RESEARCH REPORT

1. Proposal number: 2009B1917

2. Title of experiment: Thin filament in ageing: Structure and function studied by X-ray diffraction

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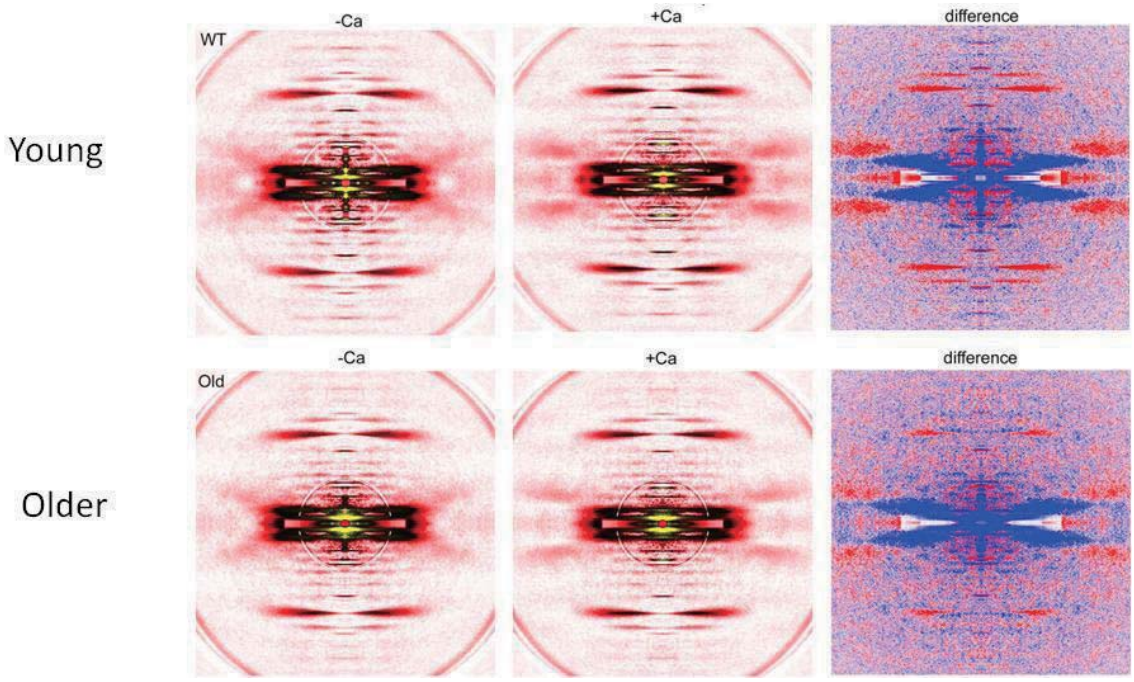
4. Beamline used: BL45XU (small-angle scattering station)

5. Research background and purpose: Falls are a major cause of morbidity and mortality in the growing population of elderly citizens. They are commonly associated to sarcopenia, i.e., condition referring to a loss of skeletal muscle mass and strength in elderly individuals.

At the muscle cell level, measurements have shown impairments in the contractile properties that may explain, in part, why older men and women suffer from muscle weakness. More specifically, an age-related decline in the ability of muscle fibres to generate force has been reported suggesting that the intrinsic characteristics related to the mechanics of myosin-thin filament interaction, i.e., cross-bridge, are altered with ageing. To date, studies have primarily focused on characterizing the myosin alterations, ignoring the thin filament changes. Consequently, the aim of the present proposal was to characterize whether and how the structure and function of the thin filament is modified in elderly individuals.

6. Experimental methodology: We performed X-ray diffraction experiments on membrane-permeabilized muscle fibres coming from biopsy sample specimens from young and older individuals. The experiments were carried out using the SPring-8 synchrotron radiation facility (Harima, Hyogo, Japan). On the day of experiment, muscle fibres were dissected and mounted in arrays of 30 fibres. X-ray diffraction patterns were recorded for each array of 30 membrane permeabilized fibres in relaxing (low [Ca^{2+}]) and activating (high [Ca^{2+}]) solutions by using a cooled CCD (charge-coupled device) camera (C4880, Hamamatsu Photonics, 1000 × 1018 pixels) in combination with an X-ray image intensifier (V5445P, Hamamatsu Photonics). The wavelength was 0.09 nm, and the specimen-to-detector distance was ~2 m. To compensate for the relatively small dynamic range of the detector, absorber masks made of aluminum and copper were placed at the center of the image intensifier. The exposure time was ~2 s, and usually several to tens of patterns were summed to obtain a final image to be analyzed. The four quadrants of the image were folded after correction for the fiber inclination, and the background was subtracted.

7. Research results: More than 30 arrays of 30 fibres were included. Briefly, in young fibres during activation, with full overlap (2.70 μm), the 2nd actin layer line (ALL) is enhanced along with the 6th and 7th by the addition of calcium (Red and blue colors indicate the area with increased and decreased intensities after activation, respectively). In older cells, the enhancement of these ALLs looks weaker, but is not significantly different. Further experiments are needed to draw a reliable conclusion.



8. Current/future issues: All the data are promising but need to be confirmed by further experiments.

9. Status of publication: More experiments using X-ray diffraction are needed before submitting the data to a scientific journal.

10. Key words: Ageing, muscle, thin filament, structure and function.