The aim of the experiments was to determine structural changes in contracting muscle fibres caused by a transition from isometric contraction at a constant length to steady shortening when muscle produces near maximal mechanical work.

During beamline session SC-3112 we made use of the set-up that was successfully tested during beamline SC-2639 session in year 2009 (Tsaturyan et al, Biophys. J. 2011). Two series of experiments with bundles of 2-3 permeabilized fibres from rabbit *psoas* muscles were performed. The 2D x-ray diffraction patterns collected during isometric contraction were compared to those during steady-state ramp shortening under load of 0.31-0.4P_0 or 0.61-0.68P_0 (P_0 is isometric tension). The range of 0.3P_0 to 0.7P_0 corresponds to maximal work production of a skeletal muscle. The samples were positioned vertically to improve the spatial resolution of the neighbour layer line reflections. The beam was focused vertically on the detector placed 2.25 m off the sample. The x-ray flux was limited to 2×10^{13} photons/s and 80 mM of dithiothreitol DTT was added to the activating solution to minimize the radiation damage.

From 2 to 20 runs of the protocol were performed with each of 14 samples until damage or isometric post-T-jump tension decreased by 15% compared to its value after the 1st run. In some experiments instantaneous stiffness was measured with fast (~0.14 ms long) step stretches applied during isometric contraction and at the end of ramp shortening just after the x-ray frames (Fig. 1*A*). Both diffraction patterns were collected during 10 ms long exposures at near physiological temperature of 32-34°C achieved with a joule temperature jump (T-jump, top records in Fig. 1). Step shortening was applied before the ramps to accelerate the transient that precedes the steady-state shortening (Fig. 1*A*). To eliminate possible effects of ATP depletion and/or accumulation of the products of its hydrolysis, ADP and inorganic phosphate, the sequence of the x-ray frames with the isometric and shortening patterns alternated (Fig. 1*A*, *B*).

Ramp shortening induced a decrease in the intensity of the M3 myosin meridional reflection and of all actin layer lines in the pattern, from A1 at ~$(37\ nm)^{-1}$ to A7 at ~$(5.1\ nm)^{-1}$. Also the intensity of the beating actin-myosin layer line at ~$(10.3\ nm)^{-1}$ decreased sharply (Fig. 2). Changes in the intensities of the x-ray reflections caused by steady shortening at a ~P_0/3 load were more pronounced than those at a ~2P_0/3 load. A significant difference was found in the intensity of the A1 layer line which did not change much during shortening at the high load while decreased dramatically at the lower load (Fig. 2). Further quantitative analysis with fibre-to-fibre statistics and modeling (Koubassova et al., 2008; Tsaturyan et al. 2011) is needed to draw more solid quantitative conclusions from the data.

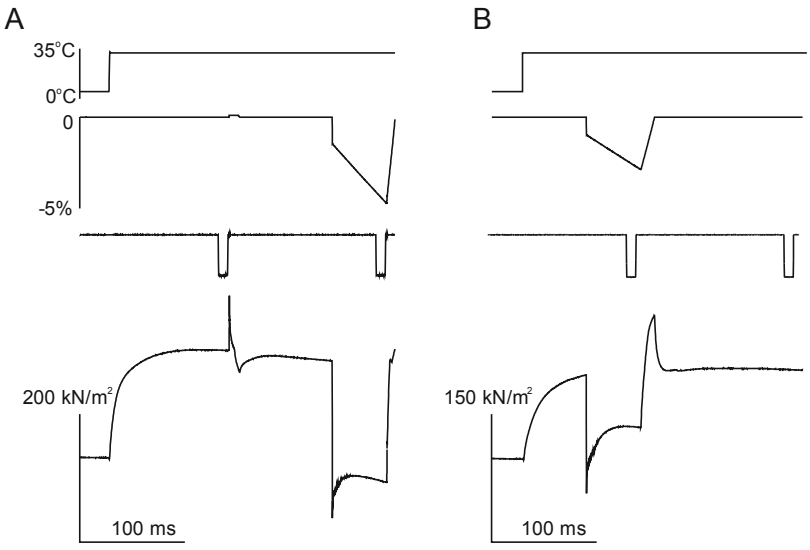

Fig. 1. Typical examples of experimental records for two series of experiments with steady shortening at low (*A*) and high (B) load. Records from top to bottom: temperature; changes in muscle fibre length (in % of its value at sarcomere length 2.45 μm); signal of opening of the fast x-ray shutter recorded with a pin diode and tension. Step stretches of 0.2% of muscle length completed in 0.14 ms were applied just after the end of the x-ray frames in *A* to measure instantaneous stiffness.

