to investigate the mechanisms of cardiotoxicity for imatinib, sorafenib and sunitinib in cardiac H9c2 cells and for sunitinib in mice.

Methods

For the in vitro experiments, we exposed H9c2 cells for 24h with increased concentrations of TKI (from 1 to 100 μM). We also treated mice with sunitinib for two weeks at 7,5 mg/kg/d.

Results

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

Conclusion

Mitochondrial dysfunction may represent a toxicological mechanism of cardiotoxicity associated with sunitinib.

P4-16

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

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Background

Truncation mutations in MYBPC3 are a common cause of hypertrophic cardiomyopathy (HCM) leading to reduced cMyBP-C in cardiac sarcomeres, but functional effects of reduced cMyBP-C are not well understood.

Objective

We sought to determine acute effects of reduced cMyBP-C in permeabilized myocytes.

Method

We used a novel "cut and paste" approach to reduce and replace cMyBP-C N'-terminal domains (C0-C7) in myocytes from "Spy-C" mice that express a TEV protease site and a "SpyTag" embedded between domains C7 and C8. TEV protease was used to "cut" cMyBP-C and then recombinant C0-C7 was "pasted" using the split protein "SpyCatcher".

Results

Similar to genetic cMyBP-C knockout, acute loss of cMyBP-C increased apparent cross-bridge cycling rates (ktr). However, loss of cMyBP-C also dramatically increased the appearance of spontaneous auto-oscillatory contractions (SPOC) that were sustained during Ca^{2+} activation and eliminated by add back of C0-C7.

Conclusions

Results suggest a novel role for cMyBP-C to modulate oscillatory waves of contraction across sarcomere ensembles and provide new insights into mechanisms by which reduced cMyBP-C causes cardiac dysregulation.

Poster Session 5. Molecular Motors

P5-1

Autoregulatory functions of myosin 16 domains

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

Based on spectroscopic experiments we found, that Myo16Ank is able to bind to the motor domain and increase its actin-activated ATPase activity. It can also enhance the motility speed of actin filaments duing the in vitro motility assay. The prolin-rich Myo16Tail is supposed to bind profilin, but we found no direct interaction between them using anisotropy measurements. Meanwhile, we also tested the interaction between Myo16Tail and Myo16Ank domains, which showed a moderate affinity (KD ~ 2,7 μM).

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

P5-2

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

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

P5-3

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

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Background

In myosin essential light chain (ELC), there are two residues, Thr65 and Ser193 that can be phosphorylated. It is however unclear what functional role of the ELC phosphorylation is in skeletal muscle.

The aim was to study effects of the ELC phosphorylation on the interaction of skeletal myosin with actin.

Material and methods

Three recombinant human myosin ELC constructs with mutations T65D, S193D, and T65D/S193D, imitating natural ELC phosphorylation were produced, and the native ELC in rabbit skeletal myosin were replaced with these mutants. We measured the sliding velocity of F-actin or regulated thin filaments over myosin with all these ELC constructs and its pCa50 in an in vitro motility assay and an average step size of myosin in an optical trap.

Results

The T65D mutant significantly decreased the sliding velocity of thin filaments and its pCa50 as compared with non-mutated ELC construct. However, with the mutants S194D and T65D/S193D it was the same as that with the recombinant native ELC. None of the ELC mutants changed the myosin step size significantly.

We conclude that the phosphorylation of Thr65 and Ser193 of ELC renders differently directed effects on the actin-myosin interaction in skeletal muscle: the T65D mutation inhibits the interaction and the S193D mutation eliminates this inhibition.

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P5-4

Controlled surface silanization for actin-myosin based nanodevices

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The surface hydrophobicity is critical for fine tuning heavy meromyosin (HMM) driven actin filament sliding velocity in nanodevices based on the in vitro motility assay. The suggested mechanism of HMM binding is via the motor domains to surfaces of low contact angle and via the tail domain to surfaces of high contact angle. Here, we tested these ideas further by varying the surface hydrophobicity of trimethylchlorosilane (TMCS) derivatised SiO2, deposited by chemical vapour deposition. A wider range of contact angles (10-85°) than in previous studies was obtained by varying the deposition chamber pressure and silanization duration.

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

Poster Session 6. Smooth Muscle Contraction and Pathology

P6-1

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

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

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

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

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

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

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P6-2

The 'catch' smooth muscle contains small fusiform cells: stem cells, sensors or else?

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Introduction

Small fusiform eosinophilic and osmiophilic bodies in the anterior byssus retractor muscle of Mytilus have not been studied.

Objective

Assess by ultrastructure these peculiar bodies.

Method

3-8 and 30 mm long muscles originated from commercial packings and Whitley Bay shoreline rocks were excised and processed after in situ fixation with buffered sea water glutaraldehyde solution.

Results

These fusiform bodies (2 µm diam- 150 µm long) have an eccentric nucleus. They appear either filled by ribosomes and haphazardly-organized thick filaments or, as in large ABRMs, as tiny spindle-shaped structures with an outermost region of typical contractile machinery, endoplasmic reticulum and mitochondria as well as gap junctions and desmosomes with adjacent myofibers. High magnification shows these bodies constitute by numerous 5-6 nm filaments gently twisted all along the peculiar structure. In many places, coiled-coil filaments seem to progress into building thick filaments.

Conclusion

These preliminary data indicate these bodies to either represent sort of stem cells distributed throughout the muscles that could provide an understanding about paramyosin formation or do these cells sorts of interoceptor cells [1]? These peculiar structures in smooth muscles should be of interest for further investigations.

[1] Gilloteaux J. Naturwissenschaften, 1971; 58: 271-272.

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Poster Session 7. Thin Filament and Actin-Binding Proteins

P7-1

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

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The tropomyosin (Tpm) isoforms α (Tpm 1.1) and γ (Tpm 3.12) are expressed in fast and slow human skeletal muscles, respectively, while β-Tpm (Tpm 2.2) is expressed in both fast and slow muscles. This results in formation of αα- and γγ-homodimers (ββ-homodimers are unstable and occur rarely) of dimeric Tpm molecules as well as αβ- and γβ-heterodimers. The properties of αα-homodimer are well studied, whereas nothing is known about the properties of γγ-homodimer and γβ-heterodimer. Using differential scanning calorimetry, we showed that the thermal stability of γγ-homodimer is much higher than that of αα-homodimer, and ββ-homodimer is the least stable Tpm. The stability of γβ-heterodimer is much lower than that of γγ-homodimer, and the thermal unfolding of αβ-heterodimer is similar to that of αα-homodimer. Sliding velocity of regulated thin filaments containing either Tpm γγ-homodimers or γβ-heterodimers moving over fast or slow skeletal myosin measured in an in vitro motility assay was significantly less than that of the filaments with αα- or ββ-homodimers, or αβ-heterodimers. Both γγ- and γβ-Tpm dimers significantly decreased the calcium sensitivity of the sliding velocity over fast myosin but increased it over slow myosin. We conclude that the Tpm γ-chain is one of essential factors that determine the properties of slow muscles.

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P7-2

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

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

P7-3

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

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

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

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

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

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

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P7-4

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

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

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

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

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P7-5

How actin-binding and α-actinin-binding of Zasp52 contribute to myofibril assembly in Drosophila

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In sarcomeres, α-actinin crosslinks thin filaments and anchors them at the Z-disc. Drosophila Zasp52, a PDZ and LIM domain-containing protein of the Alp/Enigma family, also localizes at Z-discs, and interacts with α-actinin via its extended PDZ domain, thereby contributing to myofibril assembly and maintenance. Nevertheless, the detailed mechanism of Zasp52 function is unknown. We observed a strong genetic interaction between actin and Zasp52 during indirect flight muscle assembly, indicating that an interaction of Zasp52 with actin also plays a critical role during myofibril assembly. Here we show by in vitro biochemistry that Zasp52 contains an actin-binding site, which includes the extended PDZ domain and the ZM region. Furthermore, site-directed mutagenesis identifies amino acids uniquely required for α-actinin versus actin binding. In vivo rescue assays of indirect flight muscle structure demonstrate the respective contributions of actin and α-actinin binding.

P7-6

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

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

P7-7

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

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Introduction

Leiomodin-3 (LMOD3), encoded by LMOD3, is a member of the tropomodulin protein family localising predominantly near striated muscle thin filament pointed ends. LMOD3 mutations cause a severe myopathy. The severity of disease and the presence of abnormal thin filaments in patient muscle suggest that LMOD3 is critical for muscle function likely by helping to form and maintain thin filaments.

Objective

To date, little is known about LMOD3s function and the cause of weakness in LMOD3 patients. We aim to determine whether LMOD3 is directly involved in skeletal muscle contraction as well as thin filament stability.

Method

We tested the structural integrity and contractile function of intact soleus muscle and permeabilised single myofibers of Lmod3 knock-out (KO) mice.

