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ABSTRACT

45th European Muscle Conference in Montpellier, France

The following abstracts were submitted for consideration in response to the call for abstracts of the 45th EMC. The main theme of the 45th EMC was Muscle Research in Health and Disease. It was the intention of the organizers to cover a wide range of topics focusing on muscle development and function, both in physiology and pathology. The program included the following sessions: "Molecular motor and contractile function", "Mitochondrial dynamics", "Muscle cytoskeleton", "Muscle plasticity and chronic disease", "Neuromuscular disease and therapeutic approach", "Excitation–contraction coupling in cardiac muscle", "Muscle development and aging", "Skeletal muscle diseases", "Heart and heart failure", "Smooth muscle and vascular function", "Epigentics of muscle regeneration", "Molecular motor and contractile structure", "Mechanotransduction", "Excitation–contraction coupling in skeletal muscle", "Bio energetics and ROS" and "Cardiomyopathy and Heart failure". The sessions were chaired by international and French top scientists in those fields who introduced the sessions.

The abstracts were reviewed by an independent Abstracts Committee, members or the ISB and the organizers, who also edited the abstracts.

45th EMC Organizers

Olivier Cazorla and Stefan Matcki, PhyMedExp, University of Montpellier, FR

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We thank all contributors for their hard work!

Acknowledgments We are grateful to the participants and the speakers who accepted our invitation to contribute with their work to create an excellent platform to discuss the latest achievements in muscle research and to initiate new collaborations. We are grateful to the contributors of the meeting Université de Montpellier, Région Languedoc-Roussillon, AFM-Téléthon, Pôle Biosanté Rabelais, Montpellier Méditerranée Métropole, RyR-I foundation, the Journal of Muscle Research and Cell Motility, and Supporters: Cosmed, Aurora Scientific Inc., IonOptix, ADinstruments, tebu-bio.com, Dominique Dutscher, Cytoskeleton Inc.

Olivier Cazorla & Stefan Matecki

Session 01 Muscle plasticity and Chronic disease

Oral presentations

S01.01

Impact of exercise training on skeletal muscle in heart failure: a molecular point of view

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Patients with chronic heart failure (CHF), with either reduced (HFrEF) or preserved ejection fraction (HFpEF) are characterized by breathlessness, muscle weakness, and exercise intolerance, the latter being one of the most powerful predictors of prognosis. The pathophysiology and the molecular changes underlying these symptoms are complex and only partly understood. With respect to treatment, exercise training is one best option and validated in several experimental and human studies. During the last years our knowledge with respect to molecular changes elicited by exercise training in the skeletal muscle and the diaphragm has increased. During the presentation we will discuss these molecular changes in the limb and diaphragmatic muscle occurring in CHF and the impact of different training modalities on these alterations.

S01.O2-151

Involvement of the ubiquitin/proteasome signalling pathway in the oxidative stress-induced atrophy of cultured Chronic Obstructive Pulmonary Disease myotubes

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Aims: Dysfunction and atrophy of skeletal limb muscles are important extrapulmonary manifestations of chronic obstructive pulmonary disease (COPD) contributing to impaired patient exercise tolerance, worsened quality of life, and reduced survival. Oxidative stress is considered as one of the most important mechanisms leading to muscle dysfunction and atrophy, but its direct involvement in COPD muscle atrophy has not been demonstrated yet. Using an in vitro cellular model, we have previously shown that myotubes derived from quadriceps biopsies of COPD patients display atrophy and elevated oxidative stress compared to those of healthy subjects (Pomiès et al., J.Cell.Mol.Med. 2015). Our objectives were to assess the involvement of oxidative stress in COPD muscle atrophy using cultured myotubes derived from satellite cells of 12 COPD patients (mean age: 59.0 years [54.0–62.8]; FEV1: 32.0% predicted [26.8–47.5]).
Methods and Results: We have evaluated the effects of an H2O2-induced oxidative stress and of an antioxidant molecule, ascorbic acid, on the oxidative stress status, on the diameter, and on the expression of markers of the protein breakdown and protein synthesis pathways of COPD myotubes. We showed that the antioxidant treatment of COPD myotubes led to reduced ROS production ($p < 0.001$), oxidative cellular damages ($p = 0.019$), atrophy ($p < 0.001$), and ubiquitin/proteasome signalling (MuRF1: $p < 0.001$; atrogin-1: $p = 0.002$; FoxO1: $p = 0.042$), while the pro-oxidant treatment induced the opposite effects. Furthermore, use of the proteasome inhibitor, MG132, reduced the atrophy of COPD myotubes.
Conclusion: These data are therefore the first to show the involvement of oxidative stress in the atrophy of COPD peripheral muscle cells in vitro, via the ubiquitin/proteasome signalling pathway.

S01.O3-187/S01.P17-187

Morphological indices and markers of signaling pathways in skeletal muscle of patients with alcoholic myopathy

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Alcoholism is often accompanied by skeletal muscle atrophy. The aim of our study was to investigate the intracellular signaling mechanisms that control protein synthesis and degradation in alcoholic myopathy. We examined 10 male patients with an alcoholic myopathy (group A) and 7 healthy men at the same age in control group (group C). A biopsy from m. vastus lateralis was taken. The average daily dose of alcohol was responded to 160.1±10.4 ml of ethanol. We did not find any changes in CSA of fiber between these groups, but the slow-to-fast transformation in group A was observed. Reactive oxygen species level detected by DHE fluorescence staining was higher in group A than in the control group ($p < 0.05$). We analyzed the state of anabolic (complexes mTORC1/p70S6K and ERK1/2) and catabolic signaling pathways. IRS-1 and p-4E-BP protein content was decreased, but AMPK and p-eEF2 protein content was increased in group A as compared to control ($p < 0.05$). The content of p-Akt, p-GSK, p-p70S6k and p-p90RSK in group A did not differ from that in control group. Calpain-1 content and the ubiquitylation of proteins were higher in group A as compared to control ($p < 0.05$). We did not find any changes in the mRNA level of MuRF-1and MAFbx. Analysis of muscle protective heat shock proteins Hsp90 and Hsp70.did not showed any changes in the mRNA level. Thus we observed changes of some components of anabolic signaling pathways as well as the activity of some components of catabolic signaling pathways. This finding is important for the development of methods to correct the atrophic processes in skeletal muscle in alcoholic myopathy. Supported by Russian Science Foundation No. 14-15-00392.

S01.O5-277

Resveratrol enables myotube survival under nutrient stress

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Calorie restriction (CR) is the only known dietary manipulation that can increase both lifespan and healthspan in a variety of species. Underlying these increases are primarily improved metabolic health and reductions in cancer incidence. However, these benefits are associated with a concomitant loss of skeletal muscle mass, which appears to be a repercussion of CR (Sharples et al.,2015). Activation of Sirtuin1 (SIRT1) has been reported during CR, the suppression of which abrogates lifespan extensions. In the presence of inflammatory stress (mediated by increases in TNF-α) SIRT1 has been indicated as a protein fundamental to the survival and regeneration of skeletal muscle cells (Saini et al.,2012). Objectives were therefore to uncover the potential role of SIRT1 in ameliorating the atrophic effect of CR (via low glucose) in both fusing muscle cells (myoblasts) and mature myotubes. SIRT1 activity was either increased (10 μM resveratrol) or decreased (EX-527; 100 nM) using pharmacololgical interventions. Under CR conditions (LOW glucose (6.24 mM)), resveratrol supplementation was unable to rescue a reduction in fusion, as assessed biochemically [Low Glucose vs. Low Glucose + Resveratrol at 7days (44.40 ± 8.95 vs. 36.77 ± 9.28 a.U respectively, p = N.S). However, studies in myotubes revealed an increase in the number of surviving myotubes at 48 h (9.00 ± 4.08) when supplemented with resveratrol, compared to the SIRT1 inhibitor (7.25 ± 3.58; $p \leq 0.05$). This was coupled with a reduced gene expression of the E3 ubiquitin ligases when compared to inhibitor [MuRF1, MAFbx and MUSA1) −0.13, −0.15, −0.10 fold change respectively] and in p53. Changes in these genes are important in both protein degradation/atrophy (ligases) and survival (p53). Overall these studies suggest that SIRT1 activation, via resveratrol administration during periods of CR, reduces myotube atrophy and enables myotube survival via regulation of protein degradation and survival transcriptional networks.

Posters

S01.P1-107

Neuromuscular electrical stimulation prevents skeletal muscle dysfunction in adjuvant-induced arthritis rat

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Patients with rheumatoid arthritis (RA) frequently suffer from muscle weakness, which is associated with the impaired activity of daily living. Here, we investigated the effects of neuromuscular electrical stimulation (ES) training on skeletal muscle dysfunction in adjuvant-induced arthritis (AIA) rat. AIA was induced by an injection of complete Freund's adjuvant. For ES training, dorsiflexor muscles were stimulated (0.5 ms pulse, 50 Hz, 2 s on/4 s off) via peroneal nerve using surface electrodes and the

stimulation intensity was progressively increased throughout the stimulation period in order to maintain a peak torque corresponding to 60% of the initial maximum isometric torque. Each training session included ~37 contractions and was applied every other day for three weeks. Forty-eight hours after the last ES training, extensor digitorum longus (EDL) muscles were excised and used for physiological and biochemical analyses. There was a reduction in specific force production in AIA EDL muscles, which was accompanied by the aggregation of actin and desmin. These deleterious changes were prevented by ES training. Furthermore, αB-crystallin and PGC-1α, but not HSP25, SOD2, and catalase, were increased by ES training. These results suggest that ES training prevents aggregation of myofibrillar proteins and muscle weakness in EDL muscles from AIA rats. Thus, ES training can be effective in treatment of muscle dysfunction seen in patients with RA.

S01.P3-316

FoxOs deletion prevents muscle atrophy in hindlimb unloaded mice

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Muscle atrophy is a complex process whose mechanisms are not fully understood and likely vary among disuse models.
Aims: The aims of this study were to: (i) determine whether the lack of FoxOs can protect muscle mass and force in the hindlimb unloaded mice model; (ii) define the molecular events controlling the atrophic process in different muscles with special attention to which atrogenes are under FoxOs control; (iii) compare the data obtained in this disuse model with another model of inactivity such as denervation.
Methods and Results: Both wild type and transgenic mice (FoxO1,3,4−/−) were unloaded for 3 and 14 days. The following analyses were performed on gastrocnemius, tibialis and soleus muscle: cross-sectional area (CSA) of muscle fibres (on muscle cryosections); functional analysis of isolated muscles and single muscle fibers; atrogenes expression (by real time PCR); pathways and markers involved in muscle mass maintenance (pAKT, pS6, p4EBP1, pAMPK and PGC1α, by western blot). Our data show that: (i) FoxOs inhibition completely spares muscle mass and force in hindlimb unloaded mice; (ii) atrogenes under FoxOs regulation are different in hindlimb unloading and in denervation; (iii) differences in atrogenes regulation occur among muscles in the same disuse model.
Conclusions: Our findings clearly indicate that the key role of FoxOs varies among catabolic models supporting the concept that activation of atrophy program is differentially regulated.

S01.P4-314

The role of the Warburg effect-associated enzymes Phgdh and Pkm2 in myotube hypertrophy

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A key component of the growth strategy of cancer cells is the Warburg effect. The Warburg effect refers to an increased glycolytic rate by which cancer cells provide glycolytic intermediates and other metabolites as substrates for biosynthetic reactions such as amino acid, nucleotide and lipid synthesis. Mechanisms do not evolve to promote cancer so we investigated whether the two Warburg effect-associated enzymes Phgdh and Pkm2 are expressed in skeletal muscle and whether their loss-of-function reduces growth factor-induced skeletal muscle hypertrophy. IGF-1 induced a 37% myotube hypertrophy and a 51% increase in medium lactate concentration. Blocking glycolysis by 2 deoxyglucose (2DG) caused a 30% myotube atrophy, reduced medium lactate concentration by 56%, and ablated IGF-1-induced hypertrophy. This latter could not be explained by a reduced phospho-p70S6k, enhanced activation of Ampk or elevated Murf1 and Mafbx expression. Basal mRNA levels of the Warburg mediators Phgdh and Pkm2 as well as Phgdh protein were upregulated by IGF-1 in C2C12 myotubes. Knock down of Phgdh and Pkm2 expression using siRNA reduced C2C12 myotube diameter by 27 and 41%, respectively, and ablated IGF-1 induced hypertrophy. These effects were accompanied by increased mRNA levels of Mafbx and PGC-1α. Effects of downregulation of Phgdh on myotubes size was confirmed in primary mouse myotubes. Together these results indicate that Phgdh and Pkm2 are expressed in C2C12 myotubes and that this expression is regulated by IGF-1. These Warburg effect-associated enzymes not only contribute to the regulation of growth in cancer but also contribute to C2C12 myotube hypertrophy.

S01.P5-311

On the search for 'easier' training methods: effects of high speed interval (HSIR) training on muscle function and architecture

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Aims: High intensity interval training (HIIT) is a popular method to increase aerobic fitness with small time investment, but not much is known on its impact on muscle architecture and function. Most HIIT studies use cycling (mostly concentric muscle actions). In running both concentric and eccentric muscle actions contribute. We examined the effects of High Speed Interval Running (HSIR) at two opposite inclinations (uphill vs. downhill) on muscle strength, power and architecture.
Methods: 14 active volunteers (3 women), 31.9 ± 6.9 years consented to participate in this study which had ethical approval, divided into two groups: Uphill HSIR (+10%, n = 7), and Downhill HSIR (−10%, n = 7) and trained twice per week for 8 weeks. Each session

included ten 30 s treadmill runs with a work: rest ratio 1:2 at a speed of 90% of vVO2max (speed @VO2max, re-assessed at mid-training). Vastus lateralis architecture was assessed with ultrasonography, muscle function was examined by measuring maximal voluntary contraction (MVC), rate of force development (RFD) and fatigue of the knee extensors, as well as jumping performance. A 2 × 2 (group by time) mixed method analysis, was used to assess the data.
Results: Body mass decreased, angle of peak flexor torque and resistance to fatigue increased ($p < 0.05$) while VO2max and MVC remained on average unchanged in both groups. RFD improvements were observed in half the downhill group, but no changes overall. Pennation angle (ns increase) and fascicle length (ns decrease) were not significantly changed. The Uphill group increased more on resistance to fatigue while the downhill group improved more on jumping performance.
Conclusions: The effects of inclination during HSIR training were evident in resistance to muscle fatigue and jumping performance, while changes in muscle architecture and RFD were more subtle and personalized. Overall, with less energy investment downhill HSIR might offer a practical short-term alternative to standard HIIT.

S01.P6-291

Activation of Eukaryotic Elongation Factor 2 kinase in rat m. soleus during different periods of hindlimb suspension. Cav1.1 blocking decreases eEF2k activity

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Eukaryotic elongation factor 2 (eEF2) is one of the important member of eEF family catalyzing simultaneous translocation tRNA and mRNA on a 80S ribosome. It was shown that phosphorylation of eEF2 by its specific Ca^{2+}/CaM-dependent kinase (eEF2k) decreased 10–100 folds eEF2 affinity to ribosome, which led to protein synthesis rate drop. We supposed that eEF2k is activated in rat m.soleus under hindlimb suspension (HS) in Ca^{2+}-dependent manner with Cav1.1 involvement, which reasons to eEF2 activity decrease and consequently to drop of protein synthesis rate. To test the hypothesis we divided rats into 6 groups: control group (C), hindlimb suspended (HS) groups during 1, 3, 7, 14 days and 3-day HS group with nifedipine (Cav1.1 blocker) or BAPTA-AM injections. Atrophy of m.soleus was found in 7HS and 14HS groups. We observed significant increase in eEF2k mRNA expression and its protein content in all HS groups as compared to C group. P-eEF2 (T56) level was greater in the HS groups than that in C one ($p < 0.05$). Nifedipine decreased P-eEF2 level partially in 3HS, BAPTA-AM completely prevented the rise in the level. It was shown that phosphorylation of p70S6k-related or p90rsk1-related site Ser366 of eEF2k could decrease its activity [Wang et al., 2001]. We showed that P-eEF2k (S366) content increased along with the eEF2k rise in 3HS. Nifedipine administration did not have any significant effect on this process. Unlike nifedipine, BAPTA-AM injection led to decrease in P-eEF2k (S366) level during the 3HS. We observed 40 % reduction in protein synthesis rate after 3 days of HS. Despite the reduction of P-eEF2 level in 3HS-BAPTA group the protein synthesis rate in this group was as low as in 3HS group. Thus, eEF2k is activated in m.soleus under HS which is Ca^{2+}-dependent process with involvement of Cav1.1. Decrease in eEF2 activity isn't liming event for protein synthesis rate in m. soleus during HS.

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S01.P8-224

Effects of motor deprivation on neurogenesis and muscle-derived neurotropic factors

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The positive role of exercise in individuals affected by neurologic diseases was recently brought to light (Okonkwo et al. 2014). On the contrary, both astronauts and patients affected by movement-limiting pathologies face impairment in muscle and brain performance. Moreover, recent evidences suggest that myokines released by exercising muscles affect the expression of brain derived neurotrophic factors (Phillips et al. 2014). The purpose of this work is to study how muscular inactivity affects neurogenesis and the factors that are involved in the interaction between the muscle and the neurogenic areas. We plan to study the effect of motor deprivation using a well-established ground based model: the hindlimb unloading model (HU). Four-month-old male C57BL/6 mice were randomly selected and assigned to control (CTRL) or HU groups. The unloading lasted 14 days. At the end of the suspension, gastrocnemius (GAS), soleus (SOL) muscle and brain were dissected and processed for the appropriate experimental procedure. Various neurogenic regions of the mice central nervous system (CNS) were isolated and the evaluation of the proliferative capacity of neuronal stem cells (NSCs) was performed in cultures obtained from HU and CTRL mice. Signals involved in controlling metabolism were studied in both muscles and brain. Muscle expression of fibronectin type III domain-containing protein 5 (FNDC5), which seems to play a role in regulating brain-derived neurotrophic factor (BDNF) production in CNS (Wrann et al. 2013), was also assessed. Preliminary results indicate that HU NSCs have a reduced capacity to proliferate and differentiate compared to CTRL. A reduction of PGC1alfa and FNDC5 expression in HU SOL was found. The study reveals that motor deprivation impairs neurogenesis and muscle neurotrophic factors expression.

Okonkwo, O.C., et al., 2014 Neurology, 83: 1753–60
Phillips, C. et al., 2014 Front Neurosci, 8: article 170
Wrann, C.D., et al., 2013 Cell Metab, 18: 649–59

S01.P9-220

Role of vestibular system on protein markers of muscle plasticity

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Aims: Headtilted (Het) mutant mice lack gravity sensors by vestibular deficiency in otoconia. Our objective was to determine, in skeletal muscles, if a sensorial vestibular stimulation (hypergravity) could counteract the effects of this deficiency. We investigated whether vestibular deficiency and/or hypergravity could modulate (i) protein markers of muscle plasticity (MHC) and (ii) factors of anabolic PI3K-AKT-mTor and catabolic PI3K-AKT-FOXO signalling pathways crucial in muscle protein synthesis/degradation.
Methods and Results: Four groups of mice were used: Het +/− CONT, Het−/− KO, Het +/− submitted to hypergravity (2G chronic

centrifugation during 1 month), and KO Het−/− plus centrifugation. The MHC profiles of CONT soleus was composed of 43% MHC1, 44% of MHC2A-2X and 13% of MHC2B. Het KO soleus presented an increase in MHC2B (18%) generally observed after atrophy during microgravity. After 2G, soleus muscles evolved towards a slower profile with disappearance of MHC2B. Het KO mice plus centrifugation led to an intermediate profile, without reappearance of MHC2B, but faster than 2G alone (59% MHC2A-2X; 41% MHC2A). There was a decreased activation (50%) of AKT in Het−/−, whereas it was more activated after 2G (30%). When vestibular deficiency was combined with 2G, AKT activation reached CONT values. Similar results were observed for p70S6K. Vestibular deficiency led to an increased expression of Murf-1 in Het−/− and a decreased expression after 2G that returned, when combined to vestibular deficiency, to the Murf-1 expression profile of Het−/+.

Conclusion: Hypergravity permitted, in soleus muscle, to counteract the effects provoked by the absence of gravity sensors. This model of mice otoconia deficiency would be suitable to study the effects of long-term microgravity since if we combined vestibular deficiency model with hindlimb suspension this would lead to a more complete model (absence of vestibular informations and reduction in mechanical load).

S01.P10-218

Comparison of passive gastrocnemius medialis muscle lengthening and torque in children with cerebral palsy and typically developing peers

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Aims: Children with spastic cerebral palsy (SCP) demonstrate altered muscle morphology and function (Mathewson & Lieber 2015). The aim of the study was to examine the resting joint angle (RJA), passive torque and m. gastrocnemius medialis (GM) lengthening during passive ankle dorsiflexion (DF) in children with SCP compared to typically developing peers (TD).

Methods and Results: Twelve children with SCP (GMFCS I and II, 11.9 ± 2.4 y, 148.0 ± 19.5 cm, 43.2 ± 17.9 kg) without ankle joint contracture (dorsiflexion > 0°, knees extended) and 12 TD children (11.3 ± 2.5 y, 152.0 ± 16.5 cm, 44.8 ± 15.5 kg) participated in the study. Resting ankle joint angle and ankle joint range of motion were assessed. Passive torque through the whole range of motion was measured using a dynamometer (Con-Trex Multi-joint) and the elongation of the GM starting from resting length was measured with ultrasound (Esaote Mylab 60). Variables were compared between the groups using independent one-sided t-tests (alpha = 0.05). Measurements revealed a significantly different RJA (SCP 25.7 ± 5.4°; TD 20.8 ± 4.5°) and decreased maximum DF in the SCP group (SCP—10.8 ± 9.6°; TD—18.8 ± 4.9°) during passive movement. Although muscle elongation started in greater plantar flexion in the SCP group, there was no difference in elongation of the GM muscle at 0°, 5° and 10° DF. Similarly, there was no significant difference in passive torque at 0° and 5° DF. However, at 10° DF passive torque was greater (p = 0.04) in SCP (8.7 ± 3.6 Nm) compared to TD (5.7 ± 3.1 Nm).

Conclusions: Children with SCP have a decreased maximal DF as well as RJA. Near the neutral ankle position, the elongation of GM and passive torque are similar to TD peers. However, if the ankle angle approaches its maximum, the passive torque increases rapidly, suggesting an increased stiffness of the GM in SCP children.

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S01.P11-209

High intensity exercise training improves chronic intermittent hypoxia-induced insulin resistance without autophagy modulation

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Chronic intermittent hypoxia (IH) associated with obstructive sleep apnea (OSA) is a major risk factor of cardiovascular diseases development and metabolic disorders such insulin resistance (IR). Autophagy, plays an important role in the pathophysiology of insulin resistance and high intensity training (HIT) has recently emerged as a potential therapy combined or not with the reference treatment of OSA (continuous positive airway pressure). The aim of this study was first to explore the modulation of autophagy in IH-induced IR in a tissue dependant way. In addition, we hypothesized that HIT could prevent dysregulation of autophagy induced by the expected IR induced by IH. Thirty Swiss 129 male mice were randomly assigned to 3 conditions: normoxia (N), intermittent hypoxia (IH: 21–5 % FiO2, 30s cycle, 8 h/day) or intermittent hypoxia associated with high intensity training (IH HIT) on a running treadmill. After 8 days of HIT (2x24 min, 50 to 90% of Maximal Aerobic Speed) mice were submitted to IH or N for 14 days. The HIT stopped 3 days before the end of the IH exposition in order to measure in vivo parameters: insulin tolerance test, MAS and endurance. Muscle, liver and adipose tissue were rapidly frozen to assess insulin sensitivity and autophagy markers by Western Blot or qPCR. IH induced IR which was characterized by a greater glycemia, an impaired insulin sensitivity and decreased AKT phosphorylation in adipose tissue and liver. Nevertheless, MAS and AKT phosphorylation were greater in skeletal muscle after IH exposure. Autophagy markers were not altered except for lower Bnip3 and Bnip3L ARNm expression in muscle. Insulin sensitivity and AKT phosphorylation in liver were greater in HIT associated to IH (p = 0.05), but Bnip3 protein expression in adipose tissue and liver and ARNm in muscle were lower. These findings suggest that HIT could represent a preventive strategy to limit IH-induced insulin resistance without any major change of basal autophagy.

S01.P12-196

The effect of BGP-15 in skeletal muscle regeneration of mice

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The expression of heat shock proteins (HSPs) is elevated upon exposure to a variety of stresses and limits the extent of stress-induced damage. The HSPs play a key regulatory role in cellular defense. Therefore, the aim of this work was to investigate the influence of the heat shock proteins overexpression in structural and functional aspects of skeletal muscle regeneration in mice. Young mice were treated with the HSP inducer BGP-15 [O-(3-piperidino-2-hydroxy-1-propyl) nicotinic amidoxime] (15 mg/kg/day; oral gavage) and had their tibialis anterior (TA) and extensor digitorum longus (EDL) muscles injured and evaluated after 3 and 10 days. Histological analyses showed that the muscles from mice treated with BGP-15 had apparent decrease in inflammatory process after injury compared with muscles from untreated mice in 3 days after injury. The decrease in cross-sectional area of regenerating myofibers from TA muscles evaluated at 10 days post-cryolesion was attenuated in BGP-15 treated muscles compared with the untreated ones. There was a decrease in gene expression of

collagen I and III at 10 after injury in BGP-15 treated muscles compared with the untreated ones. Finally, BGP-15 treatment prevented the reduction in maximum tetanic force of regenerating EDL muscles evaluated at 10 days post injury compared with the untreated ones. These results suggest that BGP-15, an HSP inducer, accelerates the skeletal muscle regenerative process. Bibliography: Gehrig, Sm, C Van Der Poel, Ta Sayer, Jd Schertzer, Dc Henstridge, Je Church, S Lamon, Ap Russell, Ke Davies, Ma Febbraio e Gs Lynch. Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. Nature.2012; 484(7394):394–8.

S01.P13-129

Effects of grape seed polyphenols supplementation associated with training on high fat fed rat's metabolism and performance

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Introduction: Polyphenols (PP) have been associated with improvement of insulin sensitivity and oxidative metabolism both in rodent and in obese human (1). On the other hand, exercise training is often recommended for improving metabolism and insulin resistance (2). Thus, the purpose of our study was to investigate the association of grape seed polyphenols with exercise training on metabolic mechanisms and adaptations of insulin resistant high fat diet fed rats.
Materials and methods: 40 male rats were fed ad libitum with a high-fat diet (HFD) (58% kcal from fat) during 4 weeks. Then rats were included in one of 4 groups: HFD alone (HFD), HFD and PP supplementation in drinking water (50 mg/g) (PP); HFD and exercise training on a treadmill (1 h/day; 5 days/week, 10% slope at 30 m/min) (EXO) and HFD with training and PP supplementation (EXOPP) for 8 weeks. At the end of the study endurance test, glucose tolerance test were performed and soleus insulin response was determined by measuring P-Akt/akt levels following insulin stimulation. Two way ANOVA analysis was performed; with a probability level of $p < 0.05$.
Results: An increase in systemic glucose tolerance associated with an increase in P-akt/akt ratio in soleus muscle was observed only in EXO and EXOPP groups. EXOPP had higher endurance time associated with a higher muscular and hepatic glycogen content. HydroxyacylCoA dehydrogenase activity was increased in EXO, EXOPP and PP groups.
Discussion/Conclusion: The combination of training and PP supplementation had no cumulative effect on insulin resistance but improve exercise performance by increasing lipid oxidation. Our data highlight that when insulin resistance has developed, the effect of nutritional grape polyphenol supplementation is being revealed only when associated with exercise-training.

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S01.P14-102

The effect of 6 weekly strength training on irisin level of women

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Introduction: Irisin is a recently discovered myokine hormone that is involved in health-related training effects. Therefore, the present study aimed to determine whether irisin concentration is increased after 6 week regular strength exercise training in women.
Material and methods: 19 volunter women with aged 18–25 were randomly assigned in to the strength group ($n = 9$) or control group ($n = 10$). Subjects in the training groups exercised 3 times per week for 6 weeks. Strength training consisted of 8 machine based exercises. After determining the 1 repetition maximum (1RM) load for the bench press (BP), lat pull down (LPD), shoulder press (SP), biceps curl (BC), triceps extension (TE), leg press (LP), leg extension (LE), and leg curl (LC). Physical performance provided positive control for the overall efficacy of training. Serum irisin levels were measured using ELISA. Wilcoxon test were used to assess differences on basaline and following 6 weeks metabolic parameters and irisin levels.
Results: Following 6 weeks, irisin levels in control group were significantly reduced [153.56 (104.10–229.43) vs. 129.20 (108.23–179.32) ng/ml, $P = 0.028$] whereas, in regular strength exercise training group, irisin levels were found to be increased [114.74 (108.03–155.25) vs. 158.82(103.21–320.25) ng/ml, $P = 0.021$]. In exersize group there was no significant difference serum insulin, fasting plasma glucose and also insulin resistance marker HOMA-IR levels when compared to before and after exersize. There was no correlation between irisin and metabolic parameters.
Conclusions: Regular strength exercise training can increase circulating irisin levels even though irisin levels did not correlate with any metabolic parameters.

S01.P15-292

Anabolic signalling response of unloaded rat soleus muscle to acute reloading

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A greater understanding of molecular mechanisms by which skeletal muscle is restored after a period of disuse atrophy is of importance for rehabilitation of both astronauts and patients with orthopaedic injuries. However, signalling pathways involved in the regulation of protein synthesis in mammalian skeletal muscle during an acute reloading are vaguely defined. The aim of the study was to measure the rate of protein synthesis (PS) and determine the content of the key signalling molecules implicated in the regulation of PS in rat soleus muscle during the first 24 h of reloading after disuse atrophy. Wistar rats were subjected to 14-day hindlimb unloading (HU) followed by 6- 12- and 24 h of reloading. The rate of PS was assessed using SUnSET technique. Evans Blue Dye was used as an in vivo marker of myofibre damage. A significant increase in myofibre permeability was observed following 12- and 24 h of reloading vs. HU group. The rate of PS was decreased following HU by 40% ($p < 0.05$) vs. control. In 6 h- and 12 h reloading groups the rate of PS was higher ($p < 0.05$) than in HU group. After 24 h of reloading the PS rate was elevated by 44% ($p < 0.05$) vs. control. The content of signalling proteins was determined by WB. HU induced a significant 37 and 30% decrease ($p < 0.05$) in P-p70s6k and P-4E-BP1, respectively. Following 6 h of reloading the content of P-p70s6k and P-4E-BP1 did not differ from control, however a reloading for 12 h resulted in an upregulation of both P-p70s6k and P-4E-BP1 (+18 and 38% respectively, $p < 0.05$). After 24 h of reloading we observed a slight decrease in P-p70s6k vs.

control, while P-4E-BP1 content did not alter vs. control. The content of P-p90RSK and P-GSK3-beta was reduced after HU and then completely restored by 12 h reloading. The results of the study suggest that an enhanced PS during an acute reloading may be associated with the activation of GSK3beta-eIF2B and mTORC1 pathways. The study was supported by RFBR grant # 16-34-00530a.

S01.P16-252

Dantrolene protects muscle against muscle proteolysis by chemotherapy (Taxotere)

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Many cancers induce cachexia but chemotherapy itself can also cause cachexia associated to weight loss, inflammation and muscle atrophy and that could be the limiting factor of cancer treatment. The aim of this study was to investigate the effects of Taxotere (Tax) on the early release of cytokines in circulation and on the activation of the proteolysis pathways in muscle. Anti-inflammatory effect of dantrolene (dant) was tested in order to know if dantrolene, an inhibitor of calcium release by ryanodine channels, can reduce muscular toxicity of Taxotere. Rats were divided into four groups: control (saline injected), receiving dantrolene (10mg/kg, IP), receiving Taxotere (10 mg/kg, IV) and receiving Taxotere plus dantrolene (respectively 10mg/kg, IV and 10mg/kg, P). Blood was collected 3hours after intravenous injection, then animals were sacrificed at 1dayBlood was collected and centrifuged for plasma analysis. hindlimb muscles (peroneus, soleus and extensor digitorum longus) were dissected free then frozen for further analysis. Cytokines profile, assessed by antibody array, showed that Taxotere was involved in cytokines released in circulation, from 3hours and until 1day after injection, and that this release was decreased by dantrolene. Activation of proteolysis pathway was investigated by evaluating activity (activity assay) and expression (western blot) for both calpaïnes and proteasome system (E3 ligases). The results indicated that dantrolene alleviate proteolytic effects of Taxotere on muscles.

S01.P18-185

Influence of proteasome inhibitor on m.soleus atrophy under unloading

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Skeletal muscle atrophy caused by unloading is accompanied by increased proteolysis and decreased protein synthesis. It is known, that the concentration of the free amino acids is increased under muscle unloading [Gamrin L., 1998]. We hypothesized that proteasome inhibition may decrease amino acids accumulation in skeletal muscle and prevent the atrophy. In order to test this hypothesis we have treated rats with bortezomib (a proteasome inhibitor) during 7-day hindlimb suspension. The hindlimb suspension was carried out according to Novikov-Ilyin's technique modified by Morey-Holton. 21 male Wistar rats were randomly divided into 3 groups: intact control (C), suspended group during 7 days (HS) and suspended group with bortezomib administration (0.15 mg/kg) (HSB). Phosphorylation level of key signalling proteins of the various signaling pathways was determined by Western-blotting. mRNA level of E3-ligases was estimated by RT-PCR. The rate of protein synthesis was evaluated by SUnSET technique (Goodman et al., 2011). M.soleus weight and the protein synthesis intensity in HSB and HS groups were reduced equally as compared to control (C) ($p < 0.05$). mRNA level of MuRF-1 and MAFbx, protein content of MuRF-1 and calpain-1 and the level of protein ubiquitination were increased only in HS group and did not differ in HSB from those in control group (C) ($p < 0.05$). The content of phosphorylated anabolic signaling system components (Akt/GSK3-β and mTOR/S6k/4E-BP1, as well as ERK1/2) was decreased equally in both suspended groups (HS and HSB) as compared to control (C) ($p < 0.05$). It could be concluded that the inhibition of proteasome during m.soleus unloading prevents the increase in the activity of some components of catabolic signalling pathways, and does not affect the anabolic signalling. This is not enough to reduce the rate of atrophic processes in the skeletal muscle. This work was supported by RFBR grant No. 14-04-01632.

S01.P19-178

Impact of AMPK phosphorylation on MyHCI(β) expression at the early stage of gravitational unloading

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Mechanical unloading of postural muscles results in both muscle atrophy and a slow to fast fibre type transition. The cause of such changes is a reduction in slow-type MyHC isoform expression and an increase in fast-type MyHC isoform expression. It is believed that calcineurin/NFAT signalling pathway and AMP-activated protein kinase (AMPK) are involved in the regulation of slow-type MyHC isoform. Previously we showed that AMPK phosphorylation is significantly decreased in rat soleus at the early stage of mechanical unloading. The aim of the study was to investigate the involvement of AMPK in the control of slow-type MyHC isoform expression in rat soleus muscle at the early stages of gravitational unloading. Wistar rats were subjected to 24-h hindlimb suspension (HS) being previously treated with AICAR (AMPK activator). The content of phospho-AMPK in rat soleus was determined by Western-blotting. The amount of MyHCI(β) pre-mRNA and mRNA was evaluated by RT-PCR. After 24-h HS we observed a 53% decrease ($p < 0.05$) in phospho-AMPK content vs. control group. 24-h unloading resulted in a 0.3-fold decrease in MyHCI(β) pre-mRNA expression vs. control group. An expression level of MyHCI(β) pre-mRNA in HS+AICAR group didn't differ from control. Besides, after 24-h HS we observed a 0.1 fold decrease in MyHCI(β) mRNA expression compared to control. At the early stage of unloading a decline in AMPK phosphorylation was accompanied by a decrease in the expression level of MyHCI(β) pre-mRNA. Under AICAR treatment a decrease in MyHCI(β) pre-mRNA and mRNA expression was not observed. Therefore, we can conclude that reduced AMPK phosphorylation at the early stage of unloading may be involved in the control of MyHCI(β) expression in rat soleus muscle. The study was supported by RSF grant # 15-14-00358.

S01.P20-106

Effects of electrical stimulation training on muscle atrophy and glucocorticoid signaling in colon 26 tumor bearing mice

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Cancer cachexia is a multifactorial syndrome characterized by severe skeletal muscle wasting, which is a powerful independent predictor of disease mortality. Recently, glucocorticoid signaling was shown to play a critical role in cancer-induced muscle atrophy. Here we investigated the effects of electrical stimulation (ES) training on skeletal muscle mass in a mouse model of cancer cachexia with a special focus on the role of glutamine synthetase (GS), a downstream regulator of glucocorticoid signaling. CD2F1 mice were divided into 4 groups: control (CNT), CNT+ES, C-26, and C-26+ES. Cancer cachexia was induced by a subcutaneous injection of colon 26 (C-26) cells. The ES training (60% of maximum torque, 50 Hz, 2 s on/4 s off, ~30 contractions) was performed unilaterally to the left triceps surae muscles every other day starting one day after injection of C-26. The locomotor activity was measured with an open field test two days before sacrifice. After four weeks, the weight of gastrocnemius (Gas) and soleus (SOL) muscles was decreased in C-26 group. These changes were accompanied by a marked increase in the expression of GS and reduced locomotor activity. ES training partially prevented the loss of muscle weight in SOL, but not Gas, in C-26 mice. Moreover, the GS expression in Gas muscles from C-26+ES group was lower than that in C-26 group, but was still higher than CNT group. In contrast, GS expression of SOL in C-26 mice was not affected by ES training. These findings suggest that cancer cachexia-induced muscle atrophy involves different mechanisms depending on fiber types. ES training-induced increased contractile activity could counteract the disuse-induced muscle catabolism in slow-twitch muscles, but it poorly inhibits glucocorticoid signaling in fast-twitch muscles.

Session 02 Molecular motor and Contractile function

Oral presentations

S02.O1

Myosin II-based native and synthetic machines

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In the striated muscle cell force and shortening are generated by two bipolar arrays of the motor protein myosin II in the myosin filament, during cyclical ATP-driven interactions with the actin filaments. As a collective motor, myosin II evolved specific mechano-kinetic properties that so far have been efficiently investigated only by preserving the three-dimensional myofilament lattice. Sarcomere-level mechanics with fast force-feedback and X-ray interferometry in single fibres from frog skeletal muscle (4 °C and 2.15 μm sarcomere length) show that the myosin working stroke elicited by a stepwise drop in force is 6 nm and takes 10 ms at high load and becomes larger and faster at lower load, attaining, at zero load, the crystallographic limit of ~11 nm (Reconditi et al. Nature 428:578, 2004). During steady shortening, motor ensemble kinetics provide that at high and moderate loads the number of motors scales in proportion to sarcomere load, so that each motor maintains a force of ~6 pN for a distance of ~6 nm, delivering 0.45 of the free energy of the ATP hydrolysis (Piazzesi et al. Cell 131:784, 2007). The apparent rate constant of motor detachment from actin increases in proportion to the shortening velocity up to ~2000 s-1. Values one order of magnitude smaller are found with single molecule mechanics (Capitanio et al., Nature Methods 9:1013, 2012), likely due to the lower than physiological [ATP]. Myosin II mechanics at physiological [ATP] can be studied in a sarcomere-like ensemble of motors by using a Dual Laser Optical Tweezers with a dynamic range 0.5–200 pN force and 1–10,000 nm movement (Bianco et al., Biophys J. 101:866, 2011). The myosin ensemble is brought to interact with an actin filament attached with the correct polarity to the trapped bead and up to five force-velocity points per interaction can be determined, demonstrating the ability of the synthetic bio-machine to define the power of native and engineered myosin II from striated muscle.

S02.O2-171

Myosin-induced structural changes in actin filaments during actomyosin based motion generation at different concentrations of MgATP

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Aims: Structural changes in the actin filament may be important in actomyosin based motion generation. We therefore tested whether myosin motor activity affects actin filament flexural rigidity (proportional to filament persistence length) and/or produces forces that tend to bend the filament.
Methods and Results: In vitro motility assays (IVMAs) were performed at 130 mM ionic strength and 30 degrees C using rhodamdine-phalloidin labelled actin filaments and heavy meromyosin (HMM) from fast rabbit myosin. The degree of winding of the actin filament paths was quantified by a path persistence length (Lpp), based on exponential fits to the cosine correlation function: $<\cos(\theta(s)-\theta(0))> = \exp(-s/[2*Lpp])$ (based on >200 paths) where s is the distance along the filament path and $\theta(s)$ is the tangent angle. There was a step-wise decrease of Lpp when [MgATP] was reduced below 0.2 mM, superimposed on a continuous decrease with lowered [MgATP] in the range 0.01 mM - 1 mM. This is consistent with Lpp being governed by thermal fluctuations of the myosin-free actin filament front, with reduced filament persistence length (Lpf) below 0.2 mM MgATP, and additional bending upon each myosin head attachment. Taking into account the reduced distance between actomyosin links at lowered [MgATP], quantitative analysis suggests that Lpf is 12.7±1.7 μm (mean±95 % CI) μm at [MgATP]>0.2 mM compared to 8.7±1.3 μm at lower [MgATP]. Furthermore, the analysis suggests 1.8±0.1 degrees angular change (root mean square) of the filament front upon each myosin head attachment, independent of [MgATP].
Conclusions: The results fit with structural changes in the actin filament during actomyosin based motion generation with the global actin filament structure being affected by the predominant actomyosin chemomechanical states.

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S02.O3-184

Age-dependent development of Cardiac Dysfunction in Transgenic Mice Carrying Actin E99K Mutation

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The pathogenic mechanisms underlying the apical hypertrophic cardiomyopathy (AHCM)-causing mutation E99K in cardiac actin gene

(ACTC) were probed. Skinned papillary muscle fibers (strips) from 2- and 5-mo old E99K transgenic mice were subjected to sinusoidal length perturbations to study tension transients, and the results were compared with those from age-matched non-transgenic (WT) mice. (1) At the standard activation (5 mM MgATP, 8 mM Pi, 200 mM ionic strength with K acetate, pH 7.00, 25°C), fibers from 5-mo E99K mice produced tension, stiffness, the rate of delayed tension (2πb), and magnitudes B and C, which were significantly less than those from 5-mo WT mice. In 2-mo mice, these differences were either significant (tension, stiffness, C), slight (B), or none (2πb). The rate of fast exponential advance (2πc) was not much different among 4 groups. (2) Tension and stiffness at rigor state were not significantly different among 4 groups. (3) pCa-tension study showed increased Ca sensitivity with aging in both E99K and WT groups, and pCa50 was slightly larger (~ 0.07 units) in E99K than in WT. A significant decrease in cooperativity (nH) was observed only in 5-mo E99K. The difference in pCa was larger in solutions that did not contain Pi (ΔpCa50=0.09, P=0.047) than that contained 8 mM Pi (ΔpCa50=0.04, P=0.091). (4) The ATP association constant (K1) increased with aging, and it was significantly larger in E99K than in WT; there were no differences in the rates (k2, k-2) of the cross-bridge detachment step. (5) The Pi association constant (K5) increased with aging, but there was little difference between E99K and WT; there was no difference in the rate of the force generation step (k4), but it reversal step (k-4) was slightly larger in E99K than in WT. We conclude that AHCM-causing ACTC E99K mutant resulted in progressive alterations in biomechanical parameters: changes were small at 2 months, but larger at 5 months, which correlates well with the development of AHCM.

S02.O4-219/S02.P1-219

Functional properties of αβ-tropomyosin with mutations in the α-chain are different from those of αα-tropomyosin with mutations in both α-chains

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Tropomyosin (Tpm) molecule can exist either as αα-Tpm homodimers or as αβ-Tpm heterodimers. We studied the effects of stabilizing substitutions in the central part of Tpm molecule on functional properties of αα-Tpm homodimers and αβ-Tpm heterodimers where only α-chain carried these mutations. Previously we showed that substitutions of non-canonical aminoacid residues Gly126 and Asp137 in the middle part of α-Tpm molecule by canonical Arg126 and Leu137 appreciably affect its structure and properties (Matyushenko et al., FEBS J., 2014). With an optical trap we measured the parameters of single interactions of skeletal myosin with regulated thin filaments containing Tpm and troponin at saturating Ca^{2+}. Stabilizing of the middle part of Tpm molecule did not affect step size and unitary force of myosin but decreased the step duration. Besides D137L/C190A and G126R/D137L/C190A mutations increased the duration of force events. The extent of thin filament activation was evaluated by dependence of their sliding velocity over myosin surface in an in vitro motility assay at pCa 4 on the myosin concentration. The sliding velocity of the filaments containing Tpm with stabilizing mutations was by 20–50% higher than that with control Tpm C190A. Myosin concentration required to achieve half-maximal velocity for thin filaments with with single mutations Tpms was half, and with double mutation more than 20-fold less than that for the Tpm C190A. Surprisingly, the stabilizing mutations in α-chain of heterodimers decreased the sliding velocity by 25–30% as compare to that of control αβ-Tpm. Functional properties of αβ-Tpm heterodimers with mutations in the α-chain are quite different from those of αα-Tpm homodimers with mutations in both α-chains.

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S02.O5-269/S02.P2-269

Muscle performance in response to resistance training is mainly controlled by an increase in myosin ATPase activity independent of the MHC shift

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It is generally believed that contractile and energetic properties of skeletal muscle rest mainly on myosin heavy chain (MHC) isoform content, that is primarily responsible for speed of contraction and myosin ATPase activity. Nevertheless, experimental evidences suggest that adaptive processes could occur directly at the myosin ATPase level in response to different stimuli such as endurance training, hypoxia and aging. Since resistance training is known to determine large changes in contractile activity, the aim of the present study was to detail the adaptations of the myosin ATPase activity independently of MHC isoform content, in response to resistance training, in rats. In purified myofibrils, ADP production over time was measured by Rapid Flow Quench method and High Performance Liquid Chromatography. MHC was measured by RT-qPCR. Beyond the discrete phenotypic shift from MHC1, MHC2X/2D toward MHC2A and the moderate fiber hypertrophy, a mean increase of $123 \pm 61\%$ in myosin ATPase activity was observed with the trained compared to the control group in the three muscles studied. Kinetics simulations with a simple scheme of the cross-bridge cycle suggest that adaptations responsible of the enhancement of the myosin ATPase activity come mainly from an increase in the rate constant of ADP and Pi release that occurs during the powerstroke, and to a lesser extent, from the ATP cleavage rate constant.

Posters

S02.P3-285

C-Terminal Truncation of Troponin I and Substitutions of Non-Canonical Residues in the Central Part of Tropomyosin 1.1 Disrupt Thin Filament Switched Off State in Rabbit Psoas Myofibrils

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The Ca^{2+}-dependent and highly cooperative activation of striated muscle contraction critically depends on the factors affecting Tpm-Tn

position on actin filament. Based on previously developed methods to remove/reconstitute striated muscle myofibrils with exogenous regulatory proteins [1], we investigate here the functional impact of the exchange of: (i) native fast skeletal Tn for human recombinant cTn containing either full length cTnI (cTnIFL) or truncated cTnI (cTnI1-192); (ii) native Tpm-Tn with purified skeletal Tn and recombinant Tpm1.1 either wild type (WT) or carrying one (D137L) or two (D137L/G126R) stabilizing substitutions in the central part of the molecule, decreasing its flexibility [2]. In myofibrils replaced with cTnI1-192 or D137L/G126R Tpm (15°C) force relaxation from maximal activation (pCa 4.5) to relaxing solution (pCa 9.0), was similarly prolonged (increased duration of slow phase, decreased rate of fast phase). Effects on maximal isometric tension and on rates of force activation (kACT) and redevelopment (kTR) were small (cTnI1-192) or absent (D137L or D137L/G126R Tpm). Both cTnI1-192 and Tpm substitutions strongly and additively decreased slack sarcomere length (sl) at sub-maximal activating [Ca^{2+}] and increased the steepness of the sl-passive tension relation. These effects were reversed by 10 mM BDM, suggesting that both cTnI1-192 and Tpm substitutions compromise the full inhibition of acto-myosin interactions in the absence of Ca^{2+}. This hypothesis is further supported by the significant increase of ATPase activity in relaxing solution of D137L and D137L/G126R Tpm myofibrils compared to WT Tpm. Data support the hypothesis that flexibility of the Tpm coiled-coiled structure critically modulates the turning off of the thin filament system and muscle relaxation dynamics, likely in interaction with the C-term of TnI.

