Preliminary descriptions of bone histology in an individual with a possible Klippel-Feil Syndrome, Type III from Neolithic Northern Vietnam

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1. Background

An individual (MB07H1M9 (H1M9), from the Northern Vietnamese Mán Bạc population (3,500 - 3,800 BP) was previously diagnosed to have likely suffered from Klippel-Feil Syndrome Type III (KPS III) (Oxenham *et al.,* 2009). He may have lived as part of the society for approximately 10 years after experiencing minimally paraplegia and potentially complete or intermittent quadriplegia in late childhood/early adolescence (Tilley and Oxenham, 2011). A case of childhood limb paralysis from the archaeological record has never before been assessed using hard tissue histology techniques. Here we present preliminary bone microarchitectural findings from H1M9's right humerus and femur to understand how his condition impacted skeletal physiology and growth.

Pathology context and behavioural impact

KPS III is a congenital disability. In H1M9 it was diagnosed by the manifestation of "*extreme disuse atrophy of lower and upper limbs, full ankylosis of all cervical and the first three thoracic vertebrae, a permanent torticollis, and bilateral temporomandibular joint degeneration*" (Tilley and Oxenham, 2011 p.36). This would suggest that H1M9 had severe mobility restrictions including a complete loss of voluntary lower limb movement, reduced upper body movement, and neck flexion constraints (Tilley and Oxenham, 2011, sup.). There are no case studies that directly describe the microarchitecture of humeral or femoral KPS III associated paraplegia/quadriplegia.

Bone tissue histology, paraplegia, and immobility

Bone biology is determined by the matrix resorptive cells (osteoclasts) and depositional cells (osteoblasts). A key requirement to bone health is mechanical stimulus which stimulates the nutrient rich interstitial fluid (Cowin and Cardoso, 2015). A "U" shaped relationship between mechanical stimulus and bone turnover is theorised (Fig 1). Below the minimum effective strain required to inhibit bone turnover (MES_r), osteoclastic activity is increased so that there is net bone loss (Robling et al., 2009; Fig. 1). There are only a few clinical and archaeological case studies assessing the impact of disuse (Wojda et al. 2013; Lazenby and Pfeiffer, 1993) and immobility (Schlecht *et al.,* 2012) on bone remodelling. However these do not provide information on microarchitectural growth and modelling changes.

As the onset of H1M9's immobility was experienced during ontogeny, his bone metabolism and limb growth was impacted (Olsen and Wade, 1967; Duval-Beaupere *et al*., 1983). During growth, a diaphysis re-shapes to adjust for biomechanical needs. Matrix is deposited and resorbing and this is referred to modelling drift (Fig. 2). Endosteal lamellae pockets are regions of new bone that are minimally impacted by remodelling as a result of these drifts (Maggiano et al. (2011; 2015). Bone microstructural impacts on bone modelling and drifts have not been described for those who experienced paraplegia during ontogeny.

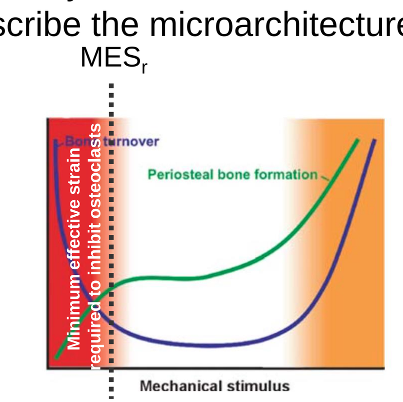


Figure 1: U shaped relationship (Robling *et al.* 2006)

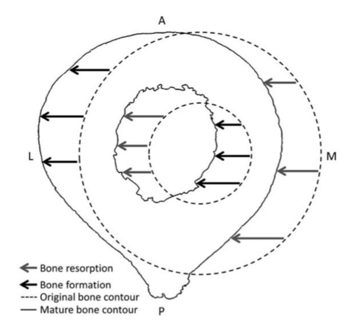


Figure 2: Modelling drifts of femur (Robling *et al.* 2006)

2. Aim

To describe the histological patterns underlying the pathology and lifestyle of individual MB07H1M9.

