



Measuring bone composition by synchrotron-based Fourier-transform infrared microspectroscopy

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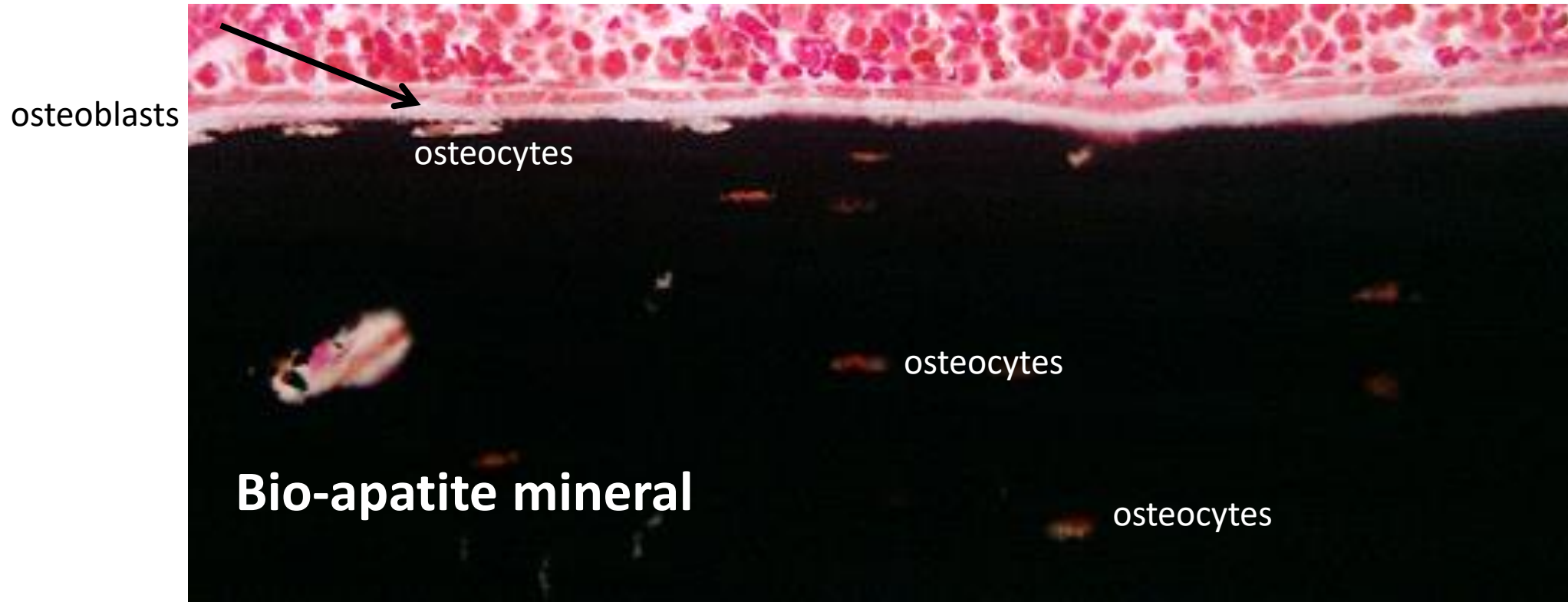
St. Vincent's Institute of Medical Research

& The University of Melbourne



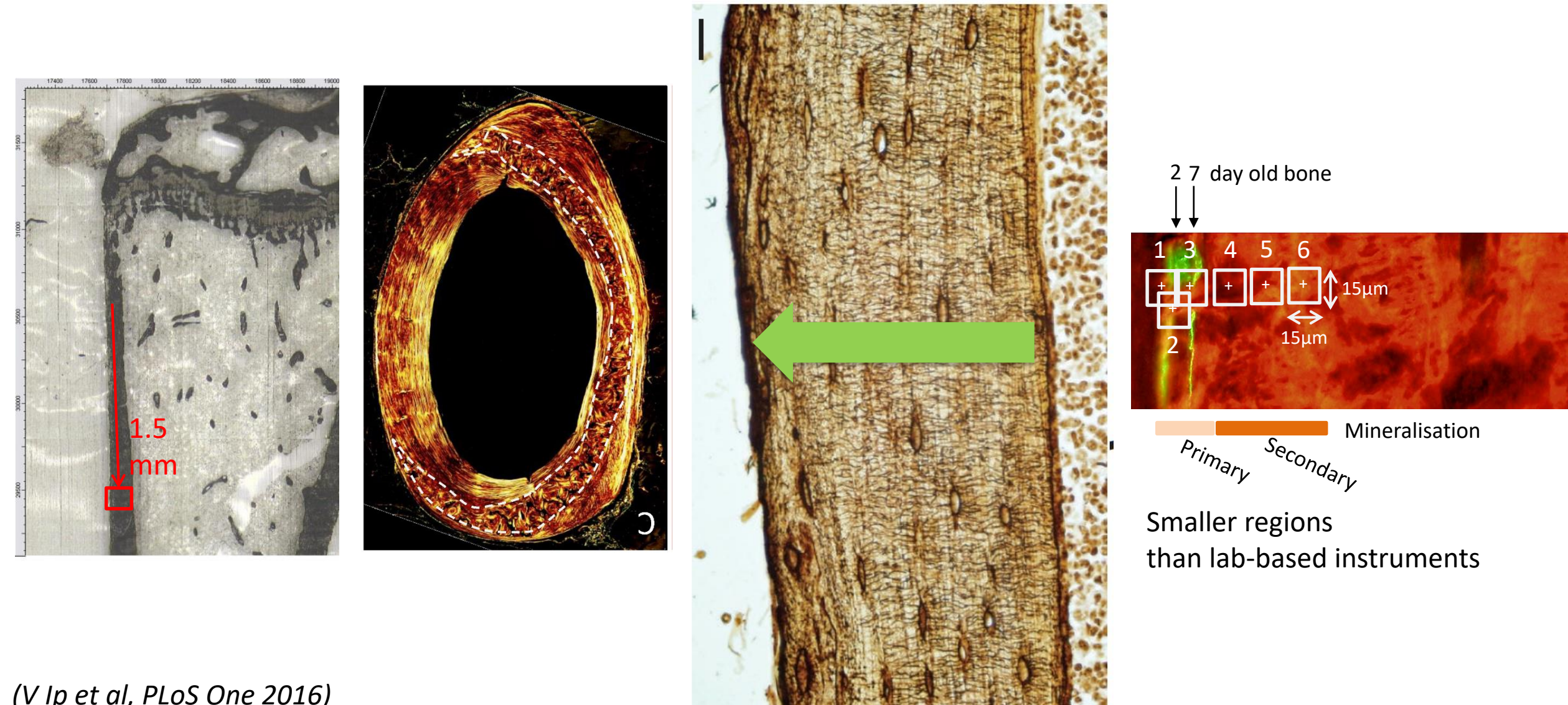
How does bone change as it becomes more mature?