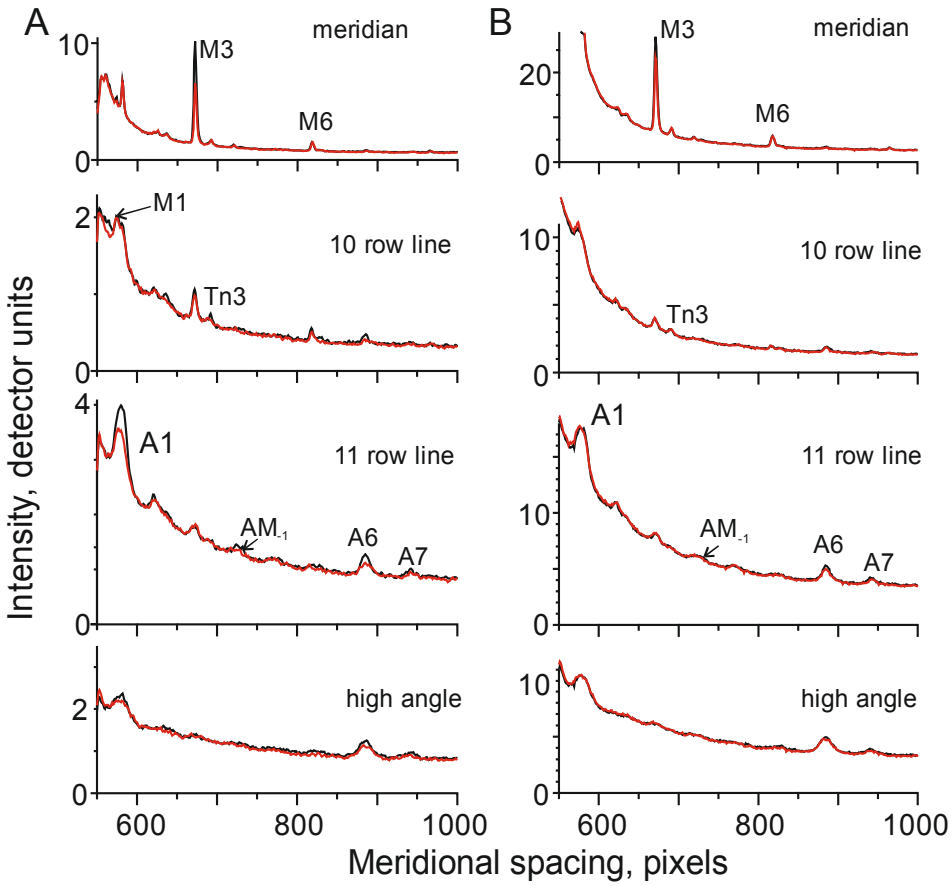

Fig. 2. Meridional profiles of the meridional and off-meridional x-ray intensities collected from two muscle samples presented in Fig. 1 (*A* and *B,* 7 and 20 runs of the protocol, respectively). Black traces correspond to isometric contraction; red records were collected during steady shortening and then corrected for the change in the fibre volume exposed to the x-ray beam. From top to bottom: intensity profiles integrated on the meridian (radial spacing of 0-0.02 nm^{-1}); the 10 row line (0.02-0.032 nm^{-1}); the 11 row line (0.032-0.06 nm^{-1}) and the high angle part of the patterns (0.06-0.09 nm^{-1}). Myosin (M) and tropomyosin (Tn) meridional reflections and actin (A) and actin-myosin beating (AM) layer lines are labeled.