Hypothesis: Cellular activity and bone tissue patterns will reflect the extent of use and strain experienced by the limb

3. MB07H1M9 & Methods

Materials (Fig. 3)

- Right posterior femoral midshaft (Miszkiewicz, 2016)
- Left anterior humeral midshaft (Pitfield et al., 2017)

Methods

- Undecalcified, unstained, thin sections (100-120µm)
- Standard histological techniques were followed to process, image, and analyse the samples (Miszkiewicz, 2016).
- Transmitted light (TL) and linear polarised light (PL) to assess lamellae and collagen patterns.
- Images taken at X4, X10, and X20 magnification (Stout and Crowder, 2012)

Bone tissue analysis

- Bone microarchitectural evaluation of growth, modelling, and remodelling follows that of Maggiano et al. (2012), Robling et al. (2006) and Maggiano et al. (2015; 2016)

Biological Profile

Age: Young Adult: 20-29
Sex: Male
Ancestry: Mixed: East Asian & Australo-Papuan
(Tilley and Oxenham, 2011; Oxenham *pers comms* 2019)

Humerus (mm)

Shaft Circumference = 45.5
Anteroposterior Diameter = 14.9
Mediolateral diameter = 10.43
Cortical Width = 3.9

Femur (mm)

Shaft Circumference = 48.5
Anteroposterior Diameter = 12.39
Mediolateral diameter = 16.48
Cortical Width = 2.25

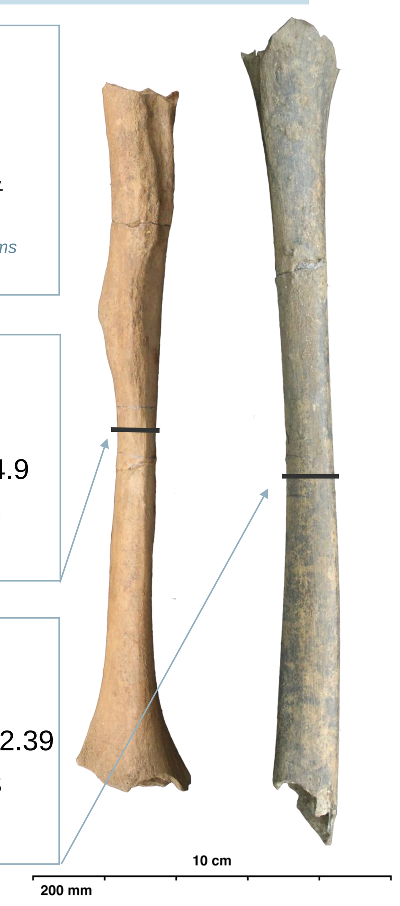
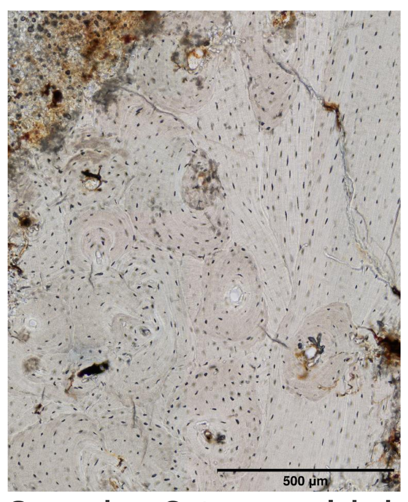


Figure 3: Left: humerus; Right: femur. Sample location indicated

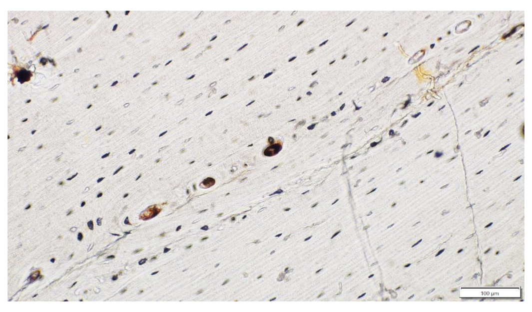
4. Results

Right Anterior Humerus

Plain Transmitted Light

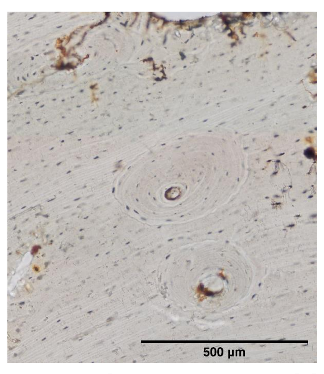


Secondary Osteon overlain by lamellae
- Early generations of targeted(?) secondary osteons Prior to modelling drifts. Biomechanical use?

