Analysis of osteocytes and cortical bone in mice

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Significance of the Topic:

Cortical bone is the main contributor to bone strength, and knockout mouse models provide information about signals controlling cortical bone development and strength. Although mice do not form a structure that contains Haversian systems, cellular mechanisms of cortical bone development are conserved between murine and human bone. Reporting of cortical phenotypes is essential when describing murine studies of bone. The osteocyte network within the cortex varies depending on bone age, and the site of measurement. Current methods for cortical analysis, including for measuring osteocyte and canalicular networks will be outlined.

Learning Objectives

1. Learn how murine cortical bone develops, how it compares with human cortical bone, and the implications for interpreting cortical bone measurements.
2. Learn a standard set of measurements of cortical bone by single-threshold micro-CT.
3. Learn why and when multiple thresholds may be useful.
4. Learn about current methods for imaging the osteocyte network in the cortex.

References (see also those listed with the figures overleaf)

Cortical bone development mechanisms:

- **Review**: Isojima T, Sims NA, Cortical bone development, maintenance, and porosity: genetic alterations in humans and mice influencing chondrocytes, osteoclasts, osteoblasts and osteocytes. *Cellular and Molecular Life Sciences.* 2021. 78:5755–5773 doi: 0.1007/s00018-021-03884-w. PMID: **34196732**

Cortical bone measurement methods:

- **Micro-CT standard analysis:** Bouxsein, M.L., et al. (2010), Guidelines for assessment of bone microstructure in rodents using micro–computed tomography. J Bone Miner Res, 25: 1468-1486. PMID: **20533309**
- **Micro-CT multiple thresholds:** Walker EC, et al. Measuring bone volume at multiple densities using micro-computed tomography. *Bio-protocol*, 11(1): e3873 (2021). PMID: 33732762
- **Micro-CT radial and longitudinal analysis** (one paper using multiple approaches): Jahaveri et al (2020), Lasting organ-level bone mechanoadaptation is unrelated to local strain. Science Advances, 6(10)eaax8301. PMID: 32181340

Osteocyte network imaging:

- **Ploton silver stain – stains osteopontin on canaliculi and cement lines:** Gaudin-Audrain, C., et al. Osteopontin is histochemically detected by the AgNOR acid-silver staining. *Histology and histopathology* 2008 Vol. 23 Issue 4 Pages 469-478. PMID: 18228204
- **Rhodamine G – infiltrates the lacuno-canalicular network** Van Tol, A.F., et al. The mechanoresponse of bone is closely related to the osteocyte lacunocanalicular network architecture. *Proc Natl Acad Sci U S A* 2020 Vol. 117 Issue 51 Pages 32251-32259. PMID: 33288694
- **Phalloidin – binds actin within cell bodies** Tiede-Lewis LAM, et al. Degeneration of the osteocyte network in the C57BL/6 mouse model of aging. Aging (Albany NY) 2017 9(10):2190-2208. PMID: **29074822**

Cortical bone development (two perspectives: from Isojima and Sims, 2020)