Results

Eccentric "damaging" contractions resulted in less force loss in KO mice, suggesting muscle integrity is not affected by LMOD3 loss. Permeabilised single myofibers showed a decrease in specific force in both fiber types. Interestingly, cross bridge cycling kinetics and active stiffness was decreased specifically in fast myofibres.

Conclusion

Our research suggests LMOD3 is directly involved in skeletal muscle contraction in fast myofibers, perhaps by regulating myosin-actin interactions. This represents a novel role for LMOD proteins and likely contributes to weakness in LMOD3 patients.

P7-8

The ATP hydrolysis mechanism of fibrous actin

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Introduction

The ATP hydrolysis of actin, which occurs only at the fibrous (F) form, is known to drive the unidirectional polymerization of the filaments. The molecular mechanism of actin ATPase was largely unknown due to the lack of the high resolution structure of F-form actin.

Purpose

To elucidate the detailed ATPase reaction mechanism of F-actin, a theoretical investigation was performed using the atomic resolution (1.2Å) X-ray crystal structures of the F-form actin with various nucleotide states we have recently determined.

Method

We employed ONIOM(DFT:MM) calculation with electronic embedding.

Results

Our analyses revealed that the ATP hydrolysis of actin proceeds via a dissociative reaction pathway, which resembled that of myosin ATPase (F. A. Kiani and S. Fischer, 2014). The attack of lytic water was concerted with the double proton transfer among two waters and the metaphosphate. In the final product, a strong H-bond was found between ADP and Pi, which may contribute to the stability of the ADP/Pi state.

Conclusion

We achieved a full exploration of actin ATPase reaction, which reasonably explained the characteristic irreversibility of the reaction.

P7-9

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

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

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P7-10

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

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

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

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

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

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

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P7-11

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

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

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

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

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P7-12

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

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

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P7-13

Actin filament multiplication for biocomputation

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

P7-14

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

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Introduction

High frequency contractions of insect flight muscle (IFM) power wing beats. Opposing muscles act reciprocally to produce resonant distortions of the thorax and rapid stretches activate the IFM, suggesting there is a length sensor. Bridges between troponin on thin filaments and the thick filaments are candidates.

Objective

The aim was to identify components of troponin bridges, to measure the binding of the tropomyosin-troponin complex (Tm-Tn) to thick filaments and the affinity of an association with myosin.

Methods

Tm-Tn and tropomyosin isoforms Tm1 and Tm2 were isolated from Lethocerus IFM or expressed in E. coli. Association with thick filaments and myosin was determined by pulldown experiments, overlay blotting and electron microscopy (EM). The affinity of Tm-Tn and Tms for myosin-S1 (S1) was measured by MST and ITC.

Results

Tm-Tn bound to thick filaments and myosin. Unexpectedly, Tm1 bound to S1 with high affinity (Kd ~65 nM) but Tm2 did not bind. EMs of Tm1-Tn associated with thick filaments showed binding with a periodicity of ~40 nm. Tm1 crosslinked thick filaments in a ladder-like structure with rungs ~40 nm apart.

Conclusion

Tn bridges are midway between the target sites on actin that bind force-producing crossbridges (39 nm apart). We suggest non-force producing bridges bind to Tm1 at the position of Tn, and Tm1 is directly pulled from an inhibitory position on actin.

P7-15

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

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Background

The mechanisms that modulate the kinetics of muscle relaxation are understudied despite their importance for muscle contractility. A prime example of the impact of impaired relaxation kinetics is nemaline myopathy caused by mutations in KBTBD13 (NEM6): in addition to weakness, patients have impaired muscle relaxation, compromising contractility and daily-life activities. The role of KBTBD13 in muscle is unknown, let alone its role in NEM6 pathology.

Methods & Results

With the use of transcranial magnetic stimulation, we established that the origin of impaired muscle relaxation kinetics in NEM6 myopathy is myogenic. The pathomechanism underlying these impaired muscle relaxation kinetics was studied using contractility assays in permeabilized muscle fibers and myofibrils isolated from NEM6 patient biopsies. We discovered that impaired muscle relaxation is sarcomere-based. By applying a combination of low-angle x-ray diffraction, super-resolution microscopy, modeling of muscle kinetics, binding- and contractility assays with bacterially-expressed KBTBD13, novel Kbtbd13-deficient and Kbtbd13R408C-knockin mouse models and a transgenic zebrafish model we showed that KBTBD13 - the protein implicated in NEM6 - is an actin binding protein.

Interpretation

Mutations in KBTBD13 slow relaxation kinetics of muscle through direct, structural effects on the actin-based thin filament. We propose that this pathomechanism is central to NEM6 pathology.

P7-16

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

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

P7-17

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

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

P7-18

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

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Background

Nebulin spans the length of the thin filament, with its C-terminus located in the z-disc and its N-terminus near the thin filament pointed-end. Mutations in nebulin, as well as in proteins that bind to nebulin cause myopathy. The pathophysiological mechanisms are incompletely understood, in part because of a lack of tools to precisely locate nebulin on the thin filament.

Objective

Develop a tool for the localization of individual nebulin molecules.

Methods

We generated a mouse in which dendra2, a photoconvertible protein, is incorporated by gene targeting into nebulin's N-terminus. Individual nebulin molecules were visualized by photoactivated localization microscopy (PALM). In PALM, dendra2 switches between fluorescent and dark states so that in every snapshot, only a small, optically resolvable fraction of dendra2 is detected.

Results

Nebulin-dendra2 mice have normal muscle weights. Western blot showed the presence of full length nebulin. IEM showed that incorporation of dendra2 does not affect nebulin's N-terminus position in sarcomeres. PALM localized individual nebulin N-termini with a resolution of 25 nm. Within a myofibril, individual nebulin N-termini show a gaussian distribution with a width at half-maximum of 90 nm.

Conclusion

These pilot results indicate the successful generation of a tool to localize individual nebulin molecules.

Poster Session 8. Motor Protein Pharmacology

P8-1

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

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

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

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

P8-2

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

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

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

We found that OM reduced in a dose-dependent manner the sliding velocity of F-actin and thin filaments reconstructed from F-actin, troponin, and tropomyosin at pCa 4. In concentration 0.1 μM, it increased the force of both ventricular and atrial myosin at maximal calcium concentration. Thus, using myosin isolated from atria and ventricle, we revealed that OM affects the force-generating ability of myosin.

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P8-3

Modulation of cardiac myosin dynamics by Omecamtiv Mecarbil

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

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

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

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

P8-4

Mavacamten Stabilizes the Super-Relaxed State of β-Cardiac Myosin: Deciphering the Mode of Action from Myosin Molecules to Muscle Fibers

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

P8-5

EPA/DHA potentiates muscle autophagy and UPS during glucocorticoid atrophy process

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Muscle atrophy occurs in many conditions including the glucocorticoids use, that acts mainly by autophagic and ubiquitin-proteasome systems. N-3 (omega-3) is world-wide consumed due its healthy properties, however, the concomitant use with glucocorticoids can increased its side-effects. Here we evaluated the N-3 influences on glucocorticoid atrophy considering IGF-1, Myostatin, ERk, AMPK pathways and UPS, autophagic/lisossomal systems. Sixty animals formed 6 groups: CT, N-3 (EPA 100mg/kg/dia for 40 days), DEXA 1.25 (vehicle for 40 days + DEXA 1.25mg/kg/day for the last 10 days), DEXA 1.25+N3 (EPA for 40 days + DEXA 1.25mg/kg/day for last 10 days), DEXA 2.5 (vehicle for 40 days + DEXA 2.5mg/kg/day for the last 10 days), DEXA 2.5+N3 (EPA for 40 days + DEXA 2.5mg/kg/day for last 10 days).

Results

N-3 associated with DEXA increases atrophy (fibers 1 and 2A), FOXO3a total, P-SMAD2/3, Atrogin-1/MAFbx (mRNA) expressions and all autophagic protein markers (P-62, LC3II, ratio LC3II/LC3I, LAMP-1 and acid phosphatase). Additionally, led to a decreased P-FOXO3a, PGC1-alpha and IRS-1 mRNA expressions.

Conclusion

N-3 supplementation can enhance muscle atrophy by DEXA in an autophagic process.

P8-6

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

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Nemaline myopathy (NM) results in muscle weakness and a poor quality of life. Here, we evaluated the acute effect of tirasemtiv – a fast skeletal muscle troponin activator - on in vitro extensor digitorum longus (EDL) muscle mechanics in the skeletal muscle α-actin-based NM mouse model (Tg.ACTA1D286G, n=14) and wildtype mice (Wt, n=14).

