# INVESTIGATING THE NANO MECHANICAL BEHAVIOUR IN THE MINERALIZED MATRIX OF METABOLIC BONE DISEASES USING SYNCHROTRON X-RAY DIFFRACTION

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## Introduction

Bone diseases such as rickets and osteoporosis cause significant reduction in bone quantity and quality, which leads to biomechanical abnormalities. While the reduction of bone in macro level can be assessed using clinical tools like DEXA and qCT, there is little quantitative knowledge of how the altered nanostructural mechanics in bone make it more prone to fracture. Understanding the nanostructural origins (Gupta et al, 2006) of increased fracture fragility in metabolic bone diseases is essential to correlate bone quality alterations in the fibrillar level to mechanical deteriorations.

## Methods

Here we demonstrate the functional link between altered bone quality and abnormal fibrillar-level mechanics by using this novel method to study a mouse model with rickets (Karunaratne et al, 2012) and glucocorticoid induced osteoporosis (GIOP) due to impaired mineralisation and remodelling process respectively. The technique uses the high brilliance of synchrotron radiation to enable real-time small angle X-ray scattering (SAXS) of the fibrillar and mineral ultrastructure in bone. During applied external loading, percentage changes in the peak positions of SAXS patterns give fibrillar- level strain as a function of applied stress. To develop a model linking nanostructural changes to altered fracture risk and deformability, we used well–defined murine models developed by N-ethylnitrosurea mutagenesis. These provide nano-mechanical parameters of bone quality, including fibril elastic modulus, maximum fibril strain and fibril to tissue strain ratio.

## Results

A significant reduction of fibril modulus and enhancement of maximum fibril strain was found in rickets and GIOP mice. We also find a much larger fibril strain/tissue strain ratio in GIOP mice (1.5) compared to the wild-type mice (0.5), which is indicative of a lowered mechanical competence at the nanoscale. Mineral content was estimated using backscattered electron imaging and the mean mineral content was lower in both GIOP and rachitic mice and was more heterogeneous in its distribution.

## Discussion

Our results are consistent with a nanostructural mechanism where incompletely mineralized fibrils show greater extensibility (eq:1) and lower stiffness, leading to macroscopic outcomes such as greater bone flexibility. The studies performed here demonstrate that the synchrotron-based in-situ X-ray nanomechanical imaging is a highly sensitive diagnostic tool, compared to BMD determination methods like DEXA, and show that small changes in mineral content or collagen deformability in metabolic diseases, when combined with abnormal nano- and microscale mineralisation, altered remodelling and possibly changes in the organic matrix structure, can lead to significant reductions in bone mechanical competence and fracture risk in metabolic bone disease conditions such as osteoporosis, osteoarthritis, and aging.

## References

Gupta et al, PNAS., 103(47):17741-6, 2006
Karunaratne et al, JBMR., 27(4):876-90, 2012

## Figures

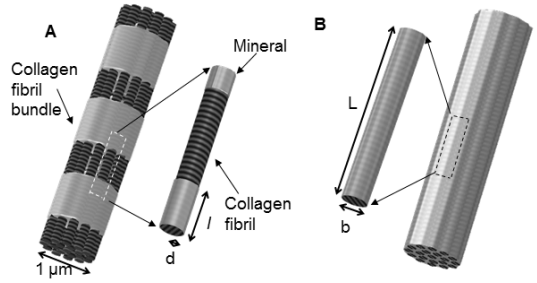


*Figure 1: (A) In this scheme partially transparent gray zones and banded zones denotes the mineral phase and collagen fibrils respectively. In rachitic mice, the collagen fibril bundles are only partly filled and covered by mineral which is also discontinuous along the length (inset showing partially mineralized fibril). (B) In wild type mice, the full length of collagen fibril bundle is covered with mineral (inset showing fully mineralized fibril).*

## Equations

$$\frac{\varepsilon_f}{\varepsilon_{f,0}} = \frac{l}{L} \times -1\left(\frac{4\left(d^2+db\right)}{b^2}\frac{E_m}{E_f}\right) + \left(1+\frac{4\left(d^2+db\right)}{b^2}\times\frac{E_m}{E_f}\right)$$