ESRF	**Experiment title:** Molecular structural basis of the effect of load on the mechanical performance of skeletal muscle	**Experiment number**: LS3147
Beamline: ID02	**Date of experiment**: from: 11/11/2022 to: 14/11/2022	**Date of report**:
Shifts: 9	**Local contact(s)**: Narayanan Theyencheri	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Dr. Luca Fusi, King's College London

*Dr. Cameron Hill, King's College London

*Dr. Michaeljohn Kalakoutis, King's College London

*Prof. Malcolm Irving, King's College London

*Miss Alice Arcidiacono, King's College London

*Dr Atsuki Fukutani, King's College London

Dr. Yanhong Wang, King's College London (remote user)

Dr. Elisabetta Brunello, King's College London (remote user)

Report:

Science background. Development of novel therapies to combat muscle weakness in disease and the elderly is limited by a poor understanding of the normal mechanisms of muscle regulation. Muscle contraction is driven by sliding between thick (myosin-containing) and thin (actin-containing) filaments, triggered by a transient increase in calcium ion concentration in the muscle cells. The calcium ions bind to the thin filaments, causing a structural change that permits binding of the myosin motor domains to actin. However, this calcium/thin filament signaling pathway does not control the strength, speed or metabolic cost of contraction[1], which depend on a second class of regulatory mechanism that controls the availability of the myosin motors themselves. In resting muscle these motors are held in helical tracks on the surface of the thick filaments, with the motor domains folded back against the filament surface, making them unavailable for actin binding. Moreover, release of the motors from this helical folded conformation is triggered mechanically, by the strain in the filament[2]. This intrinsic mechano-sensing mechanism couples the number of active motors to the external load on the muscle, maximizing the efficiency of contraction. However, the structural basis of thick filament mechano-sensing and its coupling to thin filament regulation remain unknown. The aim of LS3147 was to determine the roles of both myosin-based and thin filament regulation in the response to a change in the external load on a muscle, either a decrease in load that allows muscle shortening and work production, or an increase in load that allows muscles to work as brakes when they are stretched.

Experiments carried out during LS3417. Isolated intact extensor digitorum longus (EDL) muscles from the mouse were mounted vertically at ID02 in oxygenated Krebs solution at 27°C. X-ray diffraction patterns were collected with 2-ms time resolution on the Eiger 2X 4M detector. Sarcomere length was measured from the first-order sarcomere reflections with a muscle-to-detector distance of 31 m. In the shortening protocol, sarcomere length was set to 2.85 μm in the resting muscle. Muscles were stimulated repetitively for 120 ms; after 60 ms of fixed-end contraction, during which sarcomere length shortened to about 2.7 μm, unloaded shortening was allowed for 15 ms to sarcomere length about 2.3 μm, after which force developed at fixed

muscle length. The actin-based second layer line, reporting the activation state of the thin filament, and the low-angle myosin-based reflections that report the activation state of the thick filament and the motor conformations[3-5] were recorded with a muscle-to-detector distance of either 3.2 or 2m. The intensity of the M3 reflection associated with the axial repeat of the myosin motors decreased rapidly at the start of unloaded shortening to about 10% of its tetanus plateau value, then recovered in parallel with force redevelopment after shortening. Its spacing reached a steady value of about 14.28 nm during shortening, significantly lower than its value at rest (14.34 nm). An additional reflection with a spacing of about 14.8 nm, not detectable at rest or at the tetanus plateau, appeared during unloaded shortening. This reflection also appears transiently at the start of stimulation[6]. The first myosin-based layer line recovered to about 30% of its resting value during unloaded shortening, signalling partial reformation of the helically ordered OFF motor conformation. The second actin-based layer line, signalling the azimuthal position of tropomyosin, also decreased substantially during unloaded shortening, more than halfway towards its resting value. These results show that the activation level of both the thick and thin filaments decreases during unloaded shortening, consistent with mechano-sensing in the thick filaments, activation of thin filaments by myosin binding, and positive coupling between the regulatory states of the thin and thick filaments.

A second set of time-resolved experiments in which the same X-ray measurements were made using a ramp stretch applied at the plateau of a fixed-end contraction are currently being analysed.

References [1]Irving M 2017 *Biophys J* 113: 2579. [2]Linari M *et al*. 2015. *Nature* 528:276. [3]Piazzesi G *et al.* 2002 *Nature* 415:659, 2007. [4]Piazzesi G *et al.* 2007 *Cell* 131:784. [5]Hill C *et al*. 2021 *eLife* 10:e68211. [6]Hill *et al*. *J. Physiol* 600.17, 3983; 2022.