Osteoid deposition = predominantly type I collagen

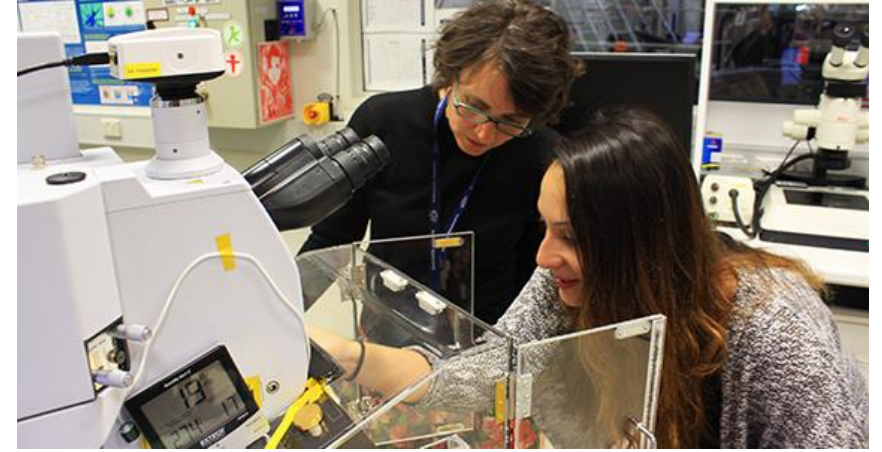
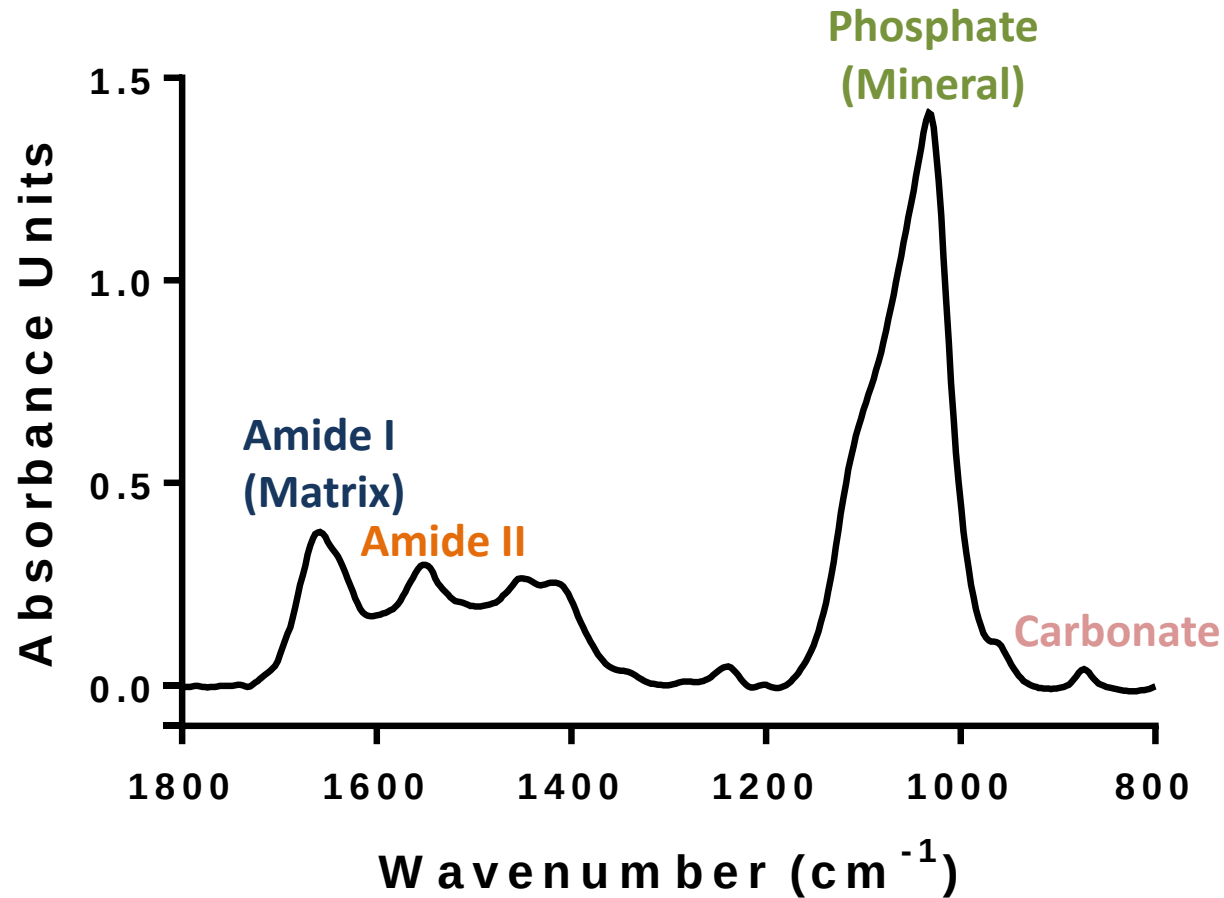


Mineralisation starts rapidly (primary) and continues slowly (secondary) until a maximum is reached (or the bone is renewed) – what are the events that control this process?

To measure bone maturation, we selected a site where bone forms and mineralises as concentric layers



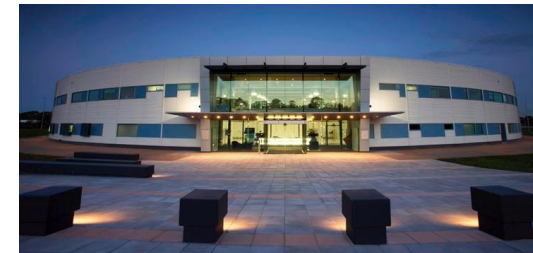
Fourier-transform infra-red microspectroscopy (FTIRM)



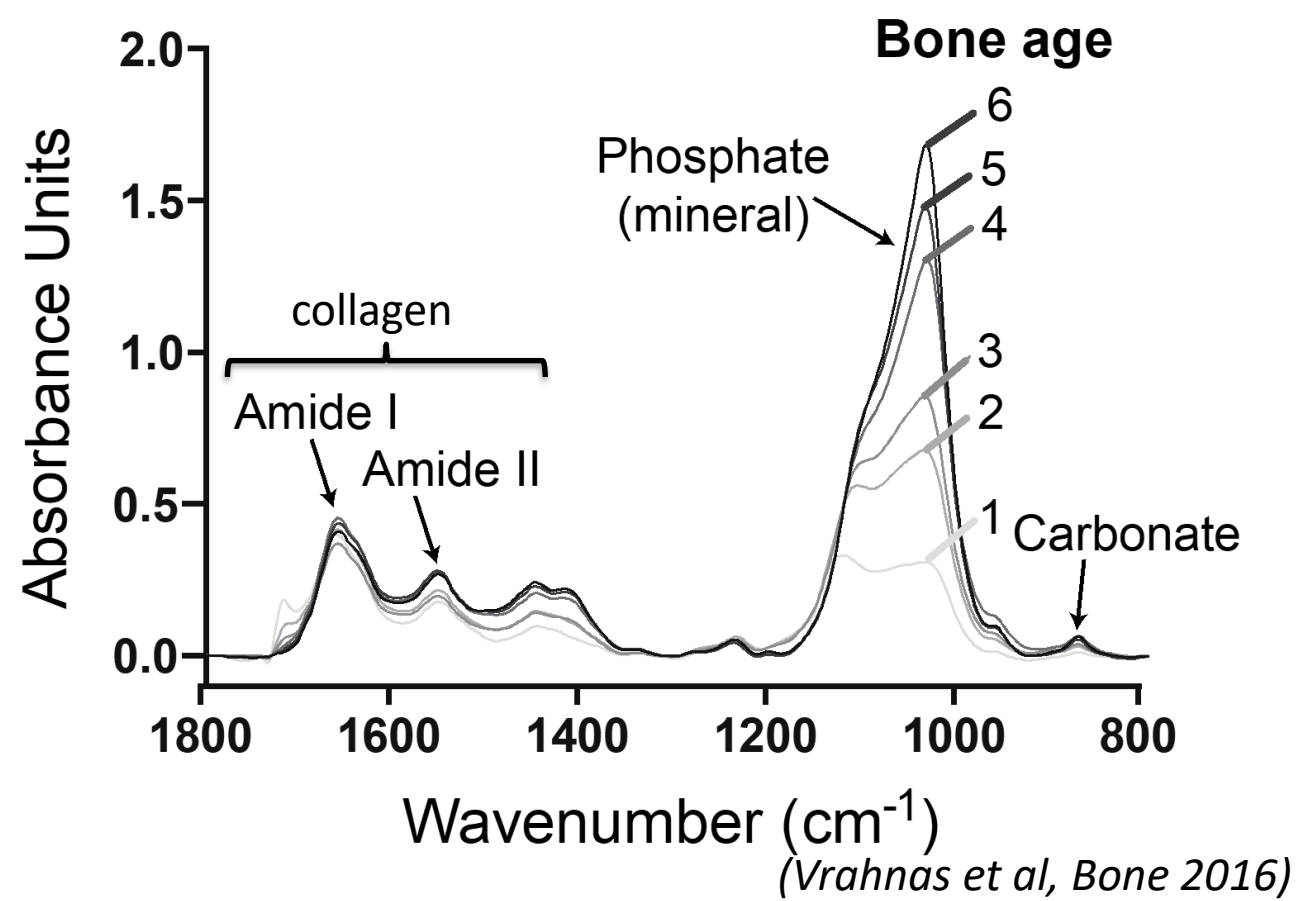
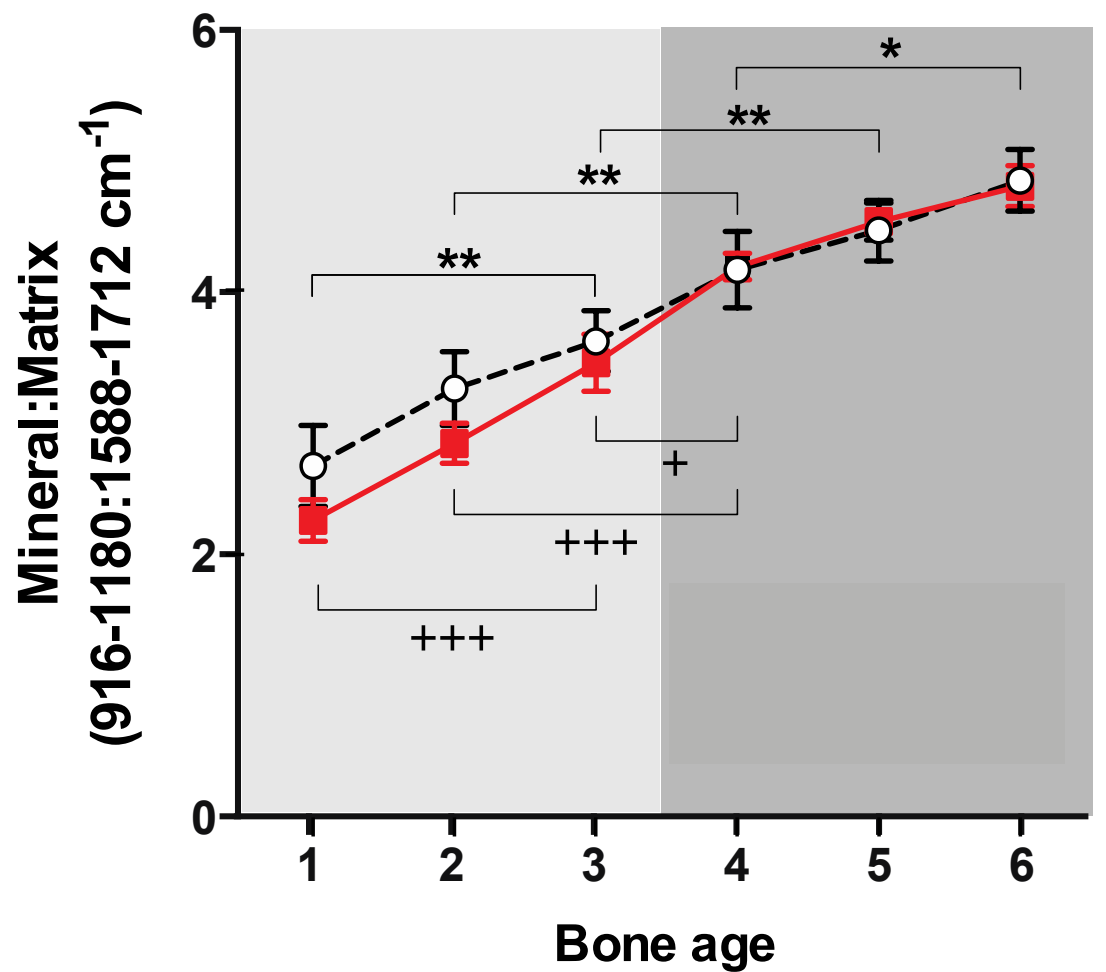
Keith Bambery



