in physical activity, diet, and other factors. We examined myofilaments' contractile characteristics and physical performance, walking speed and climbing rate, in African green vervet monkeys, housed in social groups in large indoor-outdoor enclosures and fed the same diet. Physical performance and skinned vastus lateralis (VL) muscle fiber function were investigated in four young (11 ± 1 yrs) and four old (23 ± 1 yrs) monkeys. Fiber myosin heavy chain (MHC) isoform was determined by gel electrophoresis. The old monkeys walked slower (19%) and climbed less (63%) than young monkeys ($p<0.05$). Myofiber cross sectional area (CSA) was 22% and 12% smaller for IIa and hybrid MHC, respectively, in old compared to young monkeys ($p<0.001$). Specific force (maximal Ca^{2+}-activated force normalized to fiber CSA) was 15% and 11% less for type IIa and hybrid fiber, respectively, in old compared to young monkeys ($p<0.05$). Fiber atrophy does not account for the loss in force with aging; it declined much faster than fiber CSA. Although we observed no difference in shortening velocity, the maximal power output substantially decreased in 21% of type IIa and 22% of hybrid fibers with aging ($p<0.05$). Regression modeling used to identify factors contributing to lower fiber force revealed that age is the strongest predictor ($r^2=0.31$, $p<0.001$). The diminished contractile properties measured *in vitro* correlates strongly with age-dependent decline in physical performance (waking speed: $r = 0.41$, $p < 0.001$); climbing rate: $r = 0.29$, $p < 0.001$). Our results support a detrimental effect of aging on the innate force and power generation of myofilament lattice and physical performance.

730-Pos Board B516

Slow Myosin ATP Turnover in the Super-Relaxed State in Tarantula Muscle

Edward Pate[1], Nariman Naber[2], Roger Cooke[2].
[1]WSU, Pullman, WA, USA, [2]UCSF, San Francisco, CA, USA.

We measured the nucleotide turnover activity of myosin in tarantula leg-muscle fibers by observing single turnovers of the fluorescent nucleotide analog, mantATP, as monitored by the decrease in fluorescence when mantATP is replaced by ATP in a chase experiment. We find a multi-exponential process, with approximately two-thirds of the myosin showing a very slow nucleotide turnover time constant, ~30 minutes. This slow turnover state is termed the super-relaxed state (SRX) and is a highly novel adaptation for energy conservation in an animal that spends extremely long periods of time in a quiescent state (days) employing a lie-in-wait hunting strategy. If fibers are incubated in mantADP and chased with ADP, the SRX is not seen, indicating that relaxed myosins are responsible for the SRX. Phosphorylation of the myosin regulatory light chain eliminates the fraction of myosin with the very long lifetime. The presence of the SRX measured here correlates well with the binding of myosin to the core of the thick filament in a structure known as the interacting-head motif (or J-motif) observed previously by electron microscopy. Both the structural array and the long-lived SRX require ATP, both are lost upon myosin phosphorylation, and both appear to be more stable in tarantula than in skeletal or cardiac preparations. EPR spectroscopy of a spin-labeled nucleotide bound to the motor domain of myosin in relaxed tarantula fibers likewise shows orientation that is lost when the myosin is phosphorylated. Together, the data support the hypothesis that the SRX myosin and the myosin seen in the EM of the order helical array in tarantula filaments are the same.

731-Pos Board B517

The Low Angle X-Ray Diffraction Pattern from Skinned Fibers of Rabbit Psoas Muscle: Effect of Changes in [Ca^{++}] and [Orthophosphate]

Elisabetta Brunello[1], Marco Caremani[1], Massimo Reconditi[1], Marco Linari[1], Mario Dolfi[1], Manuel Fernandez Martinez[2], Theyencheri Narayanan[2], Gabriella Piazzesi[1], Malcolm Irving[3], Vincenzo Lombardi[1].
[1]University of Florence, Florence, Italy, [2]ESRF, Grenoble, France, [3]King's College London, London, United Kingdom.

Bundles of 3-5 fibers were activated isometrically at different pCa by a temperature jump from 1°C to 12°C using a mechanical apparatus (Linari *et al*., Biophys. J. 92:2476, 2007) modified to collect the X-ray diffraction pattern. The M3 meridional reflection from the axial repeat of the myosin heads was sampled by X-ray interference between half-sarcomeres. In relaxed fibers at 12°C, the M3 reflection had a major peak at 14.56 nm and a minor peak at 14.37 nm. The ratio of peak intensities (R_{M3}) was 0.43 ± 0.06 and the spacing (S_{M3}) was 14.49 ± 0.01 nm. In relaxed fibers the intensity of the main peak reduced with increasing temperature, so that at 36°C (the physiological temperature) the 14.37 nm peak was dominant, with small satellite peaks on either side, as in resting intact fibers from frog muscle. During activation at 12°C at saturating [Ca^{++}], pCa 4.5, the intensity of the M3 reflection (I_{M3}) increased to 1.9 ± 0.4 times the relaxed value with major and minor peaks at 14.68 nm and 14.46 nm; R_{M3} was 0.62 ± 0.03 and S_{M3} was 14.59 ± 0.01 nm. Activation at pCa 5.5 or at pCa 4.5 with addition of 10 mM orthophosphate (Pi) had similar effects: force was reduced to 0.34 ± 0.10 the control value and I_{M3} to 0.56 ± 0.03; R_{M3} was 0.46 ± 0.07 and S_{M3} was 14.55 ± 0.02 nm. These results give structural support to the conclusion from mechanical experiments (Linari *et al*., 2007) that both decreasing [Ca^{++}] and increasing [Pi] reduce isometric force by a decrease in the number of force generating myosin heads with no change in force per head.

