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ABSTRACTS

Abstracts of the 41st European Muscle Conference

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The following abstracts were submitted for consideration in response to the call for abstracts to the three general thematics of the 41st EMC,

- Structure function (track 1)
- Systems biology & epigenetics (track 2)
- Clinical & technological transfer (track 3)

covering all aspects of muscle research with an emphasis given to translational research. Also, the conference programme is enriched by the participation of the European Research Networks "MUZIC" and "MYOAGE" which organise two respective symposia. Final programming was underway at the time this went to print by the 41st EMC's International Scientific Board (ISB) and the organizers (visit www.emc2012.gr). The abstracts were reviewed by an independent Abstracts Committee, members or the ISB and the organizers, who also edited the abstracts.

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We thank all contributors for their hard work! CK & GKS

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In old rats, cardiac frequency exhibited higher values than in control. Coronary flow was variable in time in aging rats in comparison with young ones where a slow decrease was observed during the experiment. Pressure developed by the left ventricle was net elevated in aging rats but with fluctuations in time. No internucleosomal fragmentation and DNA laddering displaying has been observed for this experimental model. Our electron microscopy studies have pointed out greater damage in old hearts in terms of myofiber tears, mitochondrial and sarcoplasmic reticulum disruption.

Our data have pointed out that aging rat heart has an increased reactivity towards stress conditions imposed by ischemia–reperfusion.

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Insights for the release of phosphate by myosin motors

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Force production in myosin motors is tightly coupled to transitions promoted by actin binding that allow release of phosphate followed by release of ADP. While the release of phosphate is the rate limiting step for conventional muscle myosin II, it is much faster for several unconventional myosins. Coupling structural and kinetic studies for different myosins has shown that they populate similar structural states. These studies have elucidated how certain transitions of the actin-myosin catalytic cycle, including the ATP-induced dissociation of myosin from actin and for the ATP hydrolysis step that allows priming of the lever arm. Not yet visualized however are the structural rearrangements triggered by actin binding that are coupled to force generation and product release.

While phosphate is produced in the pre-powerstroke (ADP.Pi) state, it is also trapped in this state until an actin-activated transition opens a back-door to promote its release from the active site. It is unclear, however, which of the three elements of the active site (Switch I, Switch II and the P-loop) play a role in this transition and whether the major cleft within the motor partially closes to allow this release while keeping the lever arm primed.

We have crystallized a new structural state for myosin VI in the presence of MgADP and phosphate that has an open back-door due to a Switch II rearrangement and a lever arm primed. Thus it has the expected features of the previously unseen Pi release state of myosin. Kinetic and structural studies on a number of mutants from different myosins are currently being performed to evaluate this possibility. The structure is consistent with cleft closure occurring subsequent to Pi release, which is also being tested. An interesting finding in this new structure is that the SH1 helix kinks to allow the converter to stay in the primed position, the signature of a state at the beginning of force production.

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Structural properties of actin-myosin complexes in contracting rabbit muscle fibres subjected to stretch or producing mechanical work

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Low angle time-resolved x-ray diffraction (beamline ID02, ESRF, Grenoble, France) was used to study structural properties of cross-bridges in permeabilized fibres of rabbit skeletal muscle at full activation at near physiological temperature of 31–34 °C.

Bundles of 2–3 fibres were stretched at a constant velocity of 1–1.2 length/s or allowed to shorten at a load of ca. $P_0/3$ or $2P_0/3$ where P_0 is isometric tension. Two 10–30 ms long x-ray frames were taken in each run of a protocol: during isometric contraction and at the end of the ramp stretch or shortening. The two 2D x-ray diffraction patterns were compared. Changes in fibre stiffness were measures in control experiments.

Stretch induced a decrease in the meridional M3 intensity and in the off-meridional intensities of M1, M2 myosin and A1, A2 actin layer lines and to a $\sim$30 % increase in stiffness. The data indicate that stretch unlocks stereo-specifically bound myosin heads and promotes non-stereo-specific binding to actin of detached myosin explaining high stiffness and low ATPase rate of contracting muscle during stretch. Shortening decreases stiffness, the intensities of M3, actin layer lines and of the AM_{+1} actin-myosin layer line at $\sim$10.3 nm. The intensity decrease was very small during slow shortening and more pronounced under load below $0.4P_0$.