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

Tg.ACTA1D286G mice had lower EDL muscle weights (Tg.ACTA1D286G: 9±0.3 mg vs. Wt: 12±0.4 mg, P<0.001) and muscle fiber CSA (Tg.ACTA1D286G: 1251±45 μm2 vs. Wt: 1650±116 μm2, P=0.03). Absolute force production was lower in Tg.ACTA1D286G mice. Administration of tirasemtiv (3 μM) restored absolute force levels at submaximal stimulation to those of Wt mice (20 Hz: Tg.ACTA1D286G-vehicle: 25±3 mN, Wt-vehicle: 35±3 mN (P=0.03), Tg.ACTA1D286G-tirasemtiv: 42±5 mN; 40 Hz: Tg.ACTA1D286G-vehicle: 40±5 mN, Wt-vehicle: 64±8 mN (P=0.02), Tg.ACTA1D286G-tirasemtiv: 76±10 mN).

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

P8-7

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

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

Poster Session 9. Muscle Energetics

P9-1

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

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Introduction

Malignant hyperthermia susceptibility (MHS) leads to lethal episodes under exposure to anesthetics, heat and strenuous exercise. The hyperthermic crises involves abnormal Ca^{2+} leak from SR and heat production. We recently discovered that Calsequestrin-1 knockout (CASQ1-null) mice develop a phenotype similar to MHS.

Objective

Demonstrate an association between heat generation and increased basal oxygen consumption in CASQ1-null mice.

Methods and Results

In comparison with age-matched controls, 2 months old CASQ1-null mice showed increased food consumption (29.7%) and higher basal core temperature (mean of 0.7ºC), although no differences were found in body weight. These evidences of increased metabolism were supported by: a) enhanced basal oxygen consumption during the awake period; b) elevated levels of SERCA protein and activity; and c) increased mitochondrial volume and percentage of damaged mitochondria.

Conclusion

MHS in CASQ1-null mice is associated to increased oxygen consumption and basal metabolism, possibly as a result of increased ATP demand and mitochondrial activity to support the enhanced SERCA activity, needed to reuptake the excess of cytosolic Ca^{2+}.

P9-2

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

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During long-distance races, an abrupt increase in acceleration results in a sudden energy requirement. This study investigated whether prior knowledge of increase of acceleration affected oxygen uptake (VO2) and tissue oxygenation during running.

Subjects were divided into two groups: those who had prior knowledge of acceleration increase and those who did not. They performed a velocity-incremental maximal test and two 10-min transient submaximal running tests. Submaximal tests were performed pre-acceleration (4 min) and post-acceleration (6 min). Pre-acceleration velocities corresponded with the ventilation threshold (VT); post-acceleration velocities corresponded with ⊿40% (VT + (VO2peak – VT) × 0.4).

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

There was no significant difference in variables of VO2 kinetic response between both groups. NIRS-derived deoxygenated hemoglobin was similar for both groups.

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

P9-3

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

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Background

In the mdx mouse, dystrophin deficiency leads to muscle degeneration. Corticoids and omega-3 alleviate dystrophy progression. In the young mdx mice, treadmill exercise can worsen dystrophy. Objectives: To verify the effects of treadmill exercise in the old mdx (12 months of age) and the effects of exercise associated to deflazacort (DFZ) alone or combined to omega-3.

Methods

Exercised (exe)-mdx run on a horizontal treadmill (12.4 mts/min, 15 min), 2 times/week, from 13 to 14 months of age. Exe-mdx were treated from 12 to 14 months of age with DFZ alone or combined to omega-3. Controls sedentary (sed) mdx, sed-C57BL/10 and some exe-mdx were not treated. Respiratory performance was quantified during exercise.

Results

Exercise alone or associated to therapy improved maximal oxygen consumption (VO2max: sed-mdx: 22.6 ml/kg/hr; exe-mdx: 66.2*; exe-DFZ/O3: 63.3*; C57BL/10: 61.6*; *p<0.05 compared to sed), total energy expenditure and the time to exhaustion (sed-mdx: 8 min; exe-DFZ: 14 min). Sed-mdx showed reduced performance in functional evaluations (grip strength, open field, rotarod). Exe+therapy improved functional performance (grip strength sed-mdx: 0.7 ± 0.1 gr/gr; exe-mdx: 0.9 ± 0.1*; exe-DFZ: 1.6 ± 0.1*; *p<0.05 compared to sed). Exercise apparently improved diaphragm histology and decreased creatine kinase.

Conclusion

Treadmill exercise alone or associated to corticoid/omega-3 may be beneficial to improve muscle function in aged mdx mouse.

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P9-4

Dynamic changes of energy metabolism in rat heart muscle cells

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Recent research has proved that chronic stress has a powerful impact on biochemical properties of human body. Literature data show that stress disrupts functional state of the cell and leads to or significantly intensifies various diseases, such as cardiovascular, immunity and neuron-degenerative pathologies, as well as tumor, etc.

Impact of changes caused by combined stressed model (social isolation and disrupted diurnal cycle) on the metabolism of myocardium cells has been scarcely researched. Therefore, we aimed at studying the biochemical changes leading to formation of various pathologies in the cardio-vascular system. It has been found that stress leads to a changed metabolism of the human body, portrayed by an intensified lipid peroxidation process, inhibited anti-oxidant system and lower energy metabolism. This results in an overall decline of the functional state of the organism. Under the described conditions, injection of certain nutrients positively affect the changed energy metabolism. Also, activities of antioxidant enzymes, superoxide dismutase, and catalase were diminished, indicating deterioration of the antioxidant system. In addition, there were decreased activities of mitochondrial enzymes participating in energy metabolism, indicating decreased energy levels in heart muscle cells. These results suggest the likelihood that emotional stress is a key factor that can cause a whole range of diseases of the cardiovascular system.

P9-5

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

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It is acknowledged that metabolic rate increases with decreasing elastic energy during uphill slope exercise at a constant running velocity. The purpose of this study was to investigate contribution of muscle activity at each site of the exercising muscles to increased metabolic rate during a gradient-incremental running (GIR) test. Eight middle-aged men (60±6.3 yrs) run on a treadmill the GIR test which was composed of increasing 2% gradient per 2 min from 0 to 10% at an individually chosen comfortable velocity. Oxygen uptake (VO2) and electromyogram (EMG) signals on seven sites from superficial leg and buttock muscles were measured during the GIR test. VO2 was found to linearly increase as a function of gradient. Standardized integrals of EMG data (%iEMG) of all the targeted muscles were remained a constant level until 4% gradient, and after that %iEMGs of major gluteus, vastus lateralis, biceps femoris and soleus muscles were started to increase with the elevation of gradient, while %iEMGs of the remaining three muscles did not change across over the examined gradients. The activity levels of the four muscles were considered to mainly contribute to the increased metabolic rate at higher (6%~) gradients during the GIR test. In addition, this finding suggested that activity levels of the unmeasured muscles would account partially for the increased metabolic rate at lower (~4%) gradients.

Poster Session 10. Contraction Regulation, EC Coupling

P10-1

Cardioprotective Regimen of Intermittent Hypobaric Hypoxia Affects Phosphorylated Status of Connexin 43 and Expression of Its Upstream Kinases in The Rat Heart

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

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

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

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

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

P10-2

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

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

We designed a new 3D compartmental model of the half sarcomere, which accounts for diffusion of Ca^{2+} into the three compartments and simulates its dynamics at rest and at various rates of stimulation in mice skeletal muscle fibers.

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

P10-3

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

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

P10-4

High intensity interval training (HIIT)-induced Ca^{2+} leak through RyR1 channel is involved in mitochondrial plasticity in skeletal muscle

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We recently found that, in recreationally trained volunteers, a single session of HIIT, High intensity interval training, (6 × 30s all-out cycling) induced fragmentation of the sarcoplasmic reticulum Ca^{2+} release channel (RyR1, ryanodine receptor type 1).

In this study, we tested the hypothesis that Ca^{2+} leak through modified RyR1 (the Ca^{2+} release channel) in response to high intensity interval training (HIIT) exercise may trigger mitochondrial plasticity.

We mimicked HIIT and endurance exercises in C2C12 myotubes using the C-Pace electrical device and observed RyR1 phosphorylation and release from calstabin (the small molecule stabilizing RyR1) and a lower sarcoplasmic reticulum Ca^{2+} content in response to HIIT, indicating that HIIT induced leaky RyR1. This was accompanied by greater mitochondrial biogenesis (NRF1, Tfam1, PGC1 expression), dynamics (OPA1 and DRP1 modifications) and respiration (O2 consumption in O2k Oroboros device). We confirmed these results on muscle biopsies from healthy humans submitted to HIIT exercise, as compared to a classical endurance exercise.

Taken together, our results provide, for the first time, into insight of the mechanisms by which HIIT exercise is so efficient.

