# INCREASED STIFFNESS AT THE FIBRILLAR LEVEL IN A MURINE BONE MODEL FOR AGEING

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## Introduction

Bone changes in both quality and quantity with ageing, and the changes are generally in the direction of affecting the fracture resistance mechanisms at different hierarchical levels of bone tissue [Kuhn et al, 2008], and the challenge has been to quantify what these changes are. However, there have been to date relatively few studies on the nanoscale changes in the deformation mechanisms of the mineralised bone matrix in ageing bone. Therefore it is crucial to apply multiscale imaging techniques that can quantify these alterations *in situ*. In this study, the deformation of the skeletal tissue at the material level was measured, to quantify how changes in bone quality with ageing (independent of any associated diseases like osteoporosis) may reduce mechanistic competence at the material level, independent of associated BMD reductions at organ level [Kanis, 2002]

## Methods

We combined *in situ* synchrotron imaging (SAXS/WAXD) with micromechanics to directly measure fibrillar (supramolecular) [Karunaratne et al, 2012] and mineral particle (molecular) deformation of the bone matrix when subjected to loading. In addition to *in situ* mechanical testing, cortical and trabecular micro-architectural changes as functions of ageing were studied using scanning electron microscopy (SEM) and micro-computed tomography (microCT) respectively. Human ageing was considered as a phenomenon in itself separate from any induced disease phenotype. A mouse model of premature ageing (removal of the klotho gene) [Kuro-o, 2009] was used, exhibiting multiple phenotypes very similar to those observed during human ageing.

## Results

The increase in tissue modulus in klotho mice shows that the cortical bone tissue is (~ 100 %) stiffer compared to wild-type. Macromechanical results also indicate that klotho mice bones failed at a lower tissue strains (0.75 %) compared to the wild-type bones (1.3 %). Using the nanomechanical imaging method, it was found that the effective fibril modulus was significantly higher (up to ~ 200%) in klotho mice when compared to wild-type mice. Reduced fibrillar plasticity (maximal fibril strain) was seen in klotho mice, indicating a difference in the nanoscale structural response to externally applied stress. Mineralised fibrils in klotho mice deform to lower strain values (~ 0.3 %) before reaching the plastic regime, whereas wild-type fibrils have a larger strain zone over which the stress/fibril-strain plot remains linear (~ 0.85 %).

## Discussion

In-situ tensile testing combined with nanoscale (SAXD) and microscale imaging (BSE and microCT) showed that age-related structural changes in bone are linked to increased fibrillar stiffness. These changes, adversely affecting the higher length scale mechanical and structural properties, show that bone matrix quality alterations in ageing plays a critical role in reduced mechanical competence and toughness.

## References

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