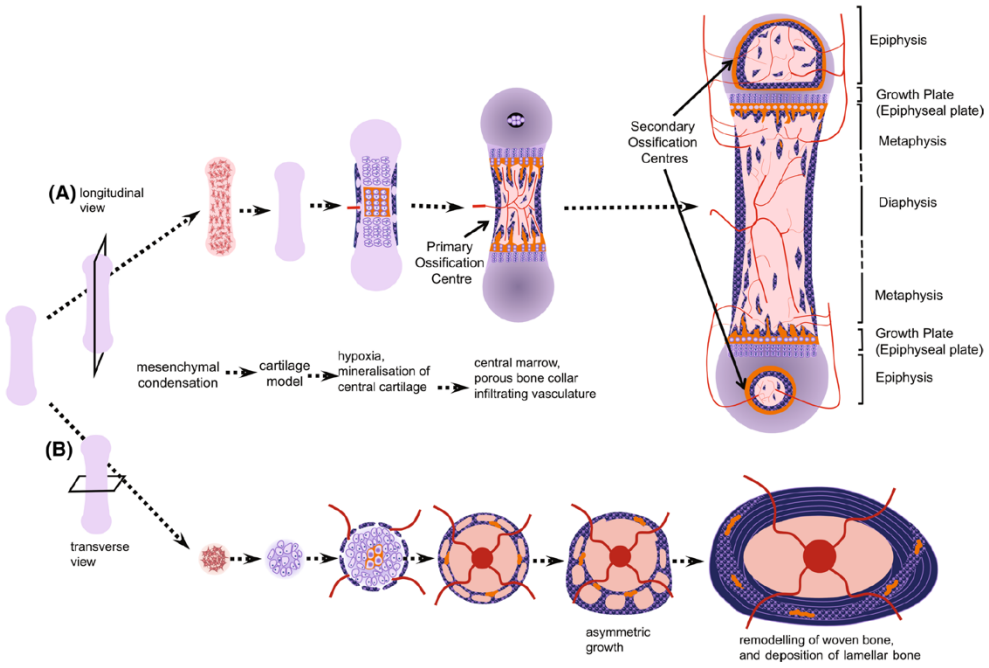


Fig. 1 Bone development, viewed as a longitudinal section (**A**) or a cross-section (**B**). During embryogenesis, mesenchymal stem cells condensate (**A**) and differentiate to form a cartilage model (anlagen) of the bone that is to form. As the bone grows, chondrocytes in the centre become hypertrophic and hypoxic, and release mineral, which accumulates within the cartilage; at the same time, new bone is deposited by osteoblasts on the perichondrium forming a porous "bone collar". Blood vessels are drawn to the primary ossification centre, bringing osteoclast precursors, which resorb a space into which marrow forms. As the marrow expands, remnants of mineralized cartilage remain (shown in orange), and woven bone continues to form at the bone collar, making a porous pre-cortical structure, with infiltrating vasculature. **A** Shows the formation of the secondary ossification centres, and the regions of the immature long bone, including the growth plate (epiphysis), diaphysis and metaphysis. Shown in **B**: under the influence of uterine and embryonic muscle activity, the bone grows asymmetrically through the formation of struts and rings of new bone. As the bone continues to grow asymmetrically after birth (modelling), this preliminary bone structure is gradually reshaped through bone resorption and bone formation. Remnants of mineralized cartilage and woven bone are gradually removed and replaced with lamellar bone (remodelling), but some remain

Standard parameters for micro-CT of femoral cortical bone (see table below from Bouxsein 2008):

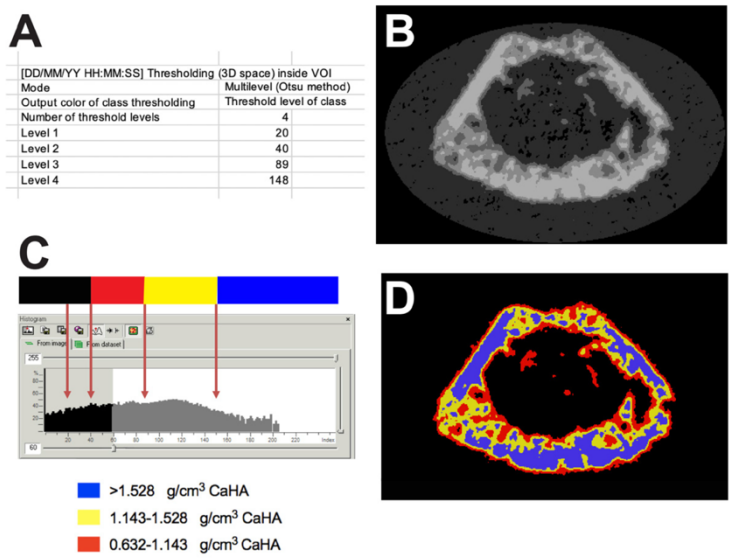
- Correct for bone length (see Bouxsein et al, 2008)
- Segmentation of trabecular and cortical bone is critical! Sometimes it is not possible.
- The diaphysis and the metaphysis are both interesting, and for different reasons!
- In addition to the below, I would add bone width: Craniocaudal diameter, mediolateral diameter
- Note that endocortical perimeter varies with complexity / segregation!
- Cortical porosity: Site, threshold and resolution is important – reality-check your data!!