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S02.P4-249

Ratio of fast and slow muscle myosin in tissue samples identified by kinetic analysis

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Cardiac myosin II exists as two isoforms - α (fast type) isoform and β (slow type) isoform. The isoforms are localised in different areas and ratios within the heart. In large mammals the atria contains approximately 90 % α with 10 % β whilst the ventricle contains approximately 85 - 90 % β with < 15 % α. During a failing human heart, the fraction of α-myosin in the ventricle falls dramatically and drug treatments that result in re-expression of α-myosin give some recovery of function. Kinetically the α-S1 has a 3 fold faster ATPase cycle and contraction velocity than the β-S1. In detail the hydrolysis step of the cross-bridge cycle is up to 10 time faster in α compared to β; the α ADP affinity for actoS1 is 10 times weaker and actin affinity is also weaker (>4 fold). Similar results are reported for both human and mouse α & β-myosin's. Preparations of cardiac myosin from tissue contain mixed isoforms and the two are difficult to separate. We report here a simple effective methods to establish the ratio of α and β myosin's (or S1) in a mixture by measuring the displacement of ADP from actomyosin in a Stopped Flow assay. ADP is displaced from actin·β-myosin at 48 s-1 whereas ADP is released at > 200 s-1 from actin·α-myosin. Thus displacing ADP from a mixture of the two myosin by an excess of ATP results in a biphasic reaction. The slow phase represents the amount of β-myosin while the fast phase reports the amount of α-myosin. In current systems the assay requires 125 μg of myosin and by miniaturising the system the assay could be performed with as little as 10 μg of myosin.

S02.P5-215

Effect of interchain disulfide cross-linking on the functional properties of tropomyosin

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Normally, the SH-groups of skeletal or cardiac α-tropomyosin (Tpm 1.1, onwards Tpm) are in a reduced state [Lehrer et al., J. Muscle Res. Cell Motil. 2011]. Disulfide cross-linking of the cardiac Tpm occurs upon human end-stage heart failure [Canton et al., J. Am. Coll. Cardiol. 2011]. It was shown with differential scanning calorimetry that this cross-linking increases the thermal stability of C-terminal part of Tpm [Kremneva et. al., Biophys. J. 2004]. We studied how disulfide bond affects the functional properties of Tpm and actin-myosin interaction using a number of approaches. With cosedimentation assay we found that the disulfide bond decreases affinity of Tpm for F-actin. Cross-linking decreased stability of Tpm–F-actin complexes as was shown by measuring the temperature dependence of dissociation of these complexes. In an in vitro motility assay the cross-linking increased the sliding velocity (Vmax) of regulated thin filaments containing F-actin, Tpm and troponin (Tn) at saturating Ca^{2+} concentration, with no appreciable effect on Ca^{2+}-sensitivity of pCa-velocity relationship. This effect highly depends on proteins, skeletal or cardiac, used in the motility assay. The most pronounced effect of the Tpm cross-linking on Vmax was observed with cardiac Tn, skeletal F-actin and cardiac myosin. This situation seems to be quite possible during cardiac hypertrophy where expression of α-skeletal actin is increased that leads to a partial replacement of cardiac α-actin isoform by skeletal one [Clement et al., Circ. Res. 1999]. Thus interchain cross-linking of SH-groups may have a significant effect on the functional properties of Tpm, and this can explain, at least partly, why this cross-linking is associated with human heart diseases.

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S02.P6-319

Alterations in actomyosin interaction and myofibrillar force under nitrosative stress conditions

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Aim: This study aimed to investigate the effect of peroxynitrite (ONOO)-derived nitrosative stress on actin and muscle contractile properties, and how it relates to muscle weakness.
Methods and Results: Nitration of myofibrils (M. psoas major, rabbit) was induced by addition of either ONOO or 3-Morpholino-sydnonimine chloride (SIN-1), and the myofibrillar forces were measured using an atomic force cantilever during activation of the myofibril (pCa 6.5 - pCa4.5). The maximal force was clearly lowered in myofibrils nitrated with either 150 uM ONOO or 10 mM SIN-1. Motility of actin was measured using an in vitro motility assay (IVMA). Nitration of isolated actin filaments were induced by addition of ONOO and were adsorbed to a heavy meromyosin coated nitrocellulose surface. The myosin-induced actin translocation was initiated by addition of 1 mM MgATP at different ionic strengths (IS). When tested at 40–130 mM IS, actin treated with

150 uM ONOO presented a sliding velocity (vf) of smooth moving actin (coefficient of variance (CV) <0.2) that was only 10–20% lower when compared to non-treated actin while actin treated with 1 μM ONOO exhibit similar vf (CV<0.2) as non-treated actin. The velocity distribution of all moving filaments (vf>0.25 μm/s, CV<0.5) shows an increase in population of slow moving filaments (<1.25 um/s) for ONOO-treated actin in relation to non-treated actin. At high IS (130 mM) this population increased from 30% (non-treated actin) to 40% (1 uM ONOO) and 70% (150 uM ONOO) while at low IS (40 mM) it increased from 55% (non-treated actin) to 75–80% of the total number of moving filaments for both ONOO concentrations.
Conclusion: The electrostatic interaction of actomyosin is altered by nitration of actin filaments. The contractile force of myofibrils is reduced by nitration. These results indicate that nitration induces muscle weakness by affecting the actomyosin interactions and the capability of myofibrils to produce force.

S02.P7-312

The extended plateau of the force-length relationship observed during fixed-end contractions is a result of sarcomere length non-uniformities

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Introduction: The shape of the descending limb of the force-length relationship in skeletal muscles is different when contractions are produced during fixed-end contractions or with clamped sarcomeres. Although the mechanism behind this difference is elusive, it has been proposed that it is associated with sarcomere length (SL) non-uniformities. The aim of this study was to investigate the force and contractile behavior of myofibrils with different numbers of sarcomeres along the descending limb of the force-length relationship.
Material and methods: Single myofibrils were isolated from the rabbit psoas, fixed to glass micro-needles, and activated at pCa 4.5 at different SLs. A video camera was used to capture the behaviour of sarcomeres during activation. SL non-uniformities along myofibrils were analyzed before, during and after the contraction, by computing differences among SLs developed over time. Myofibrils were divided in two groups for analysis: (1) myofibrils with 12 sarcomeres or less, and (2) myofibrils with more than 12 sarcomeres.
Results: The myofibrils produced less force in group 1 (38.15 nN/μm^2) than in group 2 (60.63 nN/μm^2). In both groups, a second order polynomial regression was used to fit the force decay in the descending limb of the force length relationship (r^2 ranging between 0.97 and 0.99). In group 1, force started to decay at a SL of 3.0um and reached zero at a SL of 4.2 μm, with a half-maximum force obtained at a SL of 3.8 μm (r^2 = 0.97). In group 2, force started to decay at a SL of 2.7um, and reached zero at a SL of 3.8 μm with a half-maximum force obtained at a SL of 3.5 μm (r^2 = 0.99). A larger non-uniformity of SLs was observed in group 2 (35% of sarcomeres) when compared to group 1 (20% of sarcomeres).
Discussion-Conclusion: The larger force and sarcomere length non-uniformity observed in group 2 suggests that the shape of the force length relation is partially dictated by SL non-uniformities.

S02.P8-237

Mechanical characterization of slow and fast isoforms of skeletal muscle myosin in situ

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Force and shortening in skeletal muscle are generated by ATP-driven cyclical interactions of the globular portion of the molecular motor myosin II emerging in array from the thick filament with the thin, actin-containing, filament. According to its functional task, skeletal muscle exhibits a large variation in the mechanical performances (maximum isometric force (T0), unloaded shortening velocity, maximum power output and efficiency) related to the expression of different myosin II isoforms. The study aims at defining the functional differences between myosin isoforms in situ by applying fast sarcomere-level mechanics to skinned fibres from rabbit skeletal muscle (sarcomere length 2.4 μm, temperature 12°C, 4% dextran T-500), either slow (soleus, expressing >95% type 1 myosin, T0 141 ± 13 kPa) or fast (psoas, expressing >95% type 2X myosin, T0 264 ± 8 kPa). Measurements of the half-sarcomere compliance (Chs) either in Ca^{2+}-activated fibres at T0 (39.5 ± 6.5 nm/MPa and 27.4 ± 2.5 nm/MPa in soleus and psoas fibre respectively) or in rigor (18.7 ± 0.3 and 26.8 ± 0.6 nm/MPa) show that: i, the myofilament compliance is ~15 nm/MPa in either fibre type; ii, the average strain per attached myosin motor at T0 is ± 3.3 nm for both myosin isoforms; iii, the fraction of motors at T0 is 0.46 ± 0.09 in soleus fibres and 0.29 ± 0.03 in psoas fibres; iv, the stiffness of the motor is 0.55 ± 0.12 pN/nm for the slow isoform, ~1/3 of that of the fast isoform (1.66 ± 0.17 pN/nm). Correspondingly, the force per motor of the slow isoform (1.8 ± 0.4 pN) is 1/3 of that of the fast isoform (5.4 ± 0.5 pN). These results suggest that the stiffness of the myosin motor is a determinant of the isoform-dependent functional diversity between skeletal muscles. Supported by MIUR-PRIN 2010R8JK2X and Telethon GGP12282 (Italy).

S02.P9-214

The effect of tropomyosin on the strength of actin-myosin contact

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Tropomyosin (Tpm) molecule lies in close proximity to myosin-binding site on actin filament and was shown to form hydrophobic and stereospecific interactions between actin, myosin and Tpm [Tobacman & Butters, 2000; Behrmann et al., 2012]. Here we studied the effect of Tpm on the strength of actin-myosin contact using an optical trap technique. For this 100 Hz sinusoidal oscillations of about 30 pN peak-to-peak amplitude were applied to one of two beads of dumbbell-like probe [Finer et al., 1994], formed of either F-actin or reconstructed thin filament (F-actin with Tpm and troponin complex) at saturated calcium concentration (pCa 4). In the absence of myosin head interaction with the filament the amplitude of oscillations of the both beads was almost equal. During the interaction, the oscillations of the bead in unmovable beam sharply decreased. We estimated the stiffness of the bond of myosin with F-actin or reconstructed thin filament by the ratio of the oscillation amplitude in attached and detached state. The stiffness with F-actin (1.97±0.08 pN/nm, mean±SEM; N = 32) was slightly less than that with reconstructed thin filament (2.28±0.08 pN/nm, N = 45). In the presence of Tpm the lifetime (235.1±13.7 ms) was two-fold that with bare F-actin (119.6±4.3 ms). The differences in the stiffness and lifetime values between F-actin and reconstructed thin filament are significant ($p < 0.01$, t-test). We have demonstrated that the presence of Tpm on actin filament affects the stiffness of actomyosin complex, possibly by mechanically strengthening the actin-myosin contact. This was also confirmed by an increase in the lifetime of the actin-myosin complex

in the presence of Tpm by application of the oscillation with amplitude of force exceeding force generating ability of myosin molecule.

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S02.P10-191

Inter-Sarcomere Dynamics within Skeletal Muscle Myofibrils

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Introduction: During myofibril activation and force development, there is a complex dynamics among sarcomeres arranged in-series. This inter-sarcomere dynamics is not well understood, which limits our understanding on how myofibrils produce force. To directly address this issue, we locally activated and controlled individual sarcomeres within a myofibril, allowing investigation of inter-sarcomere dynamics.
Methods: Isolated rabbit psoas myofibrils were attached between pre-calibrated micro-needles and tested at three initial average sarcomere lengths (SLi): (a) between 2.4 and 2.65μm, (b) between 2.65 and 2.9μm, and (c) above 2.9μm. A newly developed micro perfusion system was used to locally activate individual sarcomeres or groups of sarcomeres, while measuring the behavior of all sarcomeres in the myofibril.
Results: Local activation produced shortening of the target sarcomere, leading to a displacement of the adjacent sarcomeres towards the activation point. The spread of activation was continually damped, as it reduced from sarcomere to sarcomere towards the ends of the myofibril. Force produced by the myofibril, and the displacements of sarcomeres situated adjacent to local activation were larger at increasing SLi ((a) 1.01 ±0.03 μm, (b) 1.17 ±0.03μm, (c) 1.28 ±0.04μm). The displacement of adjacent sarcomeres was further increased when the myofibril was tested in rigor conditions (1.08 ±0.03μm) in comparison with relaxing conditions (0.92 ±0.04μm), suggesting that the interaction among sarcomeres is regulated by myofibril stiffness. Sarcomeres produced similar active forces (21.07 ±0.56nN/μm^2) within a myofibril at a fixed SLi.
Conclusion: Force produced by the local contraction of one sarcomere within a myofibril is spread to adjacent sarcomeres. The magnitude of this effect is dependent on SLi, suggesting a length dependent regulation of the inter-sarcomere dynamics.

S02.P11-161

Human cardiac and skeletal muscle contraction and myosin kinetics during foetal development

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Information on developing human skeletal and cardiac muscle is limited but is essential to understand muscle development and early-onset myopathies. Prior reports show weaker active force, slower activation/relaxation rates, and slower crossbridge cycling in foetal skeletal and cardiac muscle (Racca et al. ('13&'16) JPhys).

Muscle or myosin from different aged foetuses were characterized using myofibril mechanics, in vitro motility (IVM), and stopped-flow kinetics. Myosin isoforms were determined with mass-spec. Ventricle myosin crossbridge cycling slowed with age. Apparent ADP affinity (KAD) tightened almost linearly with age from 161 μM at 59 days to 98 μM at 100 days (average of −1 μM per day gestation for n = 8). This agreed with an age-related slowing in crossbridge cycling seen in IVM (Vmax 101d: 4.03 ± 0.62 μm/s; 134d: 1.98 ± 0.76 μm/s). ATP-induced actomyosin dissociation rates increased from 0.14 (s*μM)^-1 at 57 days to 0.22 at 100 days gestation. Similarly, ventricle myofibrils' force production rate was faster with increased age, as was slow-phase relaxation slope. Skeletal myosin was similar in cross-bridge cycling rate to same-age ventricle myosin (IVM 108d Vmax: 2.64 ± 0.02 μm/s), though the KAD was much greater (105d: 392 μM). KAD increased in skeletal myosin from 76 days (165 μM) to 105 days. ATP-induced actomyosin dissociation rate was faster at 76 days (0.43 (s*μM)^-1) than 105 days (0.22).

ADP affinity tightened in cardiac myosins during gestation, and ATP-induced actomyosin dissociation rate, which appeared to increase in cardiac myosin during gestation, indicated faster cross-bridge detachment in older samples (up to 105 days). Together these agree with the changes seen in prior experiments that indicate a shift in crossbridge cycling. Research of myosin heavy chain isoforms during early development is ongoing. Myosin-ATP interactions, in particular ADP release, vary widely between studied isoforms. Supported by MSCA-IF659534 to AWR.

S02.P12-82

ADP-stimulated contraction: a predictor of thin-filament activation in cardiac disease

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Background: Diastolic dysfunction is general to all hypertrophic cardiomyopathy (HCM) patients. Relaxation deficits may result from inappropriate formation of force-producing cross-bridges during diastole, due to changes in tropomyosin's position, which normally blocks actomyosin interactions in the absence of Ca^{2+}. We investigated if actomyosin blockade is altered in human disease. By measuring cardiomyocyte force-production in ADP-containing solutions (without Ca^{2+}), the ability of myosin-ADP to actin was assessed.
Methods: Force measurements were performed in single membrane-permeabilized cardiomyocytes at sarcomere length of 2.2 μm in the absence of Ca^{2+}, but in the presence of mM levels of ADP. Exogenous protein kinase A (PKA)-treatment was performed to determine whether myofilaments are sensitive to kinase treatment. Cardiac samples used were harvested from HCM patients, harboring thick- (MYH7mut, MYBPC3mut) and thin-filament (TNNT2mut, TNNI3mut) mutations, and were compared with sarcomere mutation-negative HCM (HCMsmn) and non-failing donors.
Results: Myofilament ADP-sensitivity was higher in HCM samples compared with donors, except for MYBPC3mut. Increased ADP-sensitivity in HCMsmn and MYH7mut was caused by low phosphorylation of myofilament targets, as it was normalized to donors by PKA. In MYBPC3mut samples, ADP sensitivity highly correlated with cMyBP-C protein level. Incubation of failing cardiomyocytes with cMyBP-C antibody against the thin-filament binding N-terminal

region reduced ADP-sensitivity. Troponin exchange experiments of the TNNT2mut sample corrected the abnormal actomyosin blockade.
Conclusions: Our data provides a mechanism of how phosphorylation alterations and/or expression of mutant proteins increase actomyosin interactions, that precede Ca^{2+} rise, and limit diastolic relaxation in HCM.

Session 03 Mitochondrial Dynamics

Oral presentations

S03.O1

Mitochondrial morphology-function dynamics and signaling in skeletal muscle

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Mitochondrial morphology and cristae ultrastructure are dynamic aspects of mitochondrial biology. Morphology changes are mechanistically linked to changes in mitochondrial functions. Beyond energy production, mitochondria regulate cytoplasmic calcium levels, generate reactive oxygen species, and release metabolic intermediates involved in intracellular signaling. In this presentation, we will review evidence that (i) skeletal muscle mitochondria undergo dynamic changes in morphology in response to exercise and inactivity in vivo; (ii) that genetic mitochondrial defects in patients with mitochondrial disease cause profound ultrastructural abnormalities, some never previously reported in humans tissues; and (iii) that mitochondrial dysfunction in mice cause broad alterations in the nuclear transcriptome and myofiber phenotype. These studies integrating electron microscopy, muscle physiology, and transcriptomics reveal a role for mitochondrial bioenergetics in regulating not only skeletal muscle adaptation, but also whole-body responses to stressors of various nature.

S03.O2-317

HACD1 and very long chain fatty acids: new structural and functional actors in muscle growth and metabolism

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Introduction: In dogs, humans and mice, loss-of-function mutations in the HACD1 gene cause congenital myopathies characterized by muscle weakness and exercise intolerance. HACD1 participates in the elongation of structural and regulatory very long chain fatty acids (VLCFA, $C \geq 18$).
Results-discussion: We recently proved that HACD1 function is required for normal muscle growth through the regulation of myoblasts membrane composition and fluidity, promoting myoblast fusion. Skeletal muscles are also important regulators in whole-body energy metabolism. Because HACD1 deficiency leads to a reduction in muscle mass, we hypothesized that Hacd1-knockout (KO) mice have reduced insulin sensitivity. Surprisingly, Hacd1-KO mice exhibited higher glucose tolerance and insulin sensitivity, both in normal and high fat diet conditions. Moreover, during high fat diet and despite eating more food, Hacd1-KO mice were resistant to obesity, with reduced weight gain and fat accumulation. Analysis of individual muscle fibers from Hacd1-KO mice revealed increased levels of mitochondrial beta-oxidation of fatty acids and uncoupled respiration, suggesting that the overingested fat is partially dissipated through an increased catabolic activity consuming increased levels of lipids and glucose. Lipidomic analysis revealed altered levels of mitochondria-specific lipid species, presumably impairing inner membrane organization and thus, function of the respiratory complex. In parallel, observation of mitochondrial morphology by electron and 3D confocal microscopy revealed disorganized cristae and fragmented mitochondrial network.
Conclusion: Taken together, our results demonstrate that HACD1 plays a major role in muscle growth and metabolism, and suggest that VLCFA are necessary for proper myoblast fusion, as well as mitochondrial structure and efficiency. Besides, the potential of a protective effect of HACD1 deficiency towards diet-induced obesity and insulin resistance is being explored.

S03.O3-199 / S03.P1-199

Mitochondrial morphology changes in skeletal muscle fibers correlate with sarcopenia symptoms in aged-mice.

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Aim: Sarcopenia is the degenerative loss of muscle mass and strength with aging. Within this context, although a role of mitochondrial metabolism and muscle function in the development of many diseases have been described, the role of mitochondrial dynamics in the process of muscle aging has not been studied. This work describes the timeline of mitochondrial morphology changes in skeletal muscle during the process of aging.
Materials and methods: Isolated muscle fibers from mouse flexor digitorum brevis (FDB) were obtained by enzyme digestion of the muscle. Muscle transfection was performed by intramuscular plasmid injection and electroporation of a Mito-Ds-red plasmid. Gene expression was assessed by Real time PCR. Images were taken by confocal microscopy and analyzed using ImageJ. Mitochondrial Ca^{2+} was measured after MitoPericam electroporation in a Spinning Disk microscope. Cytosolic Ca^{2+} was measured using Fluo 4-AM.
Results: Body weight and physical parameters were measured in mice grouped by age in 4 groups, 6–8 weeks old, 6–9 months, 10–14 months and 16 or more months to asses the development of sarcopenia over time. Strength was significantly decreased at age 16–21 month, weight increased in the group of 10–14 months and then significantly decreased at 16–21 months. Regarding mitochondrial morphology more fused mitochondria were observed in the group of 10–14 months, accompanied by an increase of intermiofibrilar mitochondria perpendicular to the axis of the fiber. Together with the physical changes, ATP stimulus induced cytosolic and mitochondrial Ca^{2+} transients which intensity decreases starting at age 10–14 months.
Conclusions: Our results show that there is a turning point in life where skeletal muscle loss starts correlating with a modification in mitochondrial morphology and a decrease in mitochondrial calcium uptake.
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S03.O4-147

Serine/Threonine Protein Kinase 25 (STK25) Regulates Ectopic Lipid Accumulation, Mitochondrial Function and Insulin Sensitivity in Skeletal Muscle

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Type 2 diabetes (T2D) is strongly associated with ectopic lipid deposition within nonadipose tissue, which contributes to the development of insulin resistance. Skeletal muscle plays an important role in the pathophysiology of the T2D accounting for >70% of glucose utilization. Thus, approaches that suppress ectopic lipid deposition within the skeletal muscle, and increase the responsiveness of muscle to insulin, offer the potential for the development of new anti-diabetes therapies. In the search for novel targets that contribute to the pathogenesis of T2D, we recently described STK25 as a central regulator of ectopic lipid accumulation, whole-body glucose and insulin homeostasis. We found that high-fat-fed Stk25 transgenic mice develop liver steatosis, reduced whole-body glucose tolerance, and impaired insulin sensitivity compared to wild-type littermates. Reciprocally, Stk25 knockout mice are protected against high-fat diet induced liver steatosis, systemic glucose intolerance and insulin resistance compared to wild-type littermates. The aim of this study was to assess the role of STK25 in the control of ectopic fat storage and insulin sensitivity in skeletal muscle. We found that high-fat-fed Stk25 transgenic mice display increased lipid content and impaired mitochondrial function in skeletal muscle. Correspondingly, high-fat-fed Stk25 transgenic mice have reduced endurance exercise capacity and repressed muscle insulin responsiveness. Global phosphoproteomics revealed alterations in the total abundance and phosphorylation status of different target proteins primarily located to mitochondria and the contractile elements of the sarcomere, respectively, in Stk25 transgenic vs. wild-type muscle, providing a possible molecular mechanism for the observed phenotype. In summary, our results suggest that STK25 is a new interesting regulator of the complex interplay between intramyocellular lipid storage, mitochondrial energetics and insulin action in skeletal muscle.

S03.O5-120/S03.P2-120

Mitochondrial function in diaphragm muscle fibers of mechanically ventilated critically ill patients

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Background: Diaphragm weakness in mechanically ventilated (MV) intensive care (IC) patients contributes to weaning failure and an increased duration of hospital stay and morbidity. Weakness is caused by atrophy of muscle fibers, due to increased proteolysis. Studies in animal models and in brain dead organ donors indicate that MV-induced mitochondrial dysfunction and oxidative stress are upstream regulators of proteolysis. Whether mitochondrial dysfunction and oxidative stress play a role in the pathophysiology of diaphragm weakness in IC-patients is unknown.
Aims and objectives: To gain insight in the role of the mitochondria and oxidative stress in diaphragm muscle atrophy and weakness.
Methods: Muscle fibers isolated from diaphragm biopsies of IC-patients (n = 35) and control subjects (n = 28) were analyzed by immunohistochemistry, electron microscopy, Western blotting, respirometry, and single fiber force measurements.
Results: Absolute maximal force and cross sectional area of diaphragm muscle fibers were decreased in IC-patients compared to controls, respectively, 0.34 ± 0.04 vs. 0.56 ± 0.05 mN and 2630 ± 232 vs. 3995 ± 380 μm^2. Thus, IC-patients exhibited marked weakness of diaphragm fibers. Oxidative stress markers nitrotyrosine and HNE were decreased by 60 and 23% in IC-patients compared to controls. Mitochondrial respiration was comparable between groups, but mitochondrial complex proteins were decreased by 29% in IC-patients. Subtle changes in mitochondrial morphology were observed and PGC1-α was decreased by 23% in IC-patients, whereas the ratio of pAMPK/total AMPK was increased by 63%. Compared to controls, mitochondrial fusion proteins Mfn1, Mfn2 and OPA1 were decreased in IC-patients, respectively 17, 13 and 13%, whereas mitochondrial fission protein DRP1 was increased by 21%.
Conclusion: Diaphragm fibers of IC-patients exhibit significant contractile weakness and atrophy in the absence of both oxidative stress and impaired mitochondrial respiration.

Posters

S03.P3-326

Hacd1 regulates mitochondrial network morphology and cristae structure

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Introduction: In dogs, humans and mice, loss-of-function mutations in the HACD1 gene cause congenital myopathies. HACD1 participates in the elongation of very long chain fatty acids (VLCFA, C≥18).
Results-discussion: Using our knockout mouse model (Hacd1-KO), we proved that despite a reduction in locomotor activity and an increase in food intake, deficient mice exhibited a reduced weight gain and fat accumulation. Analysis of Hacd1-KO muscle fibers revealed that mitochondrial beta-oxidation of FA and uncoupled respiration were markedly elevated, suggesting that an increased catabolic activity consumed higher levels of lipids and glucose, partially compensating the over ingested fat. Lipidomic analysis revealed altered levels of mitochondria-specific lipid species, putatively impairing the inner membrane organization and hence, function of the respiratory complex. In parallel, electron microscopy showed disorganized cristae and a reduction in the size of mitochondria. In order to precisely characterize the mitochondrial network, we performed a 3D fluorescence-based microscopy on individual muscle fibers. Myofibers from Hacd1-KO mice displayed mitochondrial fragmentation

associated with a reduction in large mitochondria (>2 μm^3) and an increase in small mitochondria (<0.5 μm^3). Interestingly, the observed morphological impairement was correlated with an unchanged expression of the main regulators of mitochondrial dynamics such as mitofusins 1 and 2, OPA1, DRP1 and FIS1. We thus hypothesize that a lower VLCFA content rather induces a modification in lipid composition of mitochondrial membranes, promoting fragmentation of the network. We are now investigating the physical properties of artificial lipids vesicles and mitochondrial membranes purified from Hacd1-KO mice.

Conclusion: Taken together, our results demonstrate that HACD1 plays a major role in muscle metabolism, at least through a VLCFA-dependent mechanism regulating proper mitochondrial structure and efficiency.

S03.P5-330

Role of RNA-binding proteins in the pathophysiology and the molecular mechanisms of oculopharyngeal muscular dystrophy (OPMD)

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Oculopharyngeal muscular dystrophy (OPMD) is a late onset disease characterized by progressive degeneration of specific muscles, leading to eyelid dropping, swallowing difficulties and limb weakness. Alanine expansions in the coding region of poly(A) binding protein Nuclear1 (PABPN1) resulting from GCG trinucleotide repeat extensions lead to the dominant autosomal inheritance of OPMD. In skeletal muscle fibers of OPMD patients, mutant PABPN1 protein aggregates as fibrillar nuclear inclusions, which are the hallmark of the disease. We have generated a Drosophila model that recapitulates muscular defects with similarities to those of OPMD (Chartier A. et al., EMBO 2006). Using this model we have shown that poly(A) tail length of mRNAs encoding mitochondrial proteins are shortened in OPMD flies and expressed at low level. This leads to mitochondrial dysfunction that could explain the earliest stages of disease progression and might be the first molecular defect in OPMD patients (Chartier A., Klein P. et al., PLOS Genet 2015). mRNA stability is controled by large RNA-binding protein complex such as P-bodies or stress granules. Among genetic suppressors of OPMD phenotype in flies, several encode RNA-binding proteins. We are now addressing the implication of these different RNA-binding proteins in OPMD.

S03.P6-149

Overexpression of Protein Kinase STK25 in Mice Exacerbates Ectopic Lipid Accumulation, Mitochondrial Dysfunction and Insulin Resistance in Skeletal Muscle

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Type 2 diabetes (T2D), characterized by hyperglycemia in the context of insulin resistance, affects at least 285 million individuals worldwide, and its prevalence is rapidly increasing. Understanding the molecular networks controlling ectopic lipid deposition and insulin responsiveness in skeletal muscle is essential to developing new pharmacological strategies to treat T2D. In the search for novel targets that contribute to the pathogenesis of insulin resistance and T2D, we recently described serine/threonine protein kinase 25 (STK25), belonging to STE20 superfamily of kinases, as a central regulator of ectopic lipid accumulation in the liver, whole-body glucose and insulin homeostasis. The aim of this study was to assess the role of STK25 in the control of ectopic fat storage and insulin sensitivity in skeletal muscle. We found that muscle of Stk25 transgenic mice displayed a lower α-oxidation rate compared to wild-type littermates. Notably, skeletal muscles isolated from both genotypes display similar fatty acid influx and incorporation of fatty acids into triacylglycerol. To assess the physiological impact of the reduced oxidative capacity observed in Stk25 transgenic muscle, we compared the endurance exercise performance of high-fat-fed Stk25 transgenic and wild-type mice by treadmill running. A markedly reduced exercise performance was found in Stk25 transgenic mice compared with wild-type littermates. Furhtermore, euglycemic-hyperinsulinemic clamp experiments with a glucose tracer were performed in high-fat-fed Stk25 transgenic and wild-type mice. Insulin-stimulated glucose uptake was significantly lower in skeletal muscles of Stk25 transgenic mice. In conclusion, our results suggest that STK25 is a new interesting regulator of the complex interplay between intramyocellular lipid storage, mitochondrial energetics and insulin action in skeletal muscle, highlighting the potential of STK25 antagonists for the treatment of T2D and related metabolic disease.

Session 04 Excitation contraction coupling in cardiac muscle

Oral presentations

S04.O1

A new excitation-contraction coupling «super-hub» mechanism in atrial myocytes

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Sarcolemmal membrane invaginations called transverse tubules (TTs) form electrochemical signaling conduits in myocytes with the junctional sarcoplasmic reticulum essential for excitation-contraction (EC) coupling. However, the exact role of sparse TTs in atrial myocytes (AM) and differences between species have been controversial, and their role in EC coupling remains unclear. We identify abundant axial membrane tubules (ATs) in 100% of AM. ATs represent deep intracellular branches connected with rare TTs. Using live cell STimulated Emission Depletion (STED) superresolution microscopy, we show that atrial ATs are significantly larger compared to TTs. To test if ATs function as central-axial Ca^{2+} signaling hubs during EC-coupling, we combined intracellular Ca^{2+} transient (CaT) imaging. High-amplitude CaTs originated directly from ATs and local onset occurred significantly earlier compared to the surface membrane. To explain the CaT heterogeneity, we performed in situ Ser-2808 and Ser-2814 phospho-epitope mapping of ryanodine receptor (RyR2) Ca^{2+} release channels. Junctional RyR2 clusters at ATs showed selectively increased phosphorylation levels in unstimulated AM. Sarcomere shortening experiments confirmed significantly larger and faster AM contractions compared to ventricular myocytes.

Genetic ablation of RyR2 phosphorylation in Ser-2808-Ala knockin mice significantly attenuated atrial sarcomere shortening and in vivo atrial but not ventricular contractile function. Our data suggest a fundamentally novel atrial EC-coupling model where AT structures with unprecedented dimensions function as signaling super-hubs. Thereby, central-axial AT structures couple rapid Ca^{2+} signaling with contractile myofilament activation. Genetic ablation of RyR2 cluster phosphorylation unmasks a previously unknown role of RyR2 phosphorylation, which may explain atrial function in health versus characteristically decreased atrial contractility in hypertrophy and heart failure.

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S04.O2-133

Meis1 regulates sympathetic target-field innervation: consequences for autonomic nervous system induced sudden cardiac death

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Background: Sudden cardiac death (SCD) are among the leading causes of premature death in the general population. Genome-wide association studies identified the transcription factor Meis1 as a risk factor for SCD and Meis1 is implicated in shaping heart morphology and in cardiomyocytes proliferation. Here, we hypothesise that Meis1 is also implicated in cardiac nervous system development and heart rhythm control.

Methods and Results: We report that specific Meis1 inactivation in mouse sympathetic neurons (HtPACRE/Meis1LoxP/LoxP) leads to SCD independently of cardiac structural defect. We showed that Meis1 is implicated in the development of cardiac sympathetic neurons, in particular in NGF/TRK1 trafficking. Using telemetric system, we record electrocardiograms in baseline condition, and after either pharmacological testing of autonomic nervous system or treadmill exercise. By heart rate variability analysis, we show that mice developed impaired sympatho-vagal regulation of cardiac rhythm. Mice exhibited atrial and/or atrioventricular conduction defects that led to spontaneous bradycardia and desynchronization, concomitant with a high occurrence of sinus arrests. Pharmacological testing revealed that mutant mice were intolerant to carbamylcholine injection which induces death in 40% of HtPACRE/Meis1LoxP/LoxP mice and, as well as to exercise tests on treadmill. During exercise, the RR decreased by 45% in WT mice ($p < 0.01$, $n = 8$), whereas a non-significant and delayed 13% decrease in the RR interval was observed in mutant mice. The maximal RR decrease in WT mice was 77±8 vs.104±3ms in mutant mice. During the recovery phase, 3 out of 4 mutant mice developed ventricular fibrillations and died.

Conclusion: Mutant mice presented profound alterations in the sympatho-vagal regulation of cardiac functions that are independent of cardiac structural phenotype, arguing for an essential role of the transcription factor Meis1 in the sympathetic nervous system development and function.

S04.O4-337 / S04.P1-337

A Mechanism of Ca^{2+} Calmodulin Regulation of the Human Cardiac Sodium Channel

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Introduction: The human cardiac sodium channel (NaV1.5) is responsible for the initial upstroke of the action potential and essential to heart function. Genetic mutations causing channel dysfunction are associated with the life threatening cardiac conditions Brugada and Long QT syndromes. Despite much investigation, successful treatment options for patients suffering from NaV1.5 dysfunction are lacking. In-depth understanding of the molecular mechanisms of channel function and regulation provides a powerful means to identify and develop novel therapeutic targets and improvements to existing treatments. To this end, we have undertaken studies of the binding of the Ca^{2+} sensing regulatory protein calmodulin (CaM) to the NaV1.5 channel inactivation gate.

Results: We discovered a previously unrecognized high affinity interaction and generated a high-resolution structural model using a combination of X-ray crystallography, NMR spectroscopy and small angle X-ray scattering. Ca^{2+} activated CaM is found to bind to two independent sites on the channel inactivation gate in an unanticipated domain configuration. The structure enabled predictions of the mechanism of malfunction for certain disease associated mutations contained within the NaV1.5 inactivation gate. Our predictions were tested using NMR analyses, which confirmed perturbations of the interaction with CaM. Our results provide a rationale and molecular mechanism for Ca^{2+} CaM modulation (Calmodulation) of NaV1.5, and sets the stage for evaluating the therapeutic potential of targeting this key regulatory interaction.

S04.O3-141

Super resolution imaging identifies fibrosis of the transverse tubules in human heart failure

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Pathological remodeling of the transverse tubules is a common feature of several heart failure etiologies. This remodeling is thought to disrupt cardiac junctions, a nano- structure essential for excitation contraction coupling, whose damage results in poor myocyte contractility. Literature studies on what drives transverse tubule remodeling have focused on changes in intracellular proteins that align the sarcolemma with the sarcoplasmic reticulum (Ca^{2+} store). However, our data from humans indicate that extra-cellular-matrix

(ECM) proteins may also be involved. Previously, only type IV collagen has been reported within the t-tubules. However, super resolution microscopy demonstrates that type I, III, IV and VI collagens are all present within the t-tubules and dramatically increase in width within the dilated t-tubules in dilated cardiomyopathy. Of particular note are the changes in distribution of the rarely studied type VI collagen. In non-failing ventricular tissue type VI collagen is largely located at the basement membrane with little interstitial labelling. However in heart failure there is a substantial accumulation within the interstitium. Interestingly, mutations in type VI collagen can cause Bethlem myopathy, and Ullrich congenital muscular dystrophy indicating an important role in muscle. We hypothesize that fibrosis as exemplified by collagen accumulation drives t-tubule remodeling in heart failure.

S04.O5-335

Aberrant biophysical properties prevented by stabilizing the RyR2 closed state and not standard β-adrenergic receptor blockade in patient-specific CPVT induced pluripotent stem cell-derived cardiomyocytes

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a highly lethal inherited arrhythmogenic disorder predominantly caused by mutations in cardiac ryanodine receptor gene (RYR2). Human induced pluripotent stem cells (hiPSCs) offer a unique opportunity for disease modeling. The goals were to derive functional cardiomyocytes (CMs) from CPVT patient via hiPSCs and test whether the novel CPVT1 mutation is associated with abnormal intracellular Ca^{2+} handling properties in CMs. Human iPSCs were generated from dermal fibroblasts from a young athletic female diagnosed CPVT resistant to metoprolol and carrying a novel heterozygous point mutation RyR2-D3638A located in a highly conserved hot-spot region. Following molecular characterization, healthy control (HC-) and CPVT-hiPSCs were differentiated into CMs. Using confocal microscopy and atomic force microscopy, their intracellular Ca^{2+} handling and mechano-biological properties were studied in resting and stress conditions. HC- and CPVT-hiPSCs expressed pluripotency markers (OCT4, NANOG, SSEA4) and had normal karyotype. Derived CMs via embryoid body (EB) formation showed typical cardiac markers such as cardiac troponin T and I, and α-actinin. At rest, there was no significant difference in any property of the spontaneous Ca^{2+} transients between HC and CPVT hiPSC-CMs while CPVT-EBs exhibit higher beat rate. Significant differences in the kinetic properties of Ca^{2+} transients as well as in the mechano-biological properties were observed under stress in agreement with the arrhythmias only induced under stress. Interestingly, higher PKA phosphorylated RyR2 at rest was observed in CPVT-EBs as well resistance to the β-adrenergic receptor blocker metoprolol in agreement with the clinical observations. Stabilizing RyR2 with a new Rycal® compound tested in clinical trials prevents those abnormalities specifically in CPVT hiPSC-CMs with no effect in HC hiPSC-CMs. Our results indicate that the novel RyR2-D3638A mutation causes sarcoplasmic reticulum Ca^{2+} leak associated with PKA hyperphosphorylation, Calstabin2 (i.e., FKBP12.6) depletion and abnormal mechano-biological properties that cannot be prevented by standard β-adrenergic receptor blockade but by stabilizing the closed state of RyR2. Our study promotes hiPSC-CMs as a suitable tool for CPVT disease modeling and testing new therapeutic compounds.

Posters

S04.P3-137

Spin-spin proton transverse relaxation times study of contraction-relaxation cycle in rat heart with experimentally hyperthyroidism

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Aim: To point out early changes in left ventricle in experimental induced hyperthyroidism(HT) using 1H NMR method for studying contraction(Co)-relaxation (Re) cycles, including equations of proton transverse relaxing times(T2) dependence on (Co) and (Re) and on extracellular [Ca+2]. By means of these equations it is possible to deduce the dynamics of intracellular protons during cardiac cycles in Control and (HT) rat heart if, the interval of time of (Co) and (Re) is known from EKG recordings.
Material and method: We used 12 adult male rats: 6 Control(C) and 6 treated (HT) with Thyroxin (i.p. 4.5mg/ kg b.w.) for 4 weeks The study includes contraction(Co)- relaxation (Re) sequences; both (Re) and (Co) cycles have been monitored at different external [Ca+2] between 0.5 2 mM. Left ventricle fragments from (C) and (HT) were kept in (Co) solution for 20–80 s and in (Re) solutions for 100 seconds. T2 measurements were donewith an Aremi Specrtometer at 25 MHz frequency using the standard sequence Carr-Purcell-Meiboom-Gill with aninterval between impulses of 8 ms.
Results: The increase in T2 in (HT) in (Re) is 5 times lower, than in(C). At 1 mM [Ca+2] in extracellular environment the highest speed of (Re) was recorded, both in (C) and in (HT). In (C) and in (HT) there is an increase in T2 in (Co) associated with amplification of [Ca+2] inductor of (Co), in both cases being present an exception: in (C) the lengthening times of (Co) is at (80 s) and in (HT) is at (40 s). The dynamics of contraction state described by T2 parameter is also deteriorated in (HT).
Conclusion: T2 parameter is a marker of myocyte physiology state which brings information about the proton exchange through myocyte membrane, related with permeability for water. High contractility in (HT) is associated with a decrease in permeability for water in myocytes induced by [Ca+2] and this may constitute a risk factor for installing diffuse ischemia

S04.P4-114

Can contractile function of myofibrils within human stem cell-derived cardiomyocytes substitute the one of myofibrils determining the pump function of the adult human heart?

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Aim: We highlighted the similarities and potential differences of beta-myosin heavy chain isoform-driven cycling cross-bridges determining the contractile function in two force-developing models in which subcellular myofibrils were generated either in vitro by human embryonic stem cell differentiation into cardiomyocytes (hESC-CMs) or in vivo, in an adult human ventricle.
Method: To observe contractile function independent of intracellular calcium (Ca) transients and of upstream cascade mechanisms modulated by hormonal activities, single demembranated hESC-CMs and adult human ventricular myofibrils bundle were directly exposed to Ca-defined identical solutions using the same micromechanical setup and we have compared steady-state and kinetic parameters from their axially-measured contractile force recordings.
Results: At maximum Ca-concentration, both contractile models developed force with similar kinetics, while at submaximal Ca-concentration, some force parameters exhibited differences which are interpreted using cross-bridge modeling. Treatment with protein kinase A did not alter cross-bridge kinetics in both contractile models, even if it changes calcium-sensitivity of isometric force.
Conclusion: These outcomes indicate the importance of biomechanical investigations at sarcomeric level of the contractile function generated by human-derived cardiomyocytes differentiated in vitro and can provide the basis for extended studies to patient-specific human pluripotent stem cell-derived cardiomyocytes carrying familiar hypertrophic cardiomyopathy-related missense mutations in sarcomeric proteins. Understanding the factors that affect contractility and promote the cardiomyocyte maturation is important for human stem cell-derived cardiomyocytes to be further used as cellular disease models, to assess novel pharmacological or genomic interventions targeting heart diseases and for clinical interests in cells transplantation.

S04.P5-261

Positive inotropic effect of IL-13 on heart is AMPc-PKA-dependent

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IL-13 is a cytokine produced during sepsis, but its pro- or anti-inflammatory effects, especially on the heart are still not clear. The aim of this study was to clarify the impact of IL-13 on heart contraction, and on voltage-dependent Na+ channels NaV1.4 and NaV1.5 which are responsible of the membrane excitability and essential for excitation/contraction coupling.

For this study, rat hearts were perfused ex vivo in a Langendorff system with IL-13 at 10ng/ml during 30min with or without inhibitors. Contractile force, heart frequency and coronary flow were recorded. Expression of NaV1.4 and NaV1.5 was analysed by western blot after membrane and cytosol protein extraction from ventricular cells. IL-13 induced an increase of the contractile force (+28.3%), and both in maximal speeds of contraction (+35.5%) and relaxation (+38.9%), but it had no effect on heart frequency and coronary flow. By using PKA or Adenyl cyclase (Ac) inhibitor we have shown that IL-13 acted by a pathway involving Ac-AMPc-PKA activation. The hearts perfused with IL-13 had more NaV1.4 (+37.4%) and NaV1.5 (+52.2%) at the membrane level. In addition, the ratios of membrane/cytosol proteins were also increased too after IL-13 perfusion for NaV1.4 (+281.4%) and NaV1.5 (+214.4%), when compared to hearts perfused without the cytokine. Moreover membrane targeting was abolished with Ac inhibition for both channels. Here we demonstrate that IL-13 has a positive inotropic effect on perfused heart. This cytokine can increase NaV1.4 and NaV1.5 membrane targeting by Ac-PKA pathway, and then increase membrane excitability. Activation of the Ac-PKA pathway can also have a stimulating effect on the calcium channels involved in excitation/contraction coupling mechanism.

S04.P6-162

Sarcoplasmic Reticulum Ca^{2+} leaks and mitochondrial reactive oxygen species: the auto-amplification loop?

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Part of the Ca^{2+} released by the Sarcoplasmic Reticulum (SR) through the type-2 ryanodine receptor (RyR2) is taking up by the mitochondria to favor ATP production. Although, mitochondrial Reactive Oxygen Species (ROS) production may affect RyR2 open probability, the consequences of a primary RyR2 dysfunction on mitochondrial metabolism remain unknown.
Aim: Thus, the aim of the present study was to determine if an increase in SR Ca^{2+} leak alters mitochondrial function.
Methods and Results: To answer this question, we used a model of calstabin2 (FKBP12.6) deficient mice, a small protein that stabilizes RyR2 close state. Under confocal microscope we observed that calstabin2 deficiency increases diastolic SR Ca^{2+} leak (Ca^{2+} sparks) as well as Ca^{2+} transients amplitude without any change in SR Ca^{2+} load. This altered Ca^{2+} homeostasis is associated with a progressive increase in RyR2 oxidation and open probability. On the other hand, the maximal respiration capacity and the mitochondrial content were increased. However the dynamic mitochondrial calcium movement, recording under whole cell patch-clamp technique, was reduced and mitochondrial ROS production measured with MitoSox red was enhanced.
Conclusion: In summary, our data demonstrate the existence of an auto-amplification loop between SR Ca^{2+} leak and mitochondrial ROS production: altered RyR2 function disturb mitochondrial ability to take up Ca^{2+}, increasing mitochondrial ROS production on a beat-to-beat basis and finally favoring RyR2 oxidation.

Session 05 Muscle development and aging

Oral presentations

S05.O1

Role of Wnt signalling in regulating satellite cell function

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Skeletal muscle is an archetypal adult stem cell model, in which maintenance, growth and repair of functionally specialised post-mitotic cells is achieved by recruitment of undifferentiated precursors. The routine needs for myonuclear turnover, together with the more

sporadic demands for myofibre hypertrophy and repair, are performed by muscle satellite cells. These resident stem cells are normally mitotically quiescent in mature muscle, and so must first be activated to undergo extensive proliferation to generate myoblasts that eventually differentiate to provide new myonuclei. Satellite cells must also self-renew to maintain the stem cell population. We have been modelling satellite cell-mediated myogenesis ex vivo using 3D scaffolds and found that three biomaterials in particular were useful for creating such scaffolds: collagen, polyethylene glycol-fibrinogen hydrogel and fibrin. We are also investigating signalling pathways that control satellite cell function with an emphasis on Wnt signalling (e.g. Figeac and Zammit (2015) Cell Signal. 27, 1652–1665). The canonical Wnt pathway acting through β-catenin promotes myogenic differentiation in satellite cells. Canonical Wnt signalling is controlled at many levels, including regulation of β-catenin degradation via the proteasome, with Axin1 and Axin2 being important scaffold proteins for the destruction complex. We find that Axin1 primarily suppresses Wnt signalling in proliferating cells, where the low levels of Axin2 are unable to completely substitute for Axin1 function. Axin1 levels then fall as cells enter myogenic differentiation to allow Wnt signalling to promote differentiation.

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S05.O2-257

Myogenic fate acquisition in cardiopharyngeal mesoderm

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Vertebrate heart and head muscle development are linked in the early embryo. Evolutionarily conserved cardiopharyngeal mesoderm (CPM), associated with the developing foregut or pharynx, has been shown to contain common progenitor cells for heart and head muscle. Addition of CPM drives elongation of the embryonic heart tube, giving rise to myocardium at the cardiac poles. CPM also gives rise to branchiomeric craniofacial skeletal muscles that activate myogenesis through different upstream regulatory programs to somite-derived muscles. Within CPM, clonally distinct subpopulations contribute to specific parts of the heart and groups of branchiomeric muscles, such as right ventricular myocardium and first arch-derived muscles of mastication or outflow tract myocardium and second arch-derived muscles of facial expression. Perturbation of CPM development results in a spectrum of cardiac and craniofacial congenital anomalies, typified by DiGeorge or 22q11.2 deletion syndrome. The transcription factor TBX1 is the major candidate gene for DiGeorge syndrome and regulates both cardiac and skeletal myogenic CPM derivatives. TBX1 is required for cardiac progenitor cell segregation to alternate arterial and venous poles of the mouse heart from a common progenitor pool in posterior CPM. Consequently loss of TBX1 results in both outflow tract and atrial septation defects, common forms of congenital heart defects, reflecting the critical contribution of CPM to growth of the embryonic heart. In addition, oesophageal, trapezius and sternocleidomastoid muscles have been identified as TBX1-dependent branchiomeric muscles. Ongoing work concerns identifying the mechanisms regulating divergent skeletal and cardiac myogenic fates in CPM and characterizing cell behavior during the dynamic process of progenitor cell deployment to the forming heart and head muscles.