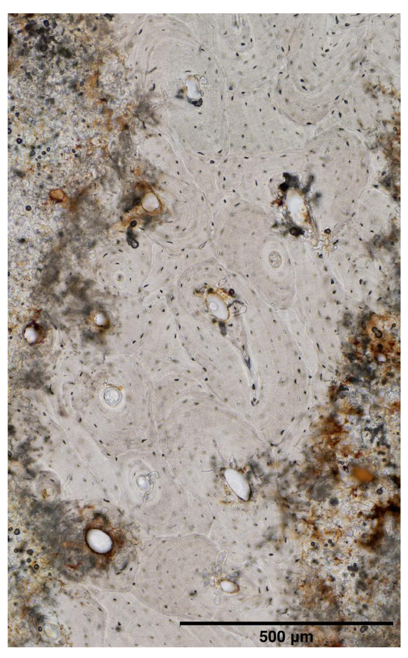


Primary Osteon
- Blood supply during growth and development of lamellae tissue

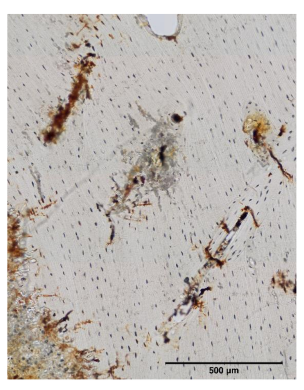
This ROI correlates with the humeral midshaft ELP in children and adults (Maggiano, 2015)



Secondary Osteon within new lamellae
- Targeted/non-targeted remodelling
- Bone metabolic and cellular activity



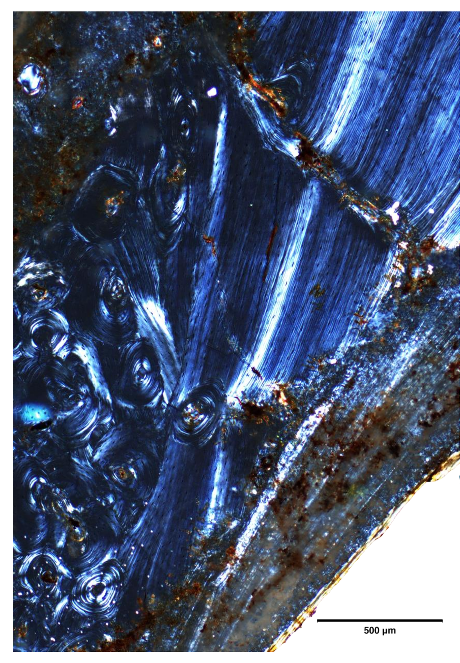
Generations of Secondary Osteon
- Targeted remodelling
- Biomechanical function in older bone?