Table 3. Definition and Description of Outcomes for Cortical Bone Morphology

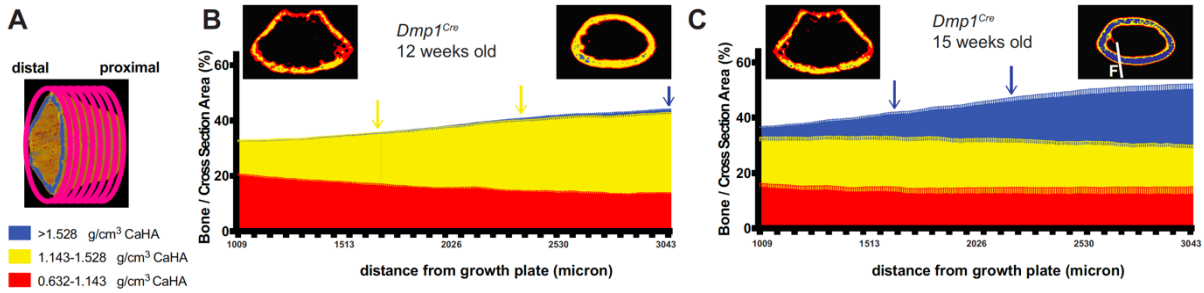
Abbreviation	Variable description	Standard unit
Tt.Ar	Total cross-sectional area inside the periosteal envelope	mm^2
Ct.Ar	Cortical bone area = cortical volume (Ct.V) ÷ (number of slices × slice thickness)	mm^2
Ma.Ar	Medullary (or marrow) area	mm^2
Ct.Ar/Tt.Ar	Cortical area fraction	%
Ct.Th	Average cortical thickness	mm
Ps.Pm	Periosteal perimeter	mm
Ec.Pm	Endocortical perimeter	mm
I_{ap}	Moment of inertia about the anteroposterior axis	mm^4
I_{ml}	Moment of inertia about the mediolateral axis	mm^4
I_{max}	Maximum moment of inertia	mm^4
I_{min}	Minimum moment of inertia	mm^4
J	Polar moment of inertia	mm^4
Ct.Po	Cortical porosity: In a given cortical region, the volume of pores (Po.V, mm^3) ÷ total volume of cortical bone compartment (Ct.V, mm^3)	%
Po.N	Pore number	n
Po.V	Total pore volume	mm^3
AvgPo.V	Average pore volume = Po.V ÷ Po.N	mm^3
Po.V.SD	Standard deviation of pore volume	mm^3
Po.Dn	Pore density = pore number (Po.N, n) ÷ total volume of cortical bone compartment Ct.V (mm^3)	mm^{-3}

Note: Variables in bold are the minimal set of variables that should be reported when describing cortical bone morphology.

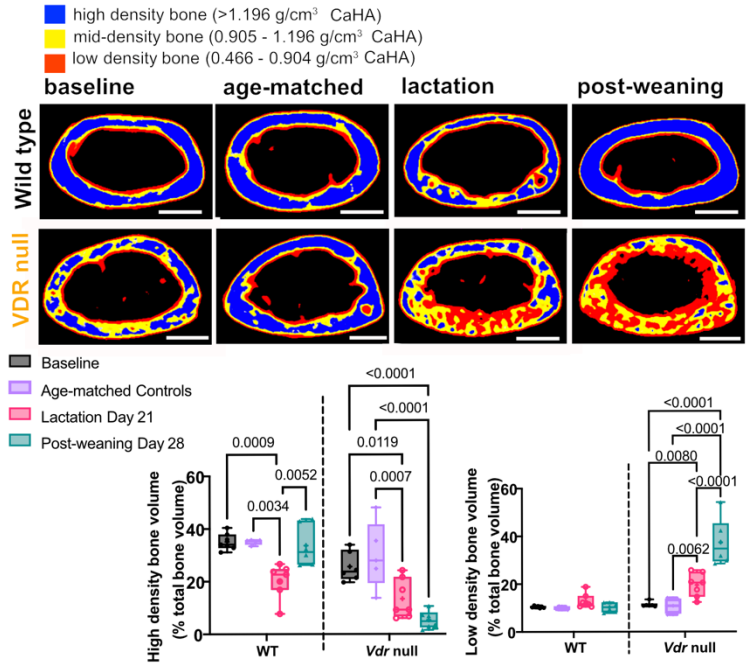
Use of multiple thresholds within the same piece of bone: Walker EC, et al. Measuring bone volume at multiple densities using micro-computed tomography. *Bio-protocol*, 11(1): e3873 (2021). PMID: 33732762