S05.O3-179

Mechanical tension and spontaneous muscle twitching precede the formation of striated muscle in vivo

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Muscles generate forces to enable body movements of higher animals. Muscle forces are produced by repetitive stereotyped acto-myosin units called sarcomeres. Sarcomeres are chained to linear myofibrils spanning across the muscle fiber. In mammalian body muscles myofibrils are aligned laterally resulting in their typical cross-striated morphology. Despite detailed textbook knowledge about the adult muscle structure, it is unclear to date how striated myofibrils are built in vivo. Recently, a tension-based model of myofibrillogenesis was proposed for specialised non-striated Drosophila flight muscles.

Results: Here, we investigate the morphogenesis of Drosophila abdominal muscles and establish these as in vivo model for striated muscle development. Using live imaging, we find that myofibrils are built simultaneously across the entire muscle fiber implying a self-organisation of the sarcomeric components to long immature myofibrils. These immature myofibrils then align laterally to build mature cross-striated myofibrils. By applying in vivo laser microlesion experiments, we demonstrate that mechanical tension across the muscle fiber precedes the formation of immature myofibrils, suggesting that mechanical tension may coordinate myofibril self-organisation in striated muscle. Interestingly, the immature myofibrils, which do not yet display a regular periodic acto-myosin pattern, readily generate spontaneous Ca^{2+} dependent contractions in vivo. The frequency of these spontaneous contractions increases during the transition to striated myofibers, correlating with precise myofibril alignment.

Conclusion: Together, these results suggest that mechanical tension and spontaneous muscle twitching are generally important to coordinate the self-organisation of different sarcomeric protein complexes across large distances in a muscle fiber. This tension-based myofibrillogenesis model provides a universal mechanism for formation of highly regular cross-striated myofibrils in vivo.

S05.O4-247

PABPN1-mediated cytoskeletal rearrangement induces aging-associated muscle wasting

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Poly(A) Binding Protein Nuclear 1 (PABPN1) is a multifunctional regulator of mRNA processing, and its expression levels specifically decline in aging muscles. An expansion mutation in PABPN1 is the genetic cause of oculopharyngeal muscle dystrophy (OPMD), a late onset and rare myopathy. Moreover, reduced PABPN1 expression correlates with symptom manifestation in OPMD. We show that altered PABPN1 expression level is an underlying cause of muscle wasting. We found that a muscle-specific mild reduction in PABPN1 levels causes muscle pathology including myofiber atrophy, thickening of extracellular matrix, myofiber-type transition, and enhanced

muscle regeneration. We show that muscle atrophy is associated with a reduction in proteasomal activity, and transition in MyHC isotope expression pattern in myofibers, whereas muscle regeneration is associated with upregulation of cytoskeletal proteins. We suggest that PABPN1 plays a central role in aging-associated muscle wasting.

S05.O5-318

Phosphorylation of muscle-specific kinase MuSK and the downstream signalling network during neuromuscular junction development

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A reciprocal exchange of signals between muscle fibres and motor neurons results in the formation of neuromuscular junctions. Muscle-specific kinase MuSK is a receptor tyrosine kinase, which is the key player during neuromuscular junction formation. Impaired MuSK signaling results in acute neuromuscular deficiencies as presented during myasthenia gravis or, even more severely, in respiratory failure and perinatal death in newborn mice lacking MuSK. MuSK is activated as tetrameric complex together with the low-density lipoprotein receptor Lrp4. Tetramerization is promoted by the binding of the motor neuron-derived heparan proteoglycan agrin to Lrp4. Signal transduction events downstream of MuSK activation induce pre- as well as postsynaptic differentiation, which, most prominently, includes the clustering of acetylcholine receptors at synaptic sites. The crucial events regulating these processes are the phosphorylation and subsequent activation of MuSK. Even though a lot is known about the signalling cascade downstream of MuSK, a complete picture of the processes that achieve a reliable and mature neuromuscular junction is missing. We have recently used a quantitative phosphoproteomics screen to identify phosphotargets of MuSK signalling. We have been able to show that transcriptional as well as cytoskeletal protein networks are activated. In on-going experiments we have been analysing proteins within these networks as well as novel MuSK phosphorylation events to better understand the complex interplay between molecular and cellular determinants of neuromuscular junction development. We expect that these studies will provide detailed mechanistic insights into the complex signaling network downstream of MuSK.

Posters

S05.P1-290

Moderate physical activity associated to soluble milk protein supplementation improve muscle force and locomotion in aged rats

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Loss of muscle mass and function associated with aging occur in sarcopenia. Consequently, adequate protein intake and physical activity are two crucial elements allowing the maintenance of muscle mass in elderly subjects. The aim of this study was to understand whether a moderate exercise associated with nutritional intervention, especially protein diets, can improve locomotion in aged rats. We investigated the effect of an 8-weeks supplementation with casein (CAS), lactoserum (LS) or soluble milk protein (PRO) on muscle force and gait analysis in aged rats Wistar RjHan (17 months). A low intensity protocol training was performed on a treadmill where each rat ran at a speed of 10m/min, 30 min/day for 8 weeks. Just after treadmill exercise, each animal received 0.85 g/day of CAS, LS or PRO. Muscle force was evaluated using the grip test (Bioseb) whereas spontaneous activity was measured with an open-field (Actimeter, Bioseb). Furthermore, an extensive gait analysis was performed using the Gait-Lab system (Gait-Lab, View Point). Results indicate that milk protein supplementation was efficient in improving muscle function and especially locomotor performances. Grip measurements demonstrated that rats from the PRO group had higher strengths and improved locomotion parameters in the open field. Using the Gait Lab system, we also demonstrated that the maximal voluntary speed was the best for PRO rats and increased by around $22.4 \pm 5{,}7\%$, $n = 8$. Furthermore, the increase in maximal voluntary speed was associated with an increase in stride frequencies. We conclude that in aged rats, increasing protein intake and low physical activity may be an effective way to improve skeletal muscle function, especially walking, but also force and skeletal muscle atrophy. Differences between casein and "fast" proteins are now well established, but this study clearly demonstrate that the intake of soluble milk proteins just after a moderate exercise is an effective way to delay sarcopenia.

S05.P2-289

Effects of aging on gait properties, spontaneous locomotion and muscle mass in the Wistar rat

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Sarcopenia, defined as an age-related skeletal muscle atrophy and weakness, leads to locomotor function decline that is associated with gait abnormalities in the elderly. Aged rats are widely used to define and determine age-related impairments. However, few aging studies have used rat models to specifically investigate gait properties, although gait may be affected by tissue and organ localized impairments. In this study, we used the Gait-Lab system (View Point, Lyon, France), an automated quantitative gait analysis system for rodents based on the CatWalk method. This system allows to obtain various spatial or temporal parameters and to analyze gait during the unforced walking in aged Wistar rats relative to younger adult rats. Short-term spontaneous motor activity was examined using open field measurements (Actimeter, Bioseb, France), and the effects on muscle mass were also determined. These experiments were performed using 19-months (Old) Wistar rats, and their functional characteristics were compared with those of 6-months old adult rodents (Adult). We found that aged rats exhibited a significant reduction in hindlimb muscle mass, as well as a decrease in spontaneous motor activity. Automated quantitative gait analysis of walking properties highlighted a reduction of around 25% in the maximum voluntary speed of travel in sarcopenic rats. This correlated closely with shorter stride lengths, as well as changes in other spatial and dynamic parameters, such as an increase in the brake time and a smaller forelimb base of support. Our analysis of gait quantitative metrics allowed for precise measurement and definition of functional consequences of various age-related dysfunctions on the walking properties of aging rats. Therefore, the scope of this rodent model of aging was improved and now allow the discrimination and the study of potential beneficial effects of therapeutic approaches on age-related locomotion impairments.

S05.P3-280

Temporal Requirement for Sonic Hedgehog during tongue muscle development

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Myogenesis requires well orchestrated singling and interactions with surrounding tissues.Tongue is a highly muscular tissue. Being located among head muscle whilst having a different embryonic origin makes tongue a very interesting muscle to study. Proper tongue formation in a specific developmental window is critical and affects the speech. In human, tongue defects can arise as either isolated or syndromic malformations. Sonic Hedgehog and Wnt pathway have been suggested to play crucial role in tongue development.However despite extensive research regarding role of shh in taste bud formation, tissue and temporal requirement of shh in whole tongue development has not been studied. Here we have used the tamoxifen inducible Cre mouse model to inhibit Shh at different time points. We assess the recombination at the cellular level in the mutant prior analyzing the effect of shh inhibition on the hybrid origins of the tongue. In this study we have shown that inhibition of shh after initiation of tongue development will lead to disruption in tongue formation and deficiency of both intrinsic and extrinsic muscles. We also demonstrate that these mice have deficiency in both neural crest as well as myogenic derived components in the tongue. Loss of shh later than E12.5 dpc however seems to have no significant effect on the tongue. This study suggests a critical temporal requirement of shh after initiation of tongue development for its proper formation. We suggest this effect is directly on neural crest cells and indirectly on myogenic precursors. These data can help us better understand the mechanism of tongue development which can later be used in the regeneration aspects of the tissue especially with regards to the invasiveness of Squamous Cell Carcinoma.

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S05.P4-250

A new model of short-time training improves performance in old mice.

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During the past three decades, endurance training was proposed to achieve autonomy of the elderly over the long term. With a more appealing strategy for elderly to adhere to an exercise program, we examined if a less time consuming training would improve performance, maximal oxygen consumption, muscle and the entire body metabolism profile. In this study, the effects of a short-term training (acceleration) were compared to a traditional endurance training over 23 months in mice C57Bl/6. The short-term training was composed in 5 sessions (over 2 weeks) of accelerated run versus 20 sessions (5 sessions per week over 4 weeks) of endurance training. The mice were divided in three groups (1) accelerated trained (ACC) mice, (2) endurance trained (ENDU) mice and (3) a control (CONT) mice. Results showed a significant improvement in performance in ACC mice: +8% Vpeak ($p < 0.05$), +10% time to exhaustion ($p < 0.01$). However the maximal respiratory capacity (VO2max) did not change. In the skeletal muscle of ACC group, the enzymatic activity of citrate synthase (CS), lactate dehydrogenase (LDH) and creatine kinase (CK) were significantly higher compared to ENDU group. In addition, the mitochondrial respiration was higher in the ACC group compared to CONT group. The metabolomics analysis of urine showed a higher taurine concentration and less branched amino acid in ACC urine. These results suggest that short-term acceleration training is more efficient to improve performance, lead to better adaptations on main energy system and a possible improvement of antioxidant defenses than endurance training in old mice.

S05.P5-244

Differential roles of STIM1 and STIM1L in skeletal muscle

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Store-Operated Ca^{2+} entry (SOCE) is a ubiquitous Ca^{2+} influx mechanism of particular importance for skeletal muscle and patients harboring mutations in SOCE genes suffer from muscular weakness and myopathies. SOCE is triggered by the Ca^{2+} depletion of the endoplasmic/sarcoplasmic reticulum (ER/SR) which initiates the oligomerization of the ER Ca^{2+} sensor STIM1 and its translocation to the plasma membrane (PM). At the PM, STIM1 opens PM Ca^{2+}-permeable channels of the Orai family allowing Ca^{2+} entry and ER/SR refilling. Stim1 or orai1 KO mice showed increased fatigability associated with decreased SR Ca^{2+} release upon repetitive stimulations, suggesting impaired SR refiling. However fatigue being a very complex process, SOCE impairment can lead to other alterations of muscle physiology that we will investigate. In human muscle SOCE is supported by 2 different isoforms of STIM1: the classical protein (STIM1) and a longer isoform (STIM1L) resulting of an alternative splicing of the stim1 gene. The respective contribution of STIM1L vs. STIM1 in muscle physiology and especially in muscle fatigue has never been investigated. To address this question, we first determined the localization of STIM1L in human adult tissue by immunostaining using an antibody directed specifically against STIM1L. Surprisingly, our results showed a preferential localization of STIM1L at the longitudinal part of the SR suggesting other role(s) of this isoform than gating PM Ca^{2+} channels. In parallel, we are working out a culture cellular system (Matrigel) to obtain mature human myotubes that we will use to study Ca^{2+} fluxes in different cellular compartments. After 10 days in culture, typical skeletal fibers striations were observed, confirming an advanced level of differentiation. Further Ca^{2+} imaging experiments after invalidation of each isoforms will allow us to better characterize the specific role of STIM1 and STIM1L in human muscle physiology and specially muscle fatigue.

S05.P6-242

Different sarcomere lengths in skeletal muscle fibres of adult and senescent humans

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Loss of muscle activity is a striking property of aging. Its underlying mechanisms are still not entirely understood. We investigated the sarcomere length as a possible determinant of changes in contractile properties of muscle fibres during aging. Biopsies were taken from pectoralis and sternocleidomastoideus muscles of adult (29–43 years) and senescent humans (71–75 years). The sarcomere lengths were measured on single skinned fibres using LASER (633 nm) diffractometry in relaxation solution. Most of the fibres were typed both by their kinetics of stretch activation under maximal Ca^{2+} activation and by their myosin heavy chain isoforms. Fibres from sternocleidomastoideus muscles exhibited considerably longer sarcomeres (mean±SD [n]; 2.59±0.25 μm [458]) than fibres from pectoralis muscles (2.14±0.25 μm [477]). Both in pectoralis and in sternocleidomastoideus muscles, the sarcomere lengths were significantly smaller in the senescent group. The values for pectoralis muscles were 2.17±0.25 μm [209] in adult and 2.12±0.24 μm [268] in senescent specimens ($p < 0.05$, t-test). The values for sternocleidomastoideus muscles were 2.65±0.23 μm [93] in adult and 2.55±0.21 μm [74] in senescent specimens ($p = 0.001$). In pectoralis muscle, the decrease with age was most evident in type I fibres. In contrast, in sternocleidomastoideus muscles, the decrease with age was most evident in type II fibres. Aging seems to be accompanied with a decrease of sarcomere length in skeletal muscles. Provided that the myofibrillar protein isoforms remain unchanged, the decrease of sarcomere length causes two effects: 1) An increase of shortening velocity, and 2) a decrease of muscle strength. The latter only occurs, if shorter sarcomeres are accompanied by a smaller zone of overlap between thick and thin filaments.

S05.P7-203

The effect of in utero inflammation on postnatal diaphragm function in preterm lambs

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Intra-uterine inflammation (eg chorioamnionitis) is associated with up to 70% of extreme preterm births and may contribute to adverse respiratory outcomes. The resilience of infants to developing respiratory failure after birth may be critically influenced by the integrity of the diaphragm. In utero lipopolysaccharide (LPS) exposure significantly impairs diaphragm function in preterm lambs. However, it is unclear whether LPS-induced diaphragm weakness persists into postnatal life, after the onset of spontaneous ventilation. We aimed to determine the effect of in utero exposure to inflammation on postnatal diaphragm function in preterm lambs. Lambs were exposed to intra-amniotic (IA) LPS (4 mg, E coli 055:B5) or saline 48h before preterm delivery at 128 d gestational age (GA; term=148 d). Lambs were managed in an intensive care environment then euthanized at 7 d postnatal age. Fetal control lambs were delivered at 135 d GA and euthanized immediately before initiating spontaneous ventilation. Longitudinal strips were dissected from the right hemi-diaphragm for in vitro assessment of contractile function. IA LPS exposure did not significantly affect contractile properties of the preterm diaphragm at 7 d postnatal age ($p > 0.05$). Interestingly, diaphragm function changed significantly with 7 d postnatal development. Both LPS naïve and LPS exposed lambs had significantly greater maximum specific force ($p < 0.001$), faster twitch contraction ($p < 0.001$) and relaxation times ($p = 0.007$) and lower relative force at stimulation frequencies of 5–40 Hz ($p < 0.05$) compared to 135 d non-breathing fetal controls.

In contrast to our hypothesis, LPS-induced diaphragm weakness that is evident at birth does not persist after 1w postnatal life in preterm lambs. Our results indicate that postnatal rather than antenatal events are the primary determinants of diaphragm function at 1w postnatal age. We propose that accelerated diaphragm development results from the onset of spontaneous breathing.

S05.P8-190

The myogenic potential of human and porcine bone marrow derived mesenchymal stem cells

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Introduction: Bone marrow derived mesenchymal stem cells (BM-MSCs) are multipotent stem cells that play important role in the formation of hematopoietic microenvironment. Importantly, they can also follow adipo-, chondro-, as well as osteogenic differentiation, and could give rise to neurons, glial cells, hepatocytes, skeletal myoblasts, or cardiomyocytes. MSCs are easy to isolate and culture in vitro, constitutively secret chemokines, and are also able to engraft injured tissues after transplantation. Moreover, MSCs are able to modulate immune cells activity and regulate cell trafficking. These features make them a very attractive tool that can be used in regenerative medicine. Until now, many studies aimed at the assessment of the myogenic potential of MSCs.
Material and methods: In present study, we aimed to induce myogenic differentiation in vitro of human and porcine BM-MSCs. We examined three models of BM-MSCs differentiation. First, we treated these cells with cytokines that are known to be present in regenerating muscle, i.e. interleukin-4 (IL-4), interleukin-6 (IL-6), and stromal derived factor-1 (SDF-1). Second, the BM-MSCs were co-cultured with C2C12 myoblasts, finally they were co-cultured with activated satellite cells isolated from regenerating muscle. To assess BM-MSCs reaction to applied treatments and culture conditions we analyzed the expression of selected genes and the ability of these cells to migrate and to form hybrid myotubes in vitro.
Results: As a result we observed changes in expression of genes encoding adhesion proteins and also the improvement of BM-MSCs to migrate and fuse with myoblasts. However, we did not observe the induction of the expression of myogenic regulatory factors in analyzed BM-MSCs.
Conclusion: Thus, BM-MSCs are able to fuse with myoblast and preconditioning of these cells could improve this process. However, they did not express myogenic regulatory factors.

S05.P9-181

Persisting muscle growth retardation due to functional alterations in heterogenic satellite cell populations

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Introduction: The number of low birth weight (LBW) piglets increased as a secondary effect of breeding. These animals often show life-long muscle growth retardation accompanied by changes in muscle composition and fat content (1). In previous experiments two functionally different satellite cell (SC) subpopulations (SP) were identified in piglets with normal birth weight (NBW). We analysed myogenic characteristics of the SC pool in LBW piglets to assess underlying mechanisms for growth retardation.
Material and methods: SCs were isolated from the longissimus dorsi and semimembranosus muscles of 4 day old piglets with trypsin digestion. SP of SCs were separated by percoll gradient centrifugation and characterized according to their proliferation, growth kinetics (xCELLigence system, ACEA Biosciences), myogenic marker expression, differentiation potential and bioenergetic profile (O2-sensitive fluorescence sensors).
Results: We found that the SC yield per gram muscle is lower in LBW piglets in comparison to NBW piglets. At the same time there seems to be no impairment in proliferative capacity, whereas the myogenic differentiation was drastically disturbed in which both subpopulations are differently affected. Protein expression analyses revealed that the level of Pax7, a marker for undifferentiated SCs (2), in LBW piglets is comparable to NBW piglets but there are considerable differences regarding differentiation markers.
Discussion: Basically, we confirmed the presence of heterogenic SC populations in LBW piglets whereas their functional deficiency may contribute to the manifestation of the muscle phenotype. Further studies, e.g. signalling pathway analyses, have to be conducted to gain a deeper insight in LBW piglets' growth retardation. Moreover, these findings can give implications to specifically interfere in muscle dysfunction diseases.

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S05.P10-144

Cell cycle control by Pax7 a new role of the old myogenic gene?

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Aims: Postnatal growth and regeneration of the skeletal muscles depends on stem cells, i.e. satellite cells. Some pathological conditions, such as muscular dystrophies, lead to the exhaustion of satellite cells pool resulting in the failure of skeletal muscle regeneration. Among the therapies aiming to improve regeneration of such affected muscles are transplantation of exogenous stem cells, such as embryonic stem cells (ESCs). However, their myogenic potential as well as factors and mechanisms regulating myogenic differentiation are still not fully characterized. Pax7 transcription factor is the key marker and regulator of embryonic myogenesis as well as skeletal muscle satellite cells maintenance. Pax7 was shown to be involved in regulation of proliferation, differentiation of myogenic cells, as well as prevention of their apoptosis. Thus, the aim of current study was to characterize the role of Pax7 in the regulation of proliferation and/or apoptosis of cells other than myogenic precursors or satellite cells.
Methods and Results: In the current study we use cells lacking Pax7 functional gene to investigate the role of Pax7 in the regulation of proliferation of differentiating ESCs and mouse embryonic fibroblasts (MEFs). By global gene expression profiling we show the differences in expression levels of some of transcripts coding cell cycle-regulators between Pax7-null and wild-type cells. However, cell cycle profile and proliferation rate are not altered in Pax7-null cells. Finally, lack of Pax7 also impacts at the percentage of proliferating and apoptotic cells, though the results differ between ESCs and MEFs.
Conclusion: Altogether, our results demonstrate the involvement of Pax7 gene in regulation of cell cycle machinery and maintenance of differentiating ESCs and MEFs. They further suggest that the function of Pax7 might depend on the type of cells analyzed.

S05.P11-123

LIX1 regulates YAP1 activity and controls the proliferation and differentiation of stomach mesenchymal progenitors

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During development, the digestive musculature arises from mesenchymal cells. In adults, these cells can undergo oncogenic transformation, leading to gastrointestinal stromal tumors. Because tumorigenesis often involves the reactivation of developmental processes, a better understanding of the molecular mechanisms driving digestive mesenchyme development could offer insight into altered mechanisms in gastrointestinal cancers. Here, we focused on the Limb Expression 1 (LIX1) gene. LIX1 is a 281 amino-acid protein. Predictive in silico studies have shown that LIX1 has a double-stranded RNA-binding domain such as DICER and DROSHA (unpublished data), suggesting that it could be involved in RNA or miRNA processing. Using the chick embryo as a model organism, we demonstrated that LIX1 (Limb expression 1) specifically defines the population of mesenchymal progenitors, thus identifying LIX1 as a marker of stomach mesenchyme immaturity (Faure et al., Development, 2015). Furthermore, using in vivo gain- and loss-of-function approaches, we showed that LIX1 is required for correct smooth muscle determination and that its expression must be finely regulated for correct differentiation to occur. We demonstrated that expression of LIX1 must be tightly regulated to allow fine-tuning of the transcript levels and state of activation of the pro-proliferative transcriptional coactivator YAP1 to regulate proliferation rates of stomach mesenchymal progenitors and their differentiation.

Our data highlight dual roles for LIX1 and YAP1 and provide new insights into the regulation of cell density-dependent proliferation, which is essential for the development and homeostasis of organs (Mckey et al., BMC Biol, 2016).

S05.P12-268

Determinants of muscle strength in haemodialysis

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Background: Uremic sarcopenia in chronic haemodialysis (HD) patients, defined as skeletal muscle weakness and decline muscle mass, has a prevalence between 20 and 50% and is associated with increased mortality risk 1. The aim of this study was to determine the clinical and biological parameters associated with the diminution of the muscle strength assessed by the isometric maximal voluntary force (MVF) of quadriceps in chronic HD patients.
Materials and methods: A belt-stabilized hand held dynamometer was used to measure the MVF of the quadriceps. Creatinine index and lean tissue index were assessed by bioelectrical impedance analysis were used to estimate muscle mass. Physical activity was assessed by the Voorrips questionnaire. Nutritional and inflammatory parameters included: serum albumin, serum high-sensitivity C-reactive protein (hs-CRP), and normalised protein catabolism rate (nPCR).
Results: One hundred fifty seven HD patients were included (103 men and 54 women). The average age was (65.8 ± 17.1), mean FMV was 89.5 ± 41.7 N.m, and the average of Voorrips score was 6.1 ± 5.4. The main determinants of MVF in multivariate analyse were lean tissue index (3.929 [2.163–5.696] p <0.001), creatinine index (7.54 [4.453–10.627] p <0.001), Voorrips score (20.729 [3.939–37.519] p = 0.017, and albumin (2.096 [0.716–3.476] p = 0.004). Increase of serum hs-CRP (p = 0.028) and nPCR decrease (p = 0.021) were the main factors responsible of serum albumin diminution.
Discussion: Physical inactivity, malnutrition and inflammation (as indicated by decrease in serum albumin), and impairment in muscle mass were independently associated with decreased muscle strength.
Conclusion: Our results strongly suggest that physical activity combined with nutritional intervention appear as promising strategies to reduce prevalence of muscle weaknessin HD patients.

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S05.P13-267

Muscle force measurement in haemodialysis

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Background: In haemodialysis, diminution of muscle strength constitutes a major prognostic factor of mortality. Currently, measurement of quadriceps isometric maximal voluntary force (MVF) represents the reference method to investigate muscle strength. However, reduction of MVF is rarely detected in these patients due to the absence of portative bedside tools in clinical practice. The purposes of this study were therefore to assess the agreement of a belt-stabilized hand held dynamometer (HHD) with the dynamometer chair (reference method), and to determine intra and inter-tester reliability of the quadriceps MVF measurements using belt-stabilized HHD in healthy subjects and in haemodialysis patients.
Materials and methods: Fifty-three healthy adult subjects (23 males, 36.5 + 12.5 y.o.) and 21 haemodialysis patients (14 males, 72.4+13.3 y.o., dialysis vintage 30 + 75.1 months) performed two sessions of MVF measurements. For each session, MVF was assessed with belt-stabilized HHD and dynamometer chair, by two independent investigators. The agreement between the two devices would be quantified using the Bland-Altman 95% limits of agreement (LOA) method and the Spearman correlation.
Results: For healthy subjects and haemodialysis patients, Spearman coefficients were 0.63 and 0.74 respectively ($p < 0.05$). In haemodialysis group, reliability was excellent for both the intra-and inter-tester reliability $R^2 = 0.85$ ($p < 0.01$) and $R^2 = 0.90$ ($p < 0.01$) respectively. In all individuals, the mean difference between the dynamometer chair and the belt-stabilised HHD was -13.07 ± 21.77 N m ($p < 0.001$). The LOA for the upper (LOAU0) and the lower (LOAL) were 29.59 and -55.73 N m, respectively.
Conclusion: In healthy subjects and in haemodialysis patientsthe belt-stabilized HHD dynamometer appearsas a valid and reliable method to measure in clinical practice isometric MVF of quadriceps.

S05.P14-221

Effect of inorganic phosphate on stress relaxation in soleus muscle fibres of adult and old mice

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Age-related changes in contractile properties are of considerable interest, but relatively little is known about the response of young or old muscle to stretch. Particularly, the decay of force may give information about the kinetics of the various cross bridge states. At the end of a ramp-and-hold stretch, force decays towards a value somewhat above the initial isometric force (force enhancement, FE). The decay (stress relaxation, SR) is best fitted with a double exponential function and a constant value for FE, where A1 and A2 correspond to a slow and a fast component, respectively*. The contributions of A1 and A2 were studied in single permeabalised soleus muscle fibres from adult (18 months) and old (32 months) mice in the absence (0 mM) or presence (15 mM) of inorganic phosphate (Pi) in the activating solution at 15°C. Fibres were stretched for 5% of their length at 1 fibre length per second, and the contributions of A1 and A2 to SR determined. With no added Pi, SR of the older fibres was faster than that of the adult fibres; the fast component A2 constituting 74+6% of SR compared to 64+9% for the younger fibres ($p < 0.005$). There were no significant differences in FE. Adding Pi slowed SR, reducing the fast A2 component more in the old than the adult fibres (to 52% in both adult and old fibres). The two components of SR may represent differences in the detachment rate of two cross bridge intermediates and the fact that added Pi increases the slow and decreases the fast component, suggests that A1 and A2 represent, respectively, cross bridges states before and after the release of Pi. This further suggests that the release of Pi from the actomyosin complex is somewhat faster in old muscle compared to the young, giving rise to faster SR and a greater proportion of the A2 component, evident when the muscle is stretched.

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S05.P15-126

Insulin-like growth factor-I and interleukin-8 augment myocilin levels and protein kinase B/Akt phosphorylation in rat primary skeletal myoblasts

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Introduction: The aim of this study was to examine and compare potential effects of IGF-I, an anabolic factor in skeletal muscle, and IL-8, a cytokine released in contracting myofibers, on expression of myocilin and phosphorylation of its downstream signaling element, protein kinase B (PKB)/Akt in rat skeletal myoblasts.
Materials and methods: Primary skeletal muscle cells (RSkMc), isolated from the limbal skeletal muscle of neonatal rats, and subjected to 11 days of differentiation were used for the study. The cells were exposed to IGF-I (final concentration – 25 nmol/l), IL-8 (final concentration – 1 ng/ml), or combination of both factors, in the entire period of differentiation. Transcript levels of myocilin were examined using qPCR technique, with Gapdh and beta-actin, serving as housekeeping genes. The cellular content of myocilin, total and phopshorylated PKB/Akt proteins were assessed by immunoblotting. Student t-test was used for comparison the results.
Results: IGF-I or IL-8 did not modify cell viability. Myotube formation in RSkMc cultures was increased by IGF-I (by 39%, $p < 0.05$), and unaltered by IL-8 acting alone or in combination with the growth factor. Myocilin transcript levels were appr. 2-fold higher under IGF-I, IL-8 and growth factor-cytokine treatment, in comparison to control values. As a consequence, cellular contents of myocilin protein were also augmented and similar in all experimental groups. IGF-I, IL-8 or both these factor combined did not affect PKB/Akt protein levels. However, PKB phosphorylation levels were markedly increased, especially in the presence of IL-8 (acting both alone and in combination).
Conclusion: IGF-I and IL-8 augment cellular content of myocilin, a protein involved in regulation of skeletal muscle size, via transcriptional mechanism. This effect can result in enhancement of PKB activity, manifested by an increased kinase phosphorylation, however it is not sufficient in stimulation of myotube formation.

Session 06 Muscle cytoskeleton

Oral presentations

S06.O1

Structural and functional roles of the giants of the striated muscle sarcomere-titin and nebulin

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Titin and nebulin are mega-dalton sized proteins that form myofilaments located in the striated muscle sarcomere. Nebulin is embedded in the Z-disk and is coextensive with the thin filament. Recent work from several laboratories has provided evidence that nebulin does not span all the way to the thin filament pointed end, bringing into question nebulin's earlier proposed role in thin filament length regulation. These findings will be discussed as well as recent work on muscles in which the size of nebulin has been altered and the effect of nebulin on thin filament length is studied. The structural and functional effects of conditionally deleting nebulin from skeletal or cardiac muscle will also be addressed. Titin is a giant protein that spans in the sarcomere from Z-disk to M-band. It's I-band spanning region functions as a molecular spring that contributes to passive stiffness of the heart with multiple stiffness adjustment mechanisms that exist within this molecular spring. Importantly, recent work from multiple laboratories has shown that these stiffness adjustment mechanisms are deranged in heart failure with preserved ejection fraction (HFpEF). Our recent studies in mice with HFpEF-like symptoms support that manipulating titin's splicing machinery through targeting the splicing factor RBM20 greatly improves diastolic function.

S06.O2-255

Ablation of palladin in adult cardiac muscle causes cardiac dilation and systolic dysfunction

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Introduction: Palladin (PALLD) belongs to the PALLD/myopalladin (MYPN)/myotilin (MYOT) family of immunoglobulin-containing proteins in the sarcomeric Z-line associated with the actin cytoskeleton. While MYPN and MYOT are expressed in striated and skeletal muscle, respectively, PALLD is ubiquitously expressed in several isoforms. The longest PALLD isoform is expressed predominantly in striated muscle and shows high structural homology to MYPN. However, while MYPN gene mutations have been associated with human cardiomyopathies, the role of PALLD in the heart has remained unknown, partly due to embryonic lethality of PALLD knockout (KO) mice. To study the role of PALLD in the heart, we generated constitutive (cPKOc) and inducible (cPKOi) cardiac specific PALLD KO mice as well as constitutive double KO mice for PALLD and MYPN (cPKOc/MKO).
Methods and Results: While echocardiographic analyses revealed development of dilated cardiomyopathy (DCM) of MYPN KO (MKO) mice starting from 4 months of age, cPKOc mice exhibited no cardiac phenotype either at basal conditions or following transaortic constriction. MKO/cPKOc mice exhibited a similar phenotype as MKO mice, suggesting that MYPN and PALLD do not have overlapping functions. In contrast, induction of PALLD KO in adult cPKOi mice resulted in progressive cardiac dilation and systolic dysfunction, associated with reduced cardiomyocyte contractility (Ionoptix analysis), increased resting tension (mechanics on isolated single myofibrils), abnormal intercalated disc ultrastructure, upregulation of markers of cardiac pathological remodeling, ERK activation, and fibrosis.
Conclusions: The development of a DCM phenotype in cPKOi mice induced at adult stage demonstrates that PALLD is essential for normal cardiac function and propose PALLD as a candidate gene for cardiomyopathy. In contrast, cPKOc mice exhibited no cardiac

phenotype, likely due to compensatory mechanisms, which do not appear to involve its closest homologue MYPN.

S06.O4-234

O-GlcNAcylation is a key modulator of cytoskeletal interactome involved in the skeletal muscle sarcomeric organization

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The striated muscle is an intricate, efficient and precise machine, composed of highly specialized myofibrils. While thin and thick filaments interact to generate the force of contraction, other proteins constitute an intricate and interconnected network, termed nowadays sarcomeric cytoskeleton, whose role is the regulation of sarcomere function to ensure its efficient working. We have recently demonstrated that O-GlcNAcylation, a particular glycosylation akin to phosphorylation, is a key modulator of sarcomeric organization since the sarcomere morphometry is modified according to the O-GlcNAcylation level of myofibrillar proteins. In particular, the morphometric changes were accompanied by changes in several protein-protein complexes involving key structural proteins. In particular, we demonstrated that the interaction between desmin and its molecular chaperon alphaB-crystallin, was modulated according to the variation of global O-GlcNAc level. We currently focused on the dynamic of O-GlcNAcylation and phosphorylation of these two key proteins of sarcomeric organization. Furthermore, through a proteomic approach performed on C2C12 myotubes, based on Click Chemistry and mass spectrometry, we have identified several key structural proteins bearing O-GlcNAc moieties, and localized O-GlcNAc sites on some of these proteins. Interestingly, some sites corresponded to mutation sites in neuromuscular disorders, in particular myofibrillar myopathies characterized by a marked disorganization of myofibrils. Taken together, all these data provided new insights for the fine understanding of the complex interactome underlying the organization of sarcomeric structure.

S06.O5-334

Trans-complementing immunoglobulin domain folding in the myomesin and obscurin-like-1 complex: implications for M-band protein network organization

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Immunoglobulin-like (Ig) domains are globular domains composed of about 100 residues, and one of most frequently utilized domain by sarcomeric modular proteins, like titin (aka connectin), obscurin and filamin. Although homology between Ig domains is not high (25 to 50%), the structures of sarcomeric Ig domains determined so far are very similar, forming a so-called "beta-sandwich" of two beta-sheets comprised of 7–10 beta strands. Recently, we determined the crystal structure of the human myomesin / obscurin-like-1(obsl1) complex formed by Ig domain 3 of obsl1 (OL3) and the short linker of myomesin between fibronectin type-3 domains 4 and 5 (MyL). The crystal structure of this complex elucidated a novel trans-complementing Ig domain fold, in which the MyL beta-strand participated in one beta-sheet formation of OL3 with beta-strands B, B', D and E via hydrogen bonds and hydrophobic interactions, whereby a complete Ig domain was folded. Mechanical forces required for detachment of the complex was measured by atomic force microscopy (AFM) and shown to be similar to, or higher than for an intact Ig domain. The structure of the complex showed the key residues on the linker of myomesin for incorporation to OL3 were not conserved in other myomesin isoform (Myom2 and 3), and the specificity of Myom1 was validated by binding assay both in vitro and in cellula. In conclusion, the intermolecular complementing Ig-fold formed by myomesin and obsl1 is an integral part of titin-obsl1-myomesin ternary protein network in M-band. Considering its firm mechanical interaction (135pN), compared to that of titin and obsl1 (30pN), the complex might contribute to the essential backbone of the M-band protein network. This work is under review.

Posters

S06.P2-339

Tetranectin labels myofibrillar disruptions after eccentric exercise in humans

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Introduction: Tetranectin is a secretory protein suggested to play a role in tissue healing processes. Here, we suggest that tetranectin is correlated to sarcomere disruption after high-force eccentric exercise and could act as a marker for myofibrillar disruptions.
Materials and methods: Eleven male students performed one bout of 300 unilateral maximal eccentric muscle actions with knee-extensors, and needle biopsies were taken from m. vastus lateralis. Immunostaining on cross-sections showed positive tetranectin labeling within myofibers, evident as early as 0.5 hours after the exercise bout, and the intracellular tetranectin staining remained prominent up to 7 days.
Results: The percentage of positive labeled myofibers in the loaded leg varied considerable between subjects (17 – 100%), compared to $<3\%$ in controls. A correlation was observed between the proportion of tetranectin positive fibers and the reduction in force-generating capacity. Positive tetranectin labeling was shown to co-localize with disruptions of sarcomere organization, visualized by double labeling against F-actin and HSP27. At the mRNA level, in situ hybridization revealed pericellular localization of tetranectin mRNA, suggesting that the protein is synthesized outside the myofibers.
Discussion: In summary, we report the novel finding of a rapid and sustained accumulation of tetranectin within damaged myofibers, bound to distinct areas and segments of damaged sarcomeres. Judging from its presence in disrupted areas we suggest a role of tetranectin in remodeling of damaged sarcomeres after overload. Furthermore, the absence of tetranectin in intact sarcomeres enables positive tetranectin staining as a useful marker for the quantification of sarcomere disruptions.

S06.P3-325

Protein phosphatase 5 regulates titin phosphorylation and function in the heart

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Background: In the N2B isoform of the giant protein titin, a cardiac-specific unique sequence (N2Bus) can be phosphorylated by various protein kinases, which reduces titin-based diastolic stiffness. Failing human hearts are hypo-phosphorylated at the N2Bus. Specific phosphatases acting on N2Bus are not known.
Methods and Results: We carried out a yeast-2-hybrid screen using human N2Bus ("bait") and a human cardiac cDNA library ("prey") and detected protein phosphatase-5 (PP5c) as a binding partner of N2Bus. The interaction was verified by GST-pulldown assays. PP5 is unique within the family of ser/thr protein phosphatases because of the presence of tetratricopeptide repeats (TPR) at the molecule's N-terminus. We found PP5 to be expressed in human and mouse hearts and elevated in failing hearts. PP5 is autoinhibited due to interaction of the TPR domain with the catalytic C-terminus. However, PP5 can be activated by proteolytic cleavage of the TPR region, through binding of the TPR domain by arachidonic acid, or via binding of heat-shock protein-90 (HSP90). In PP5-overexpressing transgenic (TG) mouse hearts, we found PP5 to co-localize with the titin-N2Bus in the sarcomeric I-band, as shown by immunofluorescence and immunoelectron microscopy. Autoradiography/back-phosphorylation assays and the detection of N2Bus-phosphosites by phospho-specific anti-titin antibodies showed that recombinant PP5 dephosphorylates recombinant human N2Bus previously phosphorylated by ERK2, PKA, or PKG. PP5 also dephosphorylated the cardiac titin isoforms, N2B and N2BA, in human heart tissue and PP5 TG mouse hearts showed reduced N2Bus phosphorylation compared to wildtype (WT) hearts. Force measurements on permeabilized single cardiomyocytes revealed increased passive stiffness in PP5 TG vs. WT hearts.
Conclusions: PP5 binds to titin N2Bus and acts as an antagonist to the protein kinase-mediated effects on titin stiffness. Inhibition of the PP5 effect on titin might benefit stiff hearts.

S06.P4-320

Nanodissection of the titin M-line complex

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Titin is a giant protein spanning between the Z- and M-lines of the sarcomere. In the M-line the C-terminal region of titin overlaps with that of oppositely oriented titin from the other half of the sarcomere. Furthermore, titin-binding proteins such as myomesin and M-protein localize in the M-line so as to form a complex. In titin molecules purified from muscle the M-line complex appears as a globular head-like structure. In titin oligomers, which are present in titin preparations, molecules bind to each other in a head-to-head orientation via the M-line complex. The exact structure and the molecular arrangement within the titin M-line complex is currently unknown. We analyzed the structure and stability of the M-line complex by investigating the properties of titin oligomers. Oligomeric state was retained, as evidenced by AFM, in 4M urea, indicating that the M-line complex is highly stable. To further explore stability and structure, we mechanically dissected the M-line complex of single surface-adsorbed titin oligomers by using AFM-based nanolithographic procedures. Titin was first deposited on mica so that the oligomers conformationally equilibrated on the surface. Then the M-line complex was dissected by pressing the cantilever tip into the center of the globular head and moving the tip sideways in predetermined directions and with constant velocity (1 μm/s) and pressing force (1 nN). Finally, we scanned the surface so as to reveal the evoked changes. Loops of filaments with lengths up to 400 nm were pulled out of the M-line complex. The thickness of the filaments was one half that of native titin, suggesting that partial unfolding may have taken place as a result of nanodissection. Our results suggest that the titin M-line complex may have a higher order three dimensional structure involving the packaging of participating filamentous molecules. Nanodissection may be used as a tool to investigate the internal structure of stable biomolecular complexes.

S06.P5-305

DCM-associated titin truncating mutations in human decrease myofibril passive tension and do not change myofibril contractility

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Dilated cardiomyopathy (DCM) was associated, in half of the cases, with mutations in genes of sarcomere proteins, from which mutations in titin gene (TTN) were accounted for about 20% of all DCM-associated mutations. We genotyped 30 explanted heart samples from fDCM patients and found 6 with TTN chain terminating mutations. The truncated titin variants were not detected by Western blotting. The total titin level, N2BA/N2B isoform ratio and total titin phosphorylation levels were not significantly different from normal donor and DCM heart samples. Titin is responsible for myofibril elasticity and supposedly play an important role in length-dependent activation. Therefore we investigated whether there was any mechanical and contractile dysfunctions caused by this mutations. We investigated the effect of two truncating DCM-associated mutations in TTN (p. R23464T fs*41 and p. Y18923*) on myofibril contractility and passive stiffness using an apparatus for single myofibril force studies. The variation of tension amplitude with sarcomere length, kinetics of force development and relaxation, and myofibril passive stiffness were investigated. The results were compared to the samples of normal donor heart and to DCM samples without mutation in TTN. The studies showed that the time course of contraction and relaxation and its dependence on sarcomere length were similar between the myofibrils containing mutation in TTN and donor heart myofibrils, while all the DCM mutations in contractile proteins TNNC1 (G159D), TNNI3 (K36Q) and MYH7 (E1426K) showed faster relaxation. Myofibrils with mutations in titin had lower passive stiffness compared to donor heart myofibrils and that was a common feature for all myofibrils isolated from DCM hearts. The Young's modulus of myofibrils from DCM hearts was about 30% lower compared to donor heart myofibrils. The decreased passive stiffness appears to be the only measureable abnormality caused by TTN chain terminating mutations.

S06.P6-266

Dynamics of transitions through the molten-globule state enhance the contractility of titin

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As the sarcomeres are stretched, the giant filamentous titin molecule extends hierarchically according to the elasticity of its segments. Although extension is thought not to involve the unfolding of globular domains under physiological conditions, there is increasing evidence in support of such a possibility. While force-driven unfolding of titin in vitro has been studied extensively, the refolding pathway and how it might contribute to the overall extensibility remains elusive. Here we manipulated mechanically pre-unfolded molecules with force-feedback optical tweezers. After quenching the force to constant 1–3 pN levels, extension fluctuated without resolvable discrete events. The folded fraction increased with decreasing quench-force levels and with increasing time spent at the given force. In constant-trap-position experiments the time- dependent force trace contained distinct fluctuations and an overall gradual increase of the average force. Thus, a titin molecule can develop force (max. 3 pN) via a dynamic, reversible rearrangement between contracted and extended structural states. Because domain unfolding is unlikely at these low forces, the fluctuations probably involve transitions between the unfolded state and a molten-globule state, and the slow force rise is due to transition, with slower kinetics, from the molten-globule to the native structure. In 4 M urea that favors the molten-globule state by destabilizing H-bonds the slow force rise disappeared but the fluctuations remained, thereby supporting our hypothesis. Furthermore, in Monte-Carlo simulations incorporating a compact molten-globule intermediate in the folding landscape all features of the force-clamp results were recovered. Because the transition through the molten-globule state shortens titin beyond that provided by a purely entropic collapse, an added contractility arises which may assist in sarcomere mechanics.

S06.P7-254

Structural Investigation and Characterisation of the Titin-Obscurin Complex from the Z-Disk

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Aims: The sarcomere is a highly complex cellular network, possessing an almost crystalline order. Previous studies revealed two separate interaction sites between the two proteins titin and obscurin: one at the M-band and one at the Z-disk. While the interaction at the M-band has been structurally determined, little is known about the interaction at the Z-disk. However, a mutation within the obscurin Ig domain 58, involved in the Z-disk-interaction, has been linked to hypertrophic cardiomyopathy (HCM). Thus, the aim of this study is a structural and molecular biological characterisation of the titin-obscurin interaction at the Z-disk.
Methods and Results: We crystallised the protein complex between the titin Ig domains Z9 and Z10 and the obscurin Ig domains 58 and 59 and were able to obtain a high-resolution crystal structure using X-ray crystallography. In order to validate the complex structure, protein-protein interactions were determined by isothermal titration calorimetry (ITC). We examined the interaction of the single titin domains with obscurin and introduced single point mutations in the interaction interface to measure possible changes of the interaction within the full length complex. We further investigated the effects of the HCM causing mutation on the interaction of titin and obscurin. Therefore we introduced the mutation into the obscurin 58 Ig domain. In a thermal shift assay we determined the thermal stability of the mutated construct and used ITC to measure the interaction between titin and the mutated obscurin, but were not able to find differences in comparison to the wild type.
Conclusion: The structure of the protein complex revealed a tight interaction of both obscurin Ig domains with the titin Ig domain Z10. ITC and thermal shift assay of the HCM causing mutation suggest no direct effects on the complex interaction. Further experiments, e.g. overexpression in mammalian cell culture, could elucidate the function of this mutation.

S06.P8-233

Muscle Z-disk: a specialized component of contractile machinery important for muscle integrity and function

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Highly ordered organisation of striated muscle cells is the prerequisite for the fast and unidirectional development of force and motion during heart and skeletal muscle contraction. Here, the sarcomeric Z-disk, which defines the lateral borders between adjacent sarcomeres and has primarily been seen as a structure important for mechanical stability, turns out to be an essential component of the contractile machinery, involved in assembly of ordered actin and myosin filaments into sarcomeres, by combining architectural, mechanosensation, mechanotransduction and signalling functions. In striated muscle cells, the Z-disk represents a highly organized three-dimensional assembly containing a huge directory of proteins orchestrated in a multi-protein complex centred on its major component α-actinin, with still poorly understood three-dimensional interaction map. On the way to elucidate the structural assembly of the Z-disk, the hierarchy of its organisation and structure-function relationships at molecular level, we are studying binary and higher order sub-complexes of several key Z-disk proteins, namely α-actinin-2, FATZ-1, myotilin, ZASP, aciculin, and filamin C. We are employing an integrative structural biology approach: by using of XL-MS, limited proteolysis and mutation analysis, we mapped interaction interfaces of selected binary complexes. By using pull-down assays, microscale thermophoresis and isothermal titration calorimetry experiments we determined their interaction affinities. By using a combination of low- and high-resolution structural biology methods (SAXS, macromolecular crystallography and electron microscopy) we obtained structural information on α-actinin-2/FATZ-1 and filamin C/FATZ-1 complexes. Finally, we tried to address physiological relevance of certain interactions by in vivo studies. Here we will present some of our recent data on aforementioned proteins and their complexes helping us to uncover their role in Z-disk maintenance and assembly.

S06.P9-205

SP-directed phosphorylation sites in Z-disk titin provide autoinhibitory control of telethonin binding

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Titin is the giant sarcomeric ruler, spanning the entire half-sarcomere to organize its assembly and to provide passive tension during contraction-relaxation. It does this by a modular organization mainly composed by Immunoglobulin-like and Fibronectin-like domains, interspaced by low-complexity linkers. However, many interesting features reside in these titin linkers, like interaction with binding partners or post-translational modifications such phosphorylation, ubiquitination and many others. In the Z-disk, two titin linkers contain a peculiar SPXR-rich motif (Zis1 and Zis5), similar to one linker at the M-band (Mis5), that constitute a potential phosphorylation platform with poorly understood physiological functions. By yeast-two-hybrid assay, Mues et al. (1998) proposed how the interaction between titin N-terminus and telethonin might be regulated by the phosphorylation of the SP motif located downstream of Zis1. We investigated this regulatory mechanism by biochemical and biophysical assays. In contrast to previous domain annotation, we found that in the titin N-terminal fragment (1–392), the predicted third Ig domain adjacent to the SP region (Z3; Labeit & Kolmerer, 1995) does not exist as a folded domain. This has fundamental implications for the attempt of building an accurate Z-disk model that necessarily has to take into account the complete extensibility of this 200 residues long linker. By FRET-based interaction assay using fluorescently tagged titin and telethonin transfected in a mammalian cell line, we demonstrated how this interaction is negatively affected by the replacement of serine residues with non-phosphorylatable alanine, supporting the regulatory mechanism proposed by the genetic screen.