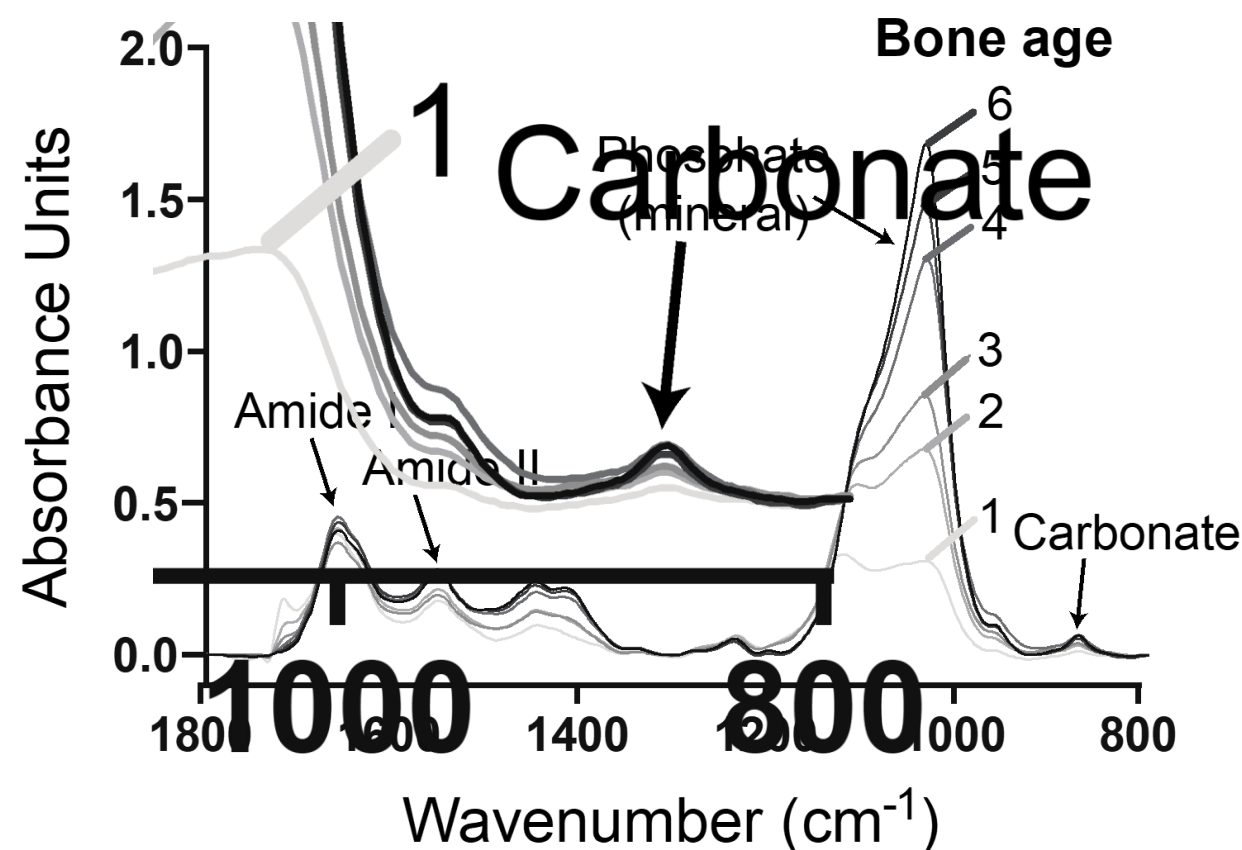
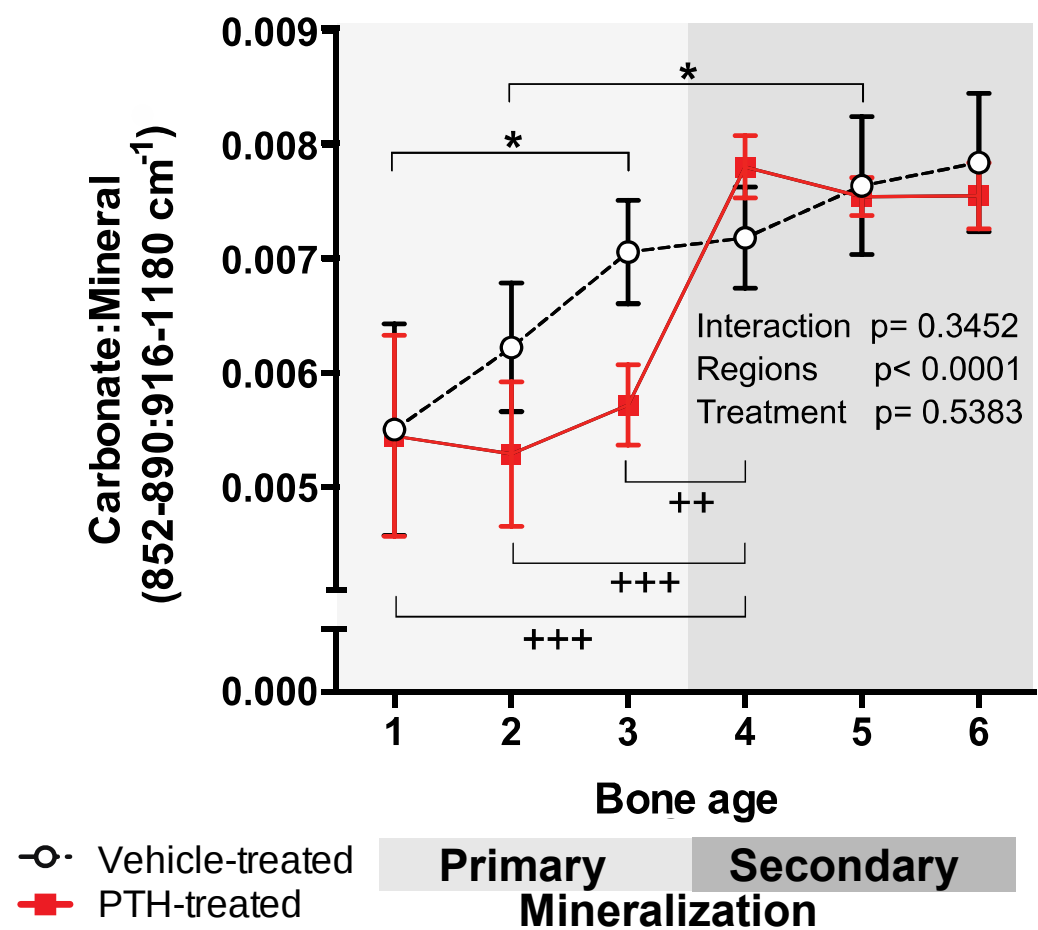
Mark Tobin