P10-5

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

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

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

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P10-6

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

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The X-ray reflections signalling the state of the thick filament in an electrically paced trabecula dissected from the ventricle of the rat heart (0.5 Hz, SL 2.15 µm, 27 °C), indicate that mechano-sensing in thick filament regulation operates also in cardiac muscle, as in skeletal muscle. During diastole most of the myosin motors lie on the surface of the thick filament packed into helical tracks in the OFF state in which they are unavailable for actin binding and ATP hydrolysis. During contraction, the myosin motors leave the OFF state in relation to the loading condition (Reconditi et al. PNAS 114, 3240–3245, 2017). Considering that in a heartbeat Ca^{2+} dependent thin filament activation is submaximal, we investigated the interdependency of the two regulatory mechanisms in electrically paced trabeculae, by recording the X-ray signals during two inotropic interventions that double the twitch force at SL 2.0 µm and external [Ca^{2+}] 1mM: either SL increase to 2.25 µm or addition to the solution of the β-adrenergic effector isoprenaline (10-7 M). In diastole none of the X-ray signals was affected by either intervention, indicating that thin-filament and thick-filament activation act independently and further supporting the idea of the thick-filament based regulation as an energetically well-suited downstream mechanism.

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P10-7

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

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Introduction

In vertebrates small transmembrane proteins, such as FXYDs (FXYD1-12), which regulate Na^+-K^+-ATPase, and phospholamban, sarcolipin, myoregulin and DWORF, which regulate sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), are fundamental to ion homeostasis in skeletal muscle and other tissues. Uncertain evolutionary history of FXYDs and regulators of SERCA led to inconsistencies in their classification across vertebrate species, thus hampering comparative studies of their functions.

Objective

To elucidate evolutionary origins and phylogenetic relationships of FXYDs and regulators of SERCA.

Methods and Results

We discovered the first FXYD homologue in sea lamprey, a basal jawless vertebrate, suggesting that FXYDs predate the emergence of fishes and other jawed vertebrates. We also found that FXYDs diversified more extensively than SERCA regulators, indicating they operate under different evolutionary constraints. Furthermore, using a combination of sequence-based phylogenetic analysis and conservation of local chromosome context, we identified 13 gene subfamilies of FXYDs and propose a revised, phylogeny-based, FXYD classification that is consistent across vertebrate species.

Conclusions

Our findings provide an improved framework for investigating functions of small transmembrane regulators of ion transport in health and disease.

P10-8

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

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

Poster Session 11. Integrative Muscle Biology

P11-1

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

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

P11-2

Protective Effects of Thai Pomegranate Juice on Oxidative Stress Induced by Ischemia-Reperfusion in Rat Skeletal Muscle.

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This study aimed to investigate the protective effects of Thai pomegranate juice (TPJ) on oxidative stress and morphological changes of rat skeletal muscle cell induced by ischemia-reperfusion (I/R). TPJ contained high total phenolic content and exhibited high antioxidant activities. Intragastric injection of low, middle, and high doses of TPJ 1h before reperfusion had an effect on antioxidant activities in rat skeletal muscle by measuring the levels of enzyme antioxidants (superoxide dismutase (SOD) and catalase), non-enzyme antioxidants (glutathione), and the marker of lipid peroxidation (malondialdehyde, MDA) in gastrocnemius muscle induced by ischemia-reperfusion (4h ischemia/2h reperfusion). The activities of SOD and catalase, and the levels of glutathione in skeletal muscle of TPJ-treated groups were significantly higher than control group. MDA levels in skeletal muscle TPJ-treated groups were significantly lower than control group. Muscle cell damage by I/R in TPJ treatment was significantly lower than control. Thus, TPJ may have protective effects against skeletal muscle tissue injury caused by I/R that are probably through antioxidant defense system against radical damage.

P11-3

Wnt7a protects skeletal muscle from cancer cachexia

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Cancer cachexia is a syndrome characterized by extensive wasting of skeletal muscle, which leads to a rapid loss of muscle strength and endurance. The reason for this muscular atrophy is an accelerated degradation of proteins via the upregulation of the ubiquitin-proteasome pathway. Concomitantly, the protein synthesis is diminished. Under normal conditions Wnt7a leads to hypertrophy of muscle fibers through the activation of the AKT/mTOR pathway.

Here, we show that the extracellular ligand Wnt7a can rescue cancer cachexia in a tissue culture model as well as in a mouse model of cancer cachexia. Addition of Wnt7a recombinant protein reverts the atrophy of primary murine or human myotubes caused by factors secreted by the tumor cells. Wnt7a also leads to an activation of the AKT/mTOR pathway in myotubes under cachectic conditions. Furthermore Wnt7a improves muscle stem cell function by overcoming the differentiation block caused by cancer cachexia. Finally we showed the rescue of skeletal muscle wasting by Wnt7a application in a cancer cachexia mouse model. We suggest that Wnt7a is a putative therapeutic target for amelioration of muscle wasting in cancer cachexia.

P11-4

Molecular architecture of Muscle Z-disc assemblies: ZASP: α-actinin-2-FATZ-1 interactome

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MFPL, Vienna

ZASP is a Z-disc protein involved in the early stage of myofibrillogenesis and it is mainly expressed in heart and skeletal muscle. It is known that ZASP acts as a mediator between cytoskeletal elements and signaling cascades and its presence in the Z-disc is essential for integrity of the sarcomere during contraction. Among the Z-disc protein network, ZASP is capable to interact with various binding partners, 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. In this project, we assume that ZASP could play a regulatory role on association of ACTN2 with other Z-disc proteins, like FATZ, Titin and Actin. We show 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 and pull-down shows the presence of titin when the ternary complex is formed.

P11-5

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

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Introduction

High-intensity interval training (HIIT) can improve physical condition and sports performance, while also stimulates systemic and local responses associated with skeletal muscle growth.

Purpose

This study investigated physiological, anaerobic and aerobic, systemic, and molecular adaptations of HIIT training in flatwater kayak athletes.

Method

Six male kayak paddlers followed an 8-week HIIT training program using a kayak ergometer. A VO2max test and blood and deltoid muscle biopsy sampling were performed prior to and 48 hrs after the completion of the 8-week program. VO2max, PSVO2max, PSVT2, PE, HRpeak, and La+2peak were measured. RT-qPCR was used to assess the mRNA expression of IGF-1Ea, IGF-1Eb, IGF-1Ec, TGF-β and VEGF-a. Plasma levels of IGF-1 and growth hormone (GH) were assayed by ELISA.

Results

HIIT protocol resulted in a significant improvement of PSVT2 ($p<0.05$). VO2max, PSVO2max, PE, La+2peak and HRpeak exhibited a trend of increase, however without reaching statistical significance ($p>0.05$). Similarly, expression of IGF-1Ea, IGF-1Eb, IGF-1Ec, TGF-β and VEGF-a, and plasma levels of GH and IGF-1showed a non-significant increase ($p>0.05$), due to a large inter-individual variability.

Conclusion

Our findings suggest that HIIT training improves PSVT2 and probably other physiological and molecular factors related to aerobic and anaerobic capacity.

P11-6

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

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

The study was funded by Russian Science Foundation grant #18-15-00107.

P11-7

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

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

P11-8

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

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Introduction

Exercise affects the homeostatic mechanisms of the human body, depending on the type, duration, intensity and frequency of exercise. Nevertheless, it is a stress stimulus for the human organism.

Purpose

The aim of this study was to determine the effects of an acute bout of moderate aerobic exercise on the Hypothalamo-Pituitary-Adrenal (HPA) axis hormonal responses in healthy adult humans.

Methods

Twelve healthy male and female volunteers (age: 30.6±4.4yrs, body mass: 77.3±12.3 kg, height: 1.77±0.07 m) performed a single bout of a 30 min aerobic exercise at 70%VO2max on a treadmill, following standard diet. Blood samples were collected before (t0), at the end of the exercise bout (t30), and 30 min later (t60) and serum adrenocorticotropic hormone (ACTH), cortisol (COR), aldosterone (ALDO) and renin (REN) were measured.

Results

ACTH and COR decreased immediately after exercise reaching a significant decrease 30 min after the completion of exercise compared to pre-exercise levels ($p<0.01$). ALDO increased at the end of exercise and 30 min after its completion, however it failed to reach significance. Renin significantly increased immediately after exercise ($p<0.05$) and remained elevated 30 min after the end of exercise.

Conclusion

This exercise regimen had beneficial effects on the stress axis thus can be recommended and prescribed for healthy and diseased populations.

Poster Session 12. Muscle Development, Regeneration and Disease

P12-1

In vivo characterization of skeletal muscle function in the Tfam KO mouse model

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Introduction
The Tfam KO mouse model of human mitochondrial myopathies has mainly been studied in vitro and in vivo data have not been reported.

Objective
The aim of the study was to assess in vivo the skeletal muscle function in the Tfam KO mouse model.

Method
Force during a fatiguing protocol (80 contractions-40 Hz) was assessed in 13-17-weeks old Tfam KO mice and control littermates (WT). Magnetic resonance (MR) images were acquired to calculate the hindlimb muscles volume (mm3). High-energy phosphate metabolites and intracellular pH were investigated using 31P-MR spectroscopy.