S06.P10-168

Nesprin-1α2 mediates MT organising centre and motor protein recruitment to the nuclear envelope to control myonuclear positioning

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Introduction: At the onset of myogenesis, the microtubule (MT) network is reorganised to facilitate the changes in cell morphology and myonuclear positioning that occur upon myotube formation. MT nucleating capacity transfers from the centrosome to the NE through recruitment of pericentriolar material components to the NE, including pericentrin and γ-tubulin. MTs then reorganise to form a longitudinal array and their associated motor proteins, dynein and kinesin-1, are recruited to the NE to drive regular myonuclear positioning. The LINC complex, comprised of SUN and nesprin proteins, resides in the NE and connects the nucleus to the cytoskeleton. Here, we provide evidence supporting a role for the LINC complex in nuclear-MT connection.
Materials and methods: We investigated the expression and subcellular localisation of nesprin isoforms during C2C12 differentiation. We used RNAi to knock down SUN and nesprin proteins to determine their impact upon pericentrin and kinesin light chain 1/2 (KLC1/2) recruitment to the NE. Finally, we examined the nesprin isoform requirement for pericentrin recruitment by co-expression of pericentrin and nesprin-1α2 in myoblasts.
Results: We found that nesprin-1 expression at the NE is induced early in myogenesis, whereas nesprin-2 is largely cytoplasmic. Furthermore, nesprin-1 is required for recruitment of both pericentrin and KLC1/2 to the NE, whilst SUN1/SUN2 act redundantly. Expression of muscle-specific nesprin-1α2 isoform is induced at the onset of myogenesis and its forced expression in myoblasts is sufficient to recruit over-expressed pericentrin to the NE.
Discussion: Our data support a model in which nesprin-1α2, anchored at the NE through redundant SUN1/SUN2 interactions, acts as a muscle-specific receptor for recruitment of the MT organising centre and motor proteins to the NE. In line with this, muscular dystrophy-associated mutations in SUN proteins cause defects in pericentrin recruitment and myonuclear positioning.

S06.P11-130

Mechanosensing defects in human muscle cell precursors carrying lamin A/C or nesprin-1 mutations

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Introduction: Linker of the Nucleoskeleton and Cytoskeleton (LINC)- complexes and A-type lamins are critical for the transmission of mechanical forces from the extracellular matrix (ECM) to the cell interior. We hypothesized that mutations in genes encoding A-type lamin (LMNA) or nesprin-1 (SYNE-1) would affect the inside to outside mechanisms by which cells adapt to a soft ECM.
Materials and Methods: We have used human myoblasts which are muscle cell precursors with LMNA or SYNE-1 mutations (hereafter named LMNA deltaK32 and Nesprin-1 deltaKASH myoblasts, respectively) responsible for severe muscular dystrophies and have investigated how these mutations affect the cell mechanosensing properties.
Results: We found that LMNA deltaK32or Nesprin-1 deltaKASH plated on soft matrix exhibited contractile stress fiber accumulation, increased focal adhesions, icreased cell spreading and reduced nuclear height compared with control (WT) myoblasts. These abnormalities were associated with increased expression of profibrotic genes including CTGF, COL1 and TGFbeta. Actin cytoskeletal defects were greatly reduced after treatment with the Rho-associated protein kinase inhibitor Y27632, but not after treatment with the myosin light chain kinase inhibitor ML7. More importantly, the protein and mRNA expression of a ROCK-dependent regulator of actin remodeling, the formin FHOD1, was significantly higher in LMNA deltaK32 and Nesprin-1 deltaKASH compared with WT cells and, siRNA-mediated depletion of FHOD1 largely rescued normal cell and nuclear morphologies in mutant cells.
Discussion and Conclusions: We conclude that functional integrity of LINC complexes is required to regulate FHOD1 activity, as well as inside to outside signaling pathways by which myogenic cells adapt their tension to a softer, more physiological environment.

S06.P13-153

Effects of TIEG1 on the structural and functional properties of skeletal muscle

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TGFb inducible early gene-1 (TIEG1) is a member of the Krüppel-like family of transcription factors (KLF10). TIEG1 was first discovered as an early response gene that regulates cellular proliferation and apoptosis. More recently, it has been shown that TIEG1 plays an important role in the growth, morphology and mechanical properties of bone and tendon tissues. TIEG1 is highly expressed in skeletal muscle but nothing is known about its biological roles in this tissue. **Aim:** To characterize the impact of TIEG1 gene deletion on the structural and functional properties of fast and slow twitch skeletal muscles.
Methods and Results: Ten congenic C57BL/6 TIEG1 global knockout (TIEG1−/−) mice (female, 3 months of age) and matching wild-type (WT) controls were used. Histological and immunohistochemical analyses were conducted on three serial transverse sections to detect the MyHC isoforms within the slow (N = 5) and fast (N = 5) muscles. In addition, passive mechanical tests (preconditioning, ramp stretch, relaxation) were performed on skinned muscle fibers (N = 25) extracted from TIEG1−/− and WT soleus (N = 5) and EDL (N = 5) muscles. The morphological results revealed a hyperplasia and hypertrophy of all fibers types (I, IIB, IIA, IIX) for TIEG1−/− muscles with an increase in the percentage of glycolytic fibers (IIX, and IIB) and a decrease in oxidative fibers (I and IIA). The mechanical tests showed a significant increase in the mechanical properties for TIEG1−/− soleus fibers (type I) and a significant decrease for TIEG1−/− EDL fibers (type IIB).
Conclusion: This study has provided new insights about the role of TIEG1 in the functional and morphological properties of fast and slow twitch skeletal muscles. TIEG1 modulates many elements of the cytoskeleton and thus plays an important role in the regulation of its dynamics and remodeling. Our data suggests that defects in TIEG1 expression and/or function may be associated with muscle disease.

Session 07 Smooth Muscle and vascular Function

Oral presentations

S07.O1

Vascular Smooth Muscle Cytoskeletal Function: Contractility and Beyond

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Smooth muscle, by definition, lacks the regular sarcomeric cytoskeletal structure of striated muscle. Recent advances in our understanding of vascular smooth muscle cytoskeletal function have lead to new functional concepts regarding the interactions of the smooth muscle actin and myosin with the co-existing non-muscle actin and myosin in these cells. A model will be presented whereby contractile force and cytoskeletal stiffness are regulated and transmitted from the contractile filaments to the extracellular matrix of the vascular wall.

These concepts may further our understanding of smooth muscle diseases and lead to the development of potential new therapeutics to cure them. Our laboratory's recent work on the smooth muscle cytoskeleton and aging-induced aortic stiffness with its negative cardiovascular consequences will be discussed in this context.

S07.O2-172

Vascular effects of ranolazine involve multiple antagonisms on Nav channels and alpha1-adrenergic receptors

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Introduction: Ranolazine is a recently developed antianginal drug used for treatment of patients with chronic stable angina. It is a selective inhibitor of the persistent cardiac Na+ current (INa), and it is known to reduce the Na+-dependent Ca^{2+} overload that occurs in cardiomyocytes during ischemia. Vascular effects of ranolazine have been reported which may involve multiple pathways. Since voltage-gated Na+ channels (Nav) present in arteries play a role in contraction, we hypothesized that ranolazine could also target these channels.
Materials and methods: We studied the effects of ranolazine on INa and intracellular calcium ($[Ca^{2+}]i$) using the patch-clamp technique and fura-2 fluorescent imaging, respectively, on cultured aortic smooth muscle cells (SMC). We also measured changes in isometric tension on rat aorta and human uterine artery in response to ranolazine. Vasoconstrictors and conditions known to specifically activate or promote INa were used.
Results: Ranolazine has vasorelaxant effects. We observed that ranolazine inhibited INa and $[Ca^{2+}]i$ increase induced by veratridine in SMC, and veratridine-induced vasoconstriction of rat aorta. We demonstrated that ranolazine inhibited the activity of TTX-sensitive voltage-gated Nav channels promoted by KCl depolarization both at sympathetic perivascular nerve terminals, through catecholamine release, and at the arterial myocytes level. Furthermore, we also observed that ranolazine antagonized the contractile response to the α-adrenergic agonist phenylephrine and its receptor binding. Thus, the vasorelaxant effects of ranolazine independent of the endothelium, were also observed in human arteries.
Conclusion: Combined alpha1-adrenergic antagonization and inhibition of SMCs Nav channels could be involved in vascular effects of ranolazine.

S07.O3-194

Effect of High-Frequency Low-Load Blood-Flow Restricted Training on Markers of Myocellular Inflammation and Stress

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Introduction: Blood- flow restricted exercise (BFRE) has been established as a low-load alternative to heavy-resistance exercise to induce increases in muscle mass and strength [1,3]. However, while indicators of muscle damage have been reported with acute BFRE, insight into the longitudinal effect of BFRE training on myocellular damage and inflammation are lacking.
Material and Methods: Ten male subjects performed 19 consecutive days (23 sessions) of low- load (20% 1RM) knee extensor exercise to failure with partial blood flow restriction (BFR) (100 mmHg), while 8 controls (CON) performed a work-matched free-flow protocol. Muscle biopsies were obtained from VL before (Pre), 8 days into the training intervention (Mid) as well as 3 days (Post3) after cessation of training. Analyses of different markers of myocellular stress and inflammation were performed including tissue pro- (M1) and anti-inflammatory (M2) macrophages (immunofluorescence) and heat shock proteins 27 and 70 (HSP27/70) (immunoblot).
Results: An increase in M1 macrophages was seen in both groups at Post3 ($p < 0.05$), while an increase in M2 macrophages was observed from Pre to Post3 with BFR training only ($p < 0.01$). No changes in HSP27 or HSP70 were observed.
Discussion: Increases in skeletal muscle tissue macrophage infiltration were observed after 19 days of high-frequency (23 training sessions performed to failure) low-load BFRE. Notably, indices of muscle inflammation were observed 3 days after cessation of training in both groups, indicating that the accumulated training stress stimulated a consistent cellular inflammatory response. Yet, the elevation in inflammatory markers ($\sim$100%) appears substantially lower compared to that reported following severe muscle overuse damage (>200%) [2].

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S07.O4-155/S07.P1-155

Essential role of smooth muscle Rac1 in bronchoconstriction and asthma-associated hyperresponsiveness

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Introduction: The molecular mechanisms regulating airway smooth muscle cells (aSMC) contraction and proliferation involved in airway hyperresponsiveness (AHR) are still largely unknown. Small GTPases of the Rho family (RhoA, Rac1 and Cdc42) play a central role in smooth muscle functions. Recently, we demonstrated that Rac1 play an essential role in the control of arterial pressure by modulating vascular SMC contraction (André et al., J Am Heart Assoc., 2014). Accordingly, we hypothesized that Rac1 could also be involved in aSMC contraction.
Methods and Results: Ex- and in vitro analysis of bronchial reactivity shows that the specific SMC deletion of Rac1 (SM-Rac1-KO) in mice prevents the bronchoconstrictor response to KCl and methacholine. Our results demonstrated that the decreased expression or activity of Rac1 in aSMC impairs bronchoconstrictor-induced rise in intracellular Ca^{2+} concentration through a mechanism involving Rac1-dependant control of PLC activity. Experiments performed in human bronchi and airways SMC reveals a similar role of Rac1 in the control of bronchoconstriction in humans. In vivo, Rac1 deficiency has no impact on the respiratory system in basal, physiological condition. However, deletion of SMC Rac1 or nebulisation of the Rac inhibitor NSC23766 prevents AHR in murine models of allergic asthma (ovalbumine and house dust mite-sensitized mice).
Conclusion: These data indicate that (1) Rac1 plays a critical role in aSMC contraction, and (2) its inhibition prevents AHR. Compared to classical bronchodilators, Rac1 inhibition presents the additional advantage to simultaneously induce bronchodilation and decrease pulmonary inflammation. Inhibition of Rac1 activity or expression may represent a novel therapeutic approach for patients with AHR associated with asthma or COPD.

S07.O5-332 / S07.P2-332

Phenotypic remodeling of perivascular adipose tissue in a rat model of metabolic syndrome: vascular consequences and beneficial impact of exercise training

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Aim: This study was designed to evaluate the impact of high fat and high sucrose diet (HFS) on perivascular adipose tissue (PVAT) and its consequence on vascular function. Whether exercise training was able to counteract this alterations has been investigated as a potential therapeutic strategy.
Methods: Rats were fed with standard (Ctrl rats) or HFS diet (HFS rats) for 15 weeks. After 6 weeks, HFS rats were randomly assigned into 2 groups: sedentary and trained group (HFS-Ex). PVAT remodeling was assessed by a proteome profiler, histological and biochemical assays. The impact of PVAT secretion on vascular function was assessed on isolated aortic rings incubated or not with PVAT secretum.
Results and discussion: We reported increased PVAT mass in HFS rats associated with increase in both white to brown adipocytes proportion and uncoupling protein 1 level. Despite no major effect of HFS diet on PVAT adipokines profile was reported, adiponectin level and secretion were reduced in PVAT of HFS rats. As a potential consequence, we observed a marked endothelial dysfunction in Ctrl aortic rings incubated with PVAT secretum of HFS rats. The use of a non-specific antioxidant (N-Acetyl cysteine) in the organ bath blunted the deleterious effect of HFS secretum on endothelial function, suggesting a redox-dependent mechanism in this phenomenon. Exercise training in HFS rats normalized PVAT mass but has no effect on the browning process. However, this strategy was able to normalize adiponectin level in PVAT of HFS rats and finally abolished the detrimental effect of its secretion on endothelial function.
Conclusion: Diet-induced metabolic syndrome leads to PVAT remodeling inducing deleterious vasoactives properties. The ability of exercise training to modulate this phenomenon could be considered as a good strategy to counteract the potential role of PVAT in the development of chronic vascular dysfunction in metabolic syndrome disease.

Posters

S07.P3-124

Impaired adaptation of training-induced blood pressure in COPD patients: implication of the muscle capillary bed?

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Targeting the early mechanisms in exercise-induced and later-appearing resting hypertension (HTA) may improve cardiovascular morbi-mortality in COPD patients. Capillary rarefaction, an early event in COPD before vascular remodeling, is a potential mechanism of exercise-induced and resting HTA. We previously observed impaired training-induced capillarization in COPD patients; this study thus compares changes in exercise blood pressure (BP) in COPD patients and matched control subjects (CS) after a similar exercise training program, in relationship with muscle capillarization. Resting and maximal exercise diastolic (DP) and systolic (SP) pressures were recorded during a standardized cardiopulmonary exercise test, and a quadriceps muscle biopsy was performed before and after training. Thirty-five CS and 49 COPD patients (FEV1=54±22 % pred.) completed a 6-week rehabilitation program and improved their symptom-limited maximal oxygen uptake (VO2SL: 25.8±6.1 vs. 27.9 mL/kg/min and 17.0±4.7 vs. 18.3 mL/kg/min; both $p < 0.001$). The improvement in muscle capillary-to-fiber (C/F) ratio was significantly greater in CS vs. COPD patients (+11±9% vs. +23±21%; $p < 0.05$). Although maximal exercise BP was reduced in CS (DP: 89±10 vs. 85±9 mmHg; $p < 0.001$ / SP: 204±25 vs. 196±27 mmHg; $p < 0.05$), it did not change in COPD patients (DP: 94±14 vs. 97±16 mmHg; $p = 0.46$ / SP: 202±27 vs. 208±24 mmHg; $p = 0.13$). The change in muscle C/F ratio was negatively correlated with maximal exercise SP in CS and patients ($r = -0.41$; $p = 0.02$).

COPD patients showed impaired training-induced BP adaptation related to a change in muscle capillarization, suggesting the possibility of blunted angiogenesis.

S07.P5-101

Blood pressure responses to isometric handgrip training in normal healthy female students

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Introduction: Regular physical activity was recommended to improve cardiovascular health and lower blood pressure among both the general population and patients with hypertension. Studies showed that deaths from strokes can be reduced by 14% by reducing systolic blood pressure by 5 mmHg, also deaths from coronary heart disease can be decreased by 9%. Regular exercise is key to preventing and treating hypertension (Chobanian et al. 2003).
Objectives: to determining the effect of isometric handgrip training program on blood pressure in sedentary young female students and to compare and find the differences between the control group and the experimental group. Methods: Thirty nine untrained female students participated in this study (Age 18.47±0.51). After taking resting measures, the experimental group were then required to perform an isometric hand grip strength contraction with one hand for 45 seconds at 30% of maximal voluntary contraction. One minute rest was given following this contraction. The left hand contraction was then performed for 45 seconds at 30% of maximal voluntary contraction. A one minutes resting period was also giving following the contraction. Four repeated isometric contraction was required (two per hand) with total of 3 minutes of exercise per session. Performing this exercise for 5 days elicited a duration of 15 minutes for the entire study. Measurements of HR and BP were taken immediately before the first 45 seconds contraction, and immediately after the 4th contraction and after 3 minutes of sitting period.
Results: There was no significant differences between the groups in terms of age, body mass, BMI,WHR, resting HR, SBP and DBP. However, there were significant differences between the groups for the maximal voluntary contraction between pre and post intervention.
Conclusion: isometric handgrip training may reduce resting heart rate and blood pressure.

Session 08 Epigenetics of muscle regeneration

Oral presentations

S08.O1

Epigenetic events in the process of muscle wasting: implications in human disease and animal models

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Muscle mass loss and impaired function are characteristic features of chronic cardiac and respiratory conditions including lung cancer and chronic obstructive pulmonary disease (COPD). Several factors and mechanisms participate in the multifactorial etiology of skeletal muscle dysfunction and mass loss of chronic disorders. Oxidative stress, ubiquitin-proteasome pathway, mitochondrial derangements, autophagy, and epigenetic regulation are biological mechanisms directly involved in enhanced protein breakdown and altered muscle function in patients with COPD and lung cancer. Recently, several epigenetic events, namely microRNA expression and histone acetylation, have been shown to be differentially expressed in the lower limb muscles of patients with COPD with and without muscle mass loss and weakness (reduced strength). Moreover, the diaphragm and especially the limb muscles of mice with lung cancer-induced cachexia also exhibited a downregulation of muscle-enriched microRNAs, with alterations in the expression of the most relevant downstream pathways involved in muscle proliferation and differentiation. Furthermore, in a mouse model of disuse muscle atrophy of the limbs, the expression of several microRNAs was also downregulated at different time-points. Modifications in the expression of the downstream regulatory pathways were also observed in the muscles of those animals. Muscle function was also reduced in the animals exposed to the different immobilization periods. These results shed light into novel mechanisms of muscle mass loss and dysfunction that offer potential avenues for research in the discovery of novel therapeutic pharmacological strategies to alleviate muscle wasting in chronic conditions.

S08.O2-338

Genetic and epigenetic regulatory control of skeletal muscle stem cells

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Introduction: Studies in the last decades have highlighted the diversity of regulatory networks that are required to establish skeletal muscle during development, and effect regeneration following injury or during disease. We are investigating this underlying diversity to assess how adult stem cells choose to divide symmetrically or asymmetrically, and the fate of the resultant daughter cells.
Material and Methods: We are using genetically modified mice, acute muscle injury, FACS and immunostaining to assess muscle stem cell fates.
Results: We manipulated Notch signaling and showed that this pathway is critical for maintaining muscle stem cell quiescence, and it is rapidly downregulated following muscle injury. During the regeneration process, the frequency of symmetric and asymmetric divisions is stereotypically regulated in myogenic cells. Following muscle injury, a subset of myogenic cells undergo asymmetric random DNA segregation. We propose that epigenetic differences exist between daughter cells, and that this is related to differential cell fates that are adopted following this asymmetric event. Importantly, the frequency of this asymmetry can be modulated by altering the microenvironment using micropattern technology.
Conclusion: Alternate cell fates correlate with asymmetric cell divisions and random DNA segregation, and these events are profoundly affected by the microenvironment.

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S08.O3-175/S08.P1-175

The role of miR-378 in sarcopenia

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Sarcopenia is defined as the loss of muscle mass and function with age that affects all individuals from approximately the 4th decade of life. The mechanisms behind sarcopenia are unclear, however they are likely to be multifactorial. MicroRNAs (miRNAs, miRs) are small non-coding RNAs that post-transcriptionally regulate gene expression. microRNAs have been shown to play key roles in muscle development and disease. We and others demonstrated a decrease in miR-378 expression in muscle with age in both rodents and humans. We were able to manipulate the expression and function of microRNA-378 in muscle of adult and old mice using microRNA mimic and antagomiR delivered via tail vein injection. Inhibition of miR-378 expression resulted in decreased muscle mass, myofibre size and function (force generation). In contrast, overexpression of miR-378 resulted in an increased muscle mass, myofibre size and function in both adult and old mice. These data suggest miR-378 plays a role in sarcopenia and may be a potential therapeutic target against sarcopenia. Further work will focus on the validation on targets of miR-378.

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S08.O4-122/S08.P2-122

Exercise reprograms muscle satellite cell proliferation and differentiation through epigenetic regulation of GREM1

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Aims: Physical exercise elicits metabolic adaptation in the muscle cell through remodeling of gene expression. Muscle satellite cells constitute precursor cells that show capacity to retain metabolic characteristics of the donor; in particular, we previously showed that eight-week endurance exercise training reprograms glucose metabolism in primary cultures of skeletal muscle cells purified from obese donors [Bourlier, JCEM 2013]. Here, we hypothesized that exercise training induces epigenetic modifications that participate in stable metabolic reprogramming of the muscle satellite cell.
Methods and Results: We analyzed the epigenome of myotube cultures collected from five middle-aged obese men before and after an eight-week exercise training program [Bourlier, JCEM 2013]. Using DNA methylation capture and small non-coding RNA (sncRNA) sequencing, we profiled genome-wide DNA methylation and sncRNA expression. While myotube cultures from trained obese men showed no difference in sncRNA expression, we detected 116 differentially methylated regions (DMRs). The second most significant DMR ($p = 9.09E-21$; $FDR = 1.73E-16$) corresponds to a regulatory region of gremlin 1 (GREM1), a gene involved in the balance between muscle cell proliferation and differentiation [Frank, J Cell Biol 2006]. GREM1 expression was increased after training and negatively correlated to methylation of the DMR, suggesting an epigenetic regulation of GREM1 expression. Overexpression of GREM1 in the myogenic mouse cell line C2C12 using the CRISPR-Cas system confirmed the role of GREM1 in muscle cell proliferation and differentiation.
Conclusion(s): Our results suggest that epigenetic regulation of GREM1 expression is a mechanism by which exercise reprograms muscle precursor cells to modulate differentiation and metabolic efficiency of the muscle cell.

S08.O5-253

A microRNA axis that regulates muscle mass and mitochondrial function in response to disease

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A loss of muscle mass and function is a common co-morbidity of a range of chronic conditions but the mechanisms that regulate these

changes remain to be fully identified. MicroRNAs are small RNAs that control the phenotype by regulating the rate of translation and degradation of RNAs. We aimed to identify miRNAs that regulate muscle mass and function in response to disease. Using a PCR screen we found that miR-542-3p was elevated in COPD patients, in cardiac patients and in those with established ICU acquired wasting. miR-542-3p was inversely associated with lung function in COPD patients and heart function in cardiac patients. In COPD patients, miR-542-3p was inversely associated with 6 minute walk distance. We also found that miR-422a was not associated with disease severity but was associated with muscle strength in COPD and cardiac patients. In patients about to undergo cardiac surgery quadriceps miR-542-3p was positively correlated and miR-422a was negatively correlated with the amount of muscle that would be lost over the following 7 days. In vitro miR-542-3p reduced the expression of the mitochondrial ribosomal protein MRSP10 and reduced the mitochondrial membrane potential. miR-542-3p also suppressed the expression of RPS22 and consistent with activation of the ribosome stress pathway increased the expression of GDF-15 (a p53 target gene). Over-expression of miR-542 in vivo reduced fibre diameter 10% in 3 days and reduced mitochondrial content. In vitro, miR-422a a suppressor of the p53 activator MLH1, inhibited miR-542-3p dependent GDF-15 expression. Methylation of the miR-422a promoter was inversely associated with muscle strength in men. Together our data suggest that in response to the stress of disease increased miR-542-3p promotes muscle wasting. This activity is opposed by the activity of miR-422a, the expression of which varies in individuals dependent on relative DNA methylation.

Posters

S08.P3-322

Do Differences Exist in Microrna Expression Profile Between Vastus Lateralis and Myotubes in COPD Cachexia?

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Quadriceps muscle weakness, muscle wasting and cachexia are common in chronic obstructive pulmonary disease (COPD) patients. Upregulation of microRNA expression may favor muscle mass growth and differentiation. We hypothesized that differences may exist in the expression of several muscle-specific microRNAs between patients with and without severe muscle wasting both in vivo (biopsies) and in vitro (myotubes). Twenty-nine patients with COPD ($n = 15$, muscle wasting, FFMI 15 kg/m^2 and $n = 14$, normal body composition, FFMI 18 kg/m^2) and 10 healthy controls (FFMI, 19 kg/m^2) were consecutively recruited. Biopsies from the vastus lateralis were obtained in all study subjects. A fragment of each biopsy was used to obtain primary cultures, in which muscle cells were first proliferated to be then differentiated into actual myotubes. In both sets of experiments (in vivo and in vitro) the following muscle-enriched microRNAs were analyzed using qRT-PCR: miR-1, miR-133, miR-206, miR-486, miR-29a, miR-27a, and miR-181a from all study subjects. While the expression of miR-1, miR-206, miR-486, and miR-29a was upregulated in the muscle biopsies of COPD patients compared to those of healthy controls, levels of all the study microRNAs in the myotubes (primary cultured cells) did not significantly differ between COPD patients and the controls. We conclude from these findings that environmental factors (blood flow, hypoxia, deconditioning) taking place in vivo (biopsies) in the muscles may account for the differences observed in microRNA expression between COPD patients and controls. In the myotubes, however, the expression of the same microRNAs did not differ between the study subjects as such environmental factors were not present. These findings suggest that therapeutic strategies should rather target environmental factors in COPD muscle weakness and mass loss as the profile of microRNA expression in myotubes is similar in patients to that observed in the healthy controls.

S08.P5-174

Skeletal muscle satellite cell niche: Cells arising from the bone marrow

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The cellular components of the specialized local environment where satellite cells reside participate in regulation of the skeletal muscle regeneration. Besides myogenic cells at various developmental stages, this niche is formed by cells of the immune system, the connective tissue and the vascular system. Unambiguous determination of their cell types and origin could contribute to improvement of cell based therapy of the skeletal muscle disorders. In our work we intravenously transplanted mouse GFP+ freshly isolated unseparated bone marrow cells into whole-body lethally irradiated immunocompetent mice 4 weeks before the cardiotoxin-induced injury of the recipients' skeletal muscles. Seven and 28 days after the toxin injection, the injured muscles were examined for presence of GFP+ cells by direct fluorescence, protein immunohistochemistry and immunogold transmission electron microscopy. Using immunohistochemistry and cell ultrastructure observation we identified cell types of the bone marrow origin located in the satellite cell niche. In the regenerating muscle 7 days after the injury GFP positivity was determined in numerous immune cells, mainly in macrophages, in some fibrogenic cells, rarely in endothelial cells of the blood vessels and in myogenic cells. Twenty-eight days after the injury, GFP+ immune cells and fibrogenic cells became less numerous, whereas infrequent GFP+ endothelial cell and muscle fibres were still detected. Our results confirmed ability of bone marrow derived cells to contribute to the cellular component of the satellite cell niche during the skeletal muscle regeneration. These cells originated not only from the hematopoietic stem cells, but obviously, also from other stem/progenitor cells residing in the bone marrow, e.g. the mesenchymal stem cells and the endothelial progenitors.

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S08.P6-143

Thyroid Hormone Receptor regulates the duration of skeletal muscle regeneration in response to acute injury

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Introduction: Thyroid hormone plays a key role in skeletal muscle development and repair. We have previously identified a 43 Kda truncated form of the nuclear receptor TRα1 (p43) which stimulates mitochondrial activity (1, 2). Using mice overexpressing specifically p43 in skeletal muscle (p43-Tg) or lacking the protein (p43−/−), we found that this receptor regulates muscle mass and the metabolic and contractile features of myofibers (3, 4). p43 overexpression decreases muscle weight whereas deletion of the protein increases muscle mass. However the molecular mechanisms regulated by p43 remain unclear as the role of the protein during muscle regeneration.
Methods: WT, p43-Tg and p43−/− mice were used in this study. Gastrocnemius muscles were used to perform western-blot and next generation mRNA sequencing (NGS). Skeletal muscle regeneration was induced by cardiotoxin injection in tibialis muscle.
Results: Using western-blot experiments, we found that in skeletal muscle, autophagy is the main pathway affected by p43. In addition, examination of the skeletal muscle transcriptional profiles by NGS technology allows us to identify new potential targets of p43. After skeletal muscle injury induced by cardiotoxin, we found that either p43 disruption or overexpression results in perturbation of muscle regeneration. We observed that mice overexpressing p43 show accelerated regeneration, whereas the absence of the protein induces a delayed but normal regeneration. In line with these observations, in vitro approach indicates that p43 plays an important role in myoblast proliferation.
Conclusion: In conclusion, we found that level of this receptor is essential for regulating the duration of skeletal muscle regeneration in response to acute injury.

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S08.P7-112

Transforming growth factor-β inhibits adipogenesis in regenerating glycerol-injured muscle

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Introduction: Fibrosis and adipogenesis are characteristic features of several muscle diseases as muscle dystrophies, inflammatory myopathies and sarcopenia, they negatively affect muscle function. Chemically-induced injuries provide good models to study the mechanism of muscular dystrophies and consequently to develop new therapies for treatment. Recently we have reported that glycerol injury induced muscle regeneration with adipogenesis and progressive deposition of intramuscular connective tissue in normal mice [1]. However, the effect of transforming growth factor-β (TGF-β) on muscle regeneration and adipogenesis is unclear.
Aim: The aim of the present study is to investigate the effect of TGF-β on muscle regeneration and adipogenesis following glycerol injury.
Methods: Mice were divided into three groups. The early treatment group was injected with TGF-β combined with glycerol. The late treatment group was injected with TGF-β at day 4 after glycerol injury. The control group was injected with glycerol only. Injections were performed into tibialis anterior muscles of adult mice. Muscle samples were collected at day 7 after glycerol injury.
Results: Early TGF-β treatment inhibited adipogenesis significantly while late treatment decreased adipogenesis. Moreover, muscle regeneration was impaired in TGF-β treated muscles compared to the glycerol-injured muscles. Furthermore, TGF-β reduced macrophages infiltration resulting in significantly larger necrotic area compared to glycerol-injured muscle. On the other hand, TGF-β injection reduced mRNA expression of both myogenic and adipogenic factors compared to the glycerol-injured muscle.
Conclusion: The inhibitory effect of TGF-β is much higher during early stages of muscle regeneration and adipogenesis.

S08.P8-140

Interleukin-8 modifies miR-338-3p expression, ephrin4A cellular content, and migratory ability of rat primary skeletal myoblasts

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1: The aim of the study was to examine the expression of miR-338-3p and its potential target ephrin4A, involved in cell migration and motility, in rat primary skeletal myoblasts exposed to IL-8. Cellular levels of ephrin4A and cell migratory ability were also investigated. 2: Primary skeletal muscle cells (RSkMc), isolated from the limbal muscle of neonatal rats, were subjected to 11 days of differentiation in the presence of IL-8 (final concentration – 1 ng/ml). MiR-338-3p and ephrin4A transcripts were examined using qPCR, with U6 (for miRNA) and Gapdh and beta-actin (for mRNA), serving as housekeeping genes. Ephrin4A protein was assessed by immunoblotting. Cell migration was examined using a wound-healing assay. A wound was simulated through a straight scratch with a sterile 100-µl pipette tip, and phase-contrast microscopy images were captured immediately (0 hour) and at 4, 8 and 12 hours after the mechanical wounding. Migration was measured as a reduction of distance between the wound edges due to the movement of cells into the cell-free zone. Student t-test was used for comparison the results. 3: The level of miR-338-3p was 1.6-fold higher ($p < 0.05$) in IL-8-treated myoblasts, in comparison to the control. Transcript level of ephrin4A was not modified by IL-8, however the cellular levels of ephrin4A was markedly increased in IL-8-treated myocytes, especially, in the presence of IGF-I (25 nmol/l). The cytokine stimulated migratory ability of differentiating myoblasts: a 1.75-fold increase over control value ($P = 0.043$) was detected after 4-h-exposure to IL-8, and a 2.-3-fold incease ($P = 0.0015$) was caused by IL-8 and IGF-I combination. 4: IL-8, a chemokine and angiogenic factor present in contracting muscles, stimulates migration of differentiating skeletal myoblasts, and this phenomenon is associated with increased ephrin4A level. MiR-338-3p, probably, does not play a critical role in the regulation of ephrin4A expression in skeletal myoblasts.

S08.P9-127

Interleukin-6 affects miR-338-3p expression, pacsin-3 cellular content, and cytoskeletal component levels and localization in rat primary skeletal myoblasts

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Introduction: The aim of the study was to examine the expression of miR-338-3p and its potential target pacsin-3, which plays important roles in cytoskeletal organization, organelle biogenesis and membrane remodeling, in rat primary skeletal myoblasts subjected to differentiation in the presence of IL-6. Cellular levels and distribution of actin, alpha-actinin and desmin were also investigated.
Material and methods: Primary skeletal muscle cells (RSkMC), isolated from the limbal muscle of neonatal rats, were subjected to 11 days of differentiation in the presence of IL-6 (final concentration – 1 ng/ml). Transcript levels of miR-338-3p, pacsin-3 and cytoskeletal components were examined using qPCR, with U6 (for miRNA) and Gapdh and beta-actin (for mRNA), serving as housekeeping genes. Protein levels of pacsin-3, actin, alpha-actinin and desmin were assessed by immunoblotting. Cellular distribution of cytoskeletal proteins were visualized by immunofluorescence and confocal microscopy. Student t-test was used for comparison the results.
Results: IL-6 did not affect the cell viability and myotube number in differentiating RSkMC cultures. The level of miR-338-3p was 3.4-fold higher ($P < 0.05$) in IL-6-treated myoblasts, in comparison to the control. Transcript levels of pacsin-3, actin, alpha-actinin and desmin were not modified by IL-6. The cellular level of pacsin-3 was slightly but significantly decreased, actin and alpha-actinin were not altered, whereas the level of desmin was increased in IL-6-treated myocytes. The presence of the cytokine caused the accumulation of alpha-actinin and desmin in perinuclear area of cells.
Conclusions: IL-6, a known proinflammatory cytokine and a physical activity-associated myokine, alters miR-338-3p and pacsin-3 levels in differentiating skeletal myoblasts. These effects are associated with cytoskeletal component modifications, such as an increase in desmin level and perinuclear distribution of alpha-actinin and desmin.

Session 09 Neuro-muscular disease and therapeutic approach

Oral presentations

S09.O1

Alternate translational initiation of dystrophin: clinical and therapeutic implications

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We recently identified an internal ribosome entry site (IRES) within exon 5 of the DMD gene that when active results in alternate translational initiation beginning within exon 6. As a result, patients carrying mutations that truncate the reading frame 5' of the IRES express an N-truncated dystrophin isoform that is highly functional, despite lacking the calponin homology domain 1 (CH1) of the actin binding domain 1 (ABD1). Consistent with genotype-phenotype correlations in Duchenne muscular dystrophy (DMD) patients, the IRES is not active in the presence of an exon 2 duplication but is active when exon 2 is deleted. We developed an AAV9.U7snRNA vector to induce skipping of exon 2, and have shown that in a DMD mouse model carrying a duplication of exon 2 (the Dup2 mouse), intramuscular (IM) or intravascular (IV) treatment results in functional and histopathologic improvement in skeletal muscle, and gene transfer at postnatal day 1 (P1) results in sustained correction of pathologic and physiologic defects. Preliminary studies in the Dup2 mouse using peptide-linked PMO antisense oligomers (PPMOs) show a similar degree of correction, suggesting multiple potential therapeutic routes to exon skipping. To model the applicability of this approach beyond exon 2 patients, we have used the same viral vector to treat human patient fibroblast-derived transdifferentiated myoblasts (FibroMyoD cells) harboring different mutations within exons 1 to 4, and shown abundant exon skipping and dystrophin expression. These results suggest that this exon-skipping approach offers a therapeutic route not only to patients with exon 2 duplications but with all mutations within the first four DMD exons, and supports the idea that early treatment of these patients will have longstanding and significant benefit resulting in a better outcome.

S09.O2-142

Gene replacement therapy as a novel approach for the treatment of oculopharyngeal muscular dystrophy

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Among triplet expansion diseases, oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant, late-onset muscle disorder characterized by progressive eyelid drooping, swallowing difficulties and proximal limb weakness. OPMD is caused by a short trinucleotide repeat expansion in the polyadenylate-binding protein nuclear 1 (PABPN1) gene that results in an N-terminal expanded polyalanine tract. PABPN1 controls several biological processes such as the length of mRNA poly(A) tails, the mRNA export from the nucleus and the alternative poly(A) site usage. OPMD is characterized by nuclear aggregates of expanded PABPN1, fibrosis and muscle atrophy. Here we demonstrate that treating mice affected by OPMD over 4 months with an AAV gene therapy strategy based on DNA-directed RNA interference (ddRNAi) to silence the endogenous expPABPN1, combined with the re-expression of a healthy sequence-optimized human PABPN1 gene, significantly reduced the amount of nuclear aggregates in affected muscles, decreased the intramuscular fibrosis, reverted the muscle strength to the level of healthy wild-type muscles and normalized transcriptome. Furthermore, although muscle atrophy was not reverted, the expression of a healthy PABPN1 markedly increased the cross sectional area of muscle fibres. Importantly the efficacy of the combined treatment was also verified in cells derived from OPMD patients. These results obtained in a relevant mammalian animal model of OPMD pave the way for the clinical application of a gene therapy approach as a treatment for OPMD patients.

S09.O3-295

Dystrophin deficient rats: a robust animal model for Duchenne muscular dystrophy studies

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Duchenne Muscular Dystrophy (DMD) is a severe muscle-wasting disorder caused by mutations in the dystrophin gene, without any curative treatment available yet. For pre-clinical evaluation of therapeutic approaches, few animal models are available. Large animal models such as dogs or pigs are expensive, difficult to handle and display important clinical heterogeneity, while mdx mice only exhibit limited chronic muscular lesions and muscle weakness. Thus, a rat model could represent a useful alternative, since rats are small animals but 10 times bigger than mice and could therefore be a better mean to study the human disease. A line of Dmd mutated-rats (Dmdmdx) was generated using TALENs. Analyzed muscles from these animals exhibited undetectable levels of dystrophin by western-blot and less than 5 % of dystrophin positive fibers by immunohistochemistry. Dmdmdx rats had significantly reduced body weight from 4 weeks. At 3 months, limb and diaphragm muscles displayed intense necrosis and regeneration. At 7 and 12 months, these muscles showed severe fibrosis and adipose tissue infiltration. From 6 weeks, muscle strength was significantly reduced and associated with muscular fatigue. Dmdmdx rats were diagnosed with decreases of spontaneous motor activity. At 3 months, echocardiography of the heart showed a significant concentric remodeling and an alteration of diastolic function. Subsequently, the heart morphology evolved into a dilated cardiomyopathy with necrotic and fibrotic tissue. A long-term study showed that life span was reduced in Dmdmdx rats. Cardiac insufficiency or dilated cardiomyopathy were frequently the direct cause of death of these Dmdmdx rats. In conclusion, Dmdmdx rats are a very promising small animal model that can now be used for pre-clinical evaluation of therapeutic approaches of DMD, particularly for testing effects on disease progression and cardiac anomalies that were previously difficult to assess using the current DMD animal models.

S09.O4-226/S09.P1-226

Generation of a mouse model of FSHD to reveal the DUX4 expression profile and dynamics

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is characterized by a descending, often asymmetric, skeletal muscle atrophy. The genetic basis of the disease is linked to DNA hypomethylation of D4Z4 macrosatellite repeats on chromosome 4q35. This results from either contractions of the D4Z4 repeat array (FSHD1) or mutations of chromatin modifiers (SMCHD1, DNMT3B; FSHD2), causing aberrant expression of the DUX4 retrogene mapped in eachD4Z4 unit. Toxic DUX4 protein is produced from the distal D4Z4 if a 3' polyA addition site (pLAM region) stabilizes the mRNA. DUX4 expression is considered the primary cause of FSHD, but its toxicity is a major issue in the development of animal models.
Aims: We aimed to generate a mouse model of FSHD to reveal the DUX4 expression profile and dynamics, but avoiding its toxicity. This mouse could give insight into FSHD pathomechanisms and provide a platform for testing potential therapeutic strategies.
Methods: In order to suppress the DUX4 ORF we constructed DUX4p-nlacZ-pLAM with the native human DUX4 promoter and pLAM region flanking a nuclear-localised (n)lacZ reporter gene. Using pronuclear injection of this reporter construct, we generated transgenic mice in which the expression dynamics of the pathogenic locus can be mapped.
Results: We first demonstrated the functionality of the DUX4p-nlacZ-pLAM construct in immortalised murine C2C12 and human myoblasts and myotubes. We then generated DUX4p-nlacZ-pLAM transgenic mice by pronuclear injection, and initial analysis of the F1 generation reveals rare nuclei containing β-galactosidase in skeletal muscle. We are now establishing multiple lines of these reporter mice and will present their detailed analysis at the meeting.
Conclusion: Through generating a transgenic reporter mouse line that carries a native human configuration of DUX4 promoter and pLAM region, we aim to create an animal model that could be used for mapping the expression profile and dynamics of DUX4.

S09.O5-307/S09.P2-307

Disease stage and muscle type influence contractile function in facioscapulohumoral dystrophy

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Introduction: Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common genetic muscle disorders. It is caused by derepression of the D4Z4 repeat array, which leads to transcription of DUX4, a transcription factor that promotes apoptosis and atrophy when mis-expressed in skeletal muscle. Because most studies in FSHD focus on the clinical and genetics aspects of the disease, there is an important gap in knowledge about the downstream effects of DUX4 on skeletal muscle contractile function in patients, and how this leads to clinical muscle weakness.
Methods: We have extensively studied the mechanical aspects of muscle contraction in a clinically well-defined group of 14 FSHD patients, as well as 12 healthy controls. Clinical evaluation and MRI were performed in all participants. Biopsies of the vastus lateralis and tibialis anterior were performed in all participants for histological analysis and single fiber studies to assess sarcomeric function.
Results: Voluntary quadriceps function is affected early in FSHD patients, who show intrinsic weakness independent of muscle size or fatty infiltration. Changes in sarcomeric function were seen in FSHD patients compared to controls, and differ depending on disease severity and muscle type. Mild disease severity was associated with type 2 hypertrophy and increased passive tension in FSHD vastus lateralis, but not in tibialis anterior. Severe disease was associated

with changes in both muscles, which consist of fiber atrophy with reduced maximum force generation and increased calcium sensitivity. **Conclusion:** FSHD muscle contractile function depends on muscle type and disease severity. Unraveling the molecular mechanisms that underlie these differences will increase our understanding of the pathophysiology of FSHD. Inherent differences between muscles must be considered when designing therapeutic trials or collecting tissue for research studies.

Posters

S09.P3-327

Development of Orally Bioavailable Therapeutics by Chloroplast Expression Counters Muscle Weakness in DMD

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Introduction: A major consequence of the heightened cycles of degeneration/regeneration in dystrophic muscle is the cumulative fibrotic replacement of muscle. Significant pro-fibrotic signals occur through the hyperactivity of the deleterious axis (ACE/Ang II/AT1R) of the renin angiotensin system (RAS). In contrast, the anti-fibrotic axis of RAS, the ACE2/Ang(1-7)/Mas pathway, represents a potential therapeutic strategy in muscular dystrophy. The goal of this study was to determine if orally bioavailable proteins in the Mas pathway would prevent pathology in dystrophic mice.
Materials and Methods: Using a novel chloroplast expression system, human ACE2 and Ang(1-7) were produced harboring an N-terminal fusion with the cholera toxin subunit B (CTB) to enable transmucosal transport into the circulation. Plant leaves were lyophilized and recombinant protein content was quantified. Male dystrophic mice (Cmah−/− × mdx) were fed 25 mg plant protein for 2 – 8 weeks. Serum was collected prior to treatment and every 2 weeks during treatment.
Results: Circulating ACE2 activity increased progressively 1.5 – 2 fold over pre-treatment values during the 8 week treatment regimen, supporting that the recombinant protein had entered the bloodstream. Following treatment, functional analysis of the diaphragm and extensor digitorum longus muscles were performed. The diaphragms from mice treated with ACE2 for 2 weeks displayed significant functional improvement compared to untreated controls, whereas the EDL muscles showed no marked improvement.
Conclusion: These results demonstrate that the use of transplastomic proteins may provide a new strategy to deliver therapeutic proteins for the muscular dystrophies.
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S09.P4-324

Effect of repeated contractions on tetanic force and rate of force development in an in vitro rat model of myasthenia gravis (MG): Importance of doublet stimulation

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Aims: MG is an autoimmune disease caused by antibodies targeting the acetylcholine receptor in the neuromuscular junction, leading to weakness and increased work-induced fatigue. To further evaluate functional consequences of the disease, this study examines effects of tubocurarine-induced MG on maximal tetanic force and the rate of force development.
Methods and Results: Isolated nerve-muscle preparations of rat soleus were mounted on isometric force transducers and incubated in Krebs-Ringer bicarbonate buffer at 30 Celsius. Contractions were elicited via nerve stimulation with 1 s trains of pulses 10 times with 1 s rest between trains (50 % duty cycle). Pulse configuration of the trains was 30 Hz constant frequency with or without an initial double pulse with 6 ms interpulse distance. MG was mimicked by pre-incubation with either 75 or 125 nM tubocurarine. Initial rate of force development was evaluated from the absolute force obtained 40 ms after the first pulse in the pulse trains. Compared to controls, muscles incubated with tubocurarine showed a significant deficit in maximal tetanic force (15±2 and 31±3 % deficit at 75 and 125 nM tubocurarine, respectively, $n = 6$ to 12, $P < 0.05$) and a much more extensive further loss of force during the 10 contractions (to respectively 70±3 and 48±4 % of initial force as compared to 91±1 % in controls). Despite this, the initial rate of force development was in all 10 contractions unaffected by pre-incubation with 75 nM tubocurarine and was only reduced from the 2nd contraction at 125 nM tubocurarine. Moreover, neither tubocurarine concentration affected the 2.3-fold potentiating of 40 ms force that was observed in control muscles when a doublet was included in the pulse train.
Conclusion: The rate of force development was well conserved even in severe MG with large reductions in maximal force and increased fatigability. This protection was especially clear when early force development was potentiated by doublet stimulation.

S09.P5-321

Myotonic dystrophy type 1 and mitochondrial encephalomyopathy in a single patient

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Introduction: Double genetics troubles are rare or underdiagnose in myotonic dystrophy type 1 (DM1). To our knowledge, coexistence of those two neuro-muscular diseases was not yet reported. The aim of this study was to present a patient with DM1 and mitochondrial encephalomyopathy.
Case report: A 49-year-old man was admitted to the Department of Neurology because of three year history of progressive bilateral ptosis. The diagnosis of myasthenia gravis was excluded. His family history of neuromuscular diseases was negative. On admission, the patient presented asymmetric ptosis, bilateral ophthalmoparesis, temporal muscles atrophy, face and limbs muscles weakness with distal atrophy of lower limbs and active myotonia. The CK serum level was normal, the lactates serum level, GGTP, ASPAT, ALAT enzymes, FSH and cholesterol were moderately elevated. On EMG, both myopathic changes and myotonic discharges were detected in most examined muscles. Muscle biopsy revealed a pattern consistent with DM1 pathology. Ragged-red fibers (RRFs) were not found. Brain MRI showed diffuse hyperintense lesions and mild cortical and subcortical atrophy. The psychological test revealed a low IQ without cognitive

function deterioration. The genetic test showed CTG expansion in DMPK gene confirming the diagnosis of DM1. Fluorescence in situ hybridization revealed multiple foci containing toxic transcripts of mutant DMPK in majority of myonuclei. Alternative splicing-specific RT-PCR assays revealed that several exons which splicing is regulated by MBNL and CELF proteins and which are specific biomarkers of DM1 were strongly misspliced in skeletal muscles biopsy sample. As the ophtalmoparesis is not characteristic in phenotype of DM1 the molecular test for mitochondrial diseases was performed and revealed multiple mtDNA deletions in the muscle tissue.
Conclusion: An individual having two separate mutations in neuromuscular disease-related genes may develop unusual phenotype.