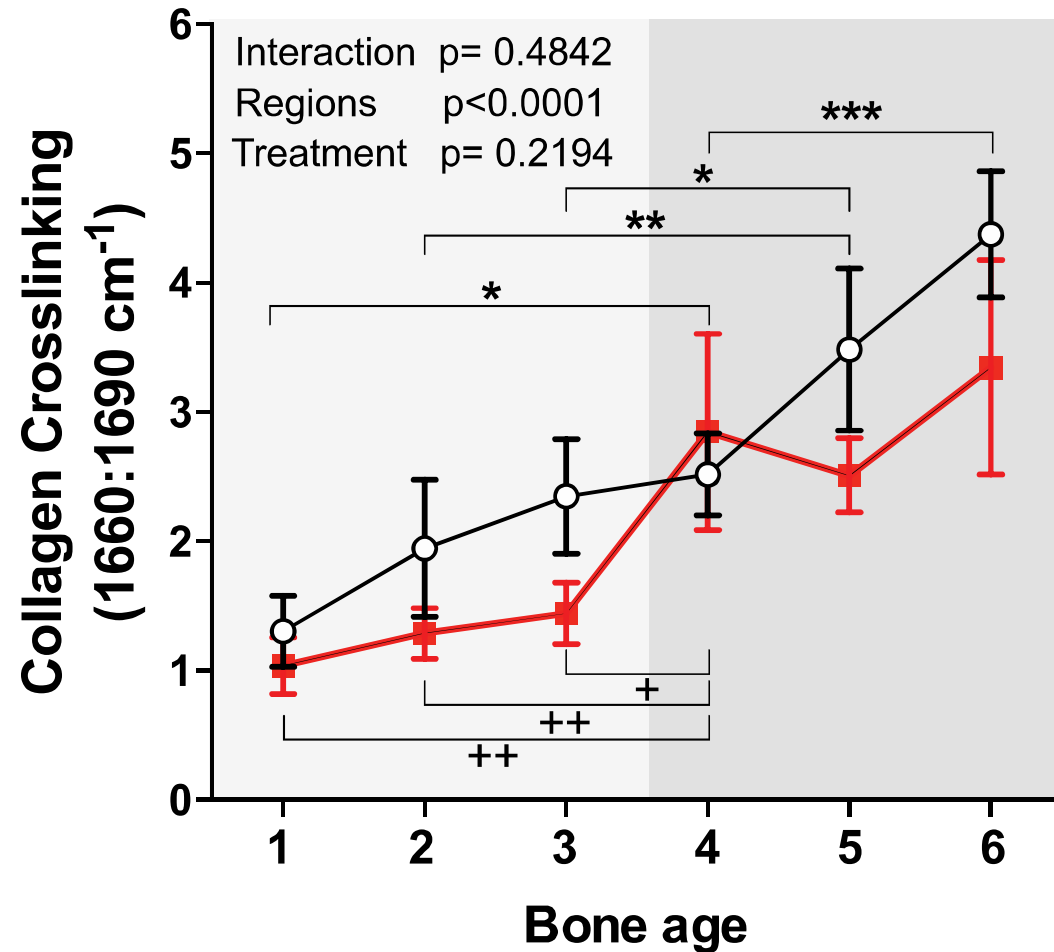
Relative heights at defined wavenumbers = quantitative information on matrix components



Increasing carbonate substitution within the apatite crystal with bone age



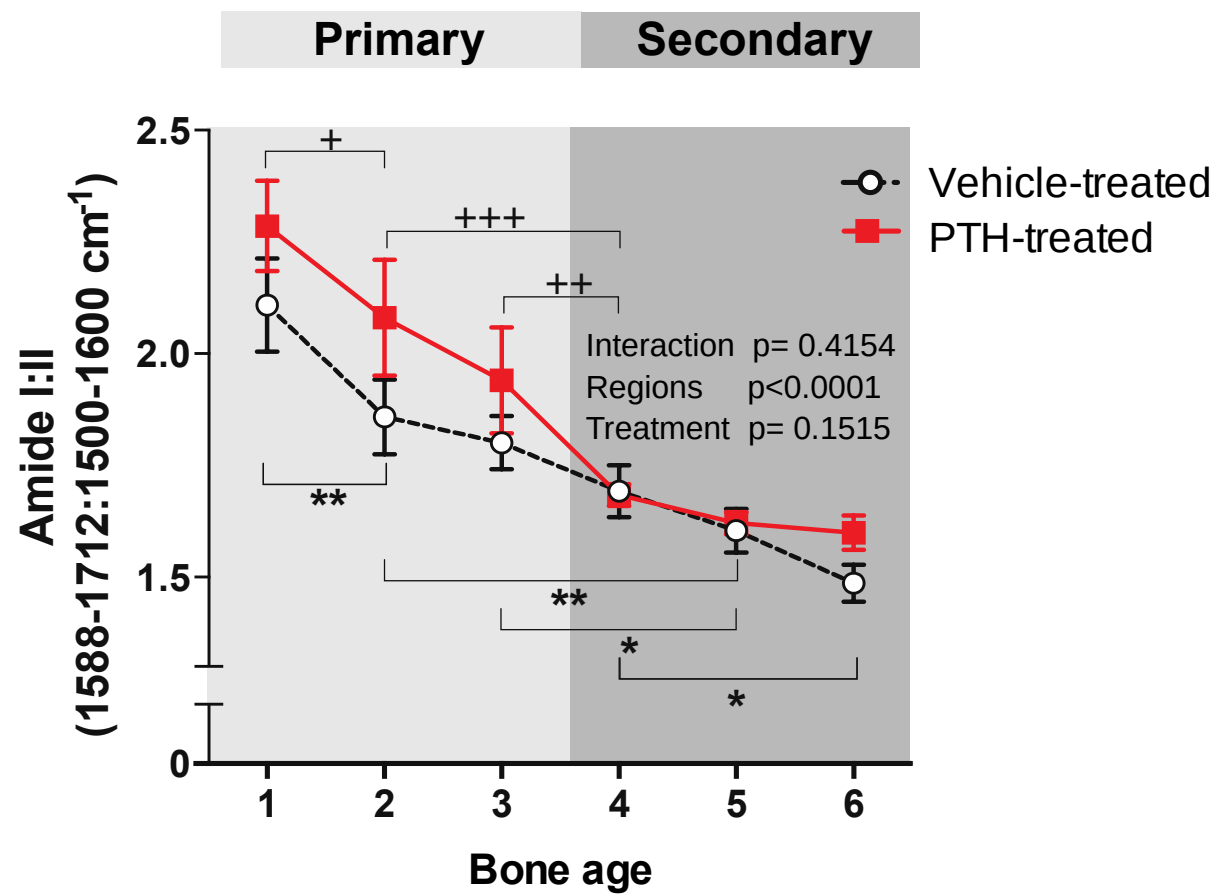
Increasing collagen crosslink maturation (Pyridinoline:deH-DHLNL) with bone age