The data show that three processes: 'roll and lock' transition of actin-myosin complexes, tilt of the myosin lever arm and the rate of detachment of strongly bound myosin heads have different strain-dependence.

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Modified EC coupling in myostatin deficient ($MSTN^{-/-}$) mice

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Myostatin, a member of the transforming growth factor β superfamily has emerged as a potent negative regulator of skeletal muscle growth. During embryogenesis, myostatin influences the differentiation and proliferation of myoblasts. $MSTN^{-/-}$ mice have increased muscle mass demonstrating that myostatin is a negative regulator of skeletal muscle development. Excessive muscle mass in $MSTN^{-/-}$ mice is associated with a profound loss of oxidative metabolic properties.

In our studies we compared $MSTN^{-/-}$ and control C57/BL6 mice. We investigated the muscle performance during moderate intensity voluntary wheel running and in grip tests. We measured force and fatigue of *musculus soleus* (SOL) and *extensor digitorum longus* (EDL). Isometric force measurements were performed during repeated activation–relaxation cycles. pCa-force relations were determined to assess the Ca^{2+}-sensitivity of force production. Changes in intracellular Ca^{2+} concentration were measured in single fibers.

In voluntary wheel running $MSTN^{-/-}$ mice performed better on average (10.8 ± 0.8 versus 7.9 ± 1.0 m/min), in maximal speed (25.4 ± 1.3 versus 16.8 ± 1.3 m/min), and in total distance (7.50 ± 0.88 versus 5.57 ± 0.53 km/day), but they spent less time in the wheel (585 ± 59 versus 622 ± 40 min/day). In grip tests $MSTN^{-/-}$ mice showed higher average force (0.24 ± 0.01 versus 0.13 ± 0.01 mN). Single twitches in EDL (102.8 ± 30.0 vs 84.5 ± 43.9 mN) and SOL (138.3 ± 45.7 vs 49.1 ± 24.8 mN) were

stronger in the control strain. pCa-force relations and calcium-transients evoked by KCl depolarization did not differ. Resting intracellular calcium concentrations were significantly elevated in $MSTN^{-/-}$ mice (464 ± 159 versus 573 ± 123 nM).

Our results from in vitro experiments do not explain our in vivo findings.

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Altered cross bridge kinetics in skeletal myofibrils from neb ΔEX55, a novel mouse model of nebulin-based nemaline myopathy

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Nemaline Myopathy is the most common non-dystrophic congenital myopathy, clinically characterized by muscle weakness. The disease is most often associated with mutations in the nebulin gene and the nebulin-based disease is referred to as NEM2. Recent work on skinned muscle fibres from NEM2 patients revealed remarkable phenotypic similarities to fibres from nebulin KO mice (Ottenheijm et al. Frontiers in Physiology, 2012). Here we investigated mechanics and kinetics of single myofibrils from a novel NEM2 mouse model (NEB Δex55) that mimics a deletion in the nebulin gene found in a large group of NEM2 patients.

We used rapid solution switching (Tesi et al., Biophys. J., 2002) to compare maximal tension and kinetics of contraction and relaxation of myofibrils isolated from frozen skeletal muscles (tibialis cranialis of neonatal mice, ~7 days) of WT and NEB Δex55 mice. Myofibrils, mounted in a force recording apparatus (15 °C), were maximally Ca^{2+}-activated (pCa 4.5) and fully relaxed (pCa 9.0).

Maximal isometric tension was markedly reduced in NEB Δex55 mouse myofibrils (49.7 ± 10.6 mN mm^{-2} $n = 11$) compared to WT (135.3 ± 16.9 mN mm^{-2} $n = 9$). The rate constant of active tension generation following maximal Ca^{2+} activation (k_{ACT}) was significantly reduced in myofibrils from the NEB Δex55 mouse model (1.46 ± 0.07 s^{-1}) compared to WT (2.75 ± 0.27 s^{-1}). Force relaxation kinetics upon Ca^{2+} removal were remarkably faster in NEB Δex55 mouse myofibrils than in WT, evidence that the apparent rate with which cross-bridges leave the force generating states is accelerated in the NEB Δex55sarcomeres.