S09.P6-313

Manipulating the muscle enviroment to improve the muscle repair and the outcome of cell mediated therapies in muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) is a genetic disease caused by lack of dystrophin and characterized by muscle wasting, chronic inflammation and progressive decrease of muscle regeneration capacity. The ability of satellite cells (MuSCs) to repair the injured tissue decline with age in the mdx mice. The constant cycles of degeneration and regeneration and the hostile microenvironment may affect the MuSCs function and exhaust their regenerative capacity. Previous work done in our laboratory showed that lack of PKCθ in mdx mice improves muscle maintenance, regeneration and performance, preventing massive inflammation and wasting. Indeed, PKCθ is highly expressed in both immune cells and skeletal muscle. PKCθ plays a unique role in T cell activation, and represents an attractive molecular target for the treatment of immune disorders. We show here that the lack of PKCθ in mdx modifies the environment in order to preserve regenerative ability of MuSCs during the different ages. Moreover, the lack of PKCθ in mdx improves the survival and the ability of transplanted stem cells to generate new muscle fibers and correct the genetic defect of the recipient. The muscle environment is composed of extracellular matrix (ECM) and local cell populations. Among them, it was shown that the fibroadipogenic progenits (FAPs), a muscle interstitial cells, contribute to muscle regeneration but also to fibroadipogenic degeneration generating myofibroblasts and adipose cells. The characterization of FAPs activity isolated from mdxθ−/− muscle suggests that the improved muscle regeneration observed depends, at least in part, to improved FAPs activity. Indeed, the reduced inflammatory environment in mdxθ−/− prevents FAPs conversion into fibro-adipocytes and increases their pro-regenerative activity. These results may contribute to the identification of new targets for cell therapies and intervention aiming to shift the balance between muscle regeneration and fibroadipogenic degeneration in DMD.

S09.P7-309

A force decrease in single fibers from the soleus muscle of mdx mice is accompanied by morphological changes but not alterations in Ca^{2+} sensitivity of the contractile apparatus

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Duchenne Muscular Dystrophy (DMD) is a progressive and lethal skeletal muscle disease caused by the absence of the protein dystrophin, which leads to muscle weakness and progressive degeneration. Although DMD affects mostly the muscle membrane and the cytoskeletal proteins, the potential effects of DMD on myosin-actin interactions and the Ca^{2+} sensitivity of the contractile apparatus remain controversial.
Aim: In this study, we analyzed the force-pCa^{2+} relation and contractile properties of permeabilized single fibers of soleus muscle of mdx mice.
Methods and Results: Soleus muscle was dissected from male C57Bl/10 (control) and C57Bl/10 mdx mice, 4–6 weeks of age. This is the period in which there are severe morphological alterations without significant muscle regeneration. Muscles were chemically permeabilized and a single fiber was dissected. The fiber was transferred to a control temperature chamber and attached between a force transducer and a length controller. Active forces were evaluated in different Ca^{2+} concentrations (pCa^{2+} 4.5, 5.5, 6.0, 6.5) at a sarcomere length of 2.5μm. Histological technique was applied to analyze the morphological alterations of fibers muscle, and immunofluorescence was used to identify the presence or absence of dystrophin. Single fibers from mdx mice showed a reduction of isometric force (pCa^{2+} 4.5) when compared to the control group. There was not a change in the force-pCa^{2+} relation, suggesting that Ca^{2+} sensitivity is not impaired in the mdx fibers. However, the decrease in force observed in mdx fibers was accompanied by significant morphological alterations, including nuclear centralization, basophilic cells and an increase of connective tissue, besides an absence of dystrophin.
Conclusion: Absence of dystrophin in mdx mice caused a significant reduction of single fiber force that was accompanied by morphological alterations of the tissue but not a change in the sensitivity of the contractile apparatus to Ca^{2+} activation.

S09.P8-304

Expanding the phenotype of BICD2 mutations towards skeletal muscle involvement

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The cargo-vesicle adaptor protein BICD2 is homologous to Drosophila bicaudal D (BicD), which is evolutionarily conserved from flies to humans. BICD2 contains five predicted coiled-coil binding domains grouped into three binding regions. The first N-terminal binding region (CC1) binds to the dynein-dynactin complex via direct interaction with the p50 subunit of dynactin, the second (CC2) binds the kinesin motor complex, and the third (CC3) interacts with the cargo-associated RAB6 GTPase. It has been suggested that BICD2 links a variety of RAB6-positive cellular cargos to the dynein motor complex and thus initiates directional cargo movement. Mutations in BICD2 cause autosomal dominant spinal muscular atrophy with lower extremity predominance 2 and hereditary spastic paraplegia. The interaction of mutated BICD2 with the dynein-dynactin complex and/or with RAB6A leads to fragmentation of the golgi apparatus, suggesting perturbations of BICD2-dynein-dynactin mediated trafficking with impairments in the development/maintenance of motor neurons. We analyzed two independent German families with clinical, genetic and muscle MRI studies and identified the mutations p.Ser107Leu and p.Thr703Met in the BICD2 gene, respectively. Immunofluorescence studies and immuno-electron microscopy showed striking impairment of dictyosome integrity, vesicle pathology and abnormal BICD2 accumulation either within the nuclei (p.Ser107Leu) or in the perinuclear regions (p.Thr703Met). Transfection studies confirmed BICD2 aggregation of different subcellular distribution. Our findings extend the phenotypic spectrum of BICD2 associated disorders, by features of a chronic myopathy and show a novel pathomechanism of BICD2 defects in skeletal muscle. Therefore, BICD2 as a key adaptor protein in trafficking of cellular cargos, seems to be important not only in neurons, but also crucial for skeletal muscle integrity and maintenance.

S09.P9-272

LGMD2T: Description of the First Algerian family with GMPPB mutations and Becker like phenotype

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Introduction: Mutations in the guanosine diphosphate mannose pyrophosphorylase B (GMPPB) gene have recently been reported to cause muscular dystrophies with hypoglycosylation of alpha-dystroglycan. Associated phenotypes range from severe congenital muscular dystrophies with structural brain involvement to limb girdle muscular dystrophy (LGMD).
Material and methods: We describe the first Algerian family with two brothers presenting a Becker like phenotype and GMPPB gene mutation. The 19 years propositus was referred to our attention with a clinical history of progressive pelvifemoral weakness and myalgia since 10 years of age and increased CK level (6850 UI). A diagnosis of Becker myopathy was first suggested to his 39 years brother.
Results: Clinical examination at the age of 15 years showed prevalent proximal lower limb moderate muscle weakness and calf hypertrophy. There was no cardiac and respiratory involvement, nor learning difficulties. His brother had similar symptoms with later onset around the age of 20 years and preserved ambulation at 39 years. Review of the muscle biopsy demonstrated normal dystrophin, sarcoglycans and decreased alpha-dystroglycan immunostaining. Next generation sequencing identified a novel homozygosis mutation c.458c>T (p.thr153lle.) in exon 5 of the GMPPB gene.
Discussion and Conclusion: These two LGMD2T patients further expand the clinical spectrum associated with this type of secondary dystroglycanopathy, in particular regarding the Becker like phenotype.

S09.P10-240

Serum osteopontin in dystrophic dogs is elevated during muscle regeneration

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Aims: Duchenne Muscular Dystrophy (DMD) is an X-linked lethal muscle disorder, characterized by progressive muscle weakness and atrophy. We found that osteopontin (OPN) is expressed in the early phase of a dystrophic dogs, Canine X-linked Muscular Dystrophy in Japan (CXMDJ) (Nakamura A et al. Sci Rep. 3:2183, 2013). OPN may have pivotal roles of inflammatory cytokine and myogenic factor in the dystrophic pathology. To explore the role of OPN as a new biomarker, we analyzed serum OPN levels in dystrophic dogs, and compared the levels to other serum markers, including serum creatine kinase (CK) and matrix metalloproteinase (MMP)-9.
Materials and Methods: Serum levels of OPN and other markers in dystrophic and wild-type (WT) dogs were monitored at different ages. Immunostaining was performed to observe OPN expression in the dystrophic muscles. Muscle injury was induced by cardiotoxin injection into tibialis cranialis muscles of WT dogs, and OPN expression was examined during subsequent muscle regeneration process.
Results and Discussion: Serum OPN levels in the dystrophic dogs were elevated compared to those in WT just before and 1 hour after a Caesarean section and at the age of 3 months, and they took a completely different pattern compared to serum CK and MMP-9. Serum OPN levels were significantly correlated with the severity of dystrophic dogs, when examined around the onset of the disease. Immunohistologically, OPN expression was detected in infiltrating macrophages, but also detected in developmental myosin heavy chain-positive regenerating muscle fibers. In particular, OPN expression in regenerating muscle fibers was well related to the serum OPN elevation pattern around three months of age. We also noticed that OPN expression was found during muscle regeneration process induced by cardiotoxin injection.
Conclusion: OPN is a promising biomarker for muscle regeneration in dystrophic dogs and can be applicable to DMD boys.

S09.P11-230

Identification and activity of ADP-sensitive purinergic receptors (P2Y) in dystrophic mouse myoblasts

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Duchenne muscular dystrophy (DMD) is the most common inherited muscle disease caused by dystrophin deficiency due to mutations in DMD gene, leading to premature death. Although undifferentiated myoblasts do not express detectable levels of dystrophin protein, mutations in the DMD gene result in numerous phenotypic changes in these cells, including affected energy metabolism, enhanced autophagy, P2X7 receptor activity and stimulated store-operated Ca^{2+} entry. Here, we describe effects of the DMD mutation on ADP-sensitive metabotropic nucleotide receptors in myoblasts derived from mdx mice. ADP is an agonist of three out of 7 P2Y receptor subfamily members identified in rodents. Among these stimulation of P2Y1 leads to an activation of phospholipase C via protein Gq-dependent pathway and Ca^{2+} release from the endoplasmic reticulum (ER), while stimulation of P2Y12 and P2Y13 inhibits adenylate cyclase in a Gi-dependent manner, although their link to other protein G subtypes has also been suggested. Stimulation of both wt and mdx myoblasts with ADP resulted in Ca^{2+} release from the ER, but response of mdx cells was substantially stronger. Treatment of myoblasts with artificial agonists and antagonists excluded P2Y1 contribution to this calcium response in either wt or mdx myoblasts, despite this receptor presence in these cells. However, a profoundly increased activity of P2Y13 and to some extent also P2Y12 were identified in mdx myoblasts, although total amounts of these proteins detected in the whole lysate as well as in the isolated plasma membrane fractions were not increased in the dystrophic cells. Inhibition of P2Y13 did not influence motility of myoblasts, which was greater in wt myoblasts than in mdx cells and did not affect cell proliferation. Therefore, cellular consequences of increased P2Y13 and P2Y12 receptor levels in dystrophic mouse myoblasts need further studies.

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Effect of Heme Oxygenase-1 on Muscle Regeneration in Duchenne Muscular Dystrophy

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Differentiation of myoblasts is dependent on miR-206,-1 and -133a/b. Recently, we showed that differentiation of murine myoblasts was inhibited by heme oxygenase-1 (Hmox1), an anti-inflammatory enzyme degrading heme to CO, iron ions and biliverdin. Inversely, differentiation of Hmox1-deficient murine primary myoblasts, showing enhanced expression of myomiRs was accelerated (Kozakowska et al, Antioxid Redox Signal 16:113–27; 2012).

In muscles of dystrophin-deficient mdx mice expression of Hmox1 is consistently increased from week 8 up to 12 months. Importantly, elevated expression of Hmox1 is observed in myeloid cells, while expression of miR-1,-133a and -133b was decreased in muscles of mdx mice. Surprisingly, expression of miR-206 was upregulated in gastrocnemius and diaphragm of mdx mice. Interestingly, single cell analysis demonstrated that in SCs (alpha7integrin+ CD34+), the expression of Hmox1 was decreased while miR-206 was increased, indicating for SC-specific regulation of Hmox1 and miR-206. Activated SCs (αalpa7integrin+ CD34-) were more abundant in mdx mice.

Mice lacking both Hmox1 and dystrophin showed significant impairment of exercise capacity on treadmill in comparison to mdx mice, aggravated muscle injury evidenced by higher level of CK and LDH, increased infiltration with inflammatory cells. Similar effects were observed in mdx mice treated with tin protoporphyrin, a pharmacological inhibitor of Hmox1 activity. Global RNA sequencing showed significant effect of dystrophin deficiency on SCs transcriptome, while lack of Hmox1 was less pronounced. Transcriptome of activated satellite cells (αalpha7i+CD34-) also differed significantly from the αalpha7i+CD34+ cells. SCs isolated from mdx mice showed disturbed and accelerated differentiation which was also affected by the lack of Hmox1.

Hmox1 exerts both satellite cells-specific effect and influences inflammation in dystrophic muscles.

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S09.P12-229

Role of P2Y2 receptor in calcium mobilization in dystrophic mouse (mdx) myoblasts and its distribution patterns in skeletal muscles

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Duchenne muscular dystrophy is X-linked genetic disorder caused by mutation in the dystrophin-encoding gene. Absence of this protein in mature muscle fibres results in their damage. Post-mitotic skeletal muscle may regenerate via satellite cells, which can proliferate, with resulting myoblasts migrating and fusing with existing muscle fibres or forming new ones. Repeated damage of the dystrophic myofibers lead to an ineffective repair, progressive and irreversible muscle dysfunction and a fatal outcome. Dystrophin appears in myotubes while it is undetectable in myoblasts. Despite this, myoblasts derived from dystrophic mice (mdx) exhibit seriously affected Ca^{2+} homeostasis partially due to enhanced level and activity of the P2X7 receptor. Here, we focus on P2Y receptors showing that altered metabotropic responses to extracellular ATP and UTP may also contribute to the abnormal calcium response in these cells. Using selective antagonists we have shown an enhanced P2Y2 activity in mdx myoblasts. Also Western blot analysis showed an increased level of P2Y2 in isolated plasma membrane fraction (but not in the whole cell lysate) of the dystrophic cells. Immunohistochemical detection of P2Y2 receptor in tibialis anterior not only confirmed a presence of this receptor in mouse muscles but also shown it to be increased around fibers in mdx mice indicating enhanced P2Y2R localization to sarcolemma. Furthermore, colocalization of P2Y2 with central nuclei of mdx fibres suggested that P2Y2R is expressed there during regeneration of injured fibers. Similar results were obtained in muscles derived from mdxbetageo mice, which are an alternative model of the Duchenne muscular dystrophy. These results suggest that differentiation, maturation and regeneration of dystrophic muscle are

accompanied by quantitative and qualitative changes of P2Y2 receptor expression and activity.

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S09.P14-158

Personalized and Specialized Therapy for Better Living with FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is the most common muscular dystrophy of adult life in Europe with a prevalence of 4/100000. This unique autosomal dominant disorder require both a genetic and an epigenetic condition involving alterations in chromatin structure at the chromosome 4q35 region that includes a polymorphic array of repeated 3.3-kb D4Z4 elements. Each D4Z4 repeat unit includes the promoter and open reading frame of Double homeobox 4 (DUX4) gene. FSHD is characterized by muscle dysfunction and loss that deeply impairs the quality of life and exercise tolerance. To date no curative therapy is available. It is now assumed that systemic inflammation and oxidative stress contribute to the clinical development of the pathology and that targeting oxidative stress through treatment with antioxidants might be an effective strategy. Over the past three years, our Department of Clinical Physiology, University Hospital (Montpellier, France), in collaboration with our laboratory INSERM U1046/CNRS UMR 9214 « PhyMedExp » has conducted a randomized double-blind placebo-controlled pilot clinical trial study designed to evaluate the synergic effect of a targeted antioxidants supplementation on muscle function of 53 patients with FSHD during 17 weeks. This antioxidants supplementation showed beneficial effect on physical performance and oxidative stress markers. However, such a supplementation requires a case-by-case posology adjustment and a long term follow up. Based on our experience, we recently developed a new long term clinical trial (NCT02622438) with primary outcome improvement of strength and as secondary outcomes decrease in oxidative stress markers and improvement of physical activity level and life quality. To develop a larger scale project, we look ahead to be part of the next H2020 european call to provide concrete practice guidelines supporting a preventive personalized treatment planning.

S09.P15-150

Contractile weakness in NEM3 patients is caused by dysfunctional sarcomeres

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Nemaline myopathy is a heterogeneous congenital non-dystrophic myopathy, characterized by muscle weakness and the presence of nemaline rods in muscle fibers. The pathophysiology of muscle weakness caused by mutations in ACTA1 (NEM3) is incompletely understood. ACTA1 encodes skeletal muscle α-actin, the main component of the sarcomeric thin filament. Hence, we hypothesized that muscle weakness in NEM3 is caused by sarcomeric dysfunction.

Contractile function of permeabilized muscle fibers (SF) and single myofibrils (MF) was determined in control (CTRL, $n = 6$) and NEM3 ($n = 14$) subjects. SF and MF were isolated and mounted between a force transducer and length motor. At sarcomere length of 2.5μm, by exposure to [Ca^{2+}] solutions, maximal tension, cross bridge cycling kinetics (Ktr) and the active stiffness were determined. Maximal tension at incremental sarcomere lengths (range 2.0–3.5μm) revealed the force-sarcomere length relationship and by fitting to a 2nd order polynomial the sarcomere length at maximal force (SLopt) and the sarcomere length at 50% of maximal force (SL50) were obtained. Actin expression was determined by western blotting.

In NEM3 patients, actin expression was similar to CTRL, but the maximal active tension of SF was reduced by 67% compare to CTRL, caused by a reduced number of bound cross bridges due to slower cross bridge cycling kinetics. No shift in the force-sarcomere length relationship was observed as SLopt and SL50 were similar. Importantly, the maximal tension of MF was also reduced, by 45%, which was accompanied by a significant reduction of cross bridge cycling kinetics.

Thus, despite a largely preserved ultrastructure, muscle fibers of NEM3 patients show significant contractile weakness in the absence of major changes in thin filament length (i.e. unaltered SLopt and SL50). The reduced contractility of single myofibrils indicates that muscle fiber weakness in NEM3 patients is partly caused by dysfunctional sarcomeres.

S09.P17-323

Low intensity training increases the infiltration of immune cells in skeletal muscle of mdx mice

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Immune cells infiltrate in dystrophic muscles during early stage of Duchenne Muscular Dystrophy (DMD) and substantially contribute to the onset and the progression of the disease. Low intensity training (LIT) rescues the phenotype of dystrophin-deficient mdx mice, an animal model of DMD. Therefore, we postulate that LIT might have anti-inflammatory properties.

To investigate this hypothesis, we assessed the infiltration of immune cells through Western blot, immunostaining and FACS analysis, in gastrocnemius muscle of 8 week-old mdx mice after 4 weeks of swimming training.

We found that CD68 positive cells, a marker of monocytes and macrophages, infiltrated in mdx muscle, and 4 weeks of swimming

increased the infiltration of these cells. FACS analysis revealed that these CD68 positive cells were F4/80 positive differentiated macrophages, rather than Ly6C positive monocytes. Further Western blot analysis showed that the number of iNOS positive inflammatory macrophages decreased in mdx mouse muscle, but the number of CD163 positive regenerative macrophages was not significantly different.

We, for the first time, found that LIT were associated with an increased number of infiltrated immune cells in dystrophin-deficient mdx muscle.

S09.P18-276

Functional characterization of the communication between muscle and nerve in Mouse models of Amyotrophic Lateral Sclerosic

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Amyotrophic Lateral Sclerosis (ALS) is a neuromuscular degenerative disease linked in the 20% of familiar cases to mutations of the SOD1 gene. The most studied mouse model of ALS carries the mutation G93A in 25 copies of the transgene. This animal model recapitulates well the human disease, and recent studies showed the primary role of skeletal muscle in the pathogenesis of ALS. To better address which element of the motor unit is initially affected by the pathology, I developed a protocol to measure, in vitro, the neuromuscular junction (NMJ) functionality of soleus sciatic preparations. This technique is based on the comparison between muscle contractile responses elicited by membrane and nerve stimulations. I investigated specimens of SOD1G93A mice at different ages, to follow the course of the pathology. To better address the role of muscle tissue in NMJ damages linked to oxidative stress, I am studying also the MLC/SOD1G93A mouse model, in which the SOD1 mutated protein is overexpressed exclusively in the skeletal muscle. Moreover, I started to study another model which carries a copy number of mutant SOD1G93A gene dropped to eight or ten. This low copy mouse has a slower progression of the pathology and could be a more appropriate model for studying the pathological processes in ALS and aid the development of therapies for earlystage treatments such as the effect of exercise training.

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S09.P19-284

Deciphering the link between Leiomodins and Kelch proteins in nemaline myopathy

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Background: Leiomodin (LMOD) 2 and 3 are members of the tropomodulin-protein family expressed in striated muscle and implicated in the nucleation and length regulation of actin filaments. Recessive loss of function mutations in LMOD3 cause severe congenital nemaline myopathy with neonatal lethality. Although LMOD2 variants have not yet been linked to human disease, an Lmod2 knock out mouse model develops a lethal, rapid-onset dilated cardiomyopathy. Three members of the Kelch protein family have been identified as genetic causes of nemaline myopathy: KBTBD13, KLHL40 and KLHL41. Kelch proteins are ubiquitination co-factors that regulate protein degradation, with recent data showing KLHL40 plays a crucial role in regulating the stability of LMOD3 and nebulin.
Aims: We characterised the tissue-specific expression of LMOD2 and LMOD3 in humans. Additionally, we closely examined the regulation of LMOD3 by KLHL40 and refined the domain within LMOD3 that confers this interaction. Lastly, we determined whether the stability of LMOD2 is also regulated by Kelch proteins.
Methods and Results: LMOD2 predominates in cardiac muscle in humans, whereas LMOD3 is expressed in both skeletal and cardiac muscle. In vitro cell culture studies demonstrated that LMOD2 and LMOD3 are both regulated by KLHL40 and KLHL41. Using LMOD3 deletion constructs we determined that regulation of LMOD3 by KLHL40 and KLHL41 is primarily conferred by the N-terminal domain (amino acids 1–100).
Conclusion: Loss of LMOD3 causes severe nemaline myopathy, via either primary causal mutations or via secondary deficiency due to KLHL40 mutations. KLHL41 also regulates the stability of LMOD3 in vitro. We are currently exploring whether disease pathogenesis in KLHL41 myopathy may also be due in part to secondary loss of LMOD3. Importantly, our data indicates cardiac LMOD2 may be also regulated by KLHL40 and KLHL41, and recommend cardiac surveillance for patients with KLHL40 and KLHL41 related nemaline myopathy.

S09.P20-248

Is the expression of connexin-based hemichannels a common feature of limb-girdle muscular dystrophies?

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Introduction: Limb-girdle muscular dystrophies (LGMD), are a group of heritable muscular diseases that can be caused by mutations in different genes, namely mutations in dysferlin, caveolin-3, anoctamin 5 or fukutin-related protein genes, among others. However, the clinical phenotype is broadly similar with some specific differences.
Aim: To find possible common features in LGMD caused by mutations in different genes, that account for the phenotype observed. With this aim, we assayed the possible presence of functional Connexin-based hemichannels (Cx HCs) in these dystrophies, which have

been recently implicated in the pathogenic mechanism of other type of muscular dystrophies, such as Duchenne muscular dystrophy, where these channels are responsible of the myofiber's apoptosis.
Methods and Results: Using immunofluorescence assays, we evaluated the presence of muscular Cxs (Cx40.1, 43 and 45) in muscular biopsies (deltoid muscle) of different patients diagnosed with LGMD caused by mutations in different genes, such as dysferlin, caveolin-3, anoctamin 5 or fukutin-related protein. Our results revealed the presence of Cx40.1, Cx43 and Cx45 in myofibers of all patients evaluated. In addition, it was analyzed the presence of functional Cx HCS in vivo with the Evans blue uptake assay, in dysferlin-deficient mice (an animal model of dysferlinopathy) of 3, 6 and 9 months of age and it was detected the presence of functionally active Cx HCs in tibialis anterior muscles from 3 months of age. However, they reach a maximal expression at 6 month, remaining elevated up to 9 month of age.
Conclusions: The results obtained strongly suggest that Cxs, through functional Cx HC, could be a common feature in LGMD. Consequently, these channels could constitute suitable candidates for future pharmacological therapies.

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S09.P21-238

Combined MRI and 31P-MRS investigations of L-tyrosine supplementation in a murine model of nemaline myopathy with actin mutation

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Aims: Nemaline myopathy (NM) is a rare neuromuscular disorder characterised by skeletal muscle weakness and the presence of rod-like bodies in the muscle fibres. No treatment is currently available for NM. Interestingly, anecdotal positive effects related to orally L-tyrosine supplementation have been reported in a small cohort of patients an in a mouse model of NM [1,2]. This study aims at further documenting the potential functional, anatomical and metabolic effects of a 4-week period of L-tyrosine supplementation in a mouse model mimicking a mild form of NM [3].
Methods and Results: Six to seven-month old transgenic male, which carry the human Asp286Gly mutation in the ACTA1 gene [4], were fed for 4 weeks with either a L-tyrosine-enriched diet (2.5%) or a standard diet (0.45%). Maximal force was assessed in response to a 100 Hz tetanic stimulation. A fatiguing protocol consisting of 80 contractions (40 Hz; 1.5 s on - 6 s off) was performed and a fatigue index, i.e. the ratio between the first five and the last five contractions, was determined. Magnetic resonance (MR) acquisitions were performed on a 4.7-T magnet. High-resolution MR images were acquired and the hindlimb muscles volume (mm3) was calculated. High-energy phosphate metabolites and intracellular pH were investigated using 31P-MR spectroscopy during the fatiguing protocol. Maximal tetanic force and the fatigue index were not different between the untreated (420 ± 12 mN and 0.22 ± 0.03) and tyrosine-treated mice (427 ± 23 mN and 0.22 ± 0.03). Both muscle volume and protocol-induced metabolic variations were also similar between the two groups. 3.
Conclusions: L-tyrosine supplementation does not rescue the phenotype of mice carrying the ACTA1(Asp286Gly) mutation.

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S09.P23-115

Surgical and rehabilitation treatment of muscular dystrophies. Evidence-based recommendations for clinical practice guidelines in Colombia

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The muscular dystrophies (MD) are genetic diseases that cause progressive weakness and degeneration of muscle. The «Guide to clinical practice for early detection, comprehensive care, monitoring and rehabilitation of patients diagnosed with muscular dystrophy,» developed recommendations for the comprehensive care of patients diagnosed with the most common MD. The GRADE methodology was used to build all the recommendations of the guide, a total of 51 structured questions were answered regarding the diagnosis and surgical treatment and rehabilitation of patients with MD. The databases used for search of information were: MedLine, Embase, Clinical Trials, LILACS, Bireme, Current Controlled Trials, The Cochrane Library, NHS Evidence, UpToDate, Clinical Evidence, TripDatabase, NCG National Guideline Clearinghouse, NeLH National Electronic Library for Health, Handbook of UK and European, CMA Infobase, Patient, GUIA SALUD, AEZQ/AQuMed, NHMRC, NICE, Guidelines International Network, NZGG New Zeland Guidelines Group, SING Scottish.

The following article includes recommendations related to the surgical and rehabilitation treatment of people with MD. Generally we recommend the spinal fixation surgery for scoliosis greater than 20 degrees, the elongation of the Achilles tendon, the scapular fixation and the tendon elongation; also the use of positive pressure devices, respiratory therapy, exercises of submaximal strengthening and aerobics, avoid prolonged immobility, the use of knee ankle foot orthotic, manual and motorized wheels chairs, previously examining the feasibility of prescribing and the patient, psychological intervention, family therapy and the use of functional scales. Spinal orthoses, high resistance exercises and leisure and recreational therapies are not recommended.

S09.P22-160

Effect of neuromuscular electrical stimulation on muscle function of patients suffering with FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease characterized by progressive weakness and atrophy of specific skeletal muscles. Patients affected by FSHD suffer from progressive muscle weakness limiting daily activities. This sedentary

lifestyle can cause a "debilitative cycle," and neuromuscular deconditioning. Recent studies have indicated the safety and the effectiveness of moderate aerobic training programs in patients with FSHD. However, these training programs have limited applicability in patients with more severe muscular weakness. Artificial strength training with neuromuscular electrical stimulation (NMES) appears to be a promising rehabilitation strategy for FSHD patients.
Objectives: this study will investigate the feasibility, safety, and effectiveness of NMES strength training to counteract quadriceps muscle weakness in patients affected by FSHD.
Materials and methods: Patients will undergo 3 months of strength training with NMES bilaterally applied to the quadriceps muscles for five 20-minute sessions per week. NMES will be delivered by means of a Kneehab® XP device, which is an innovative and clinically-proven garment-based product designed specifically to treat quadriceps muscle atrophy.
Study design: Thirty-four adult patients with FSHD will be enrolled at Montpellier University Hospital (France) in a randomized, single blind, placebo-controlled pilot clinical trial. Patients will be randomly assigned to an experimental group ($n = 17$) and to a sham group ($n = 17$), whose current intensities will be fixed beyond and below the motor threshold, respectively.
Expected Results: We expect an improvement in comfort, mobility, independence in daily living activities such as personal care. This treatment will also provide concrete recommendations on rehabilitation strategies to clinicians and families.

S09.P24-86

Physiotherapy in a patient with neuromyotonia – a case report

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Introduction: Neuromyotonia is a rare condition that affects peripheral nerves, characterized by spontaneous, continuous muscle activity resulting in visible miokymia, cramps and impaired muscle relaxation. Most studies only focus on pharmacological management of this disease.
Aims: The purpose of this study was to show effects of a non-pharmacological approach on functionality, symptoms and quality of life in one case with this syndrome.
Case Presentation: A 63 years-old Caucasian male, independent and autonomous, complaints about cramps and muscle fatigue when doing activities like walk. The symptoms appeared one year before the first day he started the intervention in this study. He had visible hypertrophy of calf muscles, on the two legs. The patient had sessions of physical therapy 2 d/wk for 8 weeks. Each session lasted about 35 minutes, and included 10 minutes of local heat, 7.5 minutes of massage on each calf muscle and 3x30 seconds of calf stretching in each leg, with a period of rest of 30 seconds. The 6-minute walk test was used to measure function and the Modified Borg Scale for Perceived Dyspnea was used to measure general level of fatigue. To measure cramps and hours of sleep two questions were asked and to measure quality of life was administered the Portuguese Short Form-12v2 Health Survey questionnaire. Outcomes were assessed at baseline and at 1,2 and 3 months after intervention started. The participant demonstrated improvements in the 6-minute walk test and in cramps frequency.
Discussion: This is the first study about the effects of physical therapy in patients with neuromyotonia. Outcomes suggest that a physical therapy program may help in improve function and reduce cramps frequency on individuals with neuromyotonia. Further investigations are needed to demonstrate its efficacy.

Session 10 Molecular motor and Contractile structure

Oral presentations

S10.O1

Structural and functional insights into the mechanism of force generation during the myosin interaction with actin

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There has been recent progress, as well as considerable controversy, in trying to understand how actin and myosin interact to trigger the release of the ATP-hydrolysis products, inorganic phosphate (Pi) and MgADP, which is coupled to the generation of force and movement. Structural insights have come from a high-resolution structure of myosin VI in a previously unseen state and from a cryo-EM 3D reconstruction of the actin-myosin-MgADP complex. The major controversy centers on understanding at what point in force generation the inorganic phosphate is released with respect to the lever arm swing, or powerstroke. The new X-ray structure was interpreted as the state that actin stabilizes to release phosphate, which was supported by mutagenesis and kinetic studies. Other kinetic studies have been performed in combination with observing the rates of the movement of the lever arm with FRET probes, and have been interpreted as demonstrating that the lever arm movement, or powerstroke, precedes the release of phosphate. Resolving this controversy is essential for understanding the mechanism of how myosin works as a chemo-mechanical transducer as well as the mechanisms underlying disease-causing mutations in myosin. These seemingly contradictory sets of data can be reconciled and provide a model for force generation by myosin on actin.

S10.O2-340

Force production by myosin motors: new avenues towards therapeutical treatment

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The actin-based motors, myosins, are critical for many cell processes, from muscle contraction to cytokinesis, cell compartments communication and sophisticated cellular functions such as hearing. Deficit in these motors can lead to a number of human genetic disorders, thus molecular motors are important potential targets for therapeutical treatment. Structures of myosin associated with small molecules provide interesting insights for the design of very specific inhibitors. Force is produced by these molecular motors by the conversion of chemical energy derived from ATP hydrolysis into mechanical energy via the interaction with their track, the actin filament. The current questions regarding the important details of force production will be presented in light of recently solved X-ray structures of myosin/drug complexes. The challenges that remain to understand how motors produce their powerstroke and how drugs can modulate or stop the motor's force generation will also be presented.

S10.O3-176

Kinetic evidence for two conformers of the active site of myosin. Transient kinetic data resurrected and reinterpreted

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Striated muscle contraction relies on specific interactions of the different states of the myosin 2 heads on the ATPase pathway with the actin filament, driving force and shortening production. Structural studies show that each myosin head has an actin binding sites and a catalytic site for ATP. Recently, Brenner and co-workers proposed that the ATP site exists in two conformers: one allowing ATP hydrolysis and the other binding ATP without hydrolysis [1]. Here, we resurrect transient kinetic data from the 1980ties with the one-headed myosin sub-fragment 1, S1, actoS1 and myofibrils that support this proposition [2–6], whereas originally we explained our data by proposing that each myosin head has two sites for ATP. The kinetics we report with myosin, actomyosin and myofibrils are based on the cold ATP chase method – a method that at once measures specifically the kinetics of the essentially irreversible binding of ATP to the ATPase site and allows to titrate the active sites as well as to detect the presence of subpopulations. The presence and potential functional relevance in vivo of the «two types of head-pathway» of Brenner and co-workers for actomyosin hydrolysis is discussed here in relation with the organization of heads on the thick filament to the aim of identifying the role of myosin motor conformation in regulating the chemo-mechanical properties of the relaxed state.

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S10.O4-286

Super-relaxed state and power generation in muscle modelling

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Recent findings show the existence of two different structures in detached, or non-force generating, muscle myosin. In the resting structure ATP consumption is inhibited, inducing the so-called super-relaxed state where myosin heads bind to the core of the thick backbone in a highly-ordered array. Before myosin heads can strongly attach to the actin filament, starting the cross-bridge cycle, they must exit from this low-energy consumption structure and project toward the thin filament, in a more disordered configuration. The transitions between these two states have been quantitatively characterised from X-ray diffraction (Linari et al. Nature 2015), and a mechanosensing mechanism for cross-bridge recruitment have been shown, where the transition between resting or OFF state (super-relaxed) and active or ON state is increasing with the tension acting on the thick filaments. Here we show, by means of a mathematical model, how such a mechanism may solve a long standing contradiction about the possible existence of a fast component in actomyosin force regeneration. Such component could reproduce, among other features, the high energy output at intermediate level of tension during the isotonic contraction. To properly fit this property, several hypotheses have been included in mathematical models during the last two decades. We have quantitatively included the observed mechanosensing mechanism on ON-OFF rate transitions in a previously proposed mathematical models of muscle contraction showing that it seems to be sufficient to reproduce the physiological power output, along with others observed properties. In the model, the tension acts a feedback for the recruitment of active myosin. This rate of recruitment acts then as a rate limiting step for the rising phase in tetanic tension.

S10.O5-287

Interference X-ray diffraction in paced intact trabeculae from rat ventricle

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Structural dynamics of the contractile proteins of the cardiac sarcomere are investigated in situ by collecting 2D X-ray diffraction patterns from intact ventricular trabeculae of the rat heart in 5–10 ms time windows during diastole-systole cycles at the ID02 beamline of the European Synchrotron Radiation Facility. Trabeculae are vertically mounted in a thermoregulated trough (27°C), perfused with oxygenated physiological solution ($[Ca^{2+}]o$ 2.5 mM), and electrically paced at a frequency of 0.5 Hz. A FReLoN CCD detector is placed either at 30 m from the preparation to measure sarcomere length (SL) or at 1.6 m to collect up to the 6th order of myosin-based meridional reflections. SL was 2.21 ± 0.02 μm during diastole, and 2.09 ± 0.01 μm at the peak of systolic force (Tp = 100 ± 12 kPa). In diastole, the pattern shows the characteristic features of resting skeletal muscle, with the first order layer line reflection (ML1), at an axial spacing of 43 nm, and the meridional reflections (M1-M6) based on the three-stranded, quasi-helical symmetry of myosin motors lying on the filament surface in their resting (OFF) state (Linari et al., Nature 228:576, 2015). The M3 reflection is sampled by X-ray interference between the two halves of the thick filament, showing a dominant peak with small satellite peaks on either side. The spacings of M3 (SM3) and M6 (SM6) are 14.356 ± 0.004 nm and 7.187 nm respectively. At Tp, the intensities of ML1 and the meridional reflections, apart from M3 and M6, are markedly reduced. SM3 and SM6 are respectively 0.9 and 1.3% larger than in diastole and M3 is split into two peaks of comparable size. These results show that, in agreement with the mechanosensing in the thick filament of skeletal muscle, force development in cardiac muscle implies increased extension of the thick filament and movement of the myosin motors by 10 nm away from the filament midpoint. Supported by MIUR-PRIN, Ente Cassa di Risparmio di Firenze and Telethon (Italy).

Posters

S10.P1-329

Loss of sarcomeric M-line organization in the diaphragm muscle of obscurin knockout mice following exercise

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We reported that skeletal muscle fibers of obscurin knockout (KO) mice present altered distribution of ankB, disorganization of the subsarcolemmal microtubule cytoskeleton and reduced localization of dystrophin at costameres, accompanied by impaired running endurance on the treadmill and increased exercise-induced sarcolemmal damage compared to wild-type animals (Randazzo et al., 2013). More recent results obtained from a combined approach of physiological, morphological, and structural studies of obscurin KO mice, revealed that the reduced endurance of obscurin KO animals on the treadmill depends on exercise intensity and age. Indeed, a mild running protocol did not evidence significant differences between control and obscurin KO mice whereas comparison of running abilities of 2, 6 and 11 month-old mice exercised at exhaustion revealed a progressive, age-dependent reduction of the exercise tolerance in KO mice. Histological analysis indicated that a heavy exercise induced leucocyte infiltration, fibrotic connective tissue deposition and hyper-contractures, mainly in the diaphragm of KO mice. Interestingly, electron microscopy revealed that in the diaphragm, but not in the hindlimb muscles, of exercised obscurin KO mice both M-line and H-zone of sarcomeres appeared wavy and less defined. These results suggest that obscurin is required for the maintenance of the morphological and the ultrastructural integrity of skeletal muscle fibers against damage induced by intense mechanical stress (Randazzo et al., submitted). Furthermore, they provide a functional support to the proposed involvement of obscurin in human genetic diseases as by the identification of mutations in the obscurin gene in human cardiac diseases and by our recent results on a potential involvement of obscurin in human muscular dystrophy.

S10.P3-263

Myosin storage myopathy in C. elegans and human cultured muscle cells

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1. Myosin storage myopathy is a protein aggregate myopathy associated with the characteristic subsarcolemmal accumulation of myosin heavy chain in muscle fibres. Despite similar histological findings, the clinical severity and age of onset are highly variable, ranging from no weakness to severe impairment of ambulation, and usually childhood-onset to onset later in life. Mutations located in the distal end of the tail of slow/ß-cardiac myosin heavy chain are associated with myosin storage myopathy. Four missense mutations (L1793P, R1845W, E1883K and H1901L), two of which have been reported in several unrelated families, are located within or closed to the assembly competence domain, which is critical for the proper assembly of sarcomeric myosin rod filaments. 2. To assess the pathogenesis leading to protein aggregation in myosin storage myopathy and to evaluate the impact of these mutations on myosin assembly and muscle function, we expressed mutated myosin proteins in cultured human muscle cells and in the nematode Caenorhabditis elegans. 3. While L1793P mutant myosin protein efficiently incorporated into the sarcomeric thick filaments, R1845W and H1901L mutants were prone to formation of myosin aggregates without assembly into striated sarcomeric thick filaments in cultured muscle cells. In C. elegans, mutant alleles of the myosin heavy chain gene unc-54 corresponding to R1845W, E1883K and H1901L, were as effective as the wild-type myosin gene in rescuing the null mutant worms, indicating that they retain functionality. 4. Taken together, our results suggest that the basis for the pathogenic effect of the R1845W and H1901L mutations are primarily structural rather than functional. Further analyses are needed to identify the primary trigger for the histological changes seen in muscle biopsies of patients with L1793P and E1883K mutations.

S10.P4-217

Investigation of bending stiffness of αα- and ββ-tropomyosin homodimers using optical trap

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Tropomyosin (Tpm) plays an important role in regulation of actin–myosin interaction in striated muscle. The TPM1 and TPM2 genes encode the α- and β-chains of Tpm, respectively. The α- and β-Tpm form αα- and ββ-homodimers and αβ-heterodimer. The αα-homodimers and αβ-heterodimer of Tpm are more stable than ββ-homodimer and, hence, most mammalian striated muscles contain both αα-homodimer and αβ-heterodimer (Boussouf ea, 2007). Expression of α- and β-Tpm in the muscle depends upon species and age of the animal and changes during pathologies. The cardiac muscle of small mammals contains solely αα-homodimer of Tpm, while the heart of large mammals also contains αβ-heterodimer. We used a two-beam optical trap to test the effect of different proportion of αβ-Tpm on the bending stiffness of reconstructed thin filaments (consisting of F-actin, Tpm and troponin complex). A measuring dumbbell-like probe was formed by a filament segment attached to two a 1 micrometer beads hold by different optical traps. One trap was static and held a bead used as a force transducer while an acousto-optical deflector moved the beam holding the second bead, capable to stretch the dumbbell. The distance between the beads was measured using image analysis of micrographs. An exact solution to the problem of bending of an elastic inextensible bar with a uniform bending stiffness and subjected to a stretch was used for analysis of the displacement-force data (Nabiev ea, 2015). We found that the bending stiffness of thin filament containing αα-homodimer was somewhat higher though insignificantly than that with ββ-homodimer ($4.0 \pm 2.3 \times 10{-}26$ N m^2 vs. $3.6 \pm 1.8 \times 10{-}26$ N m^2). Supported by RFBR grants 16-04-00688.

S10.P6-108

Effects of cardiomyopathy-associated mutations on tropomyosin function.

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Some familial cardiomyopathies correlate with mutations in proteins that regulate formation of thin filaments in cardiac cells. Mutations Lys15Asn and Arg21His in striated muscle alpha-tropomyosin are associated with dilated and hypertrophic cardiomyopathy, respectively. Molecular mechanisms of the cardiomyopathies development are not studied yet. Both mutations are located within the binding interface formed by tropomyosin and leiomodin, a protein of tropomodulin family. Tropomodulins control actin polymerization/depolymerization at the pointed end of the actin filament. Tropomyosin binds F-actin and mediates binding of leiomodin and tropomodulin to the pointed end. Together these proteins regulate length of the thin filament. Using circular dichroism, co-sedimentation and pyrene-actin polymerization assays, we investigated the effects of both mutations on tropomyosin's binding to F-actin and to different leiomodin and tropomodulin isoforms. The Lys15Asn mutation caused a decrease of tropomyosin binding to F-actin while the Arg21His mutation had no effect. Lys15Asn in tropomyosin weakens binding of both leiomodin and tropomodulin at the pointed end of actin filaments, but with a noticeably stronger effect on leiomodin affinity. The binding of leiomodin and tropomodulin at the pointed end slightly decreased in the presence of the Arg21His mutation in tropomyosin. The results provide a molecular rationale for the development of familial cardiomyopathies.

S10.P5-157

Substitutions E240K and R244G in tropomyosin have similar effects on actin-myosin interaction during ATPase cycle

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Using polarized photometry we have studied the effects of two amino acid replacements, E240K and R244G, in αfast-tropomyosin (Tpm1.1) on the position of Tpm1.1 on actin filaments and the spatial arrangement of actin monomers and myosin heads at the various mimicked stages of the ATPase cycle in the absence of troponin and calcium. E240K and R244G are located in the C-terminal, seventh actin-binding period, in f and b positions of the coiled coil heptapeptide repeat. Actin, Tpm1.1, and myosin subfragment-1 (S1) were fluorescently labeled: 1,5-IAEDANS was attached to actin and S1, 5-IAFwas bound to Tpm1.1. The labeled proteins were incorporated in ghost muscle fibers and the changes in polarized fluorescence during the ATPase cycle have been measured. The data obtained suggest that the E240K and R244G tropomyosins occupy centre-shifted position on the thin filaments, but they inhibit the ability of myosin heads to switch ON the actin monomers during different simulated stages of ATPase cycle and to form the weak-binding state with F-actin. The lack of actin activation and 'freezing' myosin heads in a strongly-bound state are the reason of reduced activity of the actomyosin Mg-ATPase observed in solution in the presence of the mutant tropomyosins. These effects suggest that similar structural changes might be the molecular basis of muscle weakness in congenital fiber type disproportion caused by substitutions E241K and R245G in Tpm3.12.

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S10.P7-308

Dimer exchange in tropomyosins occurs at 37 °C

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In muscle, tropomyosin (Tpm) can exist as a heterodimer or homodimer. How heterodimers form and if partner exchange takes place remains unknown. Yet the properties of the heterodimer (wt α-ß Tpm, or wt Tpm-Tpm* with one Tpm carrying a single point mutation) are known to be distinct from the homodimer. In non-muscle cells alternate splicing of the four Tpm genes produces up to 40 different mRNA variants, but little is known about heterodimer formation. Understanding how dimers form and the rates of exchange is therefore of potential wide implications. Previous work used Cys cross-linked Tpm dimers to fix the dimer population and allowed pure populations of dimers to be created and studied. However, the presence of the cross-link may alter the behaviour of the protein. Here we followed dimer assembly and exchange, with the use of N-terminally His-tagged Tpm, by fixing a population of dimers with a Cys cross-link and running them under non-reducing SDS PAGE. Heating a 50:50 mixture of tagged and untagged dimer at 60 °C then cooling to 4 °C gives random assembly of dimers (50 % heterodimer, 25 % of each homodimer), which indicates there is no preference for dimer formation. Dimers mixed 50:50 at 4 °C, 20 °C and 30 °C showed no exchange over 2 weeks. However at 37 °C, exchange was seen after 4 hours.

As Tpm dimers are stable up to 30 °C, then samples of defined dimers can be stored long term with little exchange. Additionally, the properties of a heterodimer can be explored without the cross-link. In the cell, free Tpm dimers will be exchanging in a few hours. The presence of Tpm binding partners would be expected to slow the exchange rate. If free Tpm is present in the cell, then exchange between dimers would result in random assembly of available Tpm isoforms. Any preference or bias in dimer formation must involve additional regulation of assembly in the cell.

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S10.P8-306

Lack of myosin VI affects skeletal muscle fiber organization

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Myosin VI (MVI), one of uncoventional myosins, is the only myosin moving backwards on actin filaments. This unique molecular motor is widely expressed in Metazoa and was shown to be involved in endocytosis, cell migration, intracellular trafficking as well as in actin cytoskeleton dynamics. Previously our group showed that in skeletal muscle MVI localized to the sarcoplasmic reticulum (SR), muscle

nuclei, and was present in postsynaptic region of the neuromuscular junction [1]. In the cardiac muscle next to the SR it was also present in the intercalated discs [1]. Moreover, we proposed a role for MVI in myotube formation (and thus in myogenesis) [2]. However, the knowledge on mechanisms of its involvement in muscle function and development is very limited. Here, we addressed this problem with the use of muscle from mice lacking MVI (MVI-KO; Snell's waltzer mice*) that were shown by others to have several defects of the hearing and central nervous systems, intestine and kidney. We used MVI-KO hindlimb muscle of adult animals and of P0 nurslings, and analyses were performed with respect to the wild type (WT) littermates. The analysis revealed abnormalities in the sarcomere organization, especially in the localization of markers of sarcolemma and sarcoplasmic reticulum. Also, organization of bungarotoxin-stained membranes of the neuromuscular junction was disturbed. Up-regulation of expression of AKAP9, one of the muscle specific binding partners [3] was observed in MVI-KO mice as well. Functional studies are in progress to determine whether and how these changes impair muscle contraction.

* Snell's waltzer mice were a gift from Dr. Folma Buss from MRC in Cambridge, UK.