(Vrahnas et al, Bone 2016)

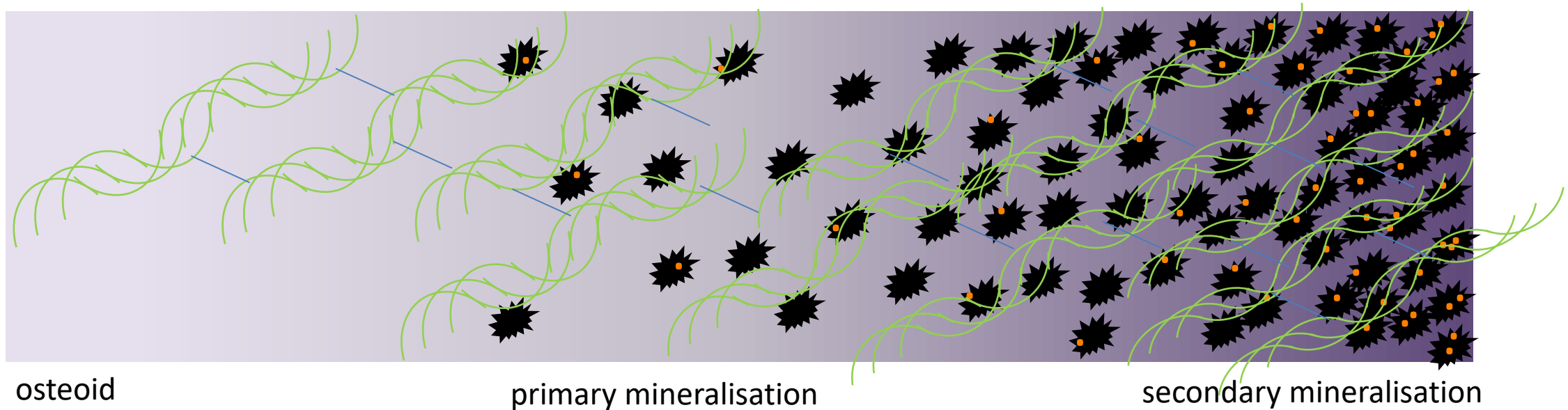
(Yamauchi, Sricholpech, Essays in Biochem 2012)

Collagen compression occurs as mineral accrues



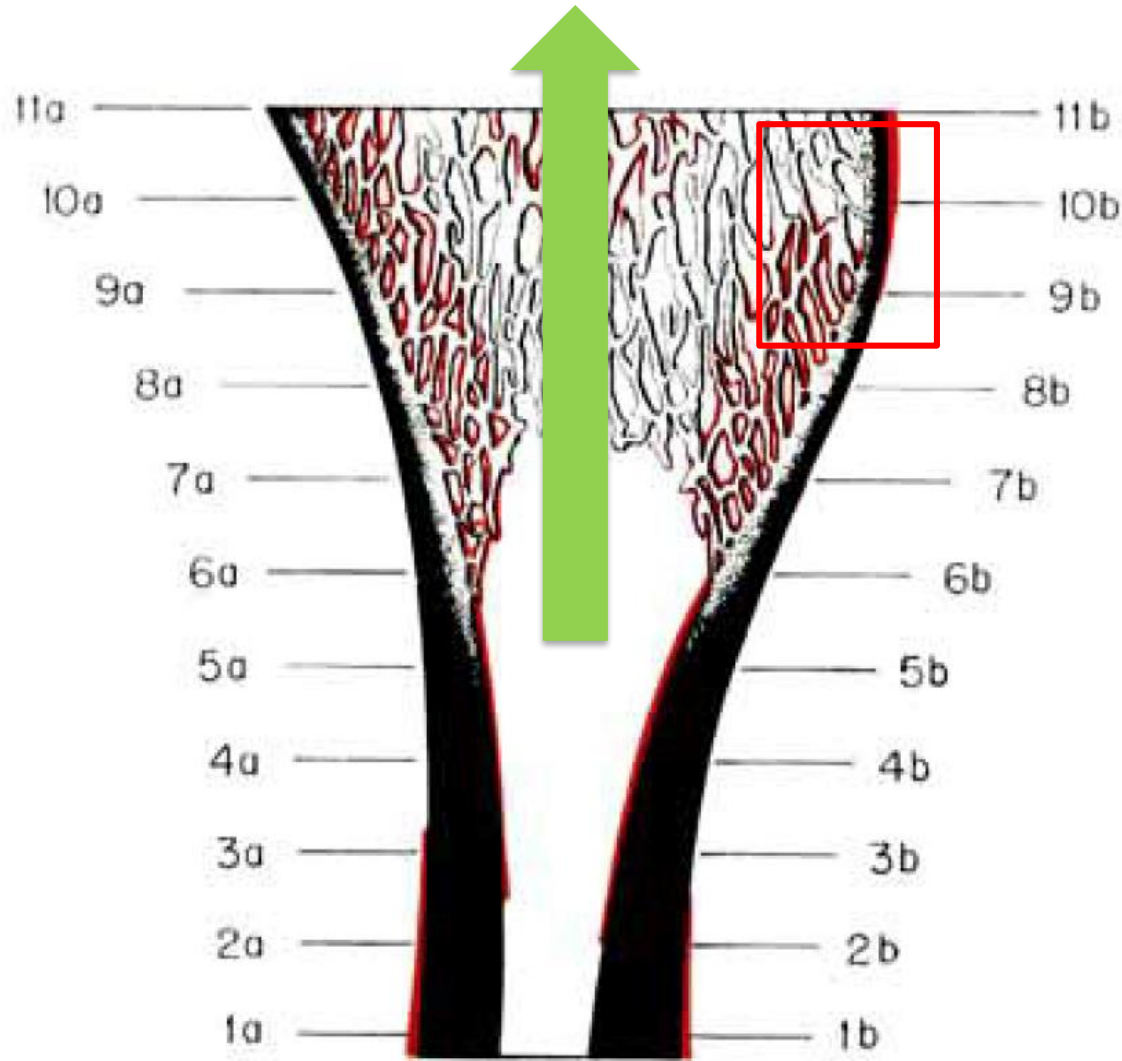
Four quantifiable steps occur simultaneously as bone matrix matures:

- Mineral crystals accumulate 
- Carbonate substitutes for phosphate within bioapatite crystals 
- Collagen compresses 
- Collagen crosslinks mature 



(Vrahnas et al, Bone 2016)