To show maturation along the metaphysis (Walker EC et al, eLife 2020 9:e56666. PMID: **32458800**

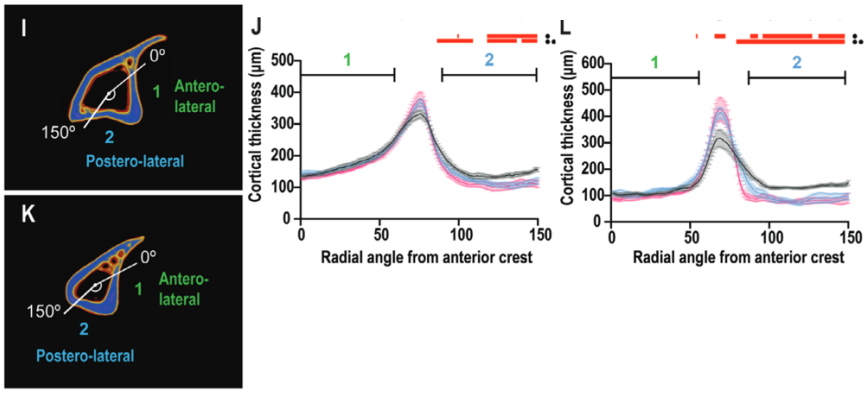


To measure under-mineralised cortical bone in lactation and vitamin-D deficiency (Ryan BA, McGregor NE et al, *J Bone Miner Res*, In Press).

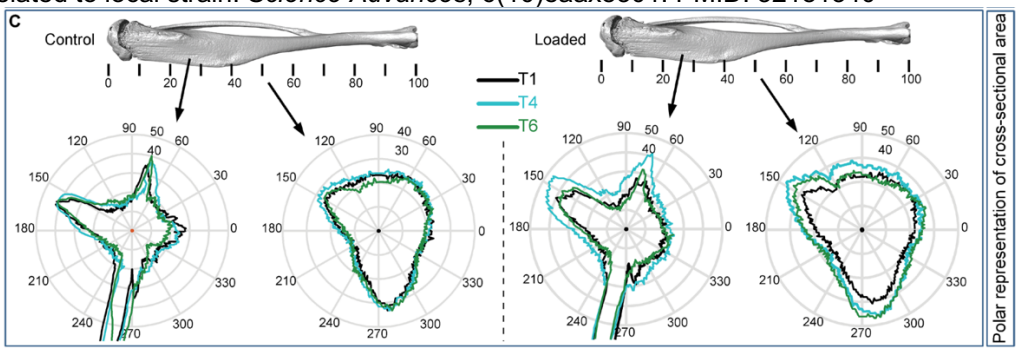


Some examples of radial bone analyses:

Shape change: Chan, ASM et al. Bone Geometry Is Altered by Follistatin-Induced Muscle Growth in Young Adult Male Mice. *JBMR Plus* 2021 Vol. 5 Issue 4 Pages e10477



Load-induced bone formation: Jahaveri et al (2020), Lasting organ-level bone mechanoadaptation is unrelated to local strain. *Science Advances*, 6(10)eaax8301. PMID: 32181340



Load-induced formation (corrected for contralateral limb): McGregor NE et al (2022), STAT3 Hyperactivation Due to SOCS3 Deletion in Murine Osteocytes Accentuates Responses to Exercise- and Load-Induced Bone Formation. *J Bone Miner Res* 37 (3): 547-558. PMID: 34870348