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S10.P10-148

Structural and functional properties of cardiac tropomyosin with cardiomyopathy-associated mutations in the troponin T-binding regions

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Tropomyosin (Tpm) is a coiled-coil actin-binding protein lying on the surface of actin filament and playing a key role in the regulation of muscle contraction. There are two regions in the Tpm molecule by which it interacts with troponin T. One of them is the area of residues 170–195, and another region is the overlap junction between N- and C-terminal ends of Tpm molecules. In our work we studied the effects of cardiomyopathic mutations L185R, E180V, I172T, M8R, K15N, I284V, M281T, and A277V in these both regions on structural and functional properties of cardiac Tpm. We applied differential scanning calorimetry (DSC) and circular dichroism (CD) to investigate effects of these amino acid substitutions on the Tpm structure. It has been shown that mutations in the region of 170–195 residues differently affect the Tpm structure. We founded that mutations L185R and E180V stabilize the C-terminal part of Tpm molecule, while mutation I172T destabilizes this part of the molecule. As for the mutations in the overlap region (M8R, K15N, I284V, and M281T), they had no significant effects on Tpm structure, except A277V mutation which increased the thermal stability of the C-terminal part of Tpm. All mutations in the overlap region decreased the viscosity of Tpm solutions. The most of mutations decreased the affinity of Tpm for actin as was shown by co-sedimentation assay; they also decreased the stability of Tpm-actin complexes except mutations A277V and E180V which increased the stability of these complexes. Mutations L185R and E180V were shown to increase the Ca^{2+}-sensitivity of the sliding velocity of regulated thin filaments in the in vitro motility assay. Thus, cardiomyopathic mutations in the troponin T-binding regions of Tpm have considerable effects on Tpm structural and functional properties, and this may explain why these mutations are associated with Hypertrophic or Dilated Cardiomyopathy. Supported by RFBR Grant 15-34-20136.

S10.P9-288

Interaction of myosin VI with its binding partners: AKAP9 and DOCK7

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Myosin VI (MVI), an unique motor protein functions in a many cellular processes such as vesicular transport, cell migration or mitosis. We showed that AKAP9 bound to MVI in C2C12 myoblasts [1] and DOCK7 interacted with MVI in neurosecretory PC12 cells [2]. AKAP9 belongs to the family of A-kinase anchor proteins (AKAPs) that are involved in regulation of protein kinase A (PKA) activity. Alterations in AKAP9 function are associated, among others, with cardiovascular disorders, for example a long QT syndrome. DOCK7 functions as a guanine nucleotide exchange factor (GEF) that activates Rac small GTP binding proteins, and is involved in neuritogenesis and Schwann cell migration. Two regions of MVI cargo domain were found to be involved in a partner recognition: a positively charged RRL motif (aa 1115–1117) and a hydrophobic WWY (aa 1191–1193) motif. We tested the nature of interaction of MVI with its interaction partners with the use of MVI tail (aa 840–1284) with mutations in either of the two regions (RRL to AAA and WWY to WLY) that were cloned into pEGFP-C3 plasmids*. The analysis revealed that endogenous AKAP9 bound to the MVI WWY region, thus via hydrophobic interaction. We plan to test which of the AKAP9 domains is engaged in the interaction and what is the physiological relevance of this interaction, especially that an increase in AKAP9 level in muscle from Snell's waltzer mice lacking MVI** was observed. DOCK7 binds to the MVI RRL region, thus via electrostatic interactions. Moreover, we showed that MVI bound to DOCK7 C-terminal domains of M2 and DHR2, involved in the protein-protein interactions and GEF activity, respectively. We also demonstrated that in PC12 cells this interaction was important for NGF-stimulated outgrowth formation.

* tail mutants and **Snell's waltzer mice are gifts from Dr. Folma Buss from MRC in Cambridge.

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S10.P11-145

Unusual aggregation of myosin subfragment 1 induced by intermolecular interactions of the N-terminal extension of essential light chain1

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We applied dynamic light scattering (DLS) to compare aggregation properties of two isoforms of myosin subfragment 1 (S1) containing different "essential" (or "alkali") light chains, A1 or A2 (A1 differs from A2 by the presence of N-terminal extension of 41 residues containing multiple Ala-Pro repeats and two pairs of Lys residues near the N-terminus). The DLS experiments were performed at low ionic strength and at relatively low temperatures, before the thermal unfolding of the protein studied by DSC. Under these conditions, we observed a significant growth in the hydrodynamic radius (Rh) of the particles for S1(A1), with no Rh changes for S1(A2). For instance, upon heating up to 40oC, i.e. before the S1 thermal denaturation, an intensive increase of the Rh value, from 16–20 nm to ~600–700 nm, was observed for S1(A1), whereas the Rh for S1(A2) remained unchanged and equal to 16–20 nm. Similar, although slightly less pronounced, difference between S1(A1) and S1(A2) was observed in the presence of ADP. In contrast, no differences were observed by DLS between these two S1 isoforms in their complexes S1-ADP-AlF4- and S1-ADP-BeFx which mimic the S1 ATPase intermediate states S1**-ADP-Pi and S1*-ATP. We propose that during the ATPase cycle the A1 N-terminal extension can interact with the motor domain of the same S1 molecule, and this can explain why S1(A1) and S1(A2) in S1-ADP-AlF4- and S1-ADP-BeFx complexes do not differ in their aggregation properties. In the absence of nucleotides (or in the presence of ADP), the A1 N-terminal extension should interact with actin and form additional actin-binding site on the myosin head. However, in the absence of actin, this extension seems to be unable to intramolecular interaction, but it probably can interact with the motor domain of another S1 molecule, and these intermolecular interactions of the A1 N-terminal extension can explain unusual aggregation properties of S1(A1). This work was supported by RFBR (Grant 15-04-03037).

Session 11 Mechanotransduction (VIDD, Shear stress, Starling's law)

Oral presentations

S11.O1

Pathophysiologic Mechanisms of Ventilator-Induced Diaphragmatic Dysfunction

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Diaphragmatic weakness is highly prevalent in the intensive care unit (ICU) population, and this phenomenon is associated with an impaired ability to successfully wean patients from mechanical ventilation (MV), along with increased mortality. A fundamental question that has arisen is how MV itself contributes to the onset of diaphragm weakness and atrophy, a condition termed Ventilator-Induced Diaphragmatic Dysfunction (VIDD). Data from both animal and human studies strongly suggest that excess mitochondrial reactive oxygen species (ROS) production in the diaphragm plays a central role in VIDD. However, the precise mechanisms responsible for the rapid development of mitochondria-derived oxidative stress during MV remain unclear. We have noted increased intramyocellular lipid accumulation in the diaphragms of patients undergoing MV, and additionally demonstrated that induction of hyperlipidemia during MV in mice worsened oxidative stress in the diaphragm. We have also documented a rapid onset of mitochondrial fission-fragmentation and activation of the pro-fission dynamin-related protein (Drp)-1 in the diaphragm within 6 hours of initiating MV in mice. This mitochondrial fragmentation preferentially involved the more abundant intermyofibrillar mitochondria that are most closely linked to contractile function, suggesting at least one possible explanation for the very rapid loss of diaphragmatic force observed with VIDD. The presence of mitochondrial fission may also help to explain why autophagy is upregulated in the diaphragm during MV, since autophagy plays a key role in eliminating damaged or fragmented mitochondria (mitophagy). Interestingly, our studies suggest that autophagy is a beneficial adaptive response which initially helps to mitigate force loss in the diaphragm during MV, possibly through removal of damaged mitochondria responsible for excessive ROS generation. The therapeutic implications of the above and other recent findings in the field will be discussed.

S11.O2-116 / S11.P1-116

Role of TRPC3 in the angiotensin II type 1 receptor-dependent slow force response to stretch in cardiomyocytes

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When a cardiac muscle is held in a stretched position, its [Ca^{2+}] transient and twitch force increase slowly over several minutes. This response is called a slow force response (SFR) to stretch. The stretch-induced release of angiotensin II has been implicated in the SFR, to raise intracellular Na+, followed by an increase in intracellular Ca^{2+} via the Na+/Ca^{2+} exchanger. TRPC 3 is known as stretch-activated non-selective cation channels, and receptor-operated cation channels regulated by angiotensin II type 1 receptor (AT1R). The involvement of TRPC3 in the SFR to stretch remains unclear. In this study, we investigated the role of TRPC3 on the SFR to stretch. Mouse ventricular myocytes were enzymatically isolated. A pair of carbon fibres was attached to each cell end. The cells were electrically stimulated in normal Tyrode solution at room temperature. Passive and active forces were calculated from the carbon fibre bending. The [Ca^{2+}] transient was measured using Fura-4F AM (an indicator of the intracellular Ca^{2+} concentration, [Ca^{2+}]i). The cell was stretched by moving a pair of carbon fibres, and then the stretch was maintained for 300 s to record the twitch force and/or [Ca^{2+}]i on the SFR to stretch. An AT1R blocker (olmesartan), a phospholipase C (PLC) inhibitor (U-73122) and a TRPC3 inhibitor (Pyr3) significantly suppressed the stretch-induced slow increase in [Ca^{2+}]i. U-73122 and Pyr3 also significantly inhibited the slow increase in [Ca^{2+}]i induced by applying angiotensin II instead of stretch. The stretch-induced slow increase in [Ca^{2+}]i was not observed in the cardiomyocytes of the TRPC3 knockout mice. Confocal imaging of heart tissues revealed that TRPC3 was partly located on the sarcolemma. A mathematical modelling simulation study showed that the SFR to stretch was reproduced by a stretch-induced Na+ and Ca^{2+} influx via TRPC on the sarcolemma. These results suggest that TRPC3, regulated by AT1R via PLC on the sarcolemma, causes the SFR to stretch.

S11.O3-128 / S11.P2-128

Leaky ryanodine receptors contribute to diaphragmatic weakness during mechanical ventilation

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Introduction: Ventilator-induced diaphragmatic dysfunction (VIDD) refers to the diaphragm muscle weakness (1) that occurs following prolonged controlled mechanical ventilation (MV). The presence of VIDD impedes recovery from respiratory failure, but the pathophysiological mechanisms accounting for VIDD are still not fully understood.
Material and methods: The cross-sectional area, force, ryanodine receptor function of human and mice diaphragm mechanically ventilated untreated and treated with S107, ICI 118551, propranolol, Trolox were compared to control.
Results: Here we show in human subjects and a mouse model of VIDD that MV is associated with rapid remodeling of the sarcoplasmic reticulum (SR) calcium release channel/ryanodine receptor (RyR1) in the diaphragm. The RyR1 macromolecular complex was oxidized, S-nitrosylated, Ser-2844 phosphorylated and depleted of the stabilizing subunit calstabin1, following MV. These post-translational modifications of RyR1 were mediated by both oxidative stress mediated by MV and stimulation of adrenergic signaling resulting from the anesthesia. We demonstrate in mouse model that these abnormal resting SR Ca^{2+} leak resulted in reduced contractile function and muscle fiber atrophy for longer duration of MV, and treatment with β-adrenergic antagonists or with S107, a small molecule drug that stabilizes the RyR1-calstabin1 interaction, prevented VIDD.
Discussion-Conclusion: Diaphragmatic dysfunction is common in MV patients and is a major cause of failure to wean patients from ventilator support. This study provides the first evidence of RyR1 alterations as a proximal mechanism underlying VIDD (i.e, loss of function, muscle atrophy), and identifies RyR1 as a potential target for therapeutic intervention.

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S11.O4-131

Common factors in the regulation of insect flight muscle and cardiac muscle of vertebrates

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The indirect flight muscle (IFM) of insects and cardiac muscle of vertebrates contract rhythmically. In both muscles, the force developed increases as the fibres are stretched (length-dependent activation, LDA). In cardiac muscle, LDA is enhanced by a rapid stretch at each beat (stretch activation) and in IFM, periodic stretch activation of opposing muscles produces the rapid contractions needed for flight. Both thick and thin filaments probably respond to stretch. Troponin bridges between the two filaments have been identified in IFM (Perz-Edwards et al, 2016) and it is likely that troponin is a stretch-sensor. Cardiac TnC and the TnCF2 IFM isoform bind one regulatory Ca^{2+} in the N-lobe. The force-pCa curve for IFM with only the TnCF2 isoform (pCa50 5.8 and co-operativity nH 3.2) is similar to that of cardiac trabeculae, suggesting the same mechanism produces the co-operative behaviour of both. TnCF1, the IFM isoform needed for stretch activation, binds a sole Ca^{2+} in the C-lobe. The force pCa curve for IFM fibres, in which TnCF1 is the predominant isoform, shows greater Ca^{2+}-sensitivity and less co-operativity (pCa 6.2 and nH 1.3). The C-terminal half of TnCF1 inhibits force generation, so the N-lobe is necessary. The force-pCa curve of IFM fibres is similar to that of cardiac trabeculae with bound C1-C2 fragment of MyBP-C, where Ca^{2+}-sensitivity is increased and co-operativity nearly eliminated. In this case, cardiac and IFM fibres achieve the same mechanical properties by different mechanisms. In order to determine if troponin could form a bridge between thin and thick filaments, we incubated a tropomyosin-troponin complex with thick filaments from myofibrils and with filaments assembled from pure myosin. TnT-TnH (TnI) bound to intact thick filaments but not to synthetic filaments. Therefore extra proteins in thick filaments are needed for the association, and this may be how troponin bridges are formed.

S11.O5-270 / S11.P3-270

Mechanical ventilation reduces the optimal length for force production in the diaphragm

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Mechanical ventilation (MV) in the intensive care unit (ICU) results in diaphragm muscle weakness and sequential weaning failure. The pathophysiological mechanisms are unclear. MV is characterized by positive end-expiratory pressure (PEEP), which forces the diaphragm into a shortened position. We hypothesized that this chronic shortening contributes to diaphragm weakening by reducing the optimal length for force generation, a reduction caused by reduced myofilament length and/or number of sarcomeres in series. Rats received controlled MV for 18 hours (PEEP: 2 cm H2O). Ultrasound revealed that MV ceased the normal cyclic changes in diaphragm length. The diaphragm was also shortened by ~19%. After MV, maximal tetanic tension of intact diaphragm strips was decreased by 46% compared to controls. The optimal length for maximal tetanic tension was ~9% shorter in MV rats compared to control rats. To investigate whether reduced length of myofilaments underlie this change in optimal length, we evaluated the force-sarcomere length (F-SL) relationship in single skinned fibers. Both the SL for maximal force and that for 50% of maximal force was comparable between MV and control rats, indicating that the SL dependence of force was not affected by MV and therefore myofilament length is not affected by MV. Finally, to study whether our findings in MV rats translate to ICU-patients who receive MV, we also investigated

the F-SL relationship of single skinned diaphragm fibers from these patients. Similarly to our findings in rats, the F-SL relation in patient fibers was comparable to control fibers. We propose that a reduction in the number of sarcomeres in series, rather than a change in the sarcomere length dependence of force, causes a reduced optimal length for force production during MV. This reduction is likely to induce weakness during weaning, when the diaphragm is forced to operate at normal muscle lengths and thus at the unfavorable descending limb of the F-SL relation.

Posters

S11.P4-328

Both tensile strain and pulsating fluid shear stress stimulate nitric oxide production and IL-6 and COX-2 gene expression in primary mouse myotubes

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During physical exercise muscle fibers are subjected to both tensile and shear stresses (Huijing and Jaspers, 2005. Recent evidence shows that nitric oxide (NO) production in C2C12 myotubes is induced by pulsating fluid shear stress (PFF) rather than by cyclic strain (CS)(Juffer et al. 2014)indicating they differentially affect mechanosensitive signaling pathways. It is unknown whether these differential effect also exists in primary myotubes and how CS and FSS affect expression of key signaling pathways and via what mechanisms. Therefore, primary mouse myoblasts were differentiated into myotubes and subjected to CS or PFF (1 h, 1 Hz). Both CS and PFF stimulated nitric oxide production by 10-fold, and interleukin- 6 (Il-6) and Cox-2 mRNA expression by 2-fold. Blocking of stretch-activated calcium channels abolished PFF-induced NO production, but did not alter PFF-induced Il-6 or Cox-2 expression, indicating that their expression was not mediated by NO and/or calcium. We conclude that in primary myotubes, unlike in differentiated C2C12 myoblasts, CS and PFF have similar mechanosensitive effects.

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S11.P5-315

Mechanosensitivity of myoblasts: Effects of pulsating fluid shear stress on mediators of proliferation, differentiation and self-renewal

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During physical exercise, muscle stem cells (MuSCs, i.e. satellite cells) are repeatedly subjected to physical stimuli. MuSCs are resident in their niche between basal lamina and sarcolemma of the myofiber. The myofascial connections between the myofibers in vivo likely cause shear stress on the MuSCs, in particular on their glycocalyx. To investigate the effects of shear loading on the expression of mediators of MuSCs proliferation and self-renewal, C2C12 myoblasts were subjected for 1h to pulsating fluid shear stress (PFSS, 1Hz). After 1h PFSS, NO production and Cfos mRNA transcript levels were increased >4-fold, while nNOS and iNOS mRNA levels were unaltered. PFSS doubled Wnt1a and IL-6 mRNA expression, while expression of COX-2, Wnt5a, Wnt10b, Vangl2, and R-spondin remained unchanged. We conclude that myoblasts are highly mechanoresponsive to PFSS causing increased transcription of factors involved in proliferation (Cfos, IL-6 and Wnt1a) rather than that of factors involved in differentiation or self-renewal.

S11.P6-245

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

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Significant improvements in modern critical have led to improved survival from critical illness, but this is associated with an increased number of patients with complications related to modern critical care. Severe muscle wasting and impaired muscle function are frequently observed and approximately 30% of mechanically ventilated and immobilized ICU patients for durations of 5 days and longer develop generalized muscle paralysis of all limb and trunk muscles, a condition known as CIM. Mechanical ventilation is a lifesaving treatment in critically ill ICU patients; however, the being on a ventilator creates dependence, and the weaning process occupies as much as 40% of the total time of mechanical ventilation. Furthermore, 20–30% of patients require prolonged intensive care due to VIDD, resulting in poorer outcomes, and greatly increased costs for health care providers. It is highly likely that a common component of ICU treatment per se is directly involved in the progressive impairment of muscle function and muscle wasting during long-term ICU treatment. The specific mechanisms underlying the muscle wasting and impaired muscle function associated with the ICU intervention are poorly understood in the clinical setting and there is compelling need for experimental animal models closely mimicking the ICU condition, including long-term exposure to mechanical ventilation and immobilization. In this project, the muscle dysfunction, which by far exceeds the loss in muscle mass in limb and respiratory muscles in patients with CIM and VIDD have been investigated in detail at the cellular and molecular levels in rodent and porcine experimental ICU models, allowing detailed studies in immobilized and mechanically ventilated animals for long duration. In addition, specific intervention strategies targeting the mechanisms underlying CIM and VIDD will be presented and the translation of these interventions to the clinic.

S11.P7-231

Investigation of skeletal muscle mass regulators after eccentric exercise-induced damage in human volunteers: myostatin,

follistatin, follistatin-like 3, Smads 2/3 and 7, activin receptor IIb, decorin

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Introduction/Aim: Exercise affects growth factors, including myostatin and its regulators and downstream signaling proteins. Smads, follistatin and follistatin-like 3 (FSTL3) all play roles in regulating muscle mass. Type I and II muscle fibres respond differently to exercise and may influence changes in proteins and mRNA measured in mixed muscle homogenate. To investigate response of muscle mass regulators to an acute bout of eccentric exercise, comparing subjects dominant in either slow type I or fast type II fibres.
Methods: 25 healthy, untrained males did one bout of plyometric jumping. Muscle biopsies and blood samples were obtained (baseline, 4 and 24 hrs post-exercise). NADH-TR staining determined fiber type. Western blotting was used to determine myostatin, activin receptor IIB, follistatin and FSTL3 protein and Smad 2/3 and 7 phosphorylation. Follistatin and decorin mRNA were determined by semi-quantitative PCR.
Results: Quadriceps force declined after eccentric exercise ($p < 0.05$). Increased serum CK confirmed muscle damage ($p < 0.001$) and was higher in volunteers with type II fibre dominance ($p < 0.01$). Myostatin, activin RIIB and FSTL3 protein did not differ at any time point. Neither did Smad 2/3 or 7 phosphorylation status change. Of significance was that the 28kDa myostatin correlated with % type I fibres. Subjects varied substantially in follistatin protein at 4 and 24 hours post-exercise, with a tendency to increase. Two bands of follistatin mRNA were identified: FST-315 (FST-344) and FST-288 (FST-317). mRNA analysis also revealed decorin splice variants A1 and A2 in skeletal muscle samples.
Conclusion: Myostatin and related proteins and signaling do not respond to muscle damage, at least not at time points investigated. Fibre type specific responses to plyometric exercise may account for inter-individual variability. This study detected two decorin and two follistatin mRNA splice variants in skeletal muscle, with FST344 at a higher level.

S11.P8-121

Effects of a chaperone co-inducer (BGP-15) on contractile properties of single fibres from soleus muscle of rats exposed to deep sedation and mechanical ventilation

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

S11.P9-119

Masseter muscle myofibrillar protein synthesis and degradation in an experimental critical iIllness myopathy model

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Introduction: Critical illness myopathy (CIM) is a debilitating common consequence of modern intensive care, characterized by severe muscle wasting, weakness and a decreased myosin/actin ratio. Limb/trunk muscles are primarily affected by this myopathy while cranial nerve innervated muscles are spared or less affected, but the mechanisms underlying these muscle-specific differences remain unknown.
Methods: In this time-resolved study, the cranial nerve innervated masseter muscle was studied in a unique experimental rat intensive care unit (ICU) model, where animals were exposed to sedation, neuromuscular blockade, mechanical ventilation, and immobilization for durations varying between 6 h and 14d. Gel electrophoresis, immunoblotting, RT-PCR and morphological staining techniques were used to analyze myosin/actin ratios, myofiber size, synthesis and degradation of myofibrillar proteins, and levels of heat shock proteins. Results obtained in the masseter muscle were compared with previous observations in experimental and clinical studies of limb muscles.
Results: Significant muscle-specific differences were observed, i.e., in the masseter, the decline in myosin/actin ratio and muscle fiber size was small and delayed. Furthermore, transcriptional regulation of myosin and actin synthesis was maintained, and Akt phosphorylation was only briefly reduced. In studied degradation pathways, only mRNA, but not protein levels of MuRF1, atrogin-1 and the autophagy marker LC3b were activated by the ICU condition. The matrix metalloproteinase MMP-2 was inhibited and protective heat shock proteins were up-regulated early.
Conclusion: These results confirm that the cranial nerve innervated masticatory muscles are less affected by the ICU-stress response than limb muscles, in accordance with clinical observation in ICU patients with CIM, supporting the model credibility as a valid CIM model.

S11.P10-117

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

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Intoduction: Ventilation-induced diaphragm dysfunction (VIDD) is a dramatic decline in diaphragm function in response to mechanical ventilation with negative consequences for health care providers and patient's quality of life, but specific treatment strategies are still lacking.
Material and methods: In this study we have used an experimental intensive care unit (ICU) model, allowing time-resolved studies of diaphragm structure and function in response to long-term mechanical ventilation and the effects of a pharmacological intervention strategy (the chaperone co-inducer BGP-15).
Results: The dramatic loss in diaphragm muscle fiber function in response to mechanical ventilation was due to post-translational protein modifications (PTMs) of myosin, but 10 days BGP-15 treatment improved diaphragm muscle fiber function dramatically ($\sim$100%), without improving diaphragm atrophy, in parallel with protection from myosin PTMs mediated via HSP72 induction, PARP-1 inhibition, and improvement of mitochondrial function and content.
Discussion-Conclusion: BGP-15 offers an efficient intervention strategy in reducing VIDD in mechanically ventilated ICU patients.

S11.P11-113

Study of disturbances in SUMO network and SUMOylated proteins in rat respiratory muscles affected by Ventilation-Induced Diaphragmatic Dysfunction (VIDD)

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Background: In spite of increasingly effective intensive care, some life-saving interventions, such as mechanical Ventilation (MV), have severe negative effects on both short and long-term prognosis among critically ill patients treated in Intensive Care Units (ICUs). One of the most relevant ICÚs side effects is named Ventilator-Induced Diaphragm Dysfunction (VIDD). This disease is characterized by a dramatic rapid, although reversible decrease in diaphragm contractile properties, which in turn, is one of the major causes of the delay and problematic weaning process from the ventilator. Final consequences of complicated weaning are increased patient morbidity, mortality and costs of care. SUMOylation is emerging as an important means to regulate cellular responses to stimuli, endogenous and exogenous stressors, both in cellular and organ levels.
Hypothesis: The decrease of muscle contractility is caused by reversible post-translational modification of proteins by covalent SUMO conjugation.
Methods and results: We used biopsies from anaesthetized, neuromuscular blocked and mechanically ventilated Sprague-Dawley female rats diaphragms. For the first time, with this study we observed a significant increase of polySUMOylated protein along mechanical ventilation. We identified by Mass-Spectrometry and validated endogenous muscle proteins conjugated by SUMO, related to calcium regulation, muscle contraction and mitochondria functions. We discovered by immunofluorescence that SUMOylated proteins are more abundant in oxidative compared to the glycolytic fibers, but after short period of MV, high levels of SUMOylated proteins were detected in all fibers, and finally, we identified some SUMO enzymes as potential VIDD biomarkers candidates.
Conclusion: Further studies on these new identified proteins along the intervention will provide the knowledge necessary for the development of pharmacological approach that can prevent VIDD and reduce the incidence of weaning problems.

S11.P12-156

Mechanical characterization of abdominal muscle fatigue. In vitro experimental model

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In mammals, abdominal wall is composed of four muscle groups: Internal Oblique (IO), External Oblique (EO), Rectus Abdominis (RA) and Transversus Abdominis (TA). The unique anatomical arrangement of these muscles has inspired descriptions and related hypotheses regarding its function as a composite-laminate structure in charge, among other tasks, for breathing, coughing, and postural control. Understanding the physical behaviour of these tissues is important from a scientific perspective and in many clinical areas for treating diseased or damaged tissue such as hernias. Previously, the authors have developed a computational model of the abdominal wall that takes into account the anisotropy, its passive and active response (1). In the present study, the in vitro fatigue behaviour of EO and RA muscles of New Zealand White rabbits has been investigated and different properties obtained to reproduce prolonged muscle activity. Five EO and RA samples were subjected to tetanic contractions for one hour and force evolution was recorded obtaining a maximal tension of 0.168 ± 0.027 and 0.099 ± 0.022 MPa for EO and RA respectively showing significant differences ($p < 0,05$). However, there were no significant differences regarding tension at the end of stimulation (0.017 ± 0.009 MPa OE and 0.018 ± 0.003 MPa RA). This response of two differente muscles could be explained because RA presents around 60% fibers type I whereas the percentage of slow fibers in OE is around 50% (2).

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Session 12 Bio energetics and ROS

Oral presentations

S12.O1

Mitochondrial dysfunction and reactive oxygen species in diseases of cardiac and skeletal muscles

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Functional and structural abnormalities of mitochondria are involved in the majority of cardiac and skeletal muscle pathologies. Indeed, mitochondrial dysfunction is causally related to both contractile impairment and loss of viability. Consequently, interventions aimed at maintaining mitochondrial function elicit a significant protection against various pathological conditions, especially in the case of injury induced by ischemia and reperfusion. These concepts will be reviewed highlighting the role of reactive oxygen species (ROS) that are both a consequence and a cause of mitochondrial derangements. In this respect, the various mitochondrial sources of ROS will be discussed focusing on monoamine oxidases. On the other hand, among the many targets of ROS that are involved in myocyte injury, the role of oxidative stress in causing both the opening of the permeability transition pore and contractile alterations will be reviewed. Finally, while the presentation will focus mostly on pathological conditions, room will also be given to the physiological roles of ROS and their involvement in endogenous self-defense mechanisms.

S12.O2-235

Serotonin induces production of superoxide anion in rat intrapulmonary arteries: role of mitochondria

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Introduction: 5-HT is a potent vasoconstrictor agonist under physiological conditions and contributes to several vascular diseases. We previously demonstrated that 5-HT, in physiological conditions, produces O2•, which is associated to contraction in rat intrapulmonary arteries (IPA). Here, we addressed the role of mitochondria in the signalling pathways associated to O2•, produced by 5-HT in IPA in physiological conditions. We also determined if such process can be mediated by other well-known vasoconstrictor agonists, namely endothelin-1 (ET-1) or phenylephrine (PHE).
Material and methods: Experiments were conducted on IPA and pulmonary arterial smooth muscle cells (PASMC) of male Wistar rats. O2•, levels were measured by electron paramagnetic resonance (EPR) and Griess reagent in rat IPA. Cultured PASMC were used to record (1) cytosolic and mitochondrial Ca^{2+} levels with Fluo-4 and Rhod-2 respectively, (2) mitochondrial O2• levels with mitosox, (3) mitochondrial membrane potential with TMRM and (4) mitochondrial respiration with a clark oxygen electrode.
Results: We showed that 5 HT increased O2•, and ONOO- levels whereas ET-1 and PHE had no effect. O2•, produced by 5-HT was blocked by rotenone, a mitochondrial complex I inhibitor whereas antimycin A, a mitochondrial complex III blocker, had no effect. We also demonstrated that, unlike ET-1, 5-HT increased both mitochondrial and cytosolic [Ca^{2+}] as well as mitochondrial O2•, levels. Rotenone blocked the mitochondrial O2•, levels produced by 5-HT. Furthermore, 5-HT depolarized mitochondria and decreased mitochondrial respiratory rate whereas ET-1 and PHE had a significantly smaller effect.
Conclusion: We have thus demonstrated, in rat IPA under physiological conditions, that the signalling pathways associated to 5-HT-induced production of O2•, involve mitochondria and especially the mitochondrial complex I. Interestingly, such process is specific to 5-HT and is not observed with ET-1 and PHE.

S12.O4-273

The role of hexokinase 2 and protein kinase B (Akt) during cardioprotective regimen of adaptation to hypoxia, ischemia and reperfusion in the left ventricle of rat heart

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1 - Adaptation to chronic hypoxia (CNH) increases the myocardial resistance to acute ischemia-reperfusion injury by affecting the mitochondrial redox balance (1,2). Hexokinase (HK) maintains mitochondrial coupling by preferential supply of ADP to the ATP synthase and thus prevents excessive formation of ROS by complex I and III of respiratory chain due to its increased association with mitochondria; thereby inhibiting the membrane permeability transition pore opening and preventing cell death (3,4). Association of HK2 with mitochondria might be induced by protein kinase B (Akt) phosphorylation and it has been sugested as cardioprotective (5,6). We aim to investigate the effect of CNH on Akt/HK2 cytoprotective pathway during ischemia and repefusion. 2 - Male Wistar rats were divided into control and CNH group (3w, pO2 0.1). Hearts were perfused according to Langendorff as follows, 20 min stabilization, 10 min ischemia, 10 min reperfusion; then used for western blotting and quantitative immunofluorescence microscopy. 3 - Our results suggests that CNH allows increasing of both HK1 and HK2 protein expression. Short ischemia increased association of HK2 with mitochondria in both experimental groups, however CNH prevented detachment of HK2 from mitochondrial membrane after reperfusion. This effect is associated with increased phosphorylation of Akt on Ser473 residue and decreased Bax/Bcl2. Moreover, adaptation to hypoxia prevents downregulation of mtCK. 4 - We conclude that cardioprotective phenotype of CNH is mediated at mitochondrial level by reinforcing anti-apoptotic processes related to the preservation of ADP availability to complex V.

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S12.O5-232

Therapeutic potential of AMP-activated protein kinase (AMPK) activators for the correction of Carnitine Palmitoyl Transferase 2 (CPT2) deficiency

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Aims: Carnitine palmitoyl transferase 2 (CPT2) is among the most common inherited defects of mitochondrial fatty acid oxidation (FAO). A frequent phenotype is an early adult-onset myopathy characterized by recurrent episodes of muscle pain, myoglobinuria and rhabdomyolysis usually triggered by prolonged exercise, fasting, fever, exposure to cold or infection. To date, there is no treatment of this disorder other than dietary management. AMPK is considered as a potential therapeutic target in many common metabolic or neurodegenerative disorders associated to mitochondrial dysfunctions.
Methods and Results: We therefore tested the therapeutic potential of AMPK activators provided by Glaxo-Smith-Kline (GSK) in myotubes from five CPT2-deficient patients. We showed that among four different GSK compounds, GSK1 was able to stimulate residual FAO capacities (3H-palmitate oxidation) in a dose- and time-dependent manner. Correction of CPT2 defect was achieved after treatment by GSK1 at 30μM for 48h. Western-blots analysis showed that GSK1 increased the amount of CPT2 mutant protein. Analysis of acylcarnitine intermediates in the culture media by MS-MS showed that CPT2-deficient myotubes exhibited, as expected, an accumulation of C16-acylcarnitines that was significantly decreased by the GSK1 treatment. Interestingly, all CPT2-deficient myotubes produced significantly more pro-inflammatory cytokines (TNFα, IL1β, IL6, IFNγ) than control cells. Furthermore, preliminary observations (immunofluorescence, western-blots, Xcellligence) suggest impaired differentiation of CPT2-deficient myotubes.
Conclusions: These initial results suggest that AMPK might represent a highly relevant therapeutic target for pharmacological correction of inborn CPT2 deficiency.

Posters

S12.P2-297

Functional responses of single psoas muscle fibres to redox imbalances

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Introduction: We have reported that in a chronic kidney disease (CKD) model, uraemic (URAEM) muscle presents with redox disturbances (1) and functional deficits (2). However, it is unclear if URAEM fibres have acquired some sensitivity to acute redox imbalances. We examined the functional responses of URAEM skeletal muscle fibres to an acute load of oxidation and/or reduction.
Materials and methods: In this approved pilot study, we used skinned psoas single fibres from an established animal model of CKD from 2 sham-operated control (CON) and 2 URAEM rabbits. We examined the effect of 10mM H2O2 and/ or 10mM DTT (3) in 2 experimental sets: A) Addition of H2O2 on fully activated fibres followed by relaxation in DTT and repeated activation ($n = 11$ CON/11 URAEM); B) Incubation with H2O2 preceded and followed by submaximal (pCa50) and maximal activation ($n = 10$ CON/13 URAEM).
Results: A) Addition of H2O2 did not affect isometric forces ($p>0.05$). Incubation with DTT caused significant ($p < 0.05$) force reductions in all fibres by ~10%, without any difference between groups ($p>0.05$); B) Incubation with H2O2 reduced ($p < 0.05$) maximally activated isometric forces for all samples by ~4%, without any difference between groups ($p>0.05$).
Conclusion: So far, we have not seen a differential functional response between URAEM and CON psoas fibres to acute oxidation and reduction. Further issues and directions will be discussed.

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S12.P3-278

Long-term cold acclimation attenuated activity of complex I of respiratory chain in left ventricle of rat heart

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1 - Despite the progress in the research and therapy, cardiovascular diseases are still the most common cause of death worldwide.. Cold acclimation or hardening has a good potential for attenuation of cardiovascular risks (1). Parameters, of mitochondrial respiration in long-term cold acclimated heart have not been studied yet. 2 – Male Wistar rats were acclimated to cold during 5 weeks and after subsequent 2-weeks recovery the left ventricles of hearts were homogenized and isolated mitochondria. We assessed mitochondrial respiration using Oxygraf Oroboros 2K, activity of selected mitochondrial enzymes by spectrophotometry and expression of mitochondrial complexes by western blotting. 3 - Our data showed significant decrease of oxygen flux in acclimated rats when energized complex I and complex II and also when palmitoylcarntine was used as a substrate. Similarly, enzyme activity of NADH-cytochrom c oxido-reductase, malate dehydrogenase and citrate synthase declined as well as protein expression of complex I. The other complexes did not change at protein and functional level. 4 - We conclude, that

attenuation of komplex I activity might prevent over-production of reactive oxygen species during ischemia reperfudion injury and might possess cytoprotective effect.

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S12.P4-260

TNF-α has a negative inotropic effect by NADPH oxidase-PKC pathway activation

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Myocardial depression, frequently observed in septic shock, is mediated by circulating molecules such as cytokines. TNF-α appears to be the most important pro-inflammatory cytokine released in the early phase of septic shock. We have previously shown that TNF-α had a negative inotropic effect on skeletal muscle and myocardium by activating a PKC pathway. Now, the aim of this study was to investigate the involvement of NADPH oxidase (Nox) on the activation of the different isoforms of PKC. Isolated rat hearts were perfused with TNF-α at 20ng/ml during 30min by using a Langendorff system. Expressions of Nox and PKC were analysed by western blot on membrane and cytosol proteins extracted from ventricular myocardium. Compared to control hearts, 20 min of TNF-α perfusion led to an increase of membrane expression of Nox2 (+51%) and of the p47phox subunit (+69%), thus resulting in Nox activation. This was also associated to an increase of PKC activity related to increased membrane translocation, with an overexpression of PKC-α (+123%) and PKC-ε (+54%) compared to control hearts. We demonstrated that TNF-a can induce PKC activation by increasing Nox2 expression and Nox activation thus leading to impaired contractility of the heart.

S12.P5-259

A set of pre-clinical tools to evaluate performance and metabolism in mice

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Introduction: The Performance & Metabolism facility in Mice (PMM) provides comprehensive, standardized or advanced, customized characterization of performance and metabolism in mouse models. We offer state-of-the-art services, consultancy and equipment for identification of anatomical, physiological, and behavioral phenotypes. PMM performs specific, on-demand experiments as well as comprehensive metabolic analyses to evaluate in vivo the energy balance in mouse. This exploration is performed under either basal condition or energy challenges using standardized techniques for the detection of phenotype in energy metabolism. It provides users with the techniques and scientific support in order to design experimental protocols as well as for the development of new techniques to investigate metabolism.

Methods and Results: Mice (n = 15) were immunized with 30 μg purified Torpedo californica AChR (TAChR) emulsified with an equal volume of CFA supplemented with mycobacterium tuberculosis 10 mg/ml to induce myastenie. - Body composition analyse This device gives precise analyzes of the body composition for fat content, lean mass and free body fluids in mice. Body composition is evaluated by NMR. Four weeks after the injection, relative lean mass was decreased (75±6 vs 78±4 %, p = 0.03). - Acute exercise We have 6 independent treadmills, allowing the VO2, VCO2 and RER (VCO2/VO2) by indirect calorimetry. We estimate the maximal capacities of the animals (VO2 peak, maximal speed, etc.), and their endurance capacity. Four weeks after the myastenie injection, absolute VO2 and relative VO2 to lean mass were decreased (respectively 149±14 vs 162±11 ml.h-1, p = 0.009 and 8.3±1.2 vs 10.9±0.7 ml.h-1.g-1 p < 0.001).

Conclusion: Besides the metabolic aspects, treadmills allow us to induce a stress that can, on one hand, reveal pathologies and/or rising adaptations, and on the other hand create muscle damage stimulating the regeneration processes.

S12.P6-251

Normal ranges of muscular lipid oxidation during exercise defined on a database of 3739 subjects

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In order to prevent obesity and metabolic disorders, the prescription of physical activity can be individualized on the basis of exercise calorimetry that allows to target it at the level of maximal fat oxidation (LIPOXmax). This training procedure improves muscle mitochondrial oxidative capacity, body composition, glucoregulation, blood lipids, and low grade inflammation in diabetic and obese patients. The aim of this work is to present a large cohort of patients who underwent in our unit an exercise calorimetry consisting of 4 steady state submaximal exercise steps on cycloergometer with calculation of carbohydrate (CHO) and fat oxidation from O2 and CO2 data with equations of calorimetry.We present a database of 3739 patients (65.4% of them being obese), 15 to 85 years old, with an average body mass index (BMI) of 32.87 kg/m2. Their average LIPOXmax is 42.83 watt (2nd and 3rd quartile ranging between 29.58 and 56.0 watt), which corresponds to 46% of maximal aerobic capacity (VO2 max), calculated according to American College of Sports Medicine (ACSM). There is a very strong correlation between LIPOXmax and theoretical VO2max predicted by Wasserman equations (r=0.445, p <0.001). Maximal lipid oxidation rate was 203.44±95 mg/min (mean± SD). There was no longer lipid oxidation above 84.81 watt (upper limit of the lower quartile: 60.39 watts and lower limit of the upper quartile: 109.23 watts), corresponding to 69% of VO2 max ACSM. The average slope of the linear relationship between CHO oxidation and power(carbohydrate cost of the watt) is 0.25 ± 0.09 mg min-1 kg-1 W-1.On the basis of this large database we can thus propose normality ranges for LIPOXmax and the other parameters of exercise calorimetry, which will be useful for targeting of exercise in obesity and diabetes.It is interesting to point out that the LIPOXmax is generally found in a quite narrow power range that can presumably be proposed for prescription even without performing calorimetry.

S12.P8-208

Increased fat and carbohydrate oxidation by exercising muscle in hypothyroid patients treated with levothyroxine

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Introduction: Subjects with hypothyroidism substituted with levothyroxine (HS) are know to be poor responders to weight reducing strategies. Since muscle energy metabolism is regulated by thyroid hormones we compared the oxidation of fat and carbohydrates (CHO) during exercise in HS versus controls.
Method: We compared 52 patients (48 women, 4 men, age 49 t years, levothyroxine dose 25–250 micrograms/day) to a control group of 2081 patients matched for sex, age, BMI and percentage of fat during an exercise calorimetry with 4 submaximal 6 minutes steps according to Pérez-Martin et al [1].
Results: At the same power intensity HS on the average oxidize more fat ($p = 0.009$) and use more oxygen ($p = 0.00019$). Lipid oxidation cuminates at the same power intensity (39,4±2,4 vs 38,6±0,3 watts) but its maximal oxidation rate is significantly higher in the HS group (10.32±0.47 vs. 9, 06±0.10 mg / min / kg muscle $p = 0.02$) and carbohydrate oxidation during the final level (1866.6 ± 77.3 vs 1705.45 ± 11.5 mg / min $p = 0.029$). The maximal lipid oxidation rate is correlated with the dose of levothyroxine ($r = 0.331$, $p < 0.05$).
Conclusion: HS patients exhibit an overall increase of energy expenditure during exercise, oxidising more lipids at mild to moderate intensities and more CHO at high intensities. This latter mechanism could result into an orexigenic effect of physical activity contributing to resistance to weight loss.

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S12.P7-227

Cytoprotective and antioxidant effects of the red alga Alsidium corallinum against hydrogen peroxide-induced toxicity in rat cardiomyocytes

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Aims: Sepsis is the manifestation of the immune and inflammatory responses to infection that may ultimately result in multiorgan failure and remains a leading cause of death. Many substances seem to be involved in myocardial dysfunction in sepsis, including toxins, cytokines and nitric oxide. Myocardial dysfunction is a well-described complication of severe sepsis, also referred to as septic cardiomyopathy. This study was designed to evaluate the in vitro characteristics and protective activity of the red alga Alsidium corallinum against hydrogen peroxide (H2O2)-induced toxicity in H9c2 cardiomyocytes.
Methods and Results: The in vitro properties and composition of A. corallinum were firstly investigated. Our results showed that the alga demonstrated the rich composition in antioxidant compounds like phenolics, flavonoids, anthocyanins, polysaccharides, chlorophylls and carotenoids. Its antioxidant activity was also confirmed using Radical-Scavenging Activity. Secondly, the H9c2 cells were pre-treated with A. corallinum extract (30 μg/ml) for 1 hour, then exposed to H2O2 40 (μmol/ml) for 4 hours. The cell viability was examined by MTT assay and the antioxidant enzymatic activities including superoxide dismutase (SOD) and glutathione peroxidase (GPx) were analysed photometrically. We demonstrated that H2O2 induced a decrease in cell viability correlating with an increase in SOD and GPx activities. Pretreatment of H9c2 cells with A. corallinum extract significantly reduce toxicity and decreased the antioxidants activities induced by H2O2.
Conclusions: These findings indicated for the first time the protective effect of A. corallinum against H2O2 induced toxicity in H9c2 cells.

S12.P9-207

Exercise test with biological samplings in the exploration of muscular pain and cramps.

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A standardized exercise test with biological samplings (ETBS) is a common approach of muscular metabolic disturbances for select patients in whom a muscular biopsy should be performed. However, the predictive value of parameters measured during this test is not well established. This study aimed at detecting which, if any, of ETBS parameters can accurately predict pathological biopsy results. We present here a database of 147 muscular biopsies performed after an exercise test which was assumed to predict a high suspicion of muscular disease because of decreased aerobic capacity (VO2max) and/or ventilatory threshold (VT), muscular pain, or biochemical parameters (lactate/pyruvate, #-OH-butyrate/oxaloacetate, myoglobin, CPK, LDH, aldolase) above the normal range. Among those 147 biopsies 34 biopsies (23.1%) evidenced an isolated abnormality in oxymetry or histology, and 17 (11.5%) in both of them. Ergometry was related to mitochondrial respiration: VO2max was correlated to Glutamate Vo ($r=0.220$ $p < 0.05$); succinate Vmax ($r=0,220$ $p < 0.05$) and ventilatory threshold (VT) was correlated to Vo Palmitoyl carnitine ($r=-0,198$ $p < 0,05$). However the large overlap for VO2max and VT between normal and pathologic cases made VO2max and VT unreliable predictors of mitochondrial respiration, as was also the kinetics of blood lactate during recovery despite its reported correlation with Vmax glutamate. Normality ranges for lactate/pyruvate and #-OH-butyrate/oxaloacetate ratio need to take into account power intensity. The blood marker with the highest sensitivity for detecting pathological cases is aldolase (77.8%), while myoglobin (92.2%) had the greatest specificity, so that the best prediction was obtained with myoglobin + creatine kinase + LDH + aldolase (sensitivity 44%; specificity: 95%; positive predictive value: 66.6%; negative predictive value: 90.2%). Thus, ETBS provides useful information for predicting abnormal biopsies, but its analysis can probably be further improved.

S12.P10-206

Sustained weight loss after low intensity endurance training is predicted by the level of maximal muscular lipid oxidation

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Among various strategies of exercise training proposed for the management of obesity and type 2 diabetes, endurance training is the most recognized procedure. When targeted with exercise calorimetry at levels where lipid oxidation is maximal (LIPOXmax), it has been shown to improve mitochondrial respiration, blood glucose control and blood lipids, low grade inflammation and body composition, even at low weekly volume. We investigated in this study its long term effects over 3 years (3 × 45 min/week) compared to low fat diet without exercise (LFD) and a control group without any exercise or diet (C). 88 subjects that continued LIPOXmax training more than 1 year (23 men, 65 women, age = 20–85 years, body mass index =23–48 kg/m^2) were compared to two matched groups (C and LFD). While C gained weight over this period, LFD and LIPOXmax group lose weight. Weight loss at 1 year was the same in exercise and diet group, but at 2 years and even more at 3 years there was a weight regain in LFD so that results were better ($p < 0.01$) in the exercise group who maintained weight loss in 80% of subjects. Average weight loss was -2.95 ± 0.37 kg after 3 months, -4.56 ± 0.68 kg after 1 year, -5.31 ± 1.26 kg at 2 years and -8.49 ± 2.39 kg at 3 years. The level at which LIPOXmax occurs is a predictor of weight loss at 1 year ($r = -0.346$ $p < 0.001$) but not at 2 and 3 years. Weight loss at 3 months is a predictor of weight loss at 1 year ($r = 0.523$ $p <$ 0,001) but not at 2 and 3 years. At 1 year subjects with LIPOX max in the lower quartile (<35% VO_{2max} $n = 23$) lose less weight than the others (-2.3 ± 0.98 vs -5.4 ± 0.83 $p = 0.05$) but this difference vanishes over time. This study shows that this low intensity exercise training maintains its weight-reducing effect 3 years while diet is no longer efficient, and that this effect is initially related to muscular ability to oxidize lipids but that metabolic and behavioral adaptations have been further developped and contribute to a long lasting effect.

S12.P11-198

Myoblasts derived from Idiopathic Inflammatory Myopathy patients show an altered mitochondrial bioenergetic profile

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Aims: Idiopathic inflammatory myopathies (IIM) are acquired diseases that progress with significant atrophy and loss of muscle force. In many cases this features persist even when the acute process of inflammation has been resolved. In this context our aim is to evaluate a possible role for mitochondrial dysfunction and an altered use of ATP in the pathophysiology of this disease, by using cellular cultures and tissue samples derived from patient biopsies.

Methods and Results: A human skeletal muscle cell line (RCMH) and primary cultured myoblasts extracted from mouse controls and IIM patients were used. To analyze the bioenergetic profile we used the extracellular flux analyzer (Seahorse Biosciences). Also, human skeletal muscle biopsies from IIM patients were used for western blot (WB) experiments. Normal and necrotizing myopathy biopsies were used for comparing purposes. Basal oxygen consumption rate, ATP linked oxygen consumption, maximal oxygen consumption and spare respiratory capacity were lower in IIM myoblasts compared to RCMH (control) myoblasts, but not between healthy mouse and IIM myoblasts. Non mitochondrial oxygen consumption rate was higher in IIM vs. RCMH myoblasts. Proton leak linked respiration was not different between both control models and IIM myoblasts. In parallel, expression levels of mitochondrial complexes (I, III, IV and V) were analyzed by WB in tissue samples. No differences were observed between control, necrotizing myoptahy and IIM patients.
Conclusions: Compared with a normal cell line, a marked mitochondrial dysfunction was observed in IIM derived myoblasts. Although, expression levels of the mitochondrial complexes was not different between control and patient samples. The alteration of the bioenergetic profile may be at the root of the muscular atrophy and loss of skeletal muscle force observed in this type of disease and so, must be analyzed as a potential therapeutic target. FONDECYT 3150623, 1151383 FONDAP 15150012.