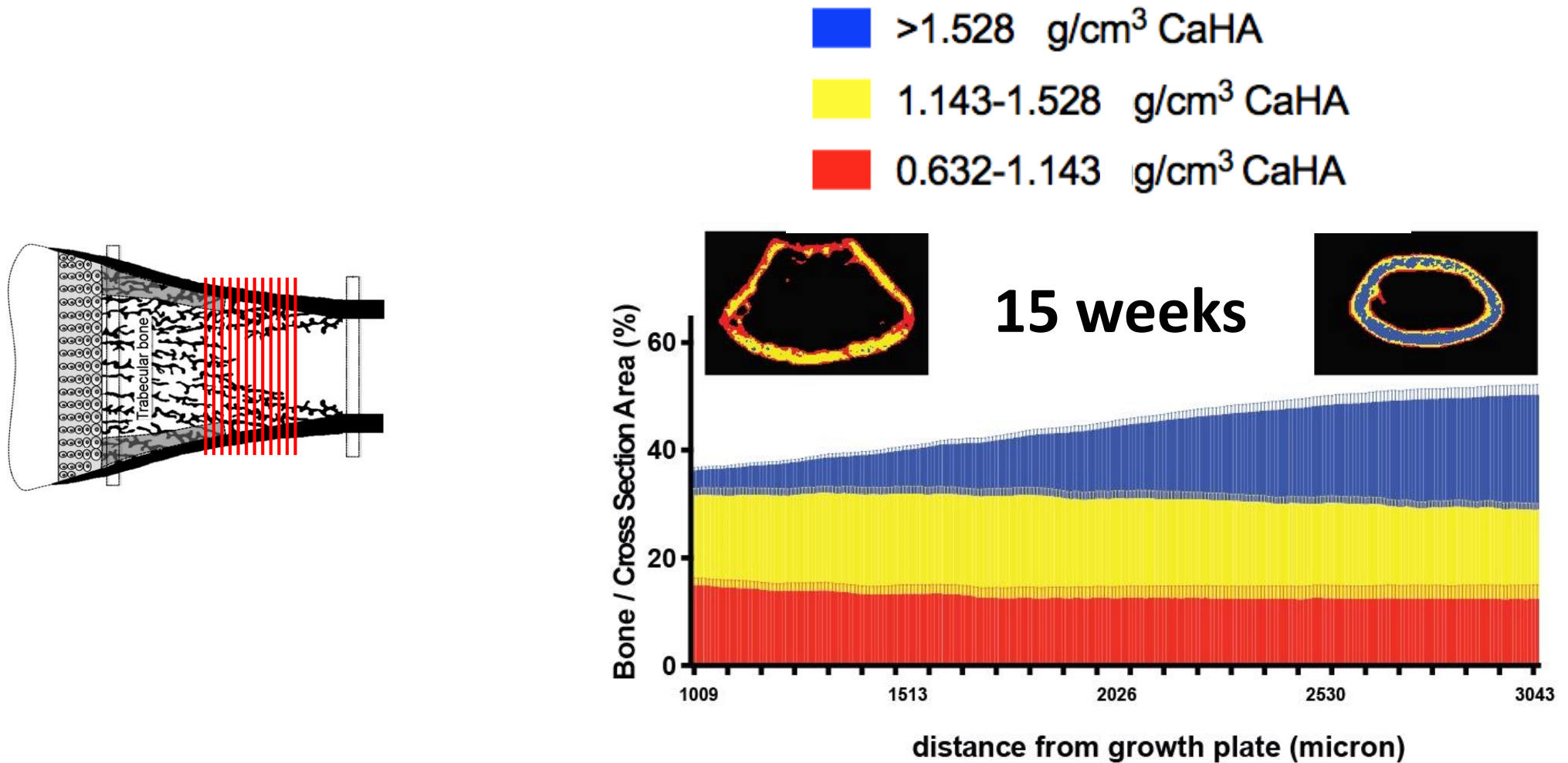
Cortical bone develops through coalescence of trabeculae



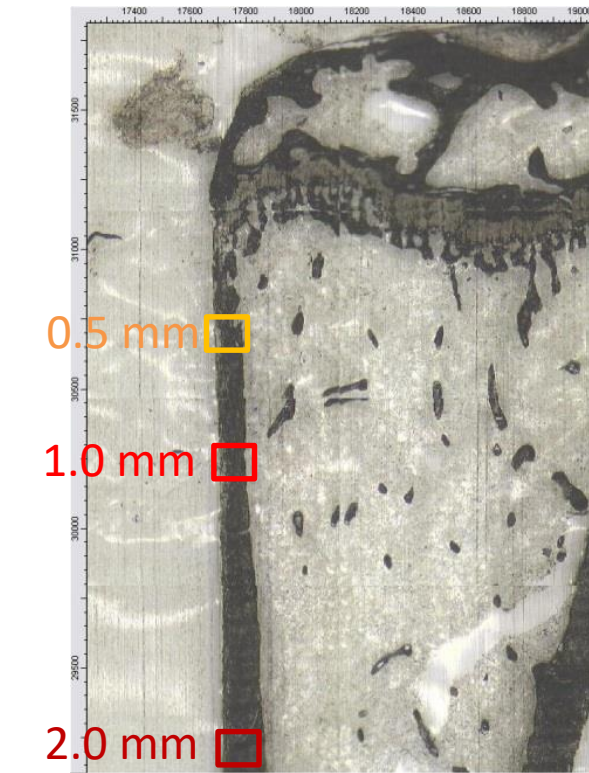
Metaphysis

Coalescence of growth plate-derived trabeculae at the periphery

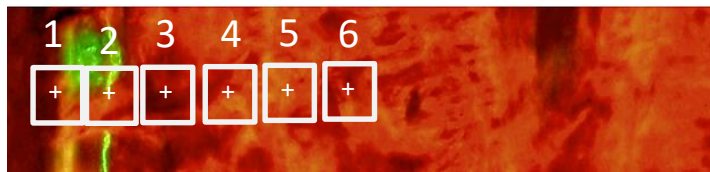
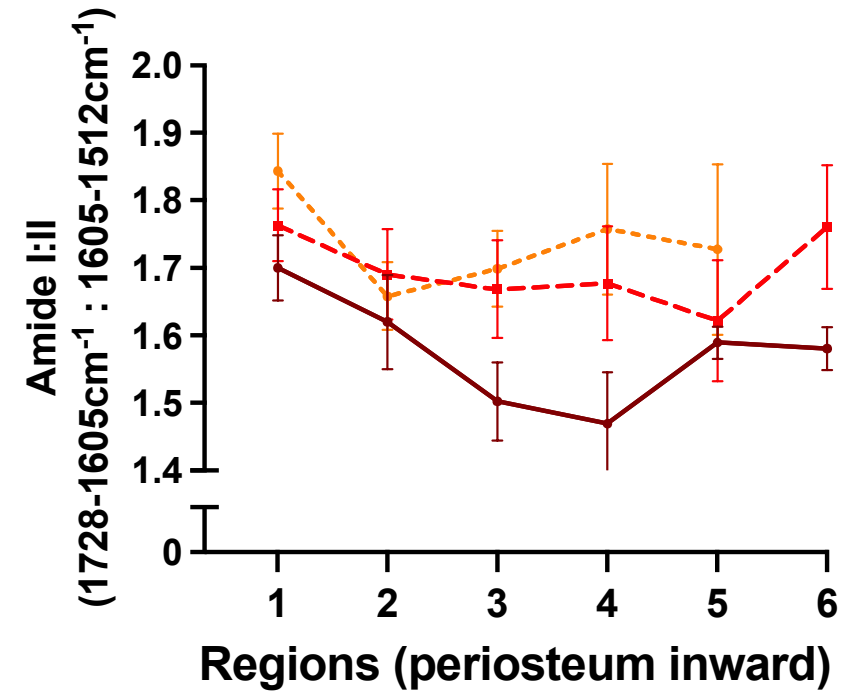
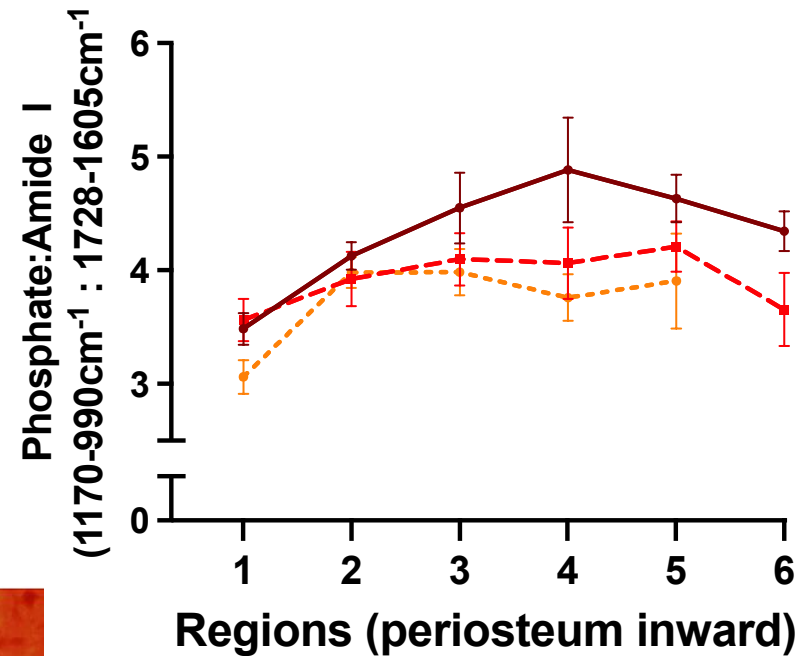
Micro-computed tomography reveals more bone of high mineral density in the most mature region



Younger bone incorporates less mineral at the periosteal edge, and doesn't reach the same maximal level



500um
1000um
2000um



Mineralisation
Primary
Secondary

New understanding of bone maturation

- Within lamellar bone, mineral crystals accumulate and incorporate more carbonate as the collagen becomes more cross-linked and compressed.
- Newly developing cortical bone has a blunted maturation profile compared to older bone further from the growth plate.

