MOLECULAR ORIGINS OF BONE MICROMECHANICS
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Introduction
Bone quality can provide a more definitive characterisation of skeletal health and utilisation of bone quality parameters to augment conventional bone fracture risk surrogate measures, such as mineral content, may provide more accurate diagnosis. A key aspect of bone quality is the consideration of material and structural contributions to mechanical performance (fracture toughness), both regulated by the bone biology environment. While the influence of the TGF-β pathway on bone fragility has been studied (Balooch, 2005), it is not clear whether TGF-β levels and related downstream targets, such as RUNX2, are regulating bone micromechanics in normal health. Here, we investigate both mRNA expression and mechanical properties via reference point indentation to elucidate this effect.

Methods
Samples were obtained from 70 day old Wistar rats, with femurs and tibias dissected out. mRNA was obtained from fragmented femur epiphyses using the TRizol reagent (Life Technologies) isolation protocol for fibrous tissue. RNA was used to analyse gene expression commonly related to osteogenesis (ALP, RUNX2 and OCN) through RT-qPCR. Expression of genes was presented at the 2(-ΔΔCt) level with reference to β-actin. Tibiae were used for reference point indentation (RPI) (Biodent, Active Life Scientific) and tested at a peak force of 8 N with a preload of 8 N for 10 cycles at 6 positions along the bone on medial, lateral and posterior sides. Indentation distance increase (IDI) and constant force displacement (creep) data was obtained. Bone samples were hydrated in Hank’s balanced salt solution before and kept hydrated throughout mechanically testing.

Results
When comparing average creep distance (mean of all indents performed on each tibia) to relative expression of ALP and RUNX2, a positive correlation was found (figure 1).

Similar, more modest, trends were found with IDI values (not shown). OCN expression displayed a negligible negative trend with IDI (R² = 0.2381).

![Figure 1: Expression of ALP and RUNX2 versus averaged creep distance. n = 4.](image)

Discussion
Quantification of gene expression show that increased levels of early osteogenic markers (ALP and RUNX2) relate to an increase in creep values. This is somewhat counter intuitive but can be explained by the sample age, where bone modelling and appositional growth is still occurring (Lelovas, 2008). As such, it is reasoned that the surface of the bone where indentations were performed consists of newer, less developed, bone that is more pliable. The slight pattern between higher OC, which initiates calcium binding during later mineralisation, and lower IDI seems to support this, as the resulting bone is more mature and has a greater resistance to applied force. The low correlation may suggest other factors, such as osteopontin, are important for mature bone micromechanics and worthy of further investigation. These initial results suggest a mechanism as to how cell signalling effects adaptation in bone micromechanics.

References