S12.P12-192

Aerobic training, a possible strategy to reduce oxidative stress and prevent malignant hyperthermia episodes

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Calsequestrin 1 knockout (CASQ 1-null) mice suffer lethal episodes when exposed to both high environmental heat or halogenated anesthetics, a phenotype similar to human malignant hyperthermia (MH) susceptibility. We previously demonstrated that excessive oxidative stress plays a key role in lethal MH crises.
Aim: Demonstrate that reduction of oxidative stress by means of aerobic training results in decrease in mortality rate in CASQ1-null mice during heat-stress.
Methods and Results: C57Bl/6 and CASQ1-null male mice had their individual maximal exercise capacity evaluated at 2 – 2.5 months of age before being subjected to aerobic training for 2 months (60% of maximal speed, 5x/week). At 4 - 4.5 months of age, all mice were then first re-evaluated and then submitted to a heat stress protocol (41°C/1h). The mortality rate of trained CASQ1-null mice dramatically decreased when compared to the untrained group. Also, during the heat stress the increase in core temperature (hyperthermia) was reduced (and the time to reach the maximum temperature doubled) when compared to the untrained CASQ1-null mice. This fact was accompanied by raised exercise aerobic capacity, although no differences in strength were detected from 2 to 4 months old. In addition, an increase of 45 and 35% on lipid peroxidation of sarcoplasmic reticulum and mitochondria membranes of gastrocnemius muscle could be seen on the C57Bl/6 × CASQ1-null comparison, being rescued after the 8 weeks of training.
Conclusions: 2 months of aerobic training: (a) reduced oxidative stress; (b) lowered increase in core temperature, and (c) prevented sudden death of CASQ1-null mice.
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S12.P13-170

Effect of ischemic preconditioning on subcellular glycogen distribution following ischemia and reperfusion of isolated rat hearts

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Aim: Ischemic preconditioning (IPC) protects against myocardial ischemia-reperfusion (IR) injury. We aimed to investigate the effect of IPC on the subcellular distribution of glycogen in response to IR in cardiomyocytes of isolated perfused rat hearts.
Methods: Hearts from 60 male Wistar rats (338±26 g) were isolated and perfused at 37 oC with a modified Krebs-Henseleit buffer and oxygenated with 95% O2 and 5% CO2. Hearts were randomly assigned to: IPC, conducted as 2 × 5 min IR, or control (Con), followed by either 0 min ischemia, 25 min ischemia, or 25 min ischemia + 15 min reperfusion. Spatially distinct subfractions of glycogen in cardiomyocytes from the left ventricle were estimated by electron microscopy: subsarcolemmal (SS); intermyofibrillar (IMF); and intramyofibrillar (Intra) glycogen. Left ventricular developed pressure (LVDP) was measured using a latex balloon connected to a pressure transducer. Data are mean values (95% CI).
Results: At baseline, the relative contribution of the three subfractions of glycogen to total glycogen was 50 (39:61)% as SS, 41 (31:50) % as IMF, and 9 (5:13) % as Intra glycogen. After 25 min of ischemia (accompanied by a large reduction in total glycogen content), the contribution of SS glycogen decreased to 39 (32:47) % ($P < 0.05$) in Con and to 38 (31:45) % ($P < 0.05$) in IPC. After reperfusion the contribution of SS glycogen was restored to baseline levels in IPC hearts (IPC: 50 (42:59) % vs Con: 40 (32:48) %; $P = 0.02$). IPC increased LVDP following IR compared with Con (77 (59:94) mmHg vs. 27 (14:40) mmHg, $P < 0.0001$), and the contribution of SS glycogen correlated positively with LVDP in Con ($r2 = 0.46$, $P = 0.04$) but not in IPC hearts ($r2 = 0.003$, $P = 0.88$).
Conclusion: Subsarcolemmal glycogen was preferentially utilized during sustained ischemia. IPC protected against IR injury and mediated a re-distribution of glycogen towards a preferential storage within the subsarcolemmal space in reperfusion.

S12.P14-164

Eucalyptus globulus extract prevents H2O2 toxicity in rat cardiomyocytes H9c2 cells

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Aim: Sepsis has been defined by consensus as a systemic inflammatory response syndrome to infection which occasionally leads to multiple organ failure including myocardial dysfunction. Reactive oxygen species seem to play an important role in this dysfunction. *Eucalyptus globulus* is an important traditional Tunisian medicine used for the treatment of inflammation and oxidative stress. We therefore investigated the effect of *E. globulus* on prevention of stress caused by H2O2 in rat cardiac myoblast H9c2 cell line.
Methods and Results: Water extract of *E. globulus* was prepared. H9c2 cardiomyocytes were pretreated with E. globulus (30 μg/ml) for 1 hour and then exposed to H2O2 (40 μM) for 4 hours. H9c2 cells were analyzed for antioxidant enzyme activities (superoxide dismutase [SOD] and glutathione peroxidase [GPx]). Cell viability determined using MTT assay after 12 h of exposure. Main results showed that cytotoxic effect of H2O2 was inhibited by *E. globulus* extract. Furthermore, the H2O2-induced increase in SOD and GPx activities were abolished with *E. globulus* extract. This could be explained by polyphenols (7.6 mg AG/gMS) and flavonoids (8 mg AG/gMS) richness of *E. globulus* extract.
Conclusion: Our findings indicated for the first time that *E. globulus* can suppress H2O2-induced toxicity in H9c2 cells. We demonstrated that the effective impact of this plant extract is mediated by the antioxidant enzymatic activities. Therefore, it could potentially serve as cardio-protective agent against oxidative stress.

S12.P15-146

Myostatin deficiency in skeletal muscle alters lipid metabolism and mitochondrial membranes lipids composition

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Inactivation or inhibition of myostatin (mstn), a member of the TGF-β superfamily, is considered as a promising treatment for various muscle-wasting disorders. Indeed, inactivation of the Mstn gene in mice, or mutations gene in different species results in a similar impressive increased muscle growth [1]. Knock-out mstn mice (KO) showed also many metabolic changes as decreased mitochondria number [2], disturbance in mitochondrial respiratory function and increased muscle fatigability [3].
Aims: Muscle membrane maintains the structure and the metabolic function of the fibre, and mitochondrial membrane, including respiratory chain complexes, are composed mainly of lipids and phospholipids. In our study, we hypothesized that changes in the muscle and mitochondrial lipid composition could exist in the KO mstn muscle, in relation with the metabolic and functional alterations.
Methods/Results: We report in KO mstn muscle a decrease of fat membrane transporter levels (FAT/CD36, FABP3, FATP1 and FATP4)associated with decreased lipid oxidative pathway (citrate synthase and βHAD activities) and decreased lipogenesis(decline in triglyceride and free fatty acids content). Interestingly, we demonstrated a decrease in mitochondrial cardiolipin(CL)content, with a decrease in PGPS and cardiolipin synthase gene expressions.
Conclusion: Overall, we demonstrate that mstn deficiency reduced lipogenesis and lipid oxidation and alters the lipid composition of muscle and mitochondrial membranes, with a decrease in CL

mitochondrial content, which plays a functional role of mitochondrial bioenergetics.

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S12.P16-139

Physiological and molecular studies of Langendorff rat heart fed on cholesterol reach diet.The impact of Procaine treatment

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Aim of study: To investigate the impact of ischemia reperfusion (IR) upon : heart rate (HR), left ventricle developed pressure (LVDP), and coronary flow (CF) and on apoptosis in rat heart with experimental hypercholesterolemia and to see the effect of Procaine treatment. We used 18 male rats aged 20 months old divided into 3 groups of 6 animal each:(A) fed on normal chow, (B) fed on chow with 2% cholesterol (lard) for 8 weeks, (C) fed on chow with 2% cholesterol associated with Procaine treatment (I.P.) Procaine 4 mg/kg body). After treatment rats were killed by cervical dislocation and hearts were removed and placed in Langendorff retrograde perfusion system with Krebs Henseleit buffer at 37C. We used a 30 minutes ischemia followed by 120 minutes reperfusion model. (HR), (CF) and (LVDP) were determined by means of a latex balloon inserted into the left ventricle and connected with a pressure transducer in groups A, B and C.TACS apoptotic laddering kit(R&D) was used to assay heart cells for apoptosis in cholesterol fed rat heart with/without Procaine treatment.
Results: Our data have pointed out that following 30 minutes ischemia, there was a significant decrease in (CF) in group (B) i.e. from 10ml after 20 minutes stabilization period to 3 ml after 120 minutes reperfusion and an increase in (HR) rate. There was a significant decrease in (LVDP) in group (B) and was relatively constant with minor fluctuations in Procaine treated rats of group (C) versus group (A).DNA laddering pattern has been observed in group B cholesterol fed rats and absent in group (C).
Conclusion: Oxidative stress following (IR) in group (B) had a negative impact upon physiological parameters of heart, and on DNA stability. Procaine in treated (Group C) seems to have a protective effect on heart physiological parameters and on DNA stability leading to a decrease in (HR) approaching the values of Controls and increasing in (CF),and in recovery of (LVDP).

S12.P17-100

Pulmonary hypertension-induced diaphragm muscle weakness was prevented by treatment with antioxidant

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Patients with pulmonary hypertension (PH) suffer from inspiratory insufficiency, which is associated with intrinsic contractile dysfunction in diaphragm muscle. Here, we examined the role of redox stress in PH-induced diaphragm weakness by using the antioxidant, EUK-134. Male Wistar rats were randomly divided into control (CNT), CNT + EUK134 (CNT + EUK), monocrotaline-induced PH (PH), and PH + EUK groups. PH was induced by a single intraperitoneal injection of monocrotaline (60 mg/kg body weight). EUK-134 (3 mg/kg body weight/day), a cell permeable mimetic of superoxide dismutase (SOD) and catalase, was daily intraperitoneally injected starting one day after induction of PH. After four weeks, diaphragm muscles were excised for mechanical and biochemical analyses. There was a decrease in specific tetanic force in diaphragm bundles from PH group. This contractile dysfunction was accompanied by an increase in 3-nitrotyrosine (3-NT) content and aggregation of actin. These changes were prevented by the administration of EUK-134. Moreover, PH diaphragm showed significant reduction in GSH: GSSG ratio and increase in NADPH oxidase 2/gp91phox, SOD2, and catalase expression. These data show that the redox stress plays a pivotal role in PH-induced diaphragm weakness. Thus, antioxidant treatment can be a promising strategy for PH patients with inspiratory failure.

S12.P19-183

Methionine supplementation affects ROS production and the bioenergetic profile of muscle progenitor cells in a bidirectional way

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Introduction: Optimal supply of L-methionine (LM) improves muscle growth, whereas over-supplementation exerts adverse effects. Changes in amino acid availability lead to alterations in mitochondrial activity resulting from changes in ROS signaling and oxidative stress. Therefore, we examined alterations of cellular bioenergetics and ROS production after supplementation of different methionine sources: LM, DL-methionine (DLM), and the methionine analogue DL-2-hydroxy-4-methylthio butanoic acid (DL-HMTBA) to muscle cells.
Material and methods: For experiments, C2C12 cells or primary muscle cells isolated from 4 day old piglets were used, and supplemented with concentrations ranging from 2.5 to 1000 μM of LM, DLM and DL-HMTBA, respectively.
Results: All supplements improved growth by increasing proliferation (DNA concentration, cell number), protein synthesis and viability. The concentration needed for maximum growth stimulation differs markedly between tested methionine sources. High-dosage supplementation led to growth retardation or cell toxicity. For DL-HMTBA this effect mainly results from increased H2O2 production during its transformation to LM. Using a fluorescence spectroscopic method, the oxygen consumption rate (OCR) was measured before and after application of mitochondrial inhibitors (Oligomycin, FCCP, Antimycin A). Significant effects on OCR, ATP synthase linked respiration and respiratory control were found after DL-HMTBA supplementation.
Discussion: The supplementation of different methionine sources changes the bioenergetic profile and ROS metabolism of muscle

progenitor cells thereby resulting in different growth behavior. Our results are in accordance with a growth promoting effect of small increases in cellular H2O2 production resulting amongst other things from adaptations in bioenergetic processes and in the cellular antioxidant defense systems.
Funding: This study was supported by Evonik Nutrition & Care GmbH (Hanau-Wolfgang; Germany).

S12.P20-118

Exercise training affects the circadian rhythmicity of muscle insulin sensitivity

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Exercise is a potent lifestyle intervention to combat the ever-increasing problem of obesity and insulin resistance. Skeletal muscle metabolism and performance display circadian rhythmicity. Perturbed circadian rhythms are associated with metabolic dysfunction and insulin resistance. Given that insulin sensitivity in skeletal muscle follows a circadian pattern we hypothesis that this pattern is important for overall metabolic function. This hypothesis is being tested in a comprehensive mouse study using isolated skeletal muscle to measure glucose uptake under basal and submaximal insulin-stimulated conditions ex vivo at four different time points during the day. Furthermore, we are examining the mechanism by which exercise training affects the circadian rhythmicity of insulin sensitivity. Our preliminary results support a circadian rhythmicity in insulin sensitivity and an effect of exercise training. Consistent with this, we have observed circadian oscillations in skeletal muscle in the signaling pathways regulating insulin- and exercise-induced glucose uptake, including AKT, AMPK and TBC1D4 phosphorylation. In conclusion, our results provide evidence that skeletal muscle insulin sensitivity exhibits circadian rhythmicity, and this rhythmicity changes in response to exercise training.

S12.P21-103

Rat performing prolonged exhaustive exercise has transient diastolic dysfunction with preserved systolic function mediated by oxidative stress

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Aim: Intense physical practices like a marathon or triathlon result in transient left ventricular (LV) dysfunction, characterized by a transient reduction of LV diastolic relaxation without changes in systolic function by unknown mechanisms. Our study aimed to investigate in vivo and in vitro, the involvement of myofilaments and their regulatory proteins in a rat model of diastolic dysfunction induced by prolonged exhaustive exercise (PEE) in comparison with control sedentary animals.
Methods and Results: Wistar rats were submitted to a PEE protocol (3 hours; 65% of maximal aerobic velocity). Systolic cardiac function evaluated in vivo by echocardiography and ex vivo in Langendorff isolated hearts was unchanged by PEE, while parameters of diastolic function were severely altered. At the cellular level, PEE had no major effect on cardiomyocytes contractile function. However, PEE was associated with a marked decrease in the calcium transient amplitude. We also observed increased myofilaments calcium sensitivity (pCa50) associated with reduced maximal tension after PEE. Post transcriptional modifications of myofilaments regulatory proteins, such as Troponin I (TnI) and Myosin-Binding Protein-C (MyBP-C) were examined by Western blot. PKA-dependent phosphorylation level of those proteins was unchanged after PEE. Finally, the implication of redox depedent post- transcriptional modifications was examined. Increasing the Antioxidant capcities before the race prevents the cardiac dysfunctions.
Conclusion: The present results show that prolonged exhaustive exercise alters diastolic function by inducing oxidative stress dependent modifications of the cardiac contractile system.

S12.P22-95

A lipid storage myopathy associated with loss of NF1

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Introduction: Neurofibromatosis Type 1 (NF1) is an autosomal dominant genetic disorder with an incidence of 1:2500 births. Although NF1 symptomology is complex and of variable penetrance, muscle weakness is a clinical challenge that has significant impacts on quality of life. Children with NF1 present with reduced muscle size, global muscle weakness, and impaired motor control. Historically, these deficits have been attributed to central nervous system dysfunction, however there is growing evidence for an underlying primary metabolic defect in NF1-deficient muscle.
Methods and Results: In this study we generated a muscle-specific Nf1 knockout mouse (Nf1MyoD−/−). Electron microscopy analysis of Nf1MyoD−/− muscle revealed normal sarcomeric structure, however, pups failed to thrive and showed abnormal intramyocellular lipid accumulation consistent with a metabolic myopathy. Lipidomics analysis using mass spectrometry revealed accumulated long-chain fatty acids and esterified cholesterols in muscle. Notably, sub species of cholesterol ester (CE18:1, CE20:2, CE22:4) were up-regulated 20-fold in Nf1 knockout samples. Furthermore, histological analysis of patient NF1 muscle tissues demonstrated corresponding intramyocellular lipid accumulation in humans.
Conclusions: These data reveal NF1 as a critical metabolic regulator in muscle and uncover novel therapeutic options. We are currently performing pre-clinical trials using pharmacological and dietary interventions aiming to rescue muscle development and function in our NF1 models.

S12.P23-84

Lipid Peroxidation and Antioxidant System Activity Changes of Rat Blood and Cardiac Muscle Cells Under Chronic Stress

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For the last few years, special attention has been given to studying the influence of stress on the development of various diseases of the

cardiovascular system. The key parameter in altered cell metabolism is the activation of lipid peroxidation (LPO). Under normal conditions, this process is necessary for the normal functioning of cells; its intensity depends on the appearance of active forms of oxygen and is connected with the degree of functionality of the antioxidant system of a cell. Based on the above, the aim of our investigation has been to determine the level of activity of LPO processes and the antioxidant system in blood and cardiac muscle cells of rats living under stress condition. The experiment was conducted on 50 adult male Wistar rats (150 ± 10 g) divided into two groups. Rats in group 1 – socially isolated rats (dark/light ratio = 23.5/0.5 h). Rats in group 2 – control group (dark/light ratio = 14/10 h). During the experiments, all rats were given water and standard laboratory chow ad libitum. The experiment was repeated four times. It has been shown that daily rhythm disorders produce psycho-emotional stress in animals and that, this is accompanied by quantitative changes in physiological parameters and hormones in the blood. In the present study, it was observed that such stress increased lipid peroxidation in blood and heart muscle cells. Also, activities of antioxidant enzymes, superoxide dismutase, and catalase were diminished, indicating deterioration of the antioxidant system. The results suggested that psycho-emotional stress was accompanied by oxidative stress, causing a reduction in the intensity of energy metabolism in cardiac muscle cells, which was further strengthened by the fact that the activity of the enzymes involved in ATP synthesis in mitochondria was reduced. Based on the results, we proposed that psycho-emotional stress is one of the factors contributing to the development of various cardiac diseases.

Session 13 Excitation-Contraction Coupling in Skeletal Muscle

Oral presentations

S13.O1

Cellular Ca^{2+}-handling in skeletal muscle fatigue, recovery and response to training

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The ability to contract becomes impaired when skeletal muscles are activated at high rates or for long periods, i.e. fatigue develops. Altered Ca^{2+}-handling has a key role in fatigue-induced functional impairments within the muscle fibres. During induction of fatigue, myofibrils become less sensitive to Ca^{2+} and Ca^{2+} release from the sarcoplasmic reticulum (SR) decreases. In addition, fatigue can induce long-lasting reductions in myofibrillar Ca^{2+} sensitivity and SR Ca^{2+} release: The relative importance of these two force-decreasing mechanisms depends on the cellular handling of reactive oxygen/nitrogen species (ROS/RNS)(1,2). ROS/RNS are also important for the fine-tuned balance between positive effects of endurance training vs. negative effects associated with overtraining (3).

My talk will summarise mechanisms underlying the acute and prolonged fatigue-induced impairments and their relation to training-overtraining.

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S13.O2-303/S13.P1-303

Assembly of Calcium Entry Units improves muscle resistance to fatigue

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Store-operated Ca^{2+} entry (SOCE) is a mechanism triggered by depletion of intracellular Ca^{2+} stores. In muscle, SOCE is important to limit fatigue during repetitive stimulation. We recently discovered that prolonged treadmill exercise promotes, in EDL muscle, formation of new junctions between sarcoplasmic reticulum and transverse-tubules which contain STIM1 and Orai1, the two main players in SOCE. We named these new junctions Calcium Entry Units (CEUs). Interestingly, in mice lacking Calsequestrin-1 (CASQ1-null) CEUs are constitutively present. AIM. Demonstrate that the presence of CEUs improves muscle resistance to fatigue by increasing SOCE activity.

Methods and Results: We used a high-frequency stimulation protocol (30 × 1s-60Hz pulses every 5 seconds) to compare fatigue resistance in EDL muscles from control WT mice, pre-exercised WT mice (1 h of treadmill running at increasing speed: from 5 m/min to 25 m/min), and CASQ1-null mice in presence or absence of extracellular Ca^{2+}, or after addition of SOCE inhibitors (BTP-2, 2-APB and SKF 96365). Results of our experiments indicate that: a) in 2.5 mM Ca^{2+} external solution, EDL muscles from pre-exercised WT and CASQ1-null mice, exhibited a significantly increased capability to maintain contractile force compared to control WT mice (residual force after 10 tetani: 61.6±3.0, 80.8±2.8 and 143.2±7.0% respectively for control WT, pre-exercised WT, and CASQ1-null EDL muscles); b) when Ca^{2+} was removed from the external solution, muscles from all the three groups of mice showed an enhanced decay of contractile force (residual force after 10 tetani: 43.5±2.8, 53.8±4.1 and 80.2±2.3% respectively for control WT, pre-exercised WT, and CASQ1-null EDL muscles); c) practically identical results were obtained when SOCE inhibitors were added to the external solution. **Conclusion:** Our data suggest that CEUs provide a preferential pathway for Ca^{2+} entry during repetitive muscle activity, likely important to limit muscle fatigue.

S13.O3-204

Excitation-Coupled Calcium Entry in fast and slow twitch muscles

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Aims: Skeletal muscle dihydropyridine receptors (Cav1.1) act as Ca^{2+} channels and voltage sensors to initiate muscle contraction by activating ryanodine receptors, the Ca^{2+} release channels of the sarcoplasmic reticulum. Cav1.1 channel activity is enhanced by a retrograde stimulatory signal delivered by the ryanodine receptor. JP45 is a junctional sarcoplasmic reticulum membrane protein interacting with Cav1.1 and the Ca^{2+} storage protein calsequestrin

(CASQ). We hypothesize that JP45 and CASQ form a signaling pathway modulating Cav1.1 channel activity.
Results: We tested this hypothesis in a variety of mouse models, including WT, JP45KO, CASQ1KO, CASQ2KO, JP45/CASQ1 double KO(DKO1), JP45/CASQ2 double KO (DKO2) and JP45/CASQ1/CASQ2 triple KO (TKO). Our results show that Ca^{2+} transient in flexor digitorum brevis (FDB) muscle from DKO1, DKO2 and TKO fibres evoked by tetanic stimulation in fibres, result from massive Ca^{2+} influx via La3+ - and nifedipine-sensitive calcium channels (1, 2) . Calcium entry enhanced by ablation of both JP45/CASQ1 and JP45/CASQ2 complexes supports tetanic force development in fast EDL- and slow -twitch Soleus muscles (1,2). We also found that CASQs interact with JP45 at [Ca^{2+}] similar to those present in the lumen of the sarcoplasmic reticulum at rest, while [Ca^{2+}] similar to those present in the SR lumen after depolarisation-induced calcium release cause the dissociation of JP45 from CASQs.
Conclusions: We conclude that the complex JP45/CASQs is a negative regulator of excitation-coupled calcium entry, and that tetanic force development in fast and slow twitch muscles is supported by the dynamic interaction between JP45 and CASQs.

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S13.O4-243/S13.P2-243

Voltage-dependent calcium release in isolated muscle fibres of mice expressing non-conducting DHP receptors

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The control of Ca^{2+} release from the sarcoplasmic reticulum (SR) in vertebrates is mediated by conformational coupling between ryanodine receptors (RyR1) and dihydropyridine receptors (DHPR) which act as voltage sensors. The DHPR also generates a slowly activating L-type Ca^{2+} inward current, with the exception of teleost fishes, which completely lack this current (Schredelseker et al., PNAS, 2010). Recently, mutation (N617D) responsible for the non-conducting DHPR in Zebrafish has been introduced into the mouse genome with the goal to identify a possible role of the L-type current in mammals (Dayal et al., Biophys.J., 2014).

We investigated transmembrane ionic current after blocking Na+, K+ and Cl- conductance and Ca^{2+} release from the SR in mature muscle fibres of N617D mice in comparison to wild-type mice. Two-electrode voltage clamp experiments were performed on enzymatically isolated toe fibres. Here we show that the slow L-type Ca^{2+} current is absent in these fully differentiated muscle cells as it was found in myotubes of the N617D mice (Dayal et al., Biophys.J., 2014). To assess details of voltage-controlled Ca^{2+} release in these fibers, we dialysed the intracellular space with an artificial solution containing 15 mM of Ca^{2+} chelator EGTA, which then dominates binding of the Ca^{2+} released by step depolarizations. Fitting the relaxation time course of fluorometric Ca^{2+} signals using a binding and transport model allowed the estimation of Ca^{2+} removal and release. The SR Ca^{2+} load was assessed by investigating the rise of myoplasmic Ca^{2+} concentration induced by 4-chloro-m-cresol in the presence of the SERCA blocker CPA. No significant differences were found in both the voltage-dependence of Ca^{2+} release and the SR Ca^{2+} load between mutant and WT muscle fibres which supports the view that L-type Ca^{2+} current has neither a role in modulating skeletal muscle excitation-contraction coupling nor in maintaining SR luminal Ca^{2+} concentration.

S13.O5 -165

The type 1 Hypokalemic Periodic Paralysis mutation R1239H induces a proton gating pore current at resting membrane potentials in mouse muscle fibers

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Missense mutations in the gene encoding Cav1.1, the α1S subunit of the L-type calcium channel, induce type 1 Hypokalemic Periodic Paralysis (HypoPP1). These mutations mainly occur at outer arginine residues in the 4th transmembrane segment of voltage-sensor domains. Very few studies have investigated the acute functional effects of these mutations because of the difficulty to express Cav1.1 in heterologous systems. In the present study we successfully used electroporation to transfer into mouse muscles cDNAs encoding turboGFP-tagged human wildtype (WT) and R1239H HypoPP1 mutant Cav1.1 in which the second outermost arginine of the S4 of domain IV is replaced by histidine. The expression profile of the two channels showed a regular striated pattern indicative of the localization of the channels in the t-tubule membrane. Measurement of the L-type Ca^{2+} current using the silicone-clamp technique showed that the maximal conductance and the voltage-dependence of the Cav1.1 channel were respectively significantly reduced and shifted towards negative potentials in R1239H-expressing fibers. Applying voltage ramps from different holding potentials in the presence of an external low-Cl-, Na+-free and K+-free solution revealed a significant higher leak conductance measured between −80 and −120 mV in R1239H-expressing fibers. Acidification of the external solution increased the leak inward current to a higher extent and reduced intracellular pH (pHi) measured with the pH indicator BCECF at a higher rate in R1239H fibers. External acidification was also found to inhibit inward rectifier K+ (Kir) channels. These data suggest that an elevated leak inward current carrying protons flows at resting membrane potentials through a gating pore generated by the R1239H mutation and that external acidification, which is known to persist after muscle exercise, could favor the onset of muscle paralysis in HypoPP1 by potentiating the proton gating pore current and inhibiting Kir channels.

Posters

S13.P3-310

Novel RyR modulators for dystrophinopathies

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Introduction: In the mdx mouse model of Duchenne muscular dystrophy (DMD), the sarcoplasmic reticulum ryanodine receptor RyR1 is abnormally nitrosylated and this leads to calstabin depletion from the protein complex and subsequent calcium leak through the channel.

RyR modulators enhance RyR1-Calstabin binding preventing calcium leak, reducing biochemical and histological evidence of muscle damage and improving muscle function.
Material and methods: In this work we have analyzed the in vivo and in vitro effect of novel RyR calcium release channel stabilizers AHK1 and AHK2, in the mdx mouse model and in human immortalized myotubes.
Results: Treatment of 1 month-old mdx mice with AHK modulators during 5 weeks reduced histological evidence of muscle damage, improved muscle function and reduced basal cytosolic calcium concentration and serum CK levels. Additionally, RNA expression profile of dystrophic samples was partially rescued after treatment with RyR modulators. Finally, we found that AHK1 and AHK2 were also able to rescue RyR-calstabin interaction in myotubes under nitro-oxidative stress.
Conclusion: Our study shows that AHK modulators ameliorate dystrophic phenotype in cellular and animal models of Duchenne muscular dystrophy. In addition, our results consolidate RyR-calstabin complex as a useful therapeutic target for drug development against muscular dystrophies as well as other disorders with subjacent nitro-oxidative stress.

Bibliography: Dudley et al., Am J Pathol 2006; Bellinger et al., Nat Med 2009; Fauconier et al., Proc Natl Acad Sci USA 2010; Vallejo-Illarramendi et al., Expert Rev Mol Med 2014.

S13.P5-262

Voltage gated sodium channels expression in muscles during sepsis

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Voltage gated sodium channels (NaV) are responsible of membrane excitability, and are involved in triggering and propagation of muscle action potential. They influence the excitation/contraction coupling. NaV 1.4 and NaV 1.5 are the mains α isoforms in skeletal muscles and in cardiomyocytes with a greater number of NaV 1.4 in muscle conversely to heart. Moreover β subunits (β1-β4), which can modulate the sodium current, are differently represented in muscle and heart. Muscle excitability is decreased during sepsis by inflammation and especially by the pro-inflammatory cytokines released, but the impact of sepsis on channel expression (α and β proteins) is still not clear. After seven days of sepsis induced by caecal ligature and perforation in rat, heart, diaphragm (Diaph), soleus (Sol) a typical slow type muscle, peroneus longus (Pl) (mixte muscle) and extensor digitorum longus (EDL) a typical fast type muscle, were dissected free for protein extraction. The quantification of NaV 1.4, NaV 1.5 and β1-β4 subunits was done by western blotting on membrane protein extracts. Heart presents less NaV 1.4, β1 and β4 subunits, but the same amount of NaV 1.5 that other muscles. After sepsis, NaV 1.4 is decreased in all muscles until −34% in the Pl, whereas sepsis has no effect on NaV 1.5 population. Sepsis has a more pronounced effect on Sol, Pl and EDL, especially for beta subunits with a decrease up to −68% for β1 in Pl and −56% of β3 in EDL. In conclusion, we have evidenced a difference in the repartition of NaV 1.4, NaV 1.5 and β1-β4 subunits in five different types of muscles in control condition. Moreover we demonstrate that sepsis has a transductionnal effect on channel population as well in α proteins type as in β subunits. These effects are depending on the type of muscle. These effects could be involved in the decreased excitability observed during sepsis.

S13.P4-300

Increased occupation of sarcomeric calcium buffers reduces required calcium release for similar troponin-c binding of subsequent activation

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Aim: To test our theory that as calcium buffers such as ATP and parvalbumin become increasingly bound with calcium, we expect to see a greater change in calcium concentration for a given calcium release over short time courses.
Methods and Results: We used a stochastic structural model of a half sarcomere and simulated two action potentials with 15 ms delay. At rest calcium concentration is 5nm, the first action potential resulted in peak calcium concentration of ∼17.2 μM within the model at 3.2 ms, and the concentration of calcium-bound ATP reached ∼56 μM (16 % of total calcium released). Between action potentials, the calcium concentration and concentration of calcium-bound ATP dropped to ∼3 μM and ∼13.5 μM, respectively. SERCA pumps are rapidly occupied by calcium but are slow to transport calcium into the SR. The peak calcium-release rate and total calcium-release were ∼74% lower during the second release than during the first release. Despite this great reduction in calcium release, peak calcium concentration was ∼16.5 μM, and the concentration of calcium-bound ATP was ∼41 μM (45% of total calcium released) following the second release. Calcium-binding to parvalbumin increased throughout the simulation. Calcium occupancy of Troponin-C peaked at 90% after the first release, dropped to 80% at the initiation of the second release, and recovered to 95% 3 ms following the peak calcium concentration of the second release.
Conclusions: Increasing the calcium-saturation of calcium buffers and SERCA pumps seems to allow the sarcomere to be more responsive to small changes in calcium ions. These findings are consistent with the positive inotropic effects seen experimentally when calcium buffers are loaded with calcium prior to muscle activation.

S13.P6-256

Supratetanic frequency and doublet stimulation affects dynamic contractility in rat EDL muscles exposed to normal and high extracellular [K+]

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Aim: In effort to produce high velocity (V), power (P) and rate of force development (RFD) fast-twitch muscle fibers are often activated at supratetanic frequencies. However, this requires that muscles can generate and propagate action potentials (AP) at supratetanic frequencies. Furthermore, fast-twitch muscles fatigue easily, which may be related to an AP dependent release of K+, limiting excitability. We studied whether muscles containing mostly fast-twitch fibers can improve contractility in response to supratetanic frequency stimulation at normal and elevated [K+].
Method: Dynamic contractions were performed in vitro in rat EDL muscles, stimulated by trains of tetanic (150Hz) or supratetanic (300Hz) frequency, or with trains of tetanic or subtetanic (50Hz) frequency initiated with/without a doublet (300Hz). Force (F), P, V

and RFD were measured during these contractions in buffers K.R. containing 4 or 11 mM K+.
Results: Raising frequency from 150 to 300Hz at 4 mM K+ increased Pmax (15 ± 3 %), RFD (23 ± 3 %) and Vmax (8 ± 1 %) significantly, but at 11 mM K+ only a small significant increase was seen for Vmax (5 ± 2 %). A 300Hz doublet initiating a 150 Hz train did not affect contractile variables at either 4 or 11 mM K+. Compared to a 50 Hz subtetanic train without doublets, the inclusion of an initial doublet in the 50 Hz train significantly increases Vmax (35 ± 3 %), Pmax (62 ± 13 %), Fmax (15 ± 3 %) and RFD (59 ± 7 %) at 4 mM K+. Smaller increases in Vmax (22 ± 2 %), Pmax (23 ± 3 %), Fmax (6 ± 2 %) and RFD (31 ± 4 %) were observed at 11 mM K+.
Conclusion: Contractility was improved by raising stimulation frequency from tetanic to supratetanic in fast-twitch muscles, but not when excitability was reduced by high extracellular [K+]. However, even at high extracellular [K+], contractility was improved when a doublet initiated a subtetanic frequency train, indicating that firing patterns with doublets are useful strategy to activate muscles even during fatigue.

S13.P7-236

Effect of brief high frequency doublet stimulation on tetanic Ca^{2+} responses measured in isolated fast interosseous fibres of the mouse

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Introduction: Skeletal muscle fibres are often exposed to motorneuron double discharges (~ 200 Hz) which enhance initial force production. Recent findings made using standard fluorescent Ca^{2+} indicator techniques suggest that initial 200 Hz doublet action potential stimulation results in a 100% increase in peak tetanic Ca^{2+} compared to control tetanic Ca^{2+} transients (no doublet), and this increased Ca^{2+} may be responsible for the enhanced force production (1). The aim of this study was to re-examine the effects of doublet stimulation on tetanic Ca^{2+} using a fast, low affinity Ca^{2+} indicator (Mag-Fluo-4) recorded with greater time resolution (μs).
Methods: Mice were euthanased and the interosseous muscles removed. Single interosseous fibres (isolated via collagenase digestion) were loaded with Mag-Fluo-4 (5μM), and maintained in Kreb's Ringer, containing the myosin inhibitor BTS (100 μM). Fibres were activated by 10 action potentials at 120 Hz with or without (control) an initial 200 Hz doublet action potential. Ca^{2+} fluorescence was captured at 10 kHz using a Zeiss 5 Live confocal microscope in line-scan mode.
Results: In this study, 200 Hz doublet stimulation did not significantly alter the amplitudes of the Ca^{2+} responses. However, doublet stimulation did increase the minimum fluorescence value between Ca^{2+} transient spikes (MFBCS) by approximately 200% compared to controls (control initial MFBCS: 61.1 ± 2.8% of 6th Ca^{2+} response; doublet: 111.3 ± 13.2% of 6th response, $p < 0.05$).
Conclusion: These results indicate that doublet stimulation rapidly increases the MFBCS in fast twitch muscle fibres, possibly leading to more rapid saturation of cytosolic Ca^{2+} binding sites and therefore, faster initiation of cross-bridge cycling and force production.

References: Cheng et al., Doublet discharge stimulation increases sarcoplasmic reticulum Ca^{2+} release and improves performance during fatiguing contractions in mouse muscle fibres. 2013, J. Physiol. 591:3739–3748.

S13.P8-211

Targeting a voltage-sensitive fluorescence biosensor to the sarcoplasmic reticulum membrane of skeletal muscle fibers

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Skeletal muscle contraction is triggered by a rise in cytosolic Ca^{2+} due to opening of the ryanodine receptors in the sarcoplasmic reticulum (SR) membrane following transverse (t-) tubule membrane depolarization. It is believed that during SR Ca^{2+} release, cationic countercurrent across the SR membrane prevents changes in SR membrane voltage. In order to test for this, we targeted voltage-sensitive FRET biosensors to the SR membrane of muscle fibers. Flexor digitorum brevis and interosseus muscles of mice were electroporated with plasmid constructs encoding voltage-sensitive FRET biosensors of the Mermaid family (Tsutsui et al., 2008, Nat Methods, 5). The biosensors Mermaid and Mermaid2 were fused to a targeting sequence consisting of either the first 306 residues of triadin (T306) or an arginine-rich sequence (ER). After 6–10 days of expression, fluorescence changes of the biosensors were studied in single isolated muscle fibers in response to voltage-clamp pulses. Upon membrane depolarizing and hyperpolarizing pulses from a holding voltage of 0 mV, Mermaid generated transient increase and decrease in the FRET ratio, respectively, consistent with detection of t-tubule voltage changes. However, upon depolarizing pulses from −80 mV a more complex response was observed, suggesting presence of some Mermaid molecules in another membrane compartment experiencing a distinct voltage change. A similar behavior was observed with ER-Mermaid2. In contrast, Mermaid-T306 did not respond to pulses from 0 mV but generated a transient decrease in the FRET ratio upon membrane depolarization from −80 mV. The amplitude of this response was voltage-dependent with a mid-voltage of activation at −30 mV. This response is consistent with hyperpolarization (luminal positive with respect to cytosol) of the SR membrane during Ca^{2+} release.

S13.P9-200

Effects of glycogen-loading on recovery process after muscle fatigue

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Introduction: The aim of present study was to investigate whether extension of exercise time associated with glycogen-loading influence on recovery process after muscle fatigue.
Methods: Male Wistar rats were divided into one of three groups: exercise-glycogen-loading (EX-GLY) group that participated in a

protocol of exercise and was allowed free access to water supplemented with 5% sucrose: exercise-control (EX-CON) group that exercised and was allowed free access to water (no sucrose): sedentary-control (SED-CON) group that did not exercise and was allowed free access to water (no sucrose). Acute nerve stimulation of rat gastrocnemius muscle was carried out via electrodes placed around the sciatic nerve of the left hind limb and the muscles from contralateral legs (right hind limb) were used as controls. Before the fatigue run, maximal tetanic forces were measured at 20 Hz and 100 Hz. The fatigue run was initiated by stimulating muscles at 20 Hz until force was reduced to 50% of the initial. Immediately after (R0), at 30 min (R30) and at 60 min (R60) after the cessation of the fatigue run, maximal tetanic forces were measured again. Control and stimulated gastrocnemius muscle were removed immediately after measures of maximal tetanic forces and the superficial region was subjected to biochemical analyses.

Results: As the result of performing glycogen-loading to rat, the glycogen content was higher ($P < 0.05$) in the EX-GLY rats than in the EX-CON rats and the SED-CON rats. In addition, glycogen-loading resulted in a significant increase ($P < 0.05$) in the fatigue run time. The run time of the EX-GLY rats was 151.5 ± 48.5 s, whereas the EX-CON rats lasted 80.0 ± 27.1 s and the SED-CON rats lasted 86.7 ± 29.6 s, respectively. On the other hands, there were no differences between the groups in any of R0, R30 and R60 both at 20 and 100 Hz.

Conclusion: It was suggested that glycogen-loading has no effect on recovery process after muscle fatigue.

S13.P10-182

Simultaneous recording of calcium transients and force production reveals modulatory effects of nitric oxide in tissue engineered muscle fibers

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Aims: It is known that NO reduces force production in skeletal muscle under physiological conditions. There are several possible mechanisms underlying the action of NO and its modulatory effect on force production, whereas it is still unclear which of these pathways is or are responsible for the observed NO effect. We designed a new strategy using the C2C12 cell line to tissue engineer muscle fibers named Myooids enabling us to overcome common issues of animal research to study NO-effects.

Methods and Results: C2C12 myoblasts were cultured in PDMS-carved wells flanked by two silk sutures in DMEM+10 % FBS until confluency. After 7 days in DMEM+2% HS, Myooid formation was finalized in DMEM+7% FBS as cell-layers detached from the wells, using the sutures as tendons. The Myooids were electrically stimulated (20V, 10ms, 1Hz) for one week. Physiological parameters of EC-Coupling in Myooids were determined via simultaneous recordings of Ca^{2+} transients and force production. Respective recordings were obtained from Myooids loaded with Fluo4-AM (10μM, 90min) by electrical stimulation (20V, 10ms) in a force transducer attached to a fluorescence microscope. Rheobase, chronaxie, force-frequency and fatigue protocols were also recorded. The NO-donor SNAP (100μM) and the unspecific NOS blocker L-NAME (5mM) were used to evaluate the effect of NO on the simultaneous recordings. Myooids were further analyzed via immunoblotting, showing that the main ECC proteins were present and modified after one week of stimulation.

Conclusion: We fully characterized molecular and physiological parameters of EC-Coupling in Myooids by simultaneously recording Ca^{2+} transients and force production under NO-modulation. Our results suggest that NO negatively modulates force production of Myooids via reducing measured biophysical parameters of the calcium transients. The next step is to combine the Myooid platform with the CRISPR/CAS9 technique providing a new model for Duchenne muscular dystrophy.

S13.P11-154

Subcellular glycogen pools are utilized in a fibre type specific manner during acute and repeated high intensity exercise in elite athletes

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Aims: The purpose of the present study was to investigate the utilization of spatially distinct subcellular pools of glycogen in type 1 and 2 fibres during acute and repeated high intensity exercise in elite athletes.

Methods: Ten elite male cross-country skiers (VO2max 5.1 ± 0.5 L·min-1) performed four self-paced roller skiing time trials (TT1–4) (average 3min 49 ± 9s) employing the classic style, with 45 min recovery between sprints. Muscle biopsies were obtained in arm muscle (m. triceps brachii) before and after the first and 4th sprint. Single muscle fibre glycogen utilization was determined by stereological analysis of transmission electron microscopy images. The subcellular glycogen locations was divided into 3 subfractions; (1) intramyofibrillar (Intra; inside the myofibrils); (2) intermyofibrillar (IMF; between myofibrils) and (3) subsarcolemmal (SS; just below the sarcolemma).

Results: Prior to exercise, type 1 fibres contained 24% more Intra glycogen than type 2 fibres, with no fibre type differences in IMF, SS or total glycogen. The first TT mediated a reduction in IMF glycogen (−19%, $P = 0.006$) and SS glycogen (−35%, $P = 0.03$) in both fibre types, but Intra glycogen decreased only in type 1 fibres (−52%, $P = 0.02$). This reduction of Intra glycogen in type 1 fibres was relatively larger than the reductions of the other subfractions ($P = 0.01$). During the TT4 only IMF glycogen was reduced in both fibre types (−31%, $P = 0.002$), demonstrating a preferential depletion of intermyofibrillar glycogen during TT4. Biochemically determined glycogen was reduced by 22 and 24% in Sprint 1 and 4, respectively.

Conclusion: Four min of exercise at a high supramaximal intensity resulted in a marked reduction of Intra glycogen solely in type 1 fibres only, whereas IMF and SS glycogen were depleted to the same extent in both fibre types. Interestingly, this difference disappeared during a subsequent fourth TT, where both fibre types preferentially utilized IMF glycogen

S13.P12-301

Force Potentiation during Repeated Eccentric Contractions in rat EDL muscles

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Aims: We recently observed force potentiation in isolated muscles during repeated eccentric contractions (ECCs). The aim of the present study was to investigate whether this potentiation was associated with a general change in the ability of the contractile apparatus to generate force when tested in isometric contractions.
Methods and Results: Preparations of intact rat extensor digitorum longus muscles were mounted at optimal length in a dynamic force transducer. Muscles were stimulated (380 ms, 80 Hz) three or four times in succession interspaced by 5 s performing ECCs or isometric contractions (ISOs). During four ISOs, peak force declined 5.5 % (95 % CI of mean: 4.8 to 6.1, n = 14) from first to last contraction. During four ECCs with an active stretch of 3 % of muscle length (constant stretch speed), however, peak force increased by 5.0 % (3.5 to 6.5, n = 21) from first to last contraction. The potentiation disappeared within five minutes of recovery without contractions. Further, the force potentiation during consecutive ECCs was not related to the magnitude of twitch potentiation, since this was similar after three ECCs and three ISOs; 39 % (136 to 142, n = 8) and 37 % (132 to 143, n = 4), respectively. Two ECCs just prior to an ISO led to the same small reduction in isometric peak force and isometric rate of force development as three ISOs in succession. Interestingly, two ISOs before an ECC was not capable of potentiating eccentric force in the same way as seen with three ECCs in succession.
Conclusions: These results suggest that the force potentiation seen during ECCs cannot be induced by isometric contractions and neither does the potentiation seem linked to the ability of the contractile apparatus to generate isometric tetanic or twitch force. Thus, the potentiation during repeated ECCs might involve an acute change in the stress-strain response of contractile or non-contractile components requiring an active stretch.

S13.P13-216

Effects of calcium on the force-velocity relationship in isolated rat soleus muscles

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Aim: In dynamically contracting muscles, increased curvature of the force-velocity (FV) curve contributes to the loss of power during fatigue. It has been proposed that this increased curvature is caused by fatigue-induced reduction in tetanic [Ca++]i. However, earlier studies conducted at 13–15°C did not support this hypothesis. Presently, though, it is still uncertain how tetanic [Ca++]i influences the curvature at near-physiological temperatures. We hypothesized that a decreased tetanic [Ca++]i would increase the curvature in isolated skeletal muscle at near-physiological temperatures.
Methods: Freshly isolated rat soleus muscles where incubated in a standard Krebs-Ringer buffer at 30°C. Contraction force and velocity were measured during electrical stimulation at a supramaximal frequency of 60Hz (baseline), and subsequently during interventions that either lowered tetanic [Ca++]i (10 μM dantrolene and submaximal stimulation at 30Hz) or increased [Ca++]i (2 mM caffeine). FV curves were obtained by fitting data on force and shortening velocity at different loads to the Hill equation. The curvature was measured as the ratio a/F0 based on the Hill equation parameters. Increased curvature was reflected in decreased a/F0.
Results: Compared to baseline, lowering tetanic [Ca++]i with dantrolene and submaximal stimulation increased a/F0 by 12 and 13% respectively. However, these interventions reduced maximal power by 51 and 41% respectively due to a decrease in maximal force and velocity. In contrast, increasing tetanic [Ca++]i with caffeine decreased a/F0 with 18%, but increased maximal power with 24% due to increases in maximal force and velocity.
Conclusion: Contrary to our hypothesis, interventions that reduced tetanic [Ca++]i caused a decrease in the curvature of the FV curves, while increasing tetanic [Ca++]i with caffeine increased the curvature. These results reject a simple causal relation between lowered [Ca++]i and an increased curvature during fatigue.

S13.P14-197

The fibre-type dependence of sag during unfused tetanic contractions can be explained by differences in cytosolic inorganic phosphate concentration

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Aims: Contraction-induced reductions in twitch duration impair summation and cause force to decline (sag) during unfused tetanic contractions in fast-twitch, but not slow-twitch skeletal muscle. We sought to determine if the fibre-type dependency of sag relates to the elevated concentrations of cytosolic inorganic phosphate (Pi) seen in slow-twitch relative to fast-twitch muscle.
Methods and Results: Fast-twitch extensor digitorum longus (EDL) muscles and slow-twitch soleus muscles were isolated from 14 female C57BL/6 mice. Each muscle was tested in two solutions applied in random order. One solution contained glucose, creating a standard Pi environment, and the other solution contained pyruvate, creating a low Pi environment. Experiments were performed at 30 degrees Celsius. Peak twitch tension was higher ($P < 0.05$) in pyruvate than glucose in the soleus (Pyruvate: 31.7±4.8 mN vs Glucose: 25.6±2.4 mN), but not the EDL (Pyruvate: 52.6±6.1 mN vs Glucose: 52.3±5.2 mN; $P>0.05$). Twitch contraction times (soleus only) and twitch half relaxation times (soleus and EDL) were larger ($P < 0.05$) in pyruvate than glucose. After 50 successive twitch contractions (5 Hz EDL, 2 Hz soleus), contraction times and half relaxation times were lower ($P < 0.05$) than initial values in all conditions. The magnitude of decrease in half relaxation time was greater ($P < 0.05$) in pyruvate than glucose in both the EDL (Pyruvate: 63.5±2.7% vs Glucose: 52.6±2.0%) and soleus (Pyruvate: 37.9±3.2% vs Glucose: 16.2±4.5%). Similarly, in both muscles, sag was greater ($P < 0.05$) in pyruvate (EDL: 50.5±1.4%, soleus: 37.8±2.7%) than glucose (EDL: 35.0±3.7%, soleus: not apparent) during unfused tetani (30 Hz EDL, 10 Hz soleus).
Conclusions: These experimental observations are in agreement with our modelled data which suggest that sag, including its fibre-type dependency, can be explained by basal differences in cytosolic Pi concentration, and contraction-induced Pi accumulation.