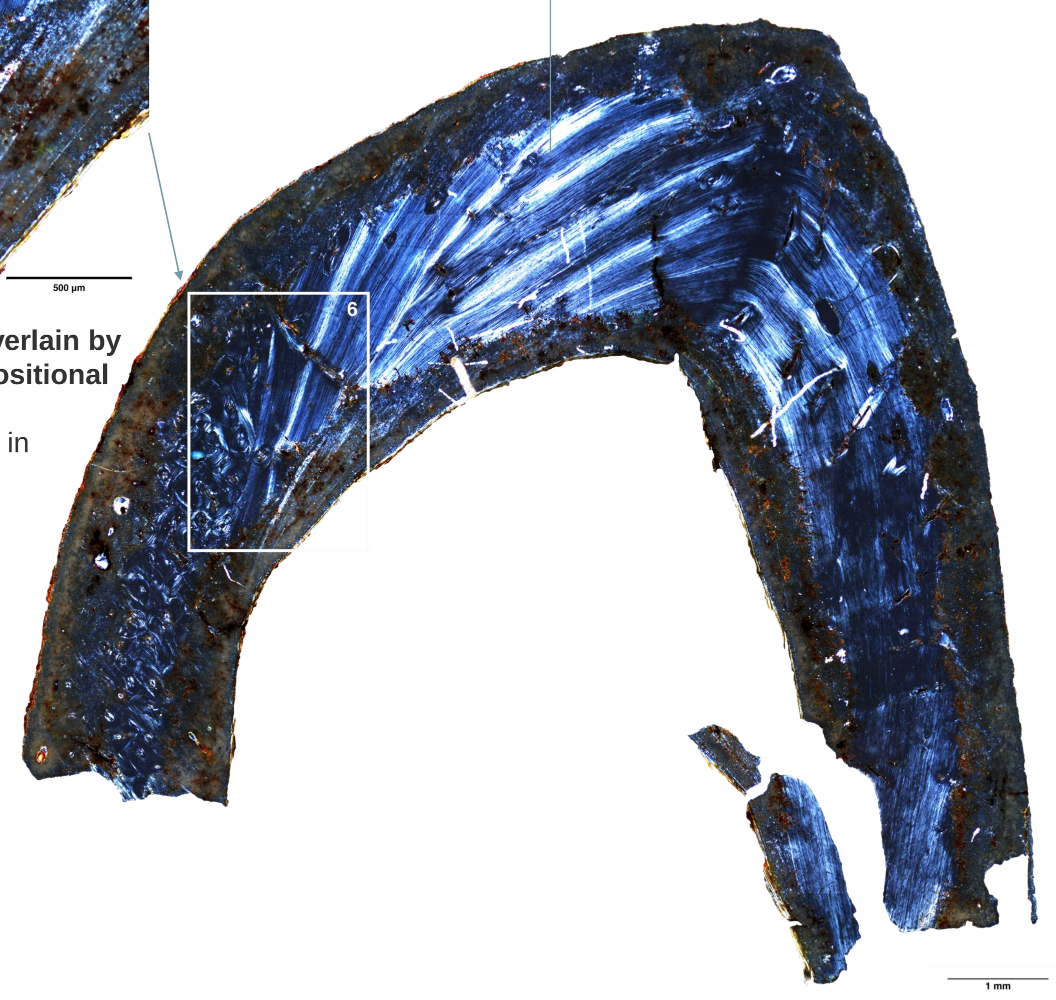
Series of Volkmann canals
- Radial vascular system
- Observed by Maggiano (2011) in the humeral ELP

Linear Polarised Light

Alternating collagen fibre orientation (anisotropy)
- Minimally remodelled ELP (Maggiano *et al*., 2015)
- Reflects a counter clockwise twist



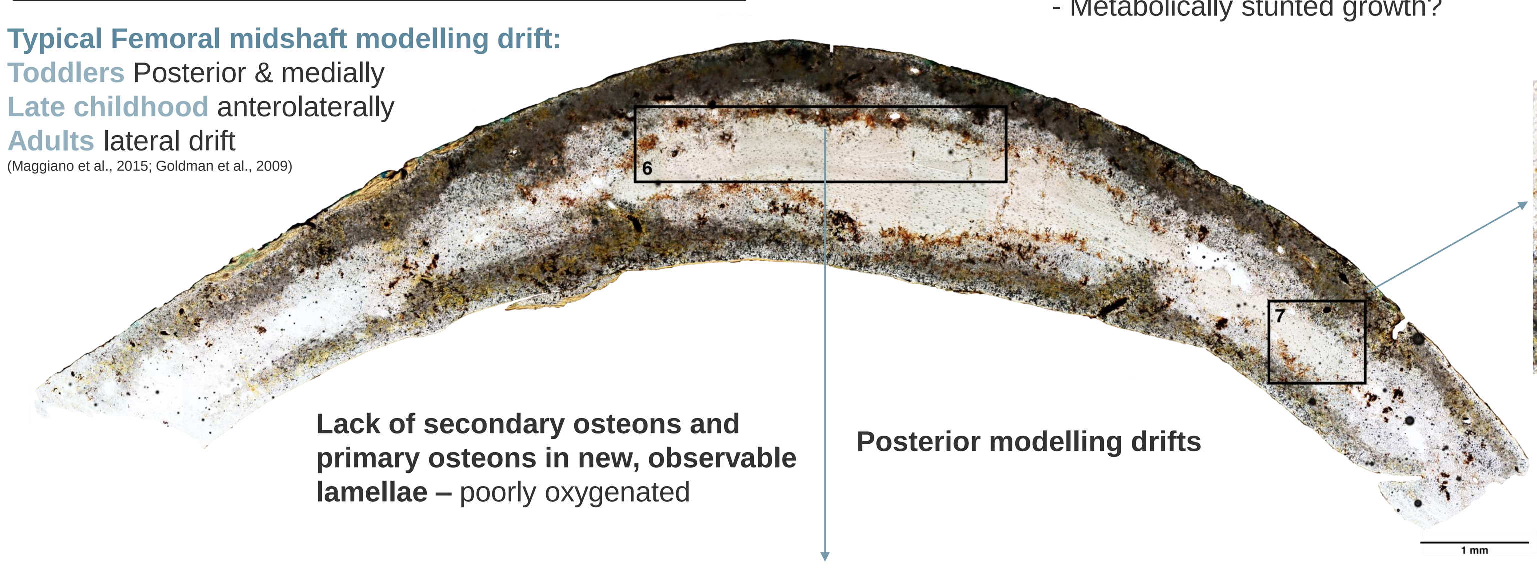
Secondary Osteon overlain by various lamellae depositional events
- Greater remodelling in "older bone"