Supported by FIRB-Futuro in Ricerca and Ente Cassa di Risparmio di Firenze (Italy), MRC (UK).

732-Pos Board B518

Structural Changes in Myosin Heads and Filaments during Unloaded Shortening and Force Redevelopment

Massimo Reconditi[1], Elisabetta Brunello[1], Marco Linari[1], Luca Fusi[1], Theyencheri Narayanan[2], Gabriella Piazzesi[1], Malcolm Irving[3], Vincenzo Lombardi[1].
[1]University of Florence, Florence, Italy, [2]ESRF, Grenoble, France, [3]King's College London, London, United Kingdom.

X-ray diffraction patterns were recorded with 5-ms time resolution at the ID02 beamline, ESRF, from single intact muscle fibers of the frog during steady shortening at the maximum velocity V_0, imposed at the plateau of an isometric tetanus (T_0), and during isometric force redevelopment following such shortening. During the first 20nm/half-sarcomere (hs) of shortening force decreased to near zero and changes in the X-ray pattern were consistent with a working stroke in actin-attached myosin heads followed by net detachment from actin (Piazzesi *et al*., *Cell* **131**:784, 2007). As V_0 shortening continued the M3 meridional reflection (associated with the conformation of the myosin heads) and its second order M6 (associated with the thick filament structure) became more like those recorded at rest. At 110 nm/hs shortening the M3 spacing and its fine structure were the same as at rest, while the M3 intensity, M6 spacing, and intensity of the first myosin layer line from the helical packing of the myosin heads, had recovered about half-way to their resting values, without sign of saturation. Isometric force redevelopment following 110 nm/hs shortening at V_0 and the associated structural changes were faster than those at the start of electrical stimulation (Reconditi et al., *PNAS*, **108**:7236, 2011). In both cases the initial force generation involves a small fraction of the myosin heads, whilst the majority are in the resting-like helically ordered conformation on the surface of the thick filament. The relationship between force and structural change is the same in the two cases for forces above 40% T_0. The rates of the structural changes at the start of stimulation are limited by the rate of thin filament activation.

Supported by MiUR and FIRB - Futuro in ricerca (Italy), MRC (UK), ESRF.

733-Pos Board B519

Sarcomere-Length Dependence of the Low Angle X-Ray Pattern from Skeletal Muscle Fibers at Rest and during Isometric Contraction

Gabriella Piazzesi[1], Massimo Reconditi[1], Elisabetta Brunello[1], Luca Fusi[2], Marco Linari[1], Manuel Fernandez[3], Theyencheri Narayanan[3], Vincenzo Lombardi[1], Malcolm Irving[2].
[1]University of Florence, Florence, Italy, [2]King's College London, London, United Kingdom, [3]European Synchrotron Radiation Facility, Grenoble, France.

X-ray patterns were recorded from bundles of 2-3 fibers of *R. Esculenta* at 4°C at rest and at the plateau of an isometric tetanus (T0) at sarcomere length (SL) 2.0 to 3.6 μm at the ID02 beamline at the European Synchrotron Radiation Facility, Grenoble. The patterns were normalized by the intensity of the 1,0 equatorial reflection at rest at each experimental SL to compensate for variation of diffracting mass with SL and between fibers. The axial diffraction pattern from resting fibers was independent of SL in the range 2.0-2.6 μm; in the range 2.6-3.0 μm the intensity and interference fine structure of the meridional M3 reflection from the axial repeat of the myosin heads along the filaments was constant, but its spacing (SM3) increased. The intensity of the first myosin layer line decreased in this SL range, indicating decreased helical ordering of the myosin heads. The intensity of the 44nm merdional reflection associated with myosin binding protein C was constant up to SL 2.7 μm, but much reduced for SL>2.7 μm. At T0, the M3 reflection intensity was smaller at longer SL, in proportion to the overlap between thick and thin filaments. The interference fine structure of the M3 was independent of SL up to 2.8 μm; at longer SL it varied between preparations and SM3 reduced with increasing SL. The SL-dependence of the M3 reflection at T0 indicates that the detached myosin heads in the non-overlap region of the thick filament are axially disordered compared with actin-attached heads in the overlap region, although the axial center of mass of the detached