Measuring bone composition in disease



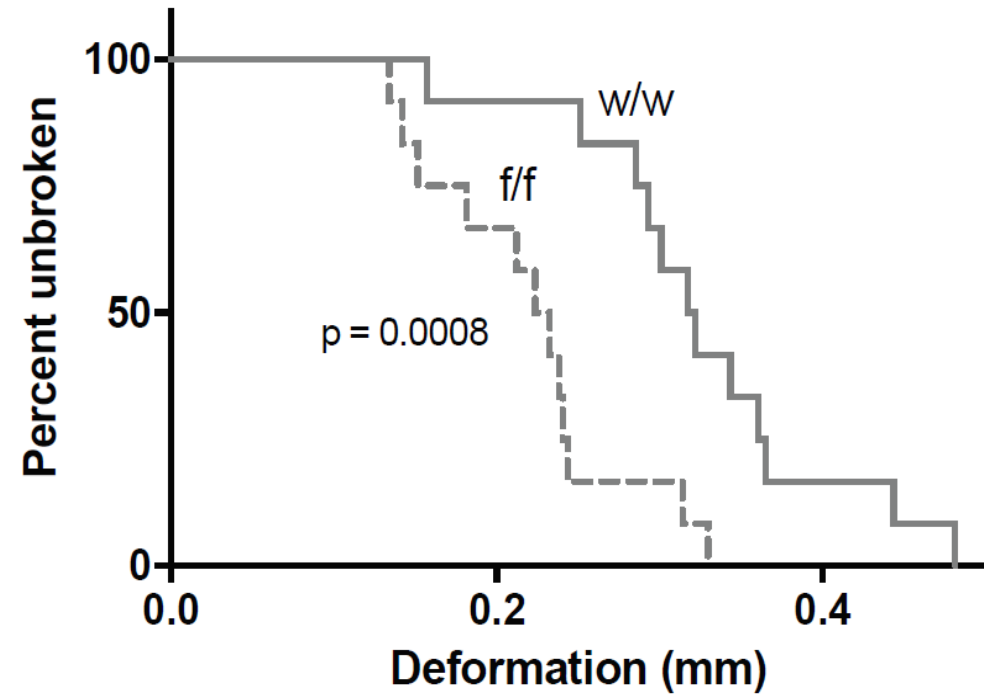
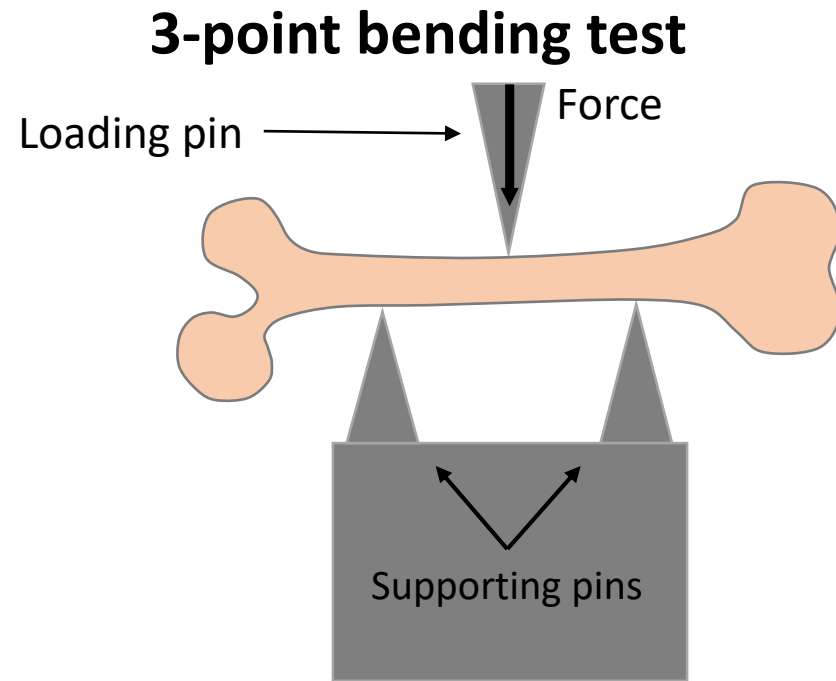
Dmp1Cre.EphrinB2^{f/f} mouse

***Dmp1Cre.EphrinB2 f/f* mouse**

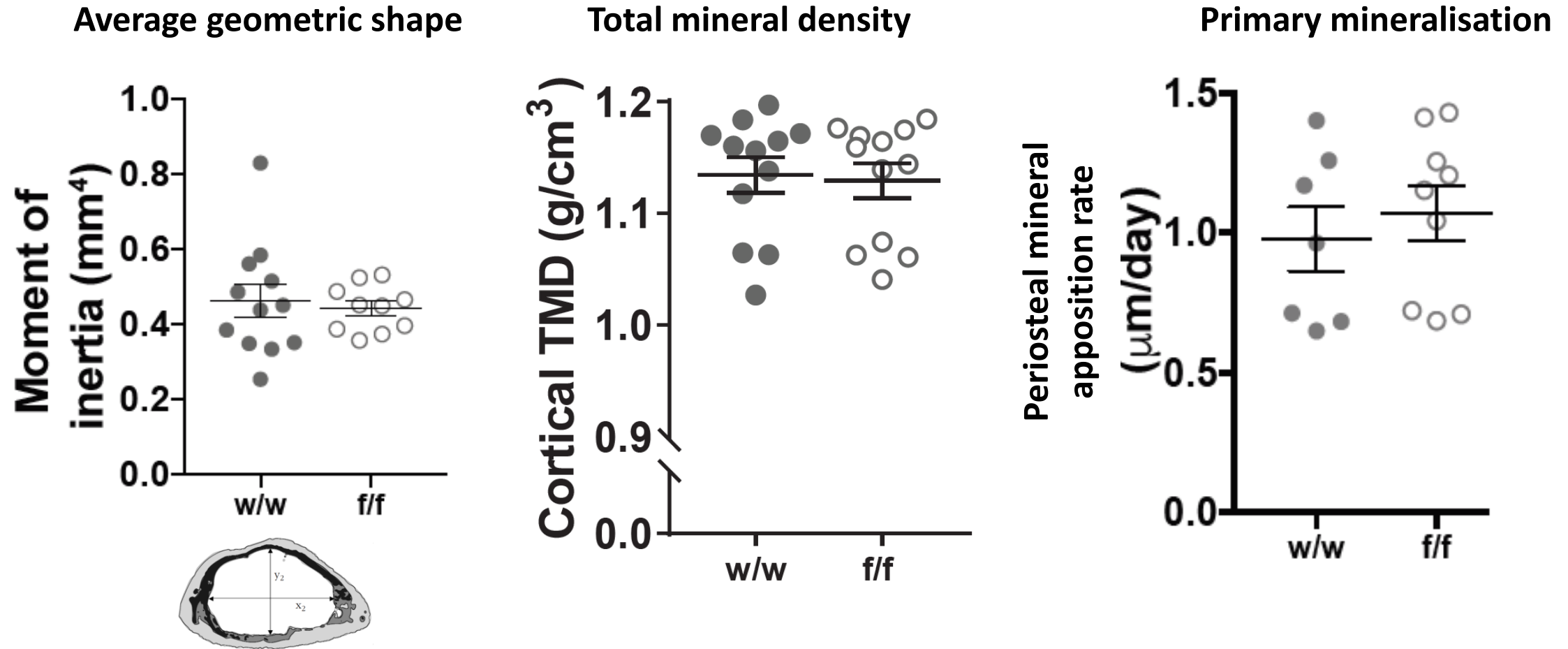
- EphrinB2 = tyrosine kinase receptor ligand
- Critical role in bone forming cells
- EphrinB2 in osteocytes?