Right Posterior Femur

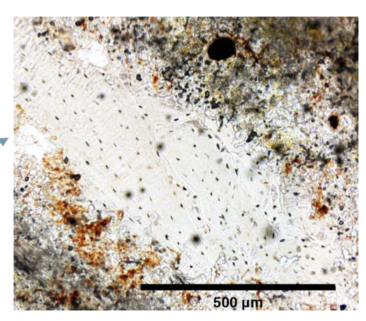
Typical Femoral midshaft modelling drift:
Toddlers Posterior & medially
Late childhood anterolaterally
Adults lateral drift
(Maggiano et al., 2015; Goldman et al., 2009)

Stunted growth reflective of early childhood
- Metabolically stunted growth?

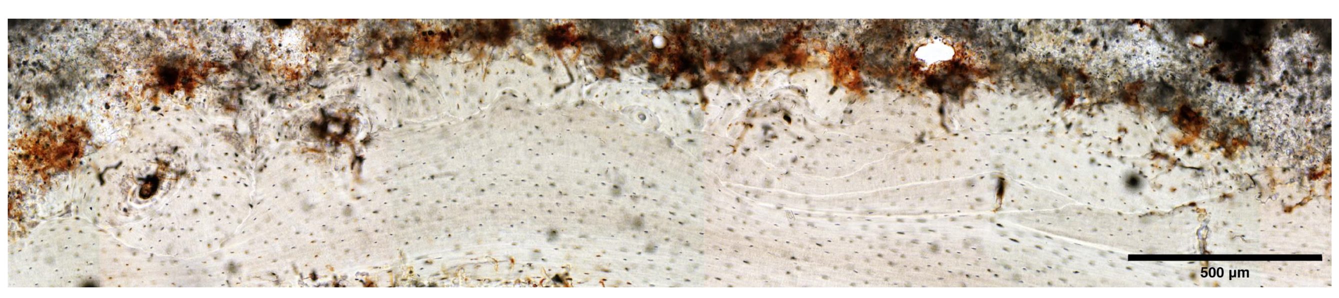


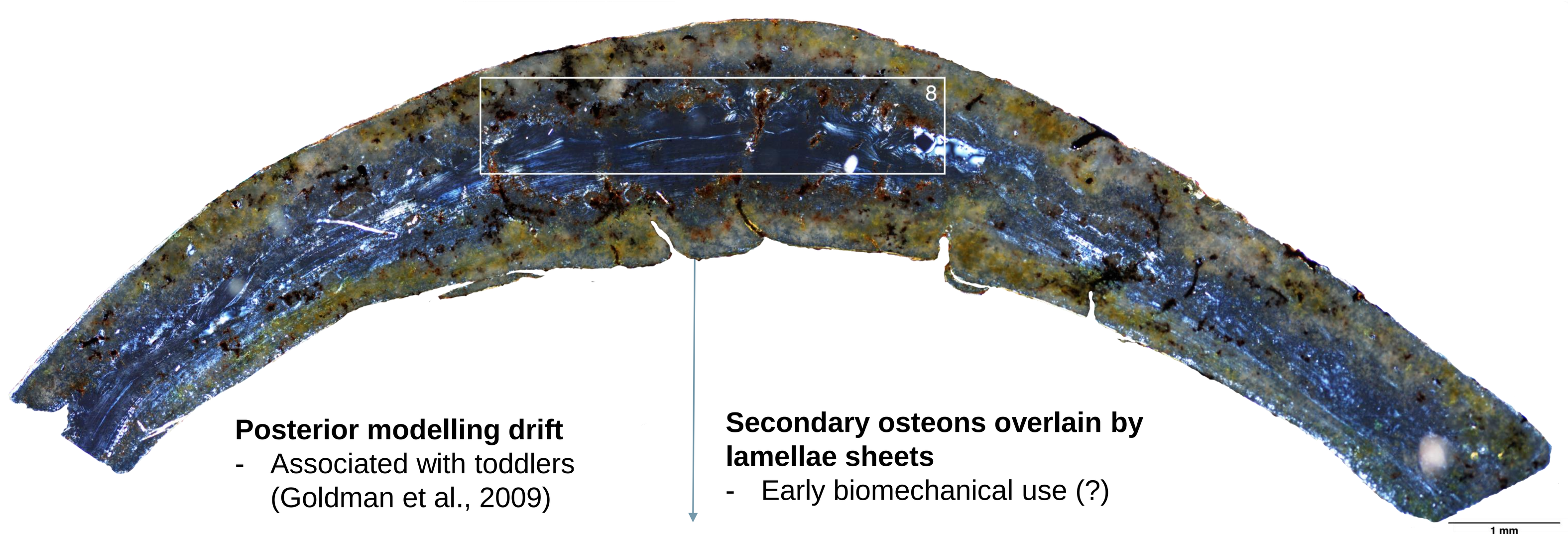
Lack of secondary osteons and primary osteons in new, observable lamellae – poorly oxygenated