Results
A 13% muscle volume reduction was measured in Tfam KO as compared to WT mice. Force produced during exercise was lower in Tfam KO (0.45±0.04 mN/mm^3) than in WT (0.68±0.03 mN/mm^3). Phosphocreatine (PCr) depletion was higher while muscle acidosis was lower during exercise in Tfam KO as compared to WT mice. The rate of PCr resynthesis after exercise was slower in Tfam KO in comparisons with WT.

Conclusion
Muscle weakness and mitochondrial dysfunction were observed in Tfam KO mice thereby indicating that this mouse model shows in vivo the main features of mitochondrial myopathies.

P12-2

Self-made electrical stimulator as a tool to govern muscle cell differentiation

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Background
Muscle atrophy is a group of disorders characterized by massive muscle wasting. It can be caused by genetic reasons, e.g. Duchene muscle dystrophy, by lack of physical activity. The healing of muscle wasting disorders is still unresolved question. Training display positive effect on pro-inflammatory cytokine, oxidative stress, myostatin expression, and the activity of the ubiquitin-proteasome system, all of them are associated with regulation of muscle wasting. While, effect of electrical pulse stimulation on muscle cells in vitro have not been well described yet. Data on the frequency, duration and strength of stimulation vary depending on the used cell model used and evaluated parameters. There are no definite protocols of electrical stimulation, leading to the development fast or slow phenotype of muscle fiber in vitro.

Goal
The goal of the present project was to develop self-made device to stimulate muscle cells electrically and to develop protocol of stimulation to promote differentiation.

Methodology
The setup represents a six-well plate, where tantalum electrodes are embedded into the lid. Desired voltage, frequency, and duration of stimulation are given by microcontroller. Murine myoblast C2C12 line was used as the cell model. Stimulation protocol was applied at the frequency 2 Hz and voltage 5 V (4 msec pulse duration) for 15 min, followed by 45 min rest. Stimuli were delivered for 72 hours. To estimate the efficiency of muscle cells differentiation under electrical stimulation, qPCR was performed. Results. Myotubes were able to contract under stimulation with the given frequency. Sarcomere and Z-disc proteins expression was markedly increased in samples underwent electrical stimulation. Increase of desmin expression was 3-fold, alpha-actinin 3-fold, myh1 15-fold, and myh4 15-fold.

Conclusion
Electrical pulse stimulation using electrodes placed in the extracellular space is a simple method to control muscle cells activation and differentiation. The present project resulted in developed equipment to deliver electrical stimuli to muscle cells. Application of self-made setup gave an additional control of the experimental parameters. Applied protocol of electrical stimulation was shown to enhance myotubes maturation towards fast phenotype. Therefore, the developed equipment could be further exploited to establish the protocol to promote muscle cell activation and differentiation.

P12-3

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

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

P12-4

Exercise prevents formation of Tubular Aggregates in ageing skeletal muscle fibers

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Background

Tubular aggregates (TAs) are clusters of ordered SR tubes found in various muscle disorders including TA myopathy (TAM), a disease linked to STIM1 or Orai1 mutations, two main players in store-operated Ca^{2+} entry (SOCE). TAs are also found in EDL fibers of aging mice where they appear positive for both STIM1 and Orai1.

Objective

Verify the functional impairment of muscles containing TAs and determine if exercise prevent their formation.

Method

Using electron microscopy and a fatiguing protocol (30x1s 60Hz pulses every 5 seconds) we studied EDL muscles from adult (4m old) and aged mice, either control (24m old) or exercised in wheel cages for 15m (9-24m of age).

Results

i) 24m old muscles exhibit a faster decay of contractile force than 4m old; ii) this force-decay is likely caused by impaired Ca^{2+} entry (suggested by experiments in Ca^{2+}-free extracellular solution); iii) exercise reduce formation of TAs (found in 7 vs. 50% of fibers in trained vs. untrained), and rescued fatigue resistance during repetitive stimulation.

Conclusion

Exercise reduces formation of TAs and improves muscle function during repetitive stimulation, possibly improving SOCE function.

P12-5

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

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Background
It's still unclear of the specific functions of Gα12/13 signaling in quiescent satellite cell and how it's worked in muscle regeneration process.

Objective
This study focuses on deciphering the specific functions and mechanism of Gα12/13 signaling in skeletal muscle regeneration process especially the potential roles of Gα12/13 in satellite cell quiescent maintenance.

Methods
We use the G12/13-Pax7CreErt2 mouse strain to conditional inactivate Gα12/13 in satellite cells by tamoxifen injections. The muscle injury was induced by IM injection of 50 μl of cardiotoxin(CTX).

Results
Gα12/13 cKO mice show severe dysregeneration phenotype in CTX induced muscle regeneration model. After 10 times tamoxifen injection, many of the nuclei central localized myofibers were observed in the TA muscle of Gα12/13 cKO mice and further immunofluorescence staining results showing that compared with wild-type, the skeletal muscle of the Gα12/13 cKO mice have dramatically increased number of the Pax7, Myod or Pax7, Ki67 double positive cells.

Conclusion
Gα12/13 is crucial to maintaining quiescent state of satellite cells and also very important to skeletal muscle regeneration process.

P12-6

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

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

P12-7

Cardiomyoblast (H9c2) molecular responses during differentiation

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

Objective
This study aimed at revealing molecular responses of H9c2 myoblasts during their differentiation towards a cardiomyocyte phenotype.

Methods
H9c2 cells were switched to differentiation and harvested at their day 0, 3, and 5 of differentiation. RT-qPCR was used to assess the mRNA expression of Cyclin D1, cardiac troponin T (cTnT) and of myogenic regulatory factors (MRFs; MyoD, Myogenin, MRF4), growth (IGF-1 isoforms: IGF-1Ea, IGF-1Eb), pro-apoptotic (Foxo, Fuca) and atrophy (Atrogin 1, Myostatin) factors. Connexin 43 (Cx43), cardiac actin (cACT), phospho(P)-ERK1/2 and P-Akt were also evaluated by western blot.

Results
Compared to day 0, The expression of MRFs, IGF-1 isoforms, pro-apoptotic factors, myostatin and cTnT increased in differentiating H9c2 and a down-regulation of Cyclin D1 and Atrogin 1 was observed ($p<0.01$). P-ERK1/2 decreased while P-Akt increased ($p<0.05$). As expected, Cx43 initially increased and subsequently decreased, and cACT was progressively detected only in differentiated cells ($p<0.01$).

Conclusion
Our findings indicate that during the differentiation of mononucleated H9c2 cells into cardiomyotubes multiple myogenic, growth, atrophy and survival factors are modulated.

P12-8

A novel DNAJB6 mutation in distal myopathy with rimmed vacuoles

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Background
Distal myopathies are a heterogeneous group of muscle diseases inherited in an autosomal dominant (AD) or autosomal recessive manner.

Objective
To identify the genetic etiology and characterize the clinicopathologic features of a novel distal myopathy.

Methods
We performed whole-exome sequencing in 1 patient with distal myopathy and the other family members. We also evaluated the pathogenicity of identified mutations using immunofluorescence, quantitative real time PCR, western blot analysis, and functional analysis in vitro.

Results
Sequencing identified a likely pathogenic a homozygous mutation, c.695_699del (p.V232 Gfs*7) in DNAJB6 in the proband, additionally four family members are heterozygous mutation in this locus. The proband had late-onset distal lower extremity weakness, mixed features on EMG, and muscle pathology demonstrating rimmed vacuoles with both TAR DNA-binding protein 43 (TDP-43) and DNAJB6 inclusions. Quantitative real time PCR, immunofluorescence and western blot analysis of the proband's muscular biopsy showed the reduction of DNAJB6 expression and TDP-43 accumulation in aggregates in the affected skeletal muscle tissue. Furthermore, immunofluorescence analyses and filter trap assay demonstrated that the c.695_699del mutation had a decreased anti-aggregation function of DNAJB6 protein.

Conclusion
This study expands the molecular spectrum of DNAJB6 mutations and also emphasizes the pathogenic role of DNAJB6 dysfunction in distal-onset myopathy. The mechanism is likely to be the decreased anti-aggregation function.

P12-9

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

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

P12-10

Nod-like receptors in C2C12 myoblasts and myotubes during differentiation and regeneration

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Nucleotide oligomerization domain (NOD)-like receptors (NLRs) are intracellular pattern recognition receptors that play critical role in responses to stress and damage by initiating immune responses and regeneration of the injured tissue. It is known that myoinjury induces inflammatory responses that has a very profound role in both myogenesis and muscle regeneration.

We aimed to investigate the expression changes of NLRs in proliferating myoblasts and differentiated myotubes of murine C2C12 skeletal muscle cells under conditions mimicking injury and regeneration.

C2C12 myoblast and myotube cells were treated with IL-1β, IFNg, LPS or myostatin. Cells were collected to analyze NLR expression using RT-Q-PCR technique and signal transduction pathways by Western blot method, while supernatant was used to measure cytokine production by ELISA method.

