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Report:

The aim of this project was to characterise the temperature dependence of the resting structure of the mammalian muscle and how it is altered by modulating lattice spacing in demembranated (skinned) fibres. Experiments were carried on intact muscle of mouse (Extensor Digitorum Longus, EDL) and skinned fibres from rabbit psoas. 20 ms X-ray frames were collected using a CCD FReLoN detector (active area 10x10 cm^2, pixel size 52μm, 8x binning in the direction perpendicular to the fibre axis) and a 3m camera length. A small beam (120 x 120 μm^2, obtained via the slits) and the high brightness of the beam allowed a high spatial resolution both with the vertical (whole muscle) and horizontal (skinned fibres) mounting of the sample.

Fibre preparation and protocol. Mice aged 4–6 weeks were sacrificed by cervical dislocation after inhalation of anaesthetic (isofluorane or CO_2) in accordance with the official regulations of the Community Council on Use of Laboratory Animals, and of the University of Florence (Official Italian Regulation No. 116/92). The EDL muscle was dissected from the hind limb using scissors and forceps under a stereomicroscope and mounted in a temperature controlled trough containing physiological solution between the lever of a motor/force transducer system 300C-LR (Aurora Scientific Inc.) and a lever carried by a micromanipulator for adjustment of the muscle length. The trough was sealed with a Perspex cover and mounted vertically at the beamline. Two mica windows close to the muscle reduced the X-ray path in water. The solution was continuously saturated with carbogen (95% O_2, 5% CO_2, pH 7.4). At each temperature tested (range 10 to 30 °C) the performance of muscle was checked by eliciting isometric contraction with electrical stimulation through two electrodes running parallel to the muscle. Demembranated bundles of fibres from rabbit psoas muscles were prepared in Florence and stored and transported to the ESRF at -20°C in relaxing solution containing 50% glycerol. Before the experiments bundles of 3-5 fibres 5-6mm long were isolated, their extremities clamped with aluminium T-clips and horizontally mounted in a drop of relaxing solution between the lever of a loudspeaker motor and a capacitance force transducer. Temperature effects were determined in control conditions and in the presence of 5% w/v dextran T500, added to the solutions to recover the interfilament spacing of intact muscle (see also report SC-3473). The thermo-regulated trough

has a Z movement that allows the plate carrying the solution drops to be lowered so that X-ray measurements can be made with the fibre in an air cavity in the centre of a temperature controlled aluminium block.

Results. In intact EDL muscle from mouse at 30°C (red line in Fig. 1, *Whole muscle*), the M3 reflection, originating from the axial repeat of the myosin heads, shows the same fine structure as in intact frog muscle, that is a main peak at 14.35 nm (MA), with small satellite peaks (LA and HA) on either side. As temperature decreases below 25 °C, the intensity of the M3 reflection (I_{M3}) decreases, showing a redistribution of peak intensity toward the LA peak and an additional peak (marked with a star) appears on the LA side. In skinned fibres (Fig. 1, *Skinned fibres, no dextran*), in the control solution in the absence of dextran, I_{M3} is much lower than in the intact muscle and does not show a clear dependence on temperature, the additional peak on LA side has a larger relative intensity with respect to I_{M3} and the redistribution of intensity between peaks is more marked so that at 10°C the LA peak at 14.5 nm becomes dominant. Addition of dextran (Fig. 1, *Skinned fibres, 5% dextran)* partially recovers I_{M3} toward the value in intact muscle, and both M3 intensity and fine structure show a temperature dependence similar to that in the intact muscle.

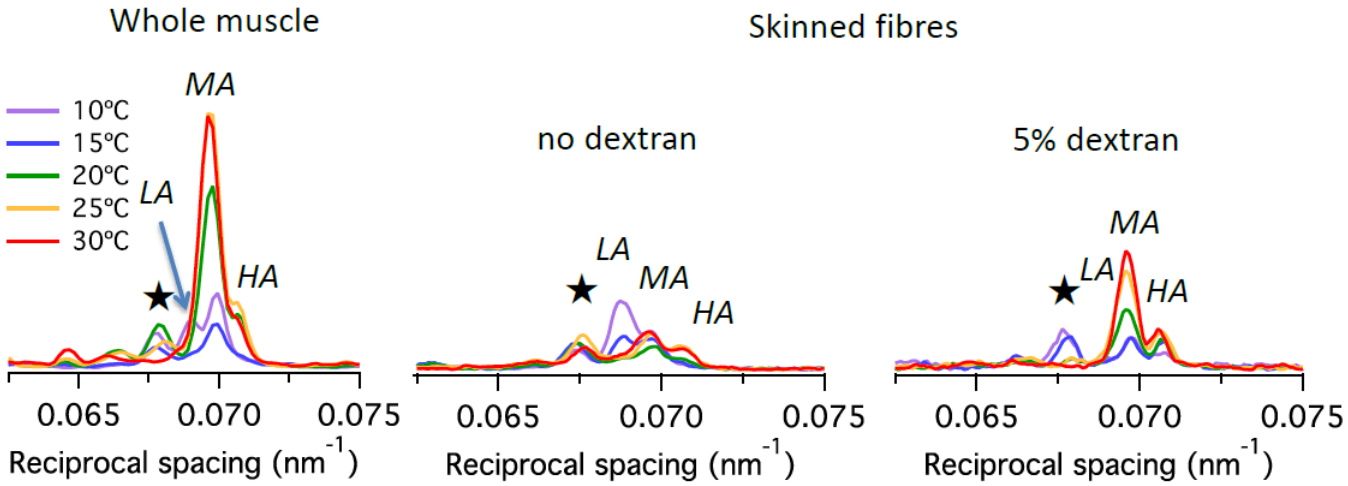

Fig. 1. Intensity profiles of M3 reflection from whole muscle and skinned fibres in the absence and presence of dextran at different temperatures as indicated in the legend.

Publication from ESRF in the last 18 months.

M. Reconditi, E. Brunello, L. Fusi, M. Linari, M. Fernandez Martinez, V. Lombardi, M. Irving, G. Piazzesi (2014), Sarcomere-length dependence of myosin filament structure in skeletal muscle fibres of the frog, *J Physiol.* doi:10.1113/jphysiol.2013.267849.

Park-Holohan S, Linari M, Reconditi M, Fusi L, Brunello E, Irving M, Dolfi M, Lombardi V, West TG, Curtin NA, Woledge RC, Piazzesi G. Mechanics of myosin function in white muscle fibres of the dogfish Scyliorhinus canicula (2012). *J. Physiol.* **590**, 1973-1988.

Abstracts

E. Brunello *et al*, *Biophys J*, 106(2): 454a (2014)
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E. Brunello *et al*. 42nd European Muscle Conference, 2013 Amsterdam (NL) *J Muscle Res Cell Motil*
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