Reduction in the rate with which cross-bridges enter the force generating state and increase in the rate of cross bridge dissociation can markedly contribute to reducing maximal tension and are expected to increase the energetic cost of tension generation of the NEB Δex55sarcomeres. The results suggest that nebulin plays a significant role in contraction regulation and that altered cross bridge kinetics contribute to NEM2 pathogenesis.

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p42/44 MAPK signaling mediates MYPT1 isoform expression

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During NO mediated vasodilatation PKG activates myosin light chain (MLC) phosphatase, which dephosphorylates the MLC to produce smooth muscle relaxation. MLC phosphatase is a trimeric enzyme consisting of a catalytic, myosin targeting (MYPT1) and 20 kDa subunits. Alternative mRNA splicing produces four MYPT1 isoforms, which differ by the presence or absence of central insert (CI) and a carboxy-terminal luecine zipper (LZ). PKG mediated activation of MLC phosphatase requires the expression of a LZ+ MYPT1 isoform, and thus, the sensitivity to NO mediated vasodilatation is mediated, in part, by LZ+/LZ- MYPT1 expression. We have reported that during heart failure, the increase in AngII is associated with an activation of p42/44 MAPK signaling and a decrease in LZ+ MYPT1 expression.

The mechanism for angiotensin induced regulation of LZ+ MYPT1 expression was assessed in cultured vascular smooth muscle cells (SMCs). For these experiments, LZ+ MYPT1 expression was determined in cultured SMCs at 24 h, 48 h and 72 h after Ang II treatment (1 μM) and compared to the control of time in culture alone. To investigate if the activation of p42/44 MAPK mediates the AngII induced decrease in LZ+ MYPT1 expression, cultured SMCs were stimulated with AngII in SMCs pretreated with a MEK inhibitor (20 μM PD98059), and the time course of p42/44 MAPK activation as well as LZ+ MYPT1 expression was determined.

AngII resulted in a time dependent activation of p42/44 MAPK. Compared to control SMCs, SMCs treated with AngII had a significant decrease in LZ+ MYPT1 expression. However pretreatment of the SMCs with a MEK inhibitor (PD98059) blocked the AngII induced activation of p42/44 MAPK and decrease of LZ+ MYPT1 expression.

These results demonstrate that LZ+ MYPT1 expression is regulated by a p42/44 MAPK signaling pathway. During heart failure, treatment with either ACE inhibitors or AngII receptor blockers will prevent the activation of p42/44 MAPK and the resulting decrease in LZ+ MYPT1 expression to preserve the sensitivity of vascular smooth muscle to NO mediated vasodilatation.

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The super relaxed state in diverse muscle types

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Recently, we observed a new state of skeletal myosin, the super relaxed state (SRX), with a very slow ATP turnover rate. Inhibition of the myosin ATPase activity in the SRX was suggested to be caused by the binding of the myosin head to the core of the thick filament in a structural motif identified earlier by electron microscopy.

The SRX also occurs in cardiac muscle. In both skeletal and cardiac muscle the SRX appears to play a similar role in relaxed cells, providing a state with a very low metabolic rate. However, in active muscle the properties of the SRX differ dramatically. We observed a rapid transition of myosin heads out of the SRX in active skeletal fibers, while the population of the SRX remained constant in active cardiac cells. This property allows the SRX to play a very different role in cardiac muscle, regulating the active force generated by cross-bridges and implying that at the level of the actomyosin interaction, cardiac muscle is regulated at both the thick- and thin-filament levels.

We have recently extended our approach to tarantula leg muscle. We find a SRX, with a very slow nucleotide turnover time constant, ~30 min. Similar to cardiac muscle, myosin in tarantula fibers can remain in the SRX upon activation, suggesting a role in the regulation of active force. The fact that two such evolutionarily distant muscles, vertebrate cardiac and arachnid exoskeletal, both display thick-filament regulation of active force involving the SRX suggests this mechanism of regulation can be anticipated to be widely distributed among muscle types.