Our results show that many of the NLRs are already expressed in these cells even without stimulation. Following stimulation, however, we observed time-dependent increase in the expression of some of the NLRs. Furthermore, interestingly, we also found NLRs that of expression was significantly reduced following cell activation.

Our data support the notion that similar to immune cells, NLRs of skeletal muscle should regulate and actively participate in immune responses in both muscle regeneration and degeneration.

P12-11

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

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Background

Alternative splicing contributes to the tissue-specific expression of the giant muscle protein titin (TTN), but there is only minor knowledge of how this tissue-specific splicing is regulated. Our goal is to identify factors involved in the splicing of Ttn exon49 solely expressed in cardiac but not in skeletal muscle cells. We hypothesize that differentially expressed RNA binding proteins regulate the tissue-specific splicing of Ttn.

Methods

The Ttn exon49 comprising pre-mRNA was attached to a streptomycin-binding aptamer, immobilized on a streptomycin affinity matrix, and incubated with either cardiac or skeletal muscle cell lysates. RNA-trapped proteins were identified by mass spectrometry and gene ontology analyses were performed.

Results and Conclusions

The titin pre-mRNA captured 134 proteins specifically from cardiac and 84 proteins specifically from skeletal muscle lysates. We show that RNA binding proteins are over-represented among these captured proteins and comprise several well-known splicing factors. Thus, our data provide a resource of candidates involved in the regulation of alternative tissue-specific Ttn splicing.

P12-12

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

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

P12-13

BCAA's may prevent muscle atrophy induced by immobilization. Influence of physical exercise

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Skeletal muscle atrophy by immobilization is a greater problem for athletes and general public to recover from, we are looking for a strategy that can prevent and/or treat this condition. The aim of our work was to study the response of the muscle to the stimulation of satellite cells (SC) by BCAAs (Branched Chain Amino Acids) plus glutamine.

Adult male Wistar rats were used. Group [A] four weeks of aerobic exercise; [B] right hindlimb has been immobilized for one week [C]; Exercise, followed by immobilization [D]; Group received BCAAs and then immobilized [E]. Group F was [E] + exercise for 4 weeks; immobilization plus four weeks of exercise [G].

At the end of experiments, gastrocnemius and soleus muscles from both hindlimbs were taken. Evaluation of the number and status of SC was made with antibodies against Pax-7, Myf-5 and c-met.

Exercise stimulated SC more intensively when it was performed after the immobilization. The supplement had a partial protective effect when it was taken during immobilization.

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P12-14

Paradoxical sleep deprivation induces differential biological response in rat masticatory muscles: inflammation and myogenesis

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The aim of this study was to evaluate if sleep deprivation (SD) induces inflammation and myogenesis in rat masticatory muscles: masseter and temporal. A total of 18 animals was distributed into three groups: Control group (CTL, n=6), subjected to SD for 96 hours (SD96, n=6) and subjected to SD for 96 hours more 96 hours of sleep recovery (SD96+R, n=6). In the group SD96 + R, no inflammatory process was evidenced to the masseter only. Upregulation of TNF-alpha production was detected in the SD96 group, while SD96 +R decreased TNF-immunoexpression for both skeletal muscles evaluated. MyoD and myogenin increased in rats submitted to SD96. Nevertheless, the levels of MyoD decreased immunoexpression. Myogenin pointed out high immunoexpresssion in SD96 + R groups. In summary, our results show that SD is able to induce inflammation and myogenesis in rat masticatory muscles, being dependent of skeletal muscle type and recovery process.

P12-15

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

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Background

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

Objectives

Goals were to characterize processes leading to simvastatin-induced myotoxicity in skeletal muscle cells and to investigate effects of insulin on the induced myopathy, on the Akt pathway and on the induction of insulin resistance.

Methods

Mouse C2C12 myotubes were treated separately or in co-treatment with simvastatin (10 microM) and insulin (10 to 100 ng/mL). Cytotoxicity assays, Western blots, PCRs and glucose uptake assays were performed after 24 hours exposure.

Results

Simvastatin induced toxicity at 10 microM. Insulin exposure prevented and rescued the cytotoxicity, reducing it by 50% with the highest insulin concentration. Simvastatin suppressed the phosphorylation of the insulin receptor, Akt and S6rp, while upregulating atrophy genes up to 2-fold and inducing ER stress. Furthermore, simvastatin induced insulin resistance by decreasing the glucose transport rate into myotubes by half. Co-treatment with insulin was able to prevent these adverse effects.

Conclusion

This study shows the importance of IR/Akt signaling in skeletal muscle and demonstrates the positive role of insulin in the prevention of myopathy and insulin resistance associated with simvastatin.

P12-16

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

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Introduction

Filamin C (FLNC) is expressed in striated muscles and localizes to Z-discs, myotendinous junctions (MTJ) and intercalated discs. Mutations in FLNC cause skeletal muscle and heart disease. The mutation p.W2710X deletes the carboxyterminal 16 amino acids from FLNC, leading to its inability to dimerize properly. Humans, heterozygous for this mutation develop myofibrillar myopathy (MFM) characterized by Z disc disintegration, myofibrillar lesions and sarcoplasmic protein aggregates. Muscle fibers of patient-mimicking knock-in mice (heterozygous p.W2711X mice) show increased myofibrillar instability.

Objective

To analyze skeletal muscle function and structure in mice homozygous for this mutation.

Results

Mice homozygous for the p.W2711X mutation are viable, fertile, and show no gross morphological abnormalities. Mutant FLNC RNA and protein is expressed at lower levels, when compared to wild-type mice. Mutant FLNC is localized in MTJ with only small quantities associated with Z-discs. Instead it is located in myofibrillar lesions that were also seen, albeit less frequently, in MFM patients and heterozygous mice. Ultrastructural studies confirmed that lesions are filamentous and structured, and not amorphous aggregates.

Conclusion

In sedentary, homozygous p.W2711X mice skeletal muscles are functional. Muscle fiber assembly and function do not depend on FLNC dimerization. The presence of multiple lesions, however, indicates a role for FLNC in Z-disc stabilization.

P12-17

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

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

The work is supported by Russian Science Foundation (№17-14-01224).

P12-18

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

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

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P12-19

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

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

P12-20

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

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

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

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

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

P12-21

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

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We previously showed that the de-differentiation of the occupied portion of muscle fibers toward Nurse cell after invasion by Trichinella spiralis is associated with increased intracellular accumulation of α-2,6-sialylated glycoproteins. The aim of this work was to analyze α-2,6-sialyltransferase expressions in mouse skeletal muscles invaded by T. spiralis. Muscle samples were collected at days 0, 10, 14, 18, 2 and 35 after invasion. Expressions of mRNA of α-2,6-sialyltransferases were analyzed by real time PCR. Immunochistochemistry was performed using rabbit polyclonal antibody against ST6GalNAc1 sialyltransferase. We found strong up-regulation of the enzyme ST6GalNac1 at day 14 after invasion that faded within the transformation of the occupied area into a Nurse cell. The enzyme ST-6GalNAc1 is not synthesized in healthy mouse muscle tissue and is rarely expressed in normal tissues. It is responsible for the formation of the cancer-associated sialyl-Tn antigen in variety of carcinomas, blocking regular carbohydrate chain elongation. The functional role of this enzyme for the Nurse cell formation of T. spiralis in muscles remain to be elucidated.

P12-22

The effect of hypothermia on muscle spasticity in a chronic spinal cord injury

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The hyperexcitability of spinal reflexes are one of the primary mechanisms of muscle spasticity after spinal cord injury (SCI). Due to the fact that the hypothermia can reduce cell mechanisms of hyperexcitability the aim of our research was to study the effect of local hypothermia on muscle spasticity after SCI.

The contusion SCI was made on the level of Th8-9 vertebrae. The motor evoked potentials induced by epidural stimulation of L2 spinal cord segment were recorded from m.tibialis and m. gastrocnemius on 3, 7, 14, 21 and 30 days after SCI.The measuring of rate-sensitive depression of reflex middle response of motor-evoked potentials was use for asses the hyperexcitability.

The obtain results shows that hypothermia did not affect on spasticity in rats on 7 and 14 th days after SCI, however it increased spasticity of m.tibialis and m. gastrocnemius on 21 and 30 days after SCI in compare with injured rats without hypothermia treatment.

It was suggested that hypothermia despite on its neuroprotective effects in an acute SCI effected on development of the spasticity in a chronic SCI.

