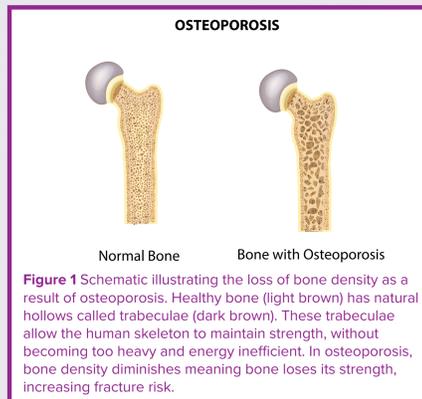


A new direction for modelling osteoporosis

What is the problem?

Osteoporosis is a major healthcare problem, which is set to rise steadily with the aging population. It is characterised by loss of bone density, both in terms of mineral (calcium hydroxyapatite) and protein (collagen) content (see Figure 1). The clinical significance of osteoporosis lies in the fractures that occur, which tend to be the result of a modest load applied quickly, such as that which would result from a fall from standing height.



What are we interested in?

It is known that bone has a greater strength in the longitudinal direction than transverse or radial directions and the risk of fracture is greatest when loads are applied outwith the principal loading axis of the bone. This is because over time, bone changes its architecture and strengthens itself along lines of stress, but remains vulnerable in other directions. However, current models for osteoporosis do not take this directionality, known as anisotropy, into account.

Pilot work has shown that treating bone with chemicals to remove collagen can replicate the increased fragility that occurs in osteoporotic bone. However, the vast majority of current research neglects the impact of collagen loss, instead focusing on the reduction in bone mineral content.

We wanted to investigate the effect that reduced protein content of bone has on the mechanical strength of bone when a force is applied in the longitudinal direction or the transverse direction in order to better understand the consequence of deficiency in the protein component.

What did we do?

We prepared matchstick sized samples (4mm x 4mm x 40mm) of bone from the outer region of the leg of cows. Half of the bone samples were from longitudinal cuts (down the length of the bone) and half were from transverse cuts (across the bone), which enabled us to see if there is a relationship between strength and direction of the bone. Of these, we left some of the bone samples untreated and removed 10% of the collagen from some and 30% of the collagen from others by incubating the samples in bleach (6% NaOCl). We were able to determine the collagen content of the samples on the basis of how much UV light the samples absorbed (see Figure 2A and Figure 3) and for how long the samples needed to be incubated in the bleach to remove the appropriate amount of collagen (see Figure 2B), using standard reference curves.

We performed four point bending tests using a large strength testing device (see Figure 4). The machine produced vast raw data which was used to calculate the mechanical performance of each sample. Two properties of particular interest that we studied were stress (force per unit area) and strain (deformity of the bone due to the force we applied). We carried out statistical analysis of the data to check that our results were meaningful and not due to chance alone.

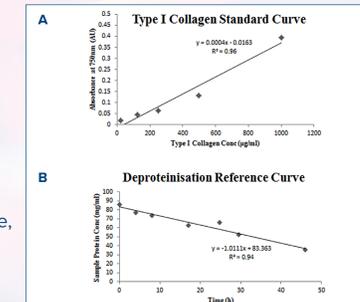


Figure 2 Standard reference curves showing (A) how the UV absorbance of collagen from cow varies with collagen concentration and (B) how the amount of collagen removed from bone varies with the incubation time in bleach (6% NaOCl).

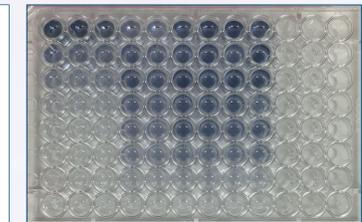


Figure 3 A 96 well plate which was used to determine the collagen concentration of the samples. Lanes one to three contain standard, known collagen concentrations. Lanes four to nine contain samples of unknown collagen concentrations. The concentration of the unknown samples was determined by comparison of their UV absorbance with the standard curve shown in Figure 2A.

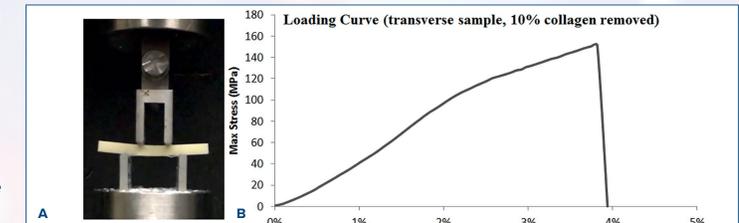


Figure 4 (A) Four point bending test using a Zwick Roell Z005 (a strength testing device). The machine applies a slowly increasing force to the sample, until the sample breaks (stress at failure point). The corresponding load curve (B) is shown, which shows the increasing strain (% deformation due to applied force) the sample is under, before it breaks at a stress of 160MPa (1MPa = 1,000,000 Newtons per m²).

What did we find?

Our results show that stress at failure (when the sample breaks) is significantly reduced in a collagen concentration-dependent manner in the longitudinal – but not transverse – direction (see Figure 5). A reduction in collagen content reduced the stress at failure. Whilst previous work has shown that bone is an anisotropic material (it has properties that vary depending on directionality), this is the first study to show that partial collagen loss affects bone significantly in the longitudinal, but not the transverse, axis.

The main limitation to this study was the relatively small number of samples used: 30 bone samples underwent mechanical testing. In order to increase our confidence in the results, more bone samples would be required to account for natural differences that occur between samples.

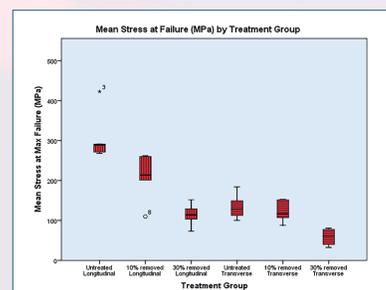


Figure 5 shows the mean (average) stress at failure for each group of samples when a force is applied. Mean stress at which failure occurred reduced significantly in line with collagen removal for the longitudinal samples. A similar pattern was seen in transverse samples, however, this trend was not statistically significant.

What does it mean?

Previous studies have concentrated on studying the effect of reduction of bone mineral content, and on directionality of bone as independent factors. This is the first study to partially remove protein and to investigate the profound effect that loading direction has on the mechanical strength of bone with collagen deficiency. We have demonstrated the importance of the protein component of bone in fracture prevention, and our findings could provide a path for newer, more physiologically representative models of osteoporosis.

Who am I?

I am a third year post-graduate medical student at the University of St Andrews, having previously obtained my MPharm in Pharmacy from the University of Strathclyde. I believe my career will follow a surgical route – I am particularly interested in Orthopaedics and trauma. The beauty of a career in medicine lies in that fact that I can combine my passions of hands-on, clinical work with patients with cutting edge, relevant research.

I would like to thank Medical Research Scotland for supporting my summer project which took place at the Department of Orthopaedics within the University of Edinburgh under the supervision of Professor Hamish Simpson and Dr Robert Wallace PhD.