Introduction
Recent studies have highlighted the functional importance of TIMP3 gene as an inhibitor of a variety of matrix metalloproteinases (MMPs) which play an important role in the formation and remodelling of bone and cartilage [Stickens et al, 2004]. TIMP3 is essential for the proper formation of mechanically competent cartilage and bones able to withstand habitual load. Mice models of TIMP3 deficient (KO) develop spontaneous osteoarthritis, collagen degradation, abnormal bone growth sooner after birth [Sahebjam et al. 2007]. These mice showed a delayed formation of secondary ossification centers in long bones and a diminution of bone length. Still unclear is the bone behaviour of TIMP3-overexpressing mice. These mice are protected from surgically-induced osteoarthritis development.

The aim of this study was to understand the coupled effects of mechanics and structure in the absence or overexpression of TIMP3. We used small angle x-ray scattering (SAXS) to measure changes in the collagen morphology and wide angle x-ray diffraction (WAXD) to directly measure strain in the mineral phase.

Methods
The ulnae of TIMP3 KO, overexpressing and normal WT mice (4 mice each group, 7 weeks old) were dissected and soft tissue removed. Bones were stored in custom-made sample holders in phosphate buffer solution. Ulnae were then mounted in a load frame positioned in beamline I22 at the Diamond Light Source synchrotron radiation facility (Oxford, UK) and were loaded in tension at a displacement rate of 1 μm/s in a custom-made rig with a 111 N load cell (RDP Electronics Ltd) while SAXS/WAXD data were collected with mechanical loading.

Scattering patterns were taken at every load step up to the tissue failure with an exposure time of 2 s. Tests were conducted at 25 °C on continuously hydrated samples. SAXS and WAXD data were collected by a high-speed Pilatus 2M detector. The tissue stress was measured by imaging the cross-sectional surface of the bone in an ESEM while the tissue strain was determined by imaging the change in spacing of horizontal lines marked on the sample’s surface. The images were analyzed with image analysis software (National Instruments). The software Fit2D permitted the conversion of the scattering data from 2D to 1D. The 2D SAXS and WAXD data were converted to 1D by radially integrating over a sector of 10° and a sector wide 20 pixels, respectively oriented parallel to the direction of loading. The fifth-order collagen peak and the (0002) HA peak were found by fitting the 1D datasets with a Gaussian and a linear function. Collagen fibrils strain and mineral strain were determined as the change in position of the corresponding peak’s center normalized by its location at zero loading.

Results
Our results showed an increase in the ratio of fibril/tissue strain testing in TIMP3 KO bones than in control. TIMP3 overexpressing bones showed instead a reduction of the fibril/tissue strain.

Discussions
Differences in fibril nanomechanics between the TIMP3 KO, overexpressing and WT bones are possibly caused by the reduced interfibrillar shear transfer due to collagen degradation in KO mouse bone and possible reduced mineralization in overexpressing bones. Future studies will investigate nanostructure and composition of these bones. This information is essential to fully understand the physiological role of TIMP3 in the maintenance of skeletal integrity.

References
Stickens et al, Develop, 1231:5883-95, 2004