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P12-23

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

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Myosin is a molecular motor indispensable for body movement and heart contractility. Apart from pure cardiomyopathy, mutations in MYH7 encoding slow/ß-cardiac myosin heavy chain can also cause skeletal muscle disease with or without cardiac involvement. Mutations mainly residing withinα-helical rod domain of MYH7 are associated with Laing distal myopathy. We developed a Drosophila melanogaster model of Laing distal myopathy in order to investigate the pathobiological mechanismsof the recurrent causative mutation (K1729del MYH7). Homozygous MhcK1728delanimals die during larval or pupal stages and both homozygous and heterozygous larvae display reduced muscle function. Heterozygous MhcK1728del adult flies and flies with exclusive expression of MhcK1728del in indirect flight and jump muscles were flightless and exhibited reduced jump abilities and a declined lifespan. The sarcomere of mutant indirect flight muscles and larval body wall muscles was disrupted with disorganised muscle filaments. Larvae homozygous, but not heterozygous, for MhcK1728del also demonstrated structural and functional impairments of heart muscle, indicating a dosage effect of the mutated allele. The phenotypes associated with MhcK1728del, including reduced jump ability, lack of flight ability and the myopathy of indirect flight and leg muscles, were fully supressed by overexpression of Abba/Thin, which has an essential role in maintaining sarcomere integrity. The data shows the first Laing distal myopathy model in Drosophila, recapitulating certain morphological phenotypic features seen in Laing distal myopathy patients with the recurrent Lys1729del mutation, which was rescued by Abba/Thin overexpression. The findings may warrant immediate clinical and molecular genetic investigation for diagnosis and possible therapeutic intervention.

P12-24

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

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

P12-25

Evaluation of recovery of motor functions spinal cord in the local delivery of methylprednisolone

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Spinal cord injury (SCI) is associated with serious neurologic disorders and limited therapeutic possibilities. The aim of the work was to assess the motor functions under the conditions of local delivery of polymer conjugates with methylprednisolone after contusion injury in rats in the acute SCI.

All experiments were performed in compliance with bioethical norms. Two experimental groups were singled out. The first group were subjected to spinal injuries at the level of Th 8-9 according to the modified A. Allen method. The second group received the application of polymer after contusion SCI, the polymer was applied to the hard shell of the spinal cord for 3 hours.

The maximum amplitude of electrical responses of the muscles of the posterior limb of the rat to epidural stimulation of the spinal cord was determined at 3 hours after SCI. Using the methylprednisolone and polymer complex therapies after SCI significant changed the Amax amplitude of all responses. The Amax of the motor response of the gastrocnemius muscle increased by 136% ($p < 0.05$), the Amax of the reflex response increased by 64% ($p < 0.05$).

The results of our study demonstrated the possibility of developing effective complexes of drugs for the treatment of acute neural trauma.

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P12-26

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

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

P12-27

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

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Background

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

Purpose

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

Methods and study desighn

We used in vitro differentiation model based on C2C12 mouse myoblasts. C2C12 cells were transduced with lentiviruses bearing either LMNA WT encoding wild type lamin A/C, lentivirus bearing LMNA G232E mutation or lentivirus bearing LMNA R482L. mutation. Confluent C2C12 cells were induced to differentiate with two types of media: pro-myogenic stimulation and mixed stimulation (combined pro-myogenic and pro-adipogenic stimulation). mRNA samples were collected at day 0, day 2 and 7 after stimulation of differentiation for expression analysis. The expression of genes that control muscle metabolism and development was analyzed: MYH3, MYF6, FABP4, ATGL, Desmin, Myomarker was analyzed. Efficiency of transduction controlled by immunostaining with antibody specific for hLMNA, lipids accumulation was detected by OilRed staining, muscle differentiation was visualized by MHC inmmunistaining.

Results

Morphology of myotubes formed by C2C12 cells bearing G232E or R482Q mutations or WT or LMNA and treated with pro-myogenic and mixed differentiation media differ significantly in their morphology: C2C12 cells bearing LMNA R482Q mutation differentiated into hypertrophied myotubes; C2C12 cells bearing both LMNA G232E and R482Q mutations, demonstrated alterations in regulation of intracellular lipid droplets development and metabolism. Furthermore, both types of mutations promoted expression of embryonic (MYH3) and neonatal (MYH8) myosins expression, but not adult myosins (MYH1, MYH4, MYH7).

Conclusions

We created an experimental model to study molecular mechanisms of R482Q mutation in LMNA gene - related disorders in lipid droplets metabolism, and muscle hypertrophy. We have also demonstrated that muscle wasting and degeneration associated with LMNA mutation G232E can be related to defects in formation, maintenance and metabolism of lipid droplets in adipose tissue and ectopic lipid depots in muscle. Both mutations prevented progression from fetal muscle development program to adult skeletal muscle differentiation in vitro.

P12-28

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

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Introduction

Reperfusion after myocardial infarction (MI) can worsen cardiac tissue damage and in vitro models of hypoxia/reoxygenation (H/R) have been developed to simulate the in vivo ischemia/reperfusion injury.

Purpose

The present study investigated the cardiomyoblast paracrine effects in H/R, by treating them with the conditioned media of mechanically loaded or unloaded cells, in vitro.

Method

H9C2 cardiomyoblasts underwent a cyclic stretching (Flexcell tension system) and then their conditioned media (secretome) was collected (stretch media, SM). Conditioned media of unstretched H9C2 was also collected (non-stretch media, NSM). H9C2 were subjected to 6 h hypoxia followed by 8 h reoxygenation (H/R) while during R, they were treated either with SM or NSM. Cell apoptosis was subsequently assessed by flow cytometry (Annexin V/PI).

Results

The percentage of early apoptotic cells decreased significantly in the SM- and NSM-treated cells compared with the non-treated cells ($p<0.01$). No significant differences ($p>0.05$) were found between the three groups in the total number of early and late apoptotic cells. Live cells exhibited a trend of increase in the SM and NSM groups ($p>0.05$).

Conclusion

Our findings suggest that H9C2 secretome is able to delay but not inhibit their apoptosis after H/R injury, independently of their mechanical load “preconditioning”.

P12-29

The effects of muscle cell aging on myogenesis

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Introduction

The process of myogenesis gradually deteriorates as skeletal muscle ages. However, less is known about the molecular responses of aged muscle cells during myogenesis.

Objective

This study investigated the effect of cell senescence on myoblast differentiation, using an in vitro model of myoblast aging.

Methods

C2C12 myoblasts were continuously cultured for 50 days, reaching passage 36 (aged myoblasts) while myoblasts passage 14 were used as controls. SA-β-gal activity was used as marker of cell ageing. Cells were switched into differentiation medium and harvested at their day 0, 2, 6 and 10 of differentiation. qRT-PCR was utilized to measure changes in expression levels of the myogenic regulatory factors (MRFs; MyoD, Myogenin, MRF4), growth (IGF-1 isoforms: IGF-1Ea, IGF-1Eb), apoptotic (Foxo, Fuca), atrophy (Murf1, Atrogin, Myostatin) and inflammatory (IL-6) factors.

Results

Compared to controls, aged differentiating myoblasts exhibited increased activity of SA-β-gal and reduced expression of MRFs and IGF-1 isoforms ($p<0.01$), along with increased expression of the apoptotic, atrophy and inflammatory factors ($p<0.05$-0.01).

Conclusion

A diminished differentiation capacity characterizes the aged myoblasts, which in combination with the induction of apoptotic and atrophy factors, indicates a disrupted myogenic process in senescent muscle cells.

P12-30

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

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

P12-31

Skeletal muscle contractile properties in experimental autoimmune encephalomyelitis mice

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Background

Neurological disorders such as multiple sclerosis (MS) can affect skeletal muscle properties.

Purpose

To investigate muscle mass and in vitro contractile properties of fast and slow hindlimb skeletal muscles in experimental autoimmune encephalomyelitis (EAE), an animal MS model.

Method

Intact m. soleus (SOL) and m. extensor digitorum longus (EDL) from 10 EAE mice (18 d after immunization) and 10 healthy mice underwent electrical stimulation to assess twitch and tetanic force, force-frequency relation, and muscle fatigue (10 min) and recovery.

Results

EAE mice were classified as mild (n=6) and severe (n=4). Severe EAE reduced EDL muscle weight (-30%, $p<0.01$) and cross sectional area (CSA, -26%, $p<0.05$), SOL tetanic force (-34%, $p<0.01$), and EDL twitch force (-30%, $p<0.05$), tetanic force (-45%, $p<0.001$) and force/CSA (-30%, $p<0.05$). Submaximal force (<25-50 Hz) was preserved and the force-frequency curve shifted leftwards in severe EAE. No differences were found during fatigue, whereas force recovery (normalized to initial force) was faster in severe EAE.

Conclusion

Severe, but not mild, EAE affects skeletal muscle properties, which is more pronounced in fast (EDL) muscle tissue.

P12-32

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

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Background

Muscle tissue is comprised of different fiber types, type I, IIa, IIB and IIx fibers. The fiber type composition is highly variable between different muscle groups and can change due to aging or training. These shifts can also be observed in several neuromuscular diseases and could play a role in disease progression. Utilization of laser microdissection (LMD) combined with mass spectrometry (MS) enables an accurate subclass specific analysis.