S13.P15-188

Investigation of calcium current properties and leak conductance in mouse muscle fibers expressing the type 1 Hypokalemic Periodic Paralysis V876E mutant calcium channel

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Missense mutations in the gene encoding Cav1.1, the α1S subunit of the L-type Ca^{2+} channel, induce type 1 Hypokalemic Periodic Paralysis (HypoPP1). Most common mutations occur at outer arginine residues in the 4th transmembrane segment of voltage-sensor domains and a current hypothesis suggests that such mutations create a gating pore at negative voltages. A mutation in the 3rd segment in domain III causing HypoPP1 has been however recently identified, but the functional effects of this mutation have never been investigated. In the present study we successfully used electroporation to transfer into mouse muscles cDNAs encoding turboGFP-tagged human wildtype (WT) and V876E HypoPP1 mutant Cav1.1 into mouse muscles. The expression profile of both channels showed a regular striated pattern indicative of their localization in the t-tubule membrane. Measurements of L-type Ca^{2+} currents using the silicone-clamp technique did not show any difference in voltage-dependence and density between V876E and WT fibers. Applying voltage ramps from different holding potentials in the presence of an external low-Cl-, Na+- and K+-free solution revealed a significant higher leak conductance measured between −80 and −120 mV in V876E fibers likely suggesting that the mutation induces a gating pore current at negative voltages. Acidification of the external solution increased the leak inward current to a higher extent and reduced intracellular pH (pHi) measured with the pH indicator BCECF at a higher rate in V876E fibers, suggesting that H+ ions flow through the gating pore. However, in the presence of external Tyrode solution, the rate of change in pHi produced by external acidification was not significantly different in V876E fibers indicating that under physiological conditions the gating pore is able to carry cations other than H+. Measurements of intracellular Na+ are currently performed to determine if Na+ is the main cation flowing through the gating pore in V876E fibers.

S13.P16-180

ER stress-altered ER-mitochondria Calcium transfer and impaired contractile function: Implications in dystrophin decifient skeletal muscle

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Aims: Besides its role in Ca^{2+} homeostasis, the sarco-endoplamic reticulum (SR/ER) is tether to mitochondria and controls protein folding. Under pathophysiological conditions the unfolded protein response (UPR) is associated with disturbance in SR/ER-mitochondria cross talk. In the present study, we investigated whether ER stress altered SR/ER-mitochondria links and Ca^{2+} handling and if its contributes to muscle damage in WT and mdx mice, the murine model of Duchenne Muscular Dystrophy (DMD).
Methods and Results: 8 hours after injection of the ER stress activator in WT mice (Tunicamycin, 1mg/kg, i.p.), the UPR markers (GRP78, IRE1a, ph-Eif2a) measured by western blot were increased. The ER/SR-mitochondria links measured by Proximity Ligation Assay and interaction of the IP3R-GRP75 complex, were significantly decreased in isolated FDB fibers after TM. Ca^{2+} imaging revealed a decrease of cytosolic Ca^{2+} transient, no change of SR Ca^{2+} load and decreased of histamine-induced mitochondrial Ca^{2+} uptake. The force generating capacity of EDL measured ex vivo dropped significantly after TM (−21% vs WT). In mdx mice, we also found higher UPR markers, a lower IP3R-GRP75 interaction and Ca^{2+} flux perturbation. Finally, we used tauroursodeoxycholic acid (TUDCA, 500mg/kg, ip) to inhibit ER stress. 4 weeks treatment tends to reduce SR Ca^{2+} load, with an increase of Ca^{2+} transients in mdx treated fibers and improvement of diaphragm contractility.
Conclusion: Our findings demonstrated that ER stress-altered ER/SR-mitochondria links, disturbed Ca^{2+} handling and muscle function in WT and mdx mice. Thus, ER stress open up a prospect of new therapeutic targets in Duchenne Muscular Dystrophy.

S13.P17-135

Altered **Ca^{2+}** homeostasis in muscle fibers from Mtm1-deficient mice and beneficial effect of pharmacological inhibition of phosphatidylinositol 3-kinase

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Mutations in the gene encoding the phosphoinositide (PtdInsP) phosphatase MTM1 are responsible for myotubular myopathy. Excitation-contraction coupling deficiency is thought to make a major contribution to the associated severe fatal muscle weakness. We previously reported that the voltage-activated Ca^{2+} release flux in Mtm1-KO muscle fibers yields decreased peak amplitude, delayed time to peak and spatial heterogeneity and that exposure of fibers to pan-PtdIns 3-kinase blockers substantially rescues these alterations. Here we further examined several subcellular features of Mtm1-KO fibers. Imaging of the fluorescence from exogenously-expressed GFP- or -mCherry-tagged peptide domains binding to the MTM1 PtdInsP substrates showed no striking distinctive spatial distribution in Mtm1-KO fibers, providing no indication for exclusive accumulation of the substrates in a given subcellular compartment. Confocal imaging in Mtm1-KO fibers of rhod-2 Ca^{2+} transients and t-tubule network with di-8-anepps revealed that locally altered t-tubule pattern correlated with defective Ca^{2+} release but the reciprocal was not systematically true, suggesting that either some t-tubule defects were undetectable or that defective t-tubule structure is not a primary disease mechanism. Confocal imaging of fluo-4 fluorescence in resting fibers revealed that Mtm1-KO fibers numerous spontaneous elementary Ca^{2+} release events, consistent with propensity of ryanodine receptor channels to open through Ca^{2+}-induced Ca^{2+} release, which could contribute to altered Ca^{2+} homeostasis. Treatment of mice with the PtdIns 3-kinase blocker wortmannin had no effect on apparent features and weight of wild type mice but significantly increased life expectancy of diseased animals, providing proof of concept for the use of PtdIns 3-kinase inhibitors in myotubular myopathy and suggesting that unbalanced PtdIns 3-kinase activity plays a critical role in the pathological process. This work was supported by AFM-Téléthon.

S13.18-125

Changes in skeletal muscle contractile properties in rat induced by acute dehydration

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Aims: It has been shown that dehydration, an altered physiologic state mainly encountered in athletes or elderly people, induces structural and enzymatic properties damages on muscle leading to alterations in its mechanical characteristics. The aim of this study was to investigate the effect of acute dehydration in the contractile properties of extensor digitorum longus (EDL) and soleus (SOL) muscles in rat.
Methods and Results: Eighteen male rats were divided in two groups: control (C group, n = 8) and 96 hours water deprivation (WD group, n = 10). Contractile properties of EDL and SOL were assessed in vitro. Twitch (Pt) and tetanus (P0) were obtained at optimal muscle length using respectively single and frequency stimulation via two silver electrodes located in the ringer bath on either side of the isolated muscle. Dehydration leads to a 15% decrease in rat body weight associated with a mean increase in hematocrit of 27%. Whatever the considered muscle, muscle weight (MW) was significantly lower in WD as compared to C group for EDL and SOL (−17 and −11% respectively). However, no modification in muscle weight to body weight ratio was observed in EDL between WD and C groups, while this ratio tends to increase in SOL. For EDL, absolute Pt and P0 were significantly lower in WD group whereas there was no difference between the two groups when normalized with respect to MW. Surprisingly, absolute Pt and P0 in SOL were respectively 43 and 25% higher in WD as compared to C group while relative force were respectively 61 and 43% higher in WD group.
Conclusions: The present study shows for the first time that acute dehydration effects differ between slow and fast twitch muscles. In EDL, the decrease in force may be explained by a decrease in muscle weight and in myofibril content. This result shows firstly that loss in SOL component certainly does not include loss in myofibrils content and secondly, which is more surprising, that contractile machinery is more effective.

S13.P19-105

Predominant cause of prolonged low-frequency force depression during recovery from in-situ fatiguing stimulation

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Aims: In skeletal muscle, after intense contractions, submaximal force production is markedly decreased, which is called prolonged low-frequency force depression (PLFFD). PLFFD seems to stem from decreased sarcoplasmic reticulum (SR) Ca^{2+} release and/or myofibrillar (myo-) Ca^{2+} sensitivity. The aim of this study was to investigate time course changes in the contribution of reduced SR Ca^{2+} release and myo-Ca^{2+} sensitivity to PLFFD during recovery.
Methods and Results: Gastrocnemius muscles from Wistar male rats were electrically stimulated at 70 Hz until force was reduced to 50% of the initial value. At 0 h, 0.5 h, 2 h, 6 h and 12 h of recovery, the muscles were excised and used for both skinned fibre and biochemical experiments. Skinned fibre experiments demonstrated that (i) the ratio of depolarization-induced force to Ca^{2+}-induced maximal force was decreased at 0 h, 0.5 h, 2 h and 6 h, (ii) caffeine threshold, a functional indicator of sarcoplasmic reticulum (SR) Ca^{2+} release channel (referred to as ryanodine receptor [RyR]), was increased at 0.5 h and 2 h, and (iii) a Ca^{2+} concentration at half-maximal force, an indicator of myo-Ca^{2+} sensitivity, was increased at 0 h and 6 h. Western blot with anti-glutathione indicated that S-glutathionylation (S-glut) of troponin I fast isoform (TnIf) was increased at 0 h, 0.5 h and 2 h, whereas it was decreased at 6 h.
Conclusion(s): The present results suggest that decreases in both SR Ca^{2+} release and myo-Ca^{2+} sensitivity lead to PLFFD immediately after fatigue induction and PLFFD is mainly ascribable to decreased SR Ca^{2+} release and reduced myo-Ca^{2+} sensitivity in the early and in the late phase of recovery, respectively.

Session 14 Cardiomyopathy and Heart failure

Oral presentations

S14.O2-302

Titin-based cardiomyocyte stiffening contributes to early adaptive ventricular remodeling after myocardial infarction

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Introduction: Myocardial infarction (MI) increases the wall stress in the viable myocardium and initiates early adaptive remodeling in the left ventricle to maintain sufficient cardiac output. Later remodeling processes include excessive fibrotic reorganization that eventually leads to development of cardiac failure. Understanding the mechanisms that support cardiac function in the early phase post-MI and to identify the processes that initiate transition to maladaptive remodeling is of major clinical interest. In this study we characterized MI-induced changes in titin-based cardiomyocyte stiffness and aimed to elucidate titin's role in ventricular remodeling of remote myocardium in the early phase after MI.
Methods and Results: Titin properties were analyzed in Langendorff-perfused mouse hearts after 20' ischemia/60' reperfusion (I/R) and mouse hearts that underwent ligature of the left anterior descending coronary artery (LAD) for 3 or 10 days. Cardiomyocyte passive tension was significantly increased one hour after I/R as well as 3 and 10 days after LAD-ligature. The increased passive tension was caused by hypophosphorylation of the N2-Bus and hyperphosphorylation of the PEVK-region of titin. Using isolated adult rat cardiomyocytes we further demonstrate that MI-induced titin stiffening could be mediated by elevated levels of the cytokine interleukine-6 (IL-6). In mice inhibition of IL-6 signaling by systemic application of IL-6 antibodies prior to ischemia significantly reduced the MI-induced increase in titin-based stiffness. We further report that remodeling processes starting about 3 days after LAD-ligature accelerate titin turnover by the ubiquitin proteasome system.
Conclusion: We conclude that titin-based cardiomyocyte stiffening improves sarcomeric stability in the remote myocardium and is an important mechanism to adapt to the increased mechanical demands after acute MI-induced myocardial injury.

S14.O3-299/S14.P1-299

Myofilaments changes in incomplete myocardial reverse remodelling after surgical removal of chronic pressure-overload

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Introduction: Incomplete myocardial reverse remodelling is a major determinant for heart failure patient worse outcome. We aim to characterize the myofilamentary changes in an animal model that mimics the structural and functional changes in myocardial reverse remodeling after chronic-pressure-overload relief.
Methods: Pressure-overload was established in 7-weeks-old-C57BL/6-mice by ascending aortic constriction. Seven weeks later a debanding(DEB) surgery was performed in half of the banding(BA) and SHAM group, resulting in BA_DEB and SH_DEB groups. Two weeks later, an echocardiographic, extracellular matrix and cardiomyocyte function analysis was performed.
Results: After debanding, left ventricle hypertrophy and cardiomyocyte hypertrophy remained increased compared to SH_DEB but similar to BA. However, contrarily to BA, BA_DEB presented higher LV cavity with normalization of wall thickness. Diastolic dysfunction normalized in BA_DEB group. However, BA_DEB animals maintained a significant elevation of IVCT indicating some degree of contractile dysfunction despite preserved ejection fraction. In BA_DEB, fibrosis and AGEs deposition normalized. Regarding myofilament function we observed a trend to higher active tension in BA and BA_DEB compared to their controls. Myofilament stiffness, assessed by passive tension-sarcomere length relation, was significantly increased in BA and normalized in BA_DEB. Interestingly, the rate of tension redevelopment was significantly decreased in BA_DEB group.
Conclusion: The relief of LV overload normalized wall thickness and diastolic function but was unable to prevent LV dilation and cardiomyocytes alterations. We showed that this mice model of reversible banding might represent an interesting model to understand the pathways associated to incomplete myocardial reverse remodeling and to LV dilation.

S14.O4-228

Cardiomyopathy-causing mutations Ile92Thr and Val95Ala in TPM1 have opposite effects on tropomyosin functions

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Tropomyosin forms homo- or heterodimeric coiled coils, which together with troponin regulate acto-myosin interactions. Mutations in TPM1 gene – Ile92Thr and Val95Ala, were found in cardiomyopathy patients. In tropomyosin Tpm1.1 both substitutions are located in the core of the coiled coil. It is striking that in spite of the close location, the two substitutions result in opposite phenotypes. While Val95Ala causes hypercontraction, Ile92Thr leads to hypocontraction of heart muscle. Using site-directed mutagenesis we introduced both mutations in human TPM1 gene. The mutant Tpm1.1, wild type Tpm1.1 and Tpm2.2 were expressed in E. coli pET system. Tpm1.1 homodimers with substitutions in both chains and cross-linked heterodimers of Tpm1.1/Tpm2.2 with substitution in one chain were tested in biochemical assays. The wild type Tpm1.1 bound to actin with Ka = 8.6 ± 0.5 μM^{-1}. Ile92Thr decreased Ka to 5.8 ± 0.5 μM^{-1}, but Val95Ala increased Ka to 10,9 ± 0.6 μM^{-1}. The differences in actin binding correlated with a probability of coiled coil formation of the actin-binding period 3 predicted by COILS. While for Val95Ala the probability was not changed, for Ile92Thr it was significantly decreased. The ATPase assay confirmed the phenotypes. The Tpm1.1-Val95Ala increased the activation in the presence of Ca^{2+} and reduced the inhibition in the absence of Ca^{2+}. Tpm1.1-Ile92Thr did not activate the actomyosin ATPase, but the inhibition was similar to the wild type Tpm1.1. Heterodimer formation with Tpm2.2 did not attenuate the effects of the mutations. Preliminary data suggested that the mutations did not change sensitivity of the tropomyosin-troponin complex to the activating Ca^{2+} concentrations. We concluded that the opposite effects of the substitutions on the regulation of contraction stem from changes in the coiled coil, which affect interactions of tropomyosin with actin.

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S14.O5-293

17β-Estradiol-Induced Interaction of Estrogen Receptor α and Human Atrial Essential Myosin Light Chain Modulates Cardiac Contractility

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Introduction and Aims: Chronic increased work demand of the human heart causes ventricular hypertrophy, re-expression of the atrial essential myosin light chain (hALC-1) and improved contractility. Although hALC-1 turned out to be an important positive inotropic regulator of the human heart, little is known about the regulation of hALC-1 expression. Here, we investigated the role of sex and/or sex hormone 17β-Estradiol (E2) on hALC-1 gene expression, the underlying molecular mechanisms, and the impact of this regulatory process on cardiac contractility.
Methods and Results: We showed that E2 attenuated the expression of hALC-1 protein in human atrial tissues of both sexes. E2 induced the nuclear translocation of estrogen receptor alpha (ERα) and hALC-1 in human ventricular AC16 cells, where they cooperatively regulate the transcriptional activity of hALC-1 gene promoter. E2-activated ERα required the estrogen response element (ERE) motif within the hALC-1 gene promoter to reduce its transcriptional activity. This inhibitory effect was significantly more pronounced in the presence of hALC-1. Yeast two hybrid screening of a human heart cDNA library revealed that ERα interacts physically with hALC-1 in the presence of E2. This interaction was confirmed by Co-IP in human atrium. Immunofluorescence analysis with human atrium tissues showed co-localization of ERα and ALC1 in a striated sarcomeric pattern. Further, we showed that chronic E2-incubation of adult mouse cardiomyocytes overexpressing hALC-1 reduced contractility.
Conclusions: Expression of the hALC-1 gene is regulated by E2/ERα, while hALC-1 acts as a co-repressor. The expression level of hALC-1 in cardiomyocytes modulates the contractility of the heart.

Posters

S14.P3-265

An investigation of Ca^{2+} regulation abnormalities underlying hypertrophic cardiomyopathy in cat hearts

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Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats affecting 1 in 6 and it also affects 1 in 500 humans. Feline HCM mirrors the human disease at the physiological, pathological and clinical level. The aim of this project is to determine whether the troponin Ca^{2+} regulation in cats is similar to that identified in humans and to ascertain if the effect of Epigallocatechin 3-gallate (EGCG) in HCM cats is similar to that found in human HCM patients.

In-vitro motility assays were performed with troponin extracted from post-mortem hearts of 7 cats diagnosed with HCM and 5 wild-type (WT) unaffected cats. Two cats showed MyBP-C haploinsufficiency, one had the MYBPC3 R820W mutation; the other cats are being sequenced. All the HCM cats have a higher Ca^{2+}-sensitivity than the WT cats (EC50 of 0.039 ± 0.007 compared to 0.061 ± 0.02) with an average increase of $x1.81\pm0.03$. This is equivalent to that found in synthetic recombinant protein systems with HCM mutations (2.3x with ACTC E99K, 1.7x with TNNT2 K280N) although not in human myectomy samples (same as from donor), probably due to secondary changes. A major phenotype of human HCM is that the relationship between troponin I phosphorylation and Ca^{2+}-sensitivity is uncoupled. This uncoupling is also observed in all the HCM cats examined (P/unP = 1.01 ± 0.03) whereas all the WT cats tested show normal coupling (P/unP = 1.79 ± 0.09). Furthermore, EGCG was tested in one WT cat and three HCM cat heart thin filament systems and was found to completely reverse the uncoupling associated with HCM in all three HCM cats tested whilst the Ca^{2+} desensitizing effect in WT cats is not as significant as that seen in humans (x1.6 increase in cats compared to x2.2 in humans). To conclude, our result further demonstrate that feline HCM is an excellent model for the human disease both at the fundamental cellular level with respect to Ca^{2+} regulation of sarcomeric proteins and at the clinical level in disease expression.

S14.P4-264

Biochemical and physiological studies of cardiac contractility in patients with mitral valvular disease

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Aim of study: To investigate the impact of ischemia reperfusion (IR) upon physiological parameters: heart rate (HR), left ventricle developed pressure (LVDP), and coronary flow (CF) and on apoptosis in rat heart with experimental hypercholesterolemia and to see the effect of Procaine treatment. 18 male rats aged 20 months old divided into 3 groups of 6 animal each: (A) fed on normal chow, (B) fed on chow with 2% cholesterol (lard) for 8 weeks, (C) fed on chow with 2% cholesterol associated with Procaine treatment (I.P.) Procaine 4 mg/kg body). After treatment rats were killed by cervical dislocation and hearts were removed and placed in Langendorff retrograde perfusion system with Krebs Henseleit buffer at 37 C. We used a 30 minutes ischemia followed by 120 minutes reperfusion model. (HR),(CF) and (LVDP) were determined by means of a latex balloon inserted into the left ventricle and connected with a pressure transducer in groups A, B and C.TACS apoptotic laddering kit(R&D) was used to assay heart cells for apoptosis in cholesterol fed rat heart with/without Procaine treatment.

Results: Our data have pointed out that following 30 minutes ischemia, there was a significant decrease in (CF) in group (B) i.e. from 10ml after 20 minutes stabilization period to 3 ml after 120 minutes reperfusion and an increase in (HR) rate. There was a significant decrease in (LVDP) in group (B) and was relatively constant with minor fluctuations in Procaine treated rats of group (C) versus group (A).DNA laddering pattern has been observed in group B cholesterol fed rats and absent in group (C).

Conclusion: Oxidative stress following (IR) in group (B) had a negative impact upon physiological parameters of heart, and on DNA stability. Procaine in treated (Group C) seems to have a protective effect on heart physiological parameters and on DNA stability leading to a decrease in (HR) approaching the values of Controls and increasing in (CF),and in recovery of (LVDP).

S14.P5-258

The impact of ischemia reperfussion upon young and old rat heart physiology and ultrastructure

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Aim of study: To investigate the impact of ischemia reperfusion (IR) upon physiological parameters: heart rate (HR), left ventricle developed pressure (LVDP), and coronary flow (CF) and on apoptosis in rat heart with experimental hypercholesterolemia and to see the effect of Procaine treatment. 18 male rats aged 20 months old divided into 3 groups of 6 animal each: (A) fed on normal chow, (B) fed on chow with 2% cholesterol (lard) for 8 weeks, (C) fed on chow with 2% cholesterol associated with Procaine treatment (I.P.) Procaine 4 mg/kg body). After treatment rats were killed by cervical dislocation and hearts were removed and placed in Langendorff retrograde perfusion system with Krebs Henseleit buffer at 37 C. We used a 30 minutes ischemia followed by 120 minutes reperfusion model. (HR), (CF) and (LVDP) were determined by means of a latex balloon inserted into the left ventricle and connected with a pressure transducer in groups A, B and C.TACS apoptotic laddering kit(R&D) was used to assay heart cells for apoptosis in cholesterol fed rat heart with/without Procaine treatment.

Results: Our data have pointed out that following 30 minutes ischemia, there was a significant decrease in (CF) in group (B) i.e. from 10 ml after 20 minutes stabilization period to 3 ml after 120 minutes reperfusion and an increase in (HR) rate. There was a significant decrease in (LVDP) in group (B) and was relatively constant with minor fluctuations in Procaine treated rats of group (C) versus group (A). DNA laddering pattern has been observed in group B cholesterol fed rats and absent in group (C).

Conclusion: Oxidative stress following (IR) in group (B) had a negative impact upon physiological parameters of heart, and on DNA stability. Procaine in treated (Group C) seems to have a protective effect on heart physiological parameters and on DNA stability leading to a decrease in (HR) approaching the values of Controls and increasing in (CF),and in recovery of (LVDP).

S14.P6-336

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

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Introduction: Duchenne Muscular Dystrophy (DMD) is associated with depressed left ventricular (LV) function. However, its effects on myofilament structure and function are poorly understood. Golden Retriever Muscular Dystrophy (GRMD) is a dog model of DMD observed in human. The objective of this study is to evaluate myofilament structure and function in dogs with spontaneous cardiac failure.
Methods and Results: We have employed synchrotron x-rays diffraction to evaluate myofilament lattice spacing at various sarcomere lengths (SL) on permeabilized LV myocardium. Our results show a negative correlation between SL and lattice spacing in both sub-epicardium (EPI) and sub-endocardium (ENDO) LV layers in control dog hearts. In the ENDO of GRMD hearts this correlation is steeper due to higher lattice spacing at short SL (1.9 m) compared with control hearts. Furthermore, cross-bridge cycling indexed by the kinetics of tension redevelopment (ktr) was faster in ENDO GRMD myofilaments at short SL compared with control hearts.
Discussion and Conclusions: We found a gradient of contractility in control dogs' myocardium that spreads across the LV wall. Interestingly, this gradient is negatively correlated with myofilament lattice spacing. At slack length increased myofilament lattice spacing in GRMD correlates with improved cross-bridge kinetics, which might explain the improved myofilament sensitivity to calcium when compared to CTRL. Consequently, the gradient of contractility across the LV wall is reduced. In conclusion, at short SL, increased lattice spacing together with improved contraction kinetics appear to be the upstream mechanisms that result in GRMD induced myofilament dysfunction.

S14.P7-331

Impact of cGMP-PKG Pathway Modulation on Titin Phosphorylation and Titin-Based Myocardial Passive Stiffness

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Rationale: The crucial contribution of the giant myofilament protein titin to diastolic stiffness and cardiomyocyte passive force (Fpassive) is dependent, in part, on titin isoform composition and phosphorylation. Phosphorylation of titin by cyclic guanosine monophosphate (cGMP)-dependent protein kinase G (PKG) lowers titin-based stiffness, thus mediating a mechanical signaling process that is disturbed in heart failure.
Objective: To elucidate which elements of the nitric oxide (NO) cGMP-PKG signaling network are critical for titin phosphorylation and stiffness in vivo.
Methods and Results: We employed genetic knockout (KO) mouse models deficient for enzymes of the cGMP-PKG pathway, including cardiomyocyte-specific deletion of the guanylyl cyclase (GC)-A receptor and cGMP-dependent PKG (cGKI), as well as global deletion of soluble GC (sGC). We assessed titin phosphorylation by immunoblotting using phosphoserine-specific titin antibodies and by mass spectrometry quantification using stable isotope labeling of amino acids in mixed cultures of heart tissue from either wild-type (WT) or KO mice. The Fpassive of single permeabilized cardiomyocytes was recorded before and after administration of PKG. In all three genetic models, all-titin phosphorylation was reduced compared to WT hearts. The important PKG-dependent phospho-S4080 site within the N2-Bus region of mouse titin was hypophosphorylated in all three KO models. Unexpectedly, mass spectrometry analysis revealed that most class 1 titin phospho-sites within the molecular spring segment, including the Ig-domain regions, were hyperphosphorylated. Only a few sites showed a phosphorylation deficit or remaining unchanged. Particularly in the cGKI model many class 1 phospho-sites were hyperphosphorylated compared to WT hearts (see figure), indicative of the presence of compensatory processes following loss of PKG; indeed, this was associated with upregulation of several kinases that phosphorylate titin and a clear rise in Fpassive in KO vs. WT cardiomyocytes. While administration of PKG lowered Fpassive of WT and KO cardiomyocytes in all models, this effect was more pronounced in the cGKI KO.
Conclusions: Multiple in vivo phosphorylated class I titin phospho-sites were identified within the molecular spring segment, some of which depended on the cGMP-PKG pathway. While cGMP-activated PKG remains an important titin-targeting kinase, many titin phospho-sites may be regulated through a network of protein kinases /phosphatases.

S14.P8-163

β2 adrenergic receptor stimulation after a myocardial infarction prolongs elevated cardiac progenitor cell number in the myocardium

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Aims: Endogenous cardiac progenitor cells (CPC) may participate in cardiac repair after a myocardial infarction (MI). The β2 adrenergic receptor pathway induces proliferation of CPC in vitro. It has not yet been investigated if stimulation of this pathway in vivo after a MI can improve CPC mediated regeneration.

Methods and Results: The expression of β1 and β2 adrenergic receptors on murine CPC in vivo and in vitro was evaluated by flow cytometry. We measured a significant increase in the percentage of CPCs expressing β1 and β2 adrenergic receptors 7 days post-MI. Twenty four hours of low serum and oxygen in vitro significantly increased the percentage of β2 adrenergic receptor expressing CPC. A functional role of β adrenergic receptors on CPC was tested in vitro by the MTT assay. Treatment with epinephrine (1 nM) or Fenoterol (1 μM), a β2 adrenergic receptor agonist, for 48 hours significantly increased CPC viability. This effect was reversed when CPC were treated in parallel with a β2 adrenergic receptor antagonist, ICI 118 551 (0.3 μM). Fenoterol treatment in vivo was then tested in a murine MI model. Fenoterol (0.25 mg/kg/day) was administered in drinking water either 1 week prior to surgery or at the time of surgery and continued until sacrifice at 7 or 21 days post-MI or sham surgery. Immunofluorescence analysis of the heart tissue for proliferation (ki-67), cardiomyocytes surface area (wheat germ agglutinin), and vessel density (isolectin) showed significant changes based on surgery but no effect based on fenoterol treatment. However, while MI induced a significant increase in the percentage of CPC at 7 days, fenoterol treatment alone maintained a significant elevated level of CPC 21 days post-surgery.
Conclusions: Our results suggest that fenoterol treatment may improve cardiac regeneration by prolonging the endogenous CPC response. Functional effects need to be evaluated to confirm the potential of this treatment.

S14.P9-152

The effects of three DCM-causing mutations A223T, F540L and F764L, on the biochemical properties of the human β-cardiac myosin motor domain

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Inherited cardiovascular diseases are serious health problems in all age groups worldwide, and are the leading cause of sudden death in young people. They affect 1 in 500 individuals and approximately 40 percent of such cardiomyopathy-causing mutations are found in the motor protein β-cardiac-myosin. Here we report detailed biochemical characterization of recombinant human β-cardiac myosin motor-domains carrying DCM (Dilated Cardiomyopathy) causing A223T, F540L or F764L. Myosins were generated in mouse C2C12 muscle cells. We compared these mutations with our previous data on the wild-type (WT) β-myosin motor (Nag et al Sci. Adv. 2015, 1;9). This is the first time the effects of these mutations on biochemical kinetics have been examined in the relevant human myosin background. All three mutations result in series of changes in the measured parameters of the affected MyHC motor domain and the alterations from the wild type are more dramatic if actin is not present. In the presence of actin all three mutants have higher affinity for ATP compared to the wild type and their maximum rates of ATP dissociation of S1 from actin are significantly slower. Interestingly the duty ratio is slightly higher in the case of all three mutants which predicts slightly higher force and a small decrease in velocity. The affinity of ADP for acto·S1 is weaker for all mutants and the rate of ADP release is also slower in the case of A223T. The affinities of the mutant motor domains for actin are also different from the wild type. The loss of affinity for the A223T mutant suggest that the cross-bridge can be disrupted more easily by load/force. The much higher affinities for the mutants F764L and F540L show that there are several different ways that lead to a DCM phenotype and we have to investigate each mutation separately to find out the leading mechanisms.

S14.P10-138

The effects of ischemic postconditioning on myocardial function following ischemia reperfusion in rats of different ages with experimental hypercholesterolemia

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Ischemic postconditioning (IPost) could decrease ischemia reperfusion(IR) injury.
Aim: To examine the effect of IPost on myocardial IR injury in young and old rats with experimental hypercholesterolemia(Hychol) on: infarct size limiting, incidence of ventricular fibrillation(VF), heart rate(HR) and on left ventricle develop pressure (LVDP).
Material and method: 24 male rats aged 6 and 37 months old divided in 4 groups of 6 rats each were fed on normal chow: A (young) and B (old) or fed on chow enriched with 2% cholesterol(lard), (young) and D (old) for 8 weeks. At the end of 8-weeks diet, serum cholesterol (Chol), triglyceride (Tg) and myocardial free fatty acid levels were measured from all groups to confirm (Hychol).After diethyl ether anesthesia the hearts were removed and perfused in Langendorff apparatus with Krebs-Henseleit buffer at 37C, subjected to 30 minutes of regional ischemia induced by coronary occlusion followed by 120 min reperfusion. Heart rate (HR) and incidence of reperfusion-induced ventricular fibrillation (VF) were monitored by ECG. IPost was induced by 5×10 cycles of 10 s coronary occlusion and 10 s reperfusion immediately at the onset of reperfusion. At the end of 120 min reperfusion, infarct size was determined by Triphenyltetrazolium chloride staining and evaluated by planimetry.
Results: IPost was effective in group(A) significantly reducing infarct size, the incidence of VF, (HR) and improved LVDP, in comparison with old rats(B). In (C)and (D), serum (Chol), (Tg) and tissue dihomo-γ-linolenic acid were significantly increased. IPost was ineffective in groups (C)and (D)in reducing infarct size, incidence of VF and (HR) and improving LVDP.
Conclusion: IPost cycles following reperfusion does not provide cardioprotection against IR injury in rats with experimental (Hychol). These data may have a clinical relevance for therapeutic protection of ischemic heart in the presence of risk factors.

S14.P11-136

Biophysical and biochemical studies of rat heart hypertrophy experimentally induced with Isoproterenol

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Aim: To study cardiac hypertrophy in male rat heart with Isoproterenol (Iso) treatment by investigating: water state and content in hypertrophied myocardium with 1H NMR method, hypertrophy redox potential, as well as apoptosis in left ventricle.

Material and method: We used 16 adult male rats, divided into two groups of 8 rats each:A Control and B treated with Iso (0.5mg/kg for 3 weeks. 10 days after the last dose the rats were sacrificed and integral myocardium to body weight; left ventricle to body weight; left ventricle to integral myocardium ratios were calculated in order to evaluate myocardial hypertrophy. Samples of integral myocardium and left ventricle were analyzed on 1H NMR Aremi Spectrometer (0.6T proton resonance at 25MHz). The estimation of T2s (bound water) and T2l (free water) proton transverse relaxation times, was done by Carr-Purcell Meiboom Gill pulse sequence. Hypertrophy redox potential was evaluated by assessing thiol groups (SH), glutation S-transferase (GST) and gamma-glutamyl transferase (GGT) from left ventricle using standard biochemical techniques. Left ventricle myocytes have been assayed for apoptosis with TACS DNA laddering kit(R&D Systems).
Results: Myocardial hypertrophy was associated with a decrease of T2l, accounting for an increase in dry mass protein content, while the total tissue water was not significantly modified. The decrease in T2s is proportional with level of left ventricle hypertrophy. There was a significant increase in GGT and Thiol groups in ISo group versus control and a decrease in GST; synthesis and utilization of GST for annihilation of free radicals was limited in ISo group. Myocyte apoptosis was pointed out by DNA fragmentation on gel electrophoresis in ISo group.
Conclusion: Protection of myocardium against oxidative stress is significantly depressed in ISo group. Our 1H NMR data were much sensitive for differential diagnosis of myocardial hypertrophy in the case of left ventricle.

S14.P12-97

Cardioprotection following injury heart failure afforded by a non-enzymatic oxygenated metabolite of omega 3 fatty acid involves Ryanodine receptor mechanism and mitochondrial function

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Introduction: cardioprotective effects of long-chain polyunsaturated fatty acids of the n-3 series (PUFAs) have been demonstrated and represent a novel approach to prevent myocardial infarctions or its consequences. Due to the abundance of double bonds, the main n-3 PUFAs; docosahexaenoic acid (C22: 6 n-3, DHA) are very sensitive to free radical oxidation and can undergo non-enzymatic spontaneous peroxidation under oxidative stress conditions as it occurs in ischemia/reperfusion. In this context, a lot of oxygenated metabolites of PUFAs like neuroprostanes (NeuroPs) are produced and used as oxidative stress biomarkers but their activities were not determined.
Materials and methods: we investigated (cardiac functions, infarct size and arrhythmias) if the pericardial delivery of NeuroPs, protects the myocardium from ischemic damages during and following an ischemia/reperfusion (IR) episode in rats.
Results: our results suggest that NeuroPs afford some cardioprotective effect during or after myocardial infarction. Compared with controls, NeuroPs-treated animals have significantly decreased infarct size (−28%) determined at the end of reperfusion and reduced ventricular arrhythmia score during reperfusion (−38%). Mechanistically, NeuroPs prevents diastolic calcium disturbances by stabilizing the FKBP12.6/ RyR2 complex (Roy et al., 2015), which can explain arrhythmias prevention during IR. Also, our results demonstrated an increase of mitochondrial membrane potential ($\Delta\Psi m$) by the application of NeuroPs. This effect was not due to an augmentation of mitochondrial respiratory chain activity but involves a diminution of the protons leak. Swelling in response to Ca^{2+} was prevented by NeuroP, indicating a decrease MPTP opening, which can be explain prevention of cell death during IR.
Conclusion: These results suggest a novel pharmacological pathway of n-3 PUFAs and suggest that their well-known cardioprotective effects are mediated by their oxygenated metabolites such as NeuroPs.

S14.P13-241

KBTBD13: a novel gene implicated in cardiomyopathy

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Aim: Patients with nemaline myopathy caused by mutations in KBTBD13 have weakness and slowness of skeletal muscles. KBTBD13 is also expressed in cardiac muscle, but a cardiac phenotype has not yet been reported. Recently, a member of a large family with a KBTBD13 mutation visited our cardiogenetic outpatient clinic because of dilated cardiomyopathy (DCM). Hence, we aimed to elucidate the consequences of KBTBD13-mutations on cardiac function.
Methods and Results: A pedigree was constructed and medical reports on cardiac characteristics were collected. Cardiological evaluation (ECG, echocardiography, ultrasound and MRI) and DNA-diagnostics of the Dutch founder mutation in KBTBD13 (c.1222C>T, p.Arg408Cys) were offered to the DCM-patient and her relatives (family 1). Next, we collected cardiac characteristics of three newly identified families with this particular mutation (family 2–4). To investigate the role of Kbtbd13 on the contractile function of the heart, we engineered a Kbtbd13-knockout mouse model and performed echocardiography at rest and during stress (i.e. administration of dobutamine) in nine month old mice. In family 1, cardiological evaluation revealed (mild) left ventricular dysfunction or DCM in four members, and pedigree analysis revealed three cases of sudden cardiac death. Strikingly, in family 2–4 at least one individual was identified with reduced left ventricular function. Functional evaluation of Kbtbd13-knockout mice by echocardiography revealed no changes in the heart rate and fractional shortening at baseline, however upon dobutamine administration Kbtbd13-knockout mice had a blunted increase in heart rate (P-interaction < 0.01) and fractional shortening (P-interaction < 0.05). No changes in diastolic parameters (E/A ratio, E/E' ratio) were observed.

Conclusion: Cardiological evaluation is recommended for patients that harbor a KBTBD13-mutation. Our data suggest that Kbtbd13-deficiency results in a mild systolic phenotype.

S14.P14-195

Development of a model of diastolic heart failure with preserved ejection fraction in the rat: functional validation in vivo

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Heart Failure (HF) has been associated for a long time with impaired systolic function of the left ventricle (LV) and reduced Ejection Fraction (EF). Recently, a sub-type of HF, characterized by diastolic dysfunction and preserved ejection fraction (HFpEF), has been identified (Bhatia RS et al.). The pathophysiology of HFpEF is unclear and there is no treatment (Owan TE et al.). Animal models must be used to investigate the cellular and molecular mechanisms, but this is a critical issue since the proposed animal models are not ideally suitable for drug testing. In this work, 20 adult male rats underwent an abdominal aortic banding (AAB) to induce a pressure overload and were compared to a control group (SHAM). Cardiac function was evaluated by echocardiography followed by a measurement of intra-ventricular pressure four weeks post-surgery. Several parameters were assessed: the ejection fraction (EF), fractional shortening (FS), early mitral inflow wave velocity peak (E), atrial contraction inflow wave velocity peak (A), isovolumic relaxation time (IRVT), and the diameter of the left atrium (LA). After four weeks, the AAB group showed a decreased EF, but without reaching the threshold of impaired systolic function (EF> 55%). Furthermore, animals had diastolic dysfunction characterized by a decrease in the ratio of diastolic filling velocity E / A (E: peak of passive filling velocity / A: peak of active atrial filling velocity), associated with an increased IVRT and a significant enlargement of the LA. This filling impairment was supported by a significant increase in diastolic intra-ventricular pressure, evaluated in vivo by inserting a microtip catheter with pressure transducer into the LV. In conclusion, AAB rats exhibited functional characteristics of HFpEF as observed clinically in humans. Our animal model provides a reliable experimental approach for studying cellular and molecular mechanisms involved in the pathogenesis of HFpEF.

S14.P15-173

Nanomechanical phenotypes in familial hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM), the most common cause of sudden cardiac death in the young, is a disease of the heart that causes the left ventricle to thicken. This anatomical feature is accompanied by hypercontractility and impaired relaxation during diastole. HCM is caused by mutations in sarcomeric genes that code for structural proteins with mechanical roles. However, the molecular mechanisms leading from mutations in sarcomeric genes to development of HCM are not well understood, which limits both the interpretation of genetic testing for diagnosis and the development of therapies. We are exploring the connection between dysregulation of protein mechanics and development of HCM. Using single-molecule atomic force microscopy, we measure the mechanical properties of mutant polypeptides that cause HCM. We have obtained preliminary evidence that mutations induce nanomechanical phenotypes in the proteins without perturbing their thermodynamical stability. To get a broader understanding on the molecular mechanisms inducing disease phenotypes, we are planning to examine several other parameters (e.g. myocardial RNA and protein levels) that may be altered by the mutations. For these determinations, we will analyze heart tissue from affected patients and/or animal models of HCM.

A

A Linke W	134, (S14.P-331)
Aagaard P	66, (S07.O-194)
Abdelwahab A	131, (S14.O-293)
Abe M	114, (S12.P-100)
Acimovic I	43, (S04.O-335)
Adami R	21, (S01.P-224)
Adolfo L	120, (S13.P-310)
Ahmadi Rastegar D	85, (S09.PY-284)
Ahmadi-noorbakhsh S	51, (S05.P-203)
Aimond F	65, (S07.O-172)
Aimond F	43, (S04.P-210)
Ainara V	120, (S13.P-310)
Ait Mou Y	133, (S14.P-336)
Aitziber I	120, (S13.P-310)
Akkad H	102, (S11.P-117)
Akkad H	102, (S11.P-119)
Al Kitani M	68, (S07.P-101)
Alain Lacampagne	98, (S11.OY-128)
Alegre-cebollada J	138, (S14.PY-173)
Alimpieva O	92, (S10.P-217)
Allard B	127, (S13.PY-135)
Allard B	120, (S13.O-165)
Allard B	126, (S13.PY-188)
Allard B	123, (S13.P-211)
Altaeva E	17, (S01.PY-187)
Altaeva E	20, (S01.P-291)
Amrutkar M	37, (S03.O-147)
Amrutkar M	40, (S03.PY-149)
Andersen E	70, (S08.OY-122)
Andersen I	68, (S07.P-110)
Andersen O	125, (S13.PY-301)
Andersson E	124, (S13.P-154)

A	
Andre G	66, (S07.OY-155)
Anegon I	76, (S09.O-295)
Angebault-prouteau C	45, (S04.PY-162)
Aoki Y	81, (S09.P-240)
Arbogast S	87, (S09.PY-160)
Arkan T	24, (S01.P-102)
Arora H	72, (S08.P-281)
Artemova N	31, (S02.P-215)
Assense A	23, (S01.P-209)
Astell C	51, (S05.P-203)
Aszer M	53, (S05.P-144)
Audebert S	57, (S06.O-234)
Avignon A	109, (S12.P-208)
Ayachi M	50, (S05.P-250)
Ayoub B	67, (S07.P-124)

B	
Baati N	113, (S12.P-146)
Bachtarzi H	75, (S09.O-142)
Bäckhed F	37, (S03.O-147)
Bäckhed F	40, (S03.PY-149)
Baddeley D	42, (S04.O-141)
Bækgaard Nielsen O	125, (S13.PY-301)
Bakker A	51, (S05.P-203)
Bakker A	122, (S13.P-236)
Bakker A	100, (S11.P-315)
Bakker A	100, (S11.P-315)
Bakker A	99, (S11.P-328)
Bang M	56, (S06.O-255)
Barclay C	122, (S13.P-236)
Barman T	90, (S10.O-176)
Barrès R	70, (S08.OY-122)
Barton E	77, (S09.P-327)
Basse A	115, (S12.PY-118)
Bastide B	22, (S01.P-220)
Bastide B	57, (S06.O-234)
Bastin J	106, (S12.O-232)
Basualto-alarcón C	111, (S12.P-198)
Battault S	67, (S07.OY-332)
Baudry C	49, (S05.P-289)
Baudry C	48, (S05.P-290)
Bausch A	47, (S05.O-179)
Bayraktar F	24, (S01.P-102)
Beedle A	58, (S06.O-334)
Beggs A	83, (S09.P-150)
Beishuizen A	38, (S03.OY-120)
Beishuizen A	99, (S11.OY-270)
Belayew A	76, (S09.OY-226)
Bellissimo C	126, (S13.PY-197)

B	
Belloum Y	21, (S01.P-246)
Belova S	26, (S01.PY-185)
Belova S	17, (S01.PY-187)
Belova S	20, (S01.P-291)
Ben Amara I	109, (S12.P-227)
Ben Saad H	109, (S12.P-227)
Ben Slama H	112, (S12.P-164)
Ben Slama H	109, (S12.P-227)
Benahmed M	80, (S09.P-272)
Benedikt Kessler B	47, (S05.O-247)
Bengtsson E	28, (S02.O-171)
Benhassine T	80, (S09.P-272)
Benoist J	106, (S12.O-232)
Benoit H	23, (S01.P-209)
Bensamoun S	64, (S06.PY-153)
Bergamelli L	119, (S13.O-204)
Bergquist J	102, (S11.P-117)
Bergström Lind	103, (S11.P-113)
Bernheim L	50, (S05.P-244)
Bershitsky S	33, (S02.PY-214)
Bershitsky S	31, (S02.P-215)
Bershitsky S	92, (S10.P-217)
Bershitsky S	29, (S02.OY-219)
Bershitsky S	131, (S14.P-296)
Berthier C	127, (S13.PY-135)
Berthier C	120, (S13.O-165)
Berthier C	126, (S13.PY-188)
Berthier C	123, (S13.P-211)
Besnard S	22, (S01.P-220)
Best H	105, (S12.OY-225)
Best H	85, (S09.PY-284)
Bevilacqua J	111, (S12.P-198)
Bevilacqua J	86, (S09.PY-248)
Bezrouk A	72, (S08.P-174)
Bianco P	60, (S06.P-266)
Bideaux P	136, (S14.P-97)
Bideaux P	134, (S14.P-163)
Bideaux P	137, (S14.PY-195)
Bigot A	63, (S06.P-130)
Bildyug N	63, (S06.PY-166)
Biliana L	79, (S09.P-313)
Billat V	50, (S05.P-250)
Birjandi A	49, (S05.P-280)
Birnbaumer L	97, (S11.OY-116)
Bisbal C	24, (S01.P-129)
Blaauw B	91, (S10.P-329)
Blanchet E	73, (S08.P-143)
Blaquière M	67, (S07.P-124)
Blaquière M	16, (S01.O-151)

B	
Blaszczyk M	55, (S05.PY-126)
Blaszczyk M	74, (S08.PY-127)
Blaszczyk M	74, (S08.PY-140)
Blondelle J	36, (S03.O-317)
Blondelle J	38, (S03.P-326)
Bo Nielsen J	84, (S09.P-111)
Boersting C	84, (S09.P-111)
Bogdanis G	20, (S01.P-311)
Boissière J	116, (S12.PY-103)
Boknik P	59, (S06.P-325)
Bolt S	133, (S14.P-336)
Boncompagni S	111, (S12.P-192)
Boncompagni S	56, (S06.O-255)
Boncompagni S	118, (S13.OY-303)
Bonhomme C	48, (S05.P-290)
Bonne G	63, (S06.P-130)
Bönnemann C	105, (S12.OY-225)
Bonnieu A	113, (S12.P-146)
Borén J	37, (S03.O-147)
Borén J	40, (S03.PY-149)
Boris D	93, (S10.P-157)
Borovikov Y	93, (S10.P-157)
Bøtker H	112, (S12.P-170)
Bottai D	21, (S01.P-224)
Bottinelli R	38, (S03.OY-120)
Bottinelli R	21, (S01.P-224)
Bottinelli R	19, (S01.P-316)
Boudet A	54, (S05.PY-267)
Boufroura F	106, (S12.O-232)
Bouilloux F	41, (S04.O-133)
Boulberdaa M	72, (S08.P-281)
Boulgobhra D	67, (S07.OY-332)
Bourlier V	70, (S08.OY-122)
Bouvard C	123, (S13.P-211)
Bozán M	111, (S12.P-198)
Braza F	66, (S07.OY-155)
Brenner B	44, (S04.P-114)
Britto F	21, (S01.P-246)
Brocca L	19, (S01.P-316)
Broholm R	68, (S07.P-110)
Bronia A	83, (S09.P-158)
Bronia A	87, (S09.PY-160)
Bronisz I	82, (S09.P-169)
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