Posterior modelling drifts



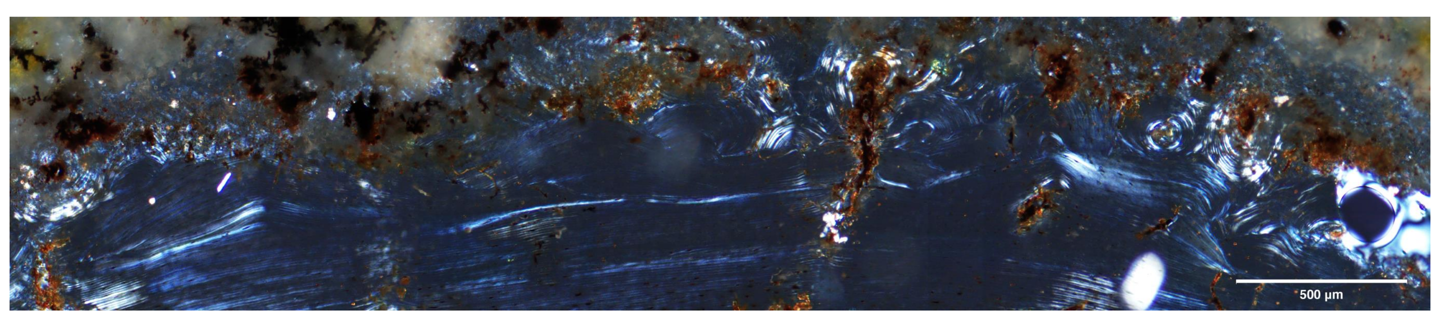
Series of lamella layers MEDIAL/LATERAL highlighting several depositional events of primary tissue





Posterior modelling drift
- Associated with toddlers (Goldman et al., 2009)

Secondary osteons overlain by lamellae sheets
- Early biomechanical use (?)



5. Discussion & Conclusion

Macroscopic and microscopic analysis highlight that bone growth may have been stunted as evidenced by atrophied limbs, thin cortices, and modelling patterns. This is most likely caused by immobility during ontogeny.

Humerus
- Regular growth and development patterns evident by anterior ELP formation associated with an adult diaphysis. However the lack of further periosteal formation, and low remodelling of the younger lamellae suggests reduced biomechanical loading.
- Present haversian vascular network (Medial/Lateral), and a vascular radial network (MEDIAL/LATERAL).
- Some secondary osteons within the ELP may highlight minimal mechanical loading, however these may be a product of mineral homeostasis and maintenance.
- Considerable remodelling in "older" bone not associated with modelling drifts. Possibly biomechanical function early in life.

Femur
- Modelling drift associated with toddler growth. Growth likely stunted by immobility from very early in life coupled with altered endocrine and metabolic functions (Olsen and Wade, 1967).
- Early remodelling events evident by Secondary osteons. Potential biomechanical function early in life.

6. References

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7. Acknowledgements

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