Methods

LMD samples from murine muscles were either used to establish an optimized workflow for proteomic analysis including: 1) sample lysis; 2) tryptic digestion 3) peptide seperation, or fiber type differentiation by flourescent staining. The optimized protocols were further used for the analysis of different fiber types (type I and type II) in male and female mice.

Results

All fiber types could be clearly distinguished by specific antibody staining. For LMD generated samples lysis with formic acid followed by an in solution digest was the most effective method identifying more than 4500 peptides. The analysis of different fiber types of male and female mice, idenfied several differential expressed proteins between the two sexes.

Conclusions

We established a fast, simple and cost effective workflow for the differentiation of fiber types and their label free quantitative analysis by LMD-MS and could by that identify gender specific differences in type I and II fibers.

P12-33

Quantification of developmental myosin fibres in muscle biopsies as a clinical biomarker for muscle disease

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Introduction / Background

The expression of developmental myosin heavy chain can be detected in newly formed regenerating skeletal myofibres 2-3 days after injury and remains for 3-4 weeks. The presence of developmental myosin heavy chain (MYH3, MHCd) provides a very specific biomarker of regenerating fibres in pathologic skeletal muscle including DMD.

Objective / Purpose

To quantify expression of developmental myosin heavy chain (MHCd) in DMD patient muscle fibres.

Method

Summit have utilised novel automated immunohistochemistry imaging algorithms, in partnership with Flagship Biosciences. The imaging technology was utilised in the Phase 2 open label study of ezutromid from muscle biopsies with results from treatment Week 24 (n=25) described.

Results

Compared to baseline, the Week 24 biopsies demonstrated a statistically significant decrease in the number of fibres expressing MHCd with a relative reduction of 23%.

Conclusion

We demonstrate that twice daily ezutromid treatment for 24 weeks resulted in a significant decrease in the number of fibres undergoing the natural repair process due to damage. These data demonstrate that unbiased quantification of MHCd positive fibres from muscle biopsy sections is a potential biomarker of muscle regeneration and importantly, of reduced muscle damage after therapeutic intervention.

P12-34

Extraocular muscles in desmin knockout mice

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

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

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

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

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

P12-35

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

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Dysferlinopathy, is a genetic disease caused by mutations in the gene encode to dysferlin, a sarcolemma protein that participates in membrane repairing. The onset is around third decade of life, as a progressive lower-limb weakness. In dysferlin-deficient (DD) mice (model of dysferlinopathies) the recovery of membrane resealing function by expression of a mini-dysferlin does not arrest progressive muscular damage, suggesting the presence of dysferlin-dependent pathogenic mechanisms unknown. In this regard, we demonstrated a persistent expression of functional connexin-based hemichannels (Cx HCs) in pathological conditions that affect skeletal muscles. Such membrane channels are permeable to Ca^{2+} and critically contribute to muscular damage. DD myofibers also showed positive immunostaining for Cxs 39, 43 and 45 and elevated Cx HCs activity concomitant with elevated resting intracellular free Ca^{2+} level compared with wt myofibers. Also, it was detected a lower performance of DD mice in rotarod type exercise compared to wt mice. All these changes were prevented in DD mice deficient in Cx43 and 45, suggesting that Cxs HCs are responsible of the pathogenic mechanism. In addition, the presence of Cxs 40.1, 43 and 45 was detected in human muscle biopsies from Chilean dysferlinopathy patients. Therefore, Cx HCs could be good candidate as a new therapeutic target.

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P12-36

MARP1 is a negative regulator of passive stretch-induced hypertrophy

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Background

MARP1 is a stress-induced protein involved in trophic signaling. Though extensively studied in the heart, MARP1's function in skeletal muscle has remained elusive. Here, we studied the role of MARP1 in skeletal muscle hypertrophy.

Methodology & results

We used unilateral diaphragm denervation (UDD), a model associated with stretch-induced hypertrophy of the denervated hemidiaphragm, and a 320-fold increase of MARP1 protein, that primarily localizes to the titin N2A-segment, without nuclear localization.

To study the role of MARP1 in stretch-induced hypertrophy, we used a knock-out mouse model for the Ankrd1 gene (MARP1 KO). Applying UDD to MARP1 KO mice, we found that that hypertrophy is increased in the absence of MARP1 (6d after UDD: 56±4% tissue mass increase vs. 43±3% in WT mice).

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

Working hypothesis

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

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

P12-37

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

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Background
Cerebral palsy (CP) is a non-progressive motor disorder that affects the posture and gait of the patients.

Aim
The aim of the present study was to elaborate whether specific signaling pathways in the muscle are correlated to increased passive stiffness and reflex activity in CP

Methods
Next Generation Sequencing of 92 candidate targets was performed in muscle biopsies from the m. Gastrochnemius muscle. In addition, stretch reflexes and passive stiffness was measured in the lower legs.

Results
Passive stiffness was significantly correlated to mRNA expressions of HSPG2 (p=0.02), PRELP (p=0.002), RYR3 (p=0.04), COL5A3 (p=0.0007), ASPH (p=0.002) and COL4A6 (p=0.03). In addition the reflex activity was significantly correlated to mRNA expressions of HSPG2 (p=0.02), LAMc1 (p=0.04) and COL4A6 (p=0.05). Subsequently children with CP showed more pathogenic variants in the mitochondria especially in COII, COIII and ATPase6.

Conclusion
The present study shows that some of the investigated targets potentially might be associated to the severity of passive stiffness and reflex activity in cerebral palsy.

P12-38

Zebrafish models of ACTA1-related myopathies recapitulate human disease

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Introduction
Mutations in the gene encoding skeletal muscle alpha actin, ACTA1, have been associated with a range of myopathies. There are no curative treatments available for patients, partly due to the lack of models that can be utilized in high-throughput approaches for discovery of effective therapeutic options.

Objective
In order to develop animal models that can be used in small molecule screens for therapeutic compounds, we developed transgenic zebrafish models that express disease-associated actin variants.

Methods
We developed transgenic zebrafish over-expressing the dominant negative mutations and using Crispr-Cas9 system knockout (KO) fish to mimic the patient pathology.

Results
Transgenic fish acta1aV165MTg demonstrated decreased muscle mass and curved bodies, impaired muscle contractility, reduced motility and accumulations of actin aggregates. In addition Acta1a knockout zebrafish line had reduced lifespan and abnormal pattern of birefringence.

Conclusions
These results show that zebrafish models of actin mutations recapitulate the human disease and have robust myopathic phenotypes that may be amenable for high-throughput chemical screening.

P12-39

Diaphragm weakness in critically ill patients is associated with upregulation of the titin-binding protein MARP1

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Critically ill patients often develop diaphragm weakness, which prolongs ventilator dependency and hospital stay. Diaphragm weakness is the result of atrophy and contractile dysfunction of diaphragm fibers, but whether the mechanosensory protein titin is involved in these pathophysiological mechanisms is unclear. In this study we explore the role of titin in the development of diaphragm weakness.

Diaphragm biopsies were obtained from critically ill and control patients. Passive stiffness of single fibers was comparable in the two groups. In addition, using gel electrophoresis and western blot, titin levels and phosphorylation status were determined, revealing no change between critically ill and control patients. We subsequently probed several titin-binding proteins, showing MARP1, a protein with low baseline presence in the diaphragm, to be strongly upregulated (34-fold) in critically ill patients. This upregulation was recapitulated in 18h mechanically ventilated (MV) rats, 99-fold increase of MARP1. To test the role of MARP1 in diaphragm weakness, we performed 6h MV on Ankrd1 knock-out (MARP1 KO) and wild type mice. Intact diaphragm mechanics showed that MV MARP1 KO mice developed 24% less contractile dysfunction compared to WT mice. Thus, titin-binding protein MARP1 might play a key role in the pathophysiology of diaphragm weakness in critically ill patients.

P12-40

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

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Background

MicroRNAs modulate gene expression post-transcriptionally and, when released from cells, provide humoral signals for tissue homeostasis.

The purpose of the study was to examine effects of IGF-I on miRNA expression and secretion in rat skeletal primary myoblasts.

Method

Microarray and qPCR analyses were used to assess and validate miRNA profiles in myoblasts, subjected to 11-day differentiation with IGF-I (25 nmol/l). Student t-test was used for comparison the results vs control.

Results

Microarray analysis revealed increased expression of 45 miRNAs and decreased expression of 20 miRNAs in myoblasts treated with IGF-I. Ten secreted miRNAs were increased and 3 miRNAs were decreased by presence of IGF-I. PCR analysis confirmed increased expression and secretion of miR-100 (fold change, FC, values: 1.78 and 1.21, respectively), miR-107 (FCs: 5.14 and 3.59), and decreased expression of miR-21 (FC: 0.6). Expression of miR-322 was increased (FC: 1.65), but its secretion was decreased (FC: 0.41) by IGF-I.

Conclusion

IGF-I, an anabolic factor for skeletal muscle, can modulate micro-environment of differentiating myoblasts through the effects on miRNA expression and secretion.

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