Dmp1Cre.EphrinB2 f/f mice develop brittle bones

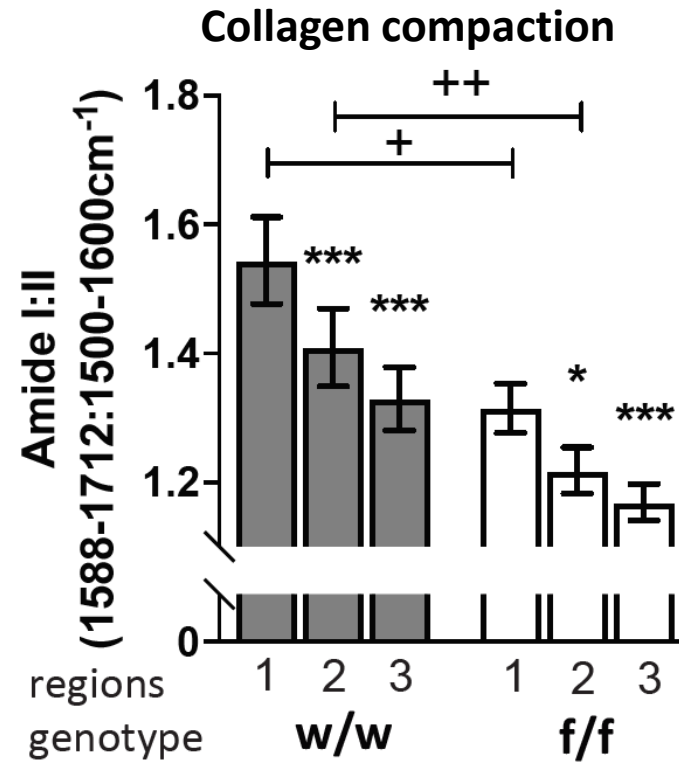
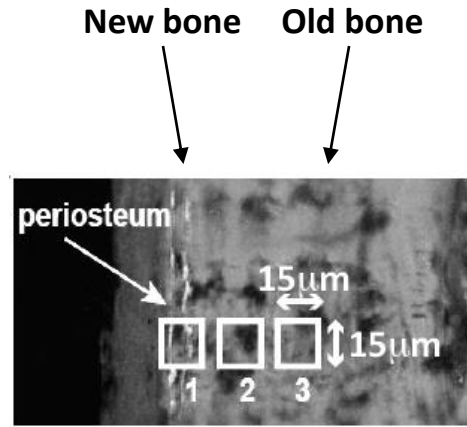


Dmp1Cre.EphrinB2 f/f mice have normal structural parameters



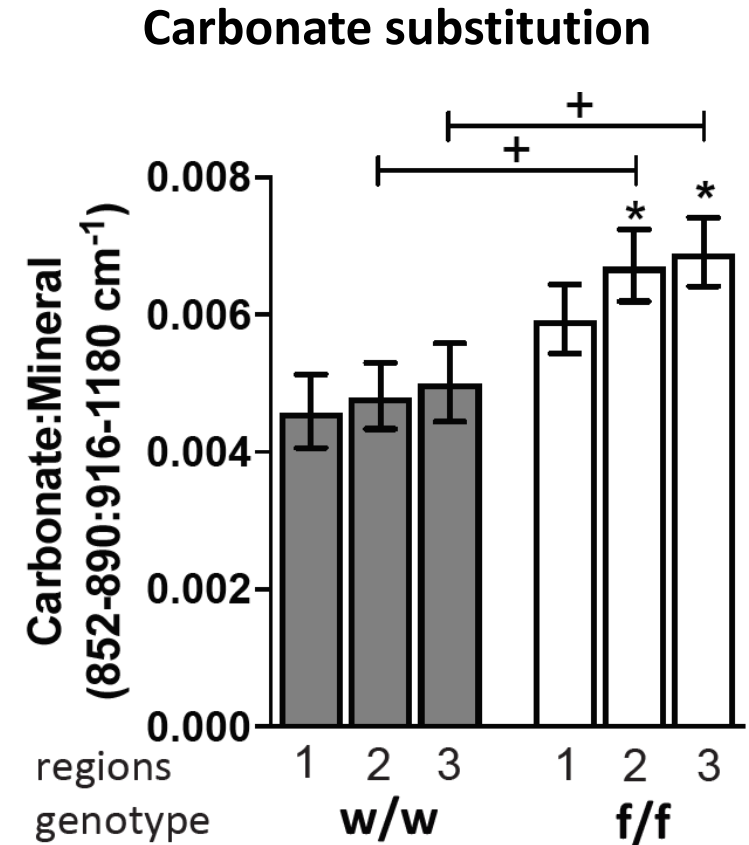
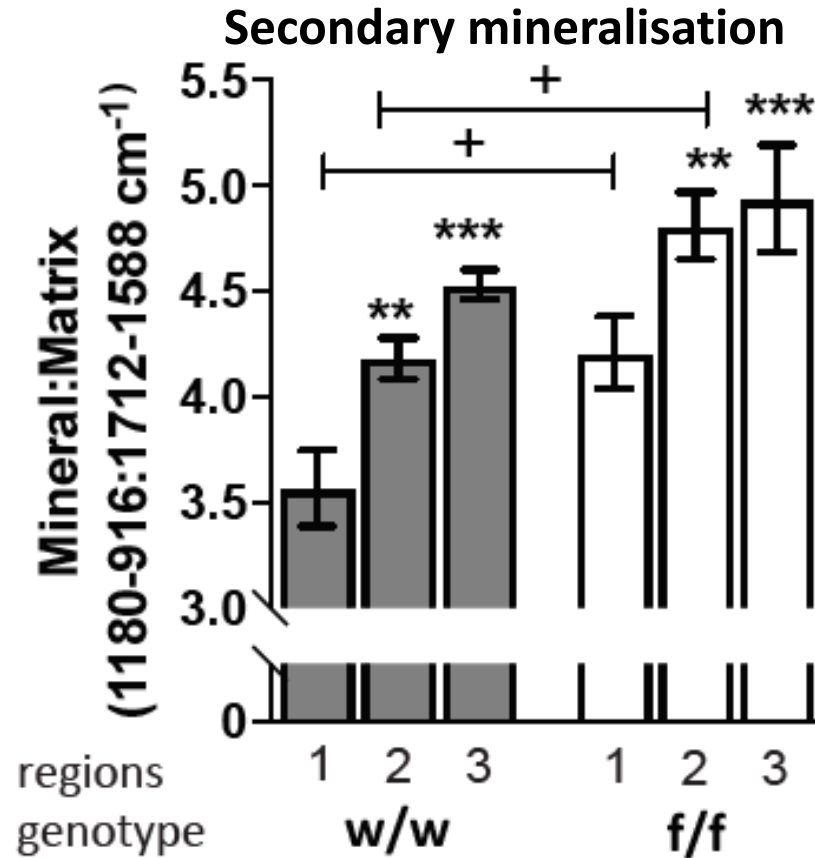
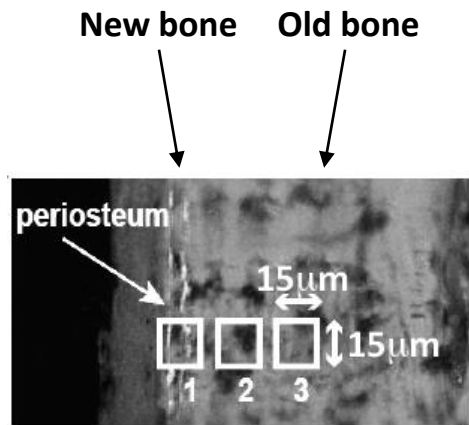
Experience of limitations of our methods that can detect structural but not material properties of bone

Dmp1Cre.EphrinB2 f/f mice exhibit higher collagen compaction



+, $p < 0.05$; ++, $p < 0.01$ genotype difference
*, $p < 0.05$; ***, $p < 0.001$ region difference
 $n = 12-13$ per group

Dmp1Cre.EphrinB2 f/f mice bones are hypermineralised and have more carbonate substitutions in the mineral



+, $p < 0.05$, genotype difference
*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, region difference
 $n = 12-13$ per group

Conclusion

- Investigating the bone material with sFTIR allowed us to describe the first pathway through which secondary mineralisation can be controlled
- Understanding how osteocytes regulate secondary mineralisation can lead to new therapeutic approaches to increase bone strength without the necessity of forming new bone

Acknowledgements



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