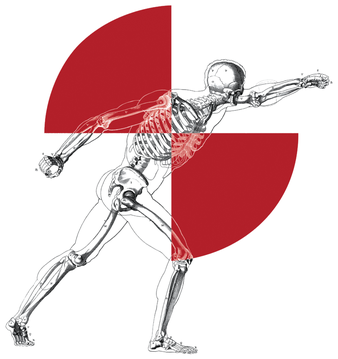

Regional Variation in Osteon Population Density at the Femoral Midshaft – Implications for the Asymptote

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INTRODUCTION

- Age estimation from the human skeleton can be conducted through a histological examination of remodeling events in cortical bone as a complement to traditional macroscopic methods, or when the necessary macroscopic elements are absent or damaged. Such techniques are largely concerned with the accumulation of osteons, the basic structural unit in cortical bone remodeling. The number of intact and fragmentary osteons is typically divided per mm^2 to calculate the osteon population density (OPD). After all primary lamellar bone is remodeled, however, new osteons remove evidence of previous ones, and OPD will then approach an asymptote and hinder age estimation.

- The OPD value at which the asymptote occurs varies by skeletal element, as does the age at which the asymptote is approached (Stout and Crowder 2012). Using data from Kerley (1965), Frost (1987) posited that the OPD asymptote at the femoral midshaft would be approximately $50/mm^2$, though this claim has never been adequately tested.

- In order to thoroughly investigate the OPD asymptote, this study examined the spatial distribution of all remodeling events across the entirety of the femoral midshaft. This was accomplished using GIS software, which has recently been recognized for its utility in visualizing patterns in human bone microstructure (Rose *et al*. 2012).

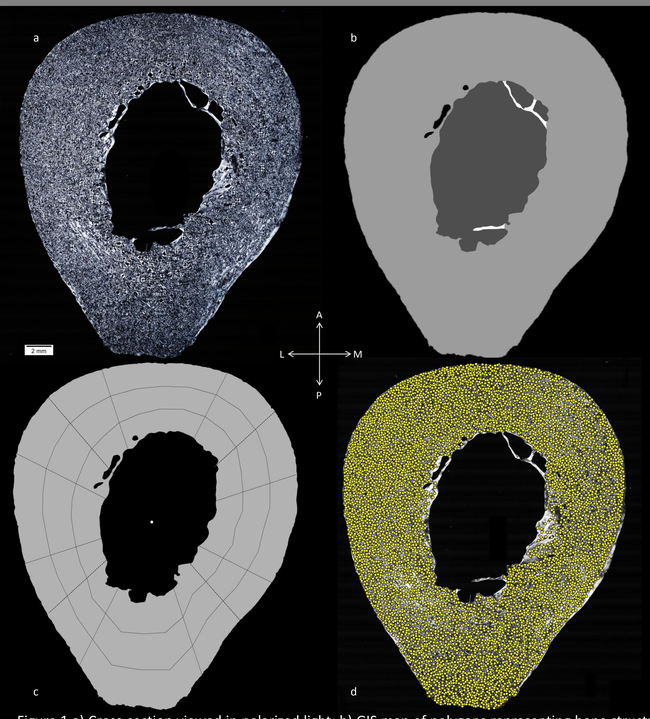

Figure 1 a) Cross-section viewed in polarized light; b) GIS map of polygons representing bone structure (light gray = cortical area, dark gray = medullary area, white = trabeculae, black = resorption spaces; c) GIS map of cortical area segmented: dashed and solid radiating lines represent APML quadrants and eighths, respectively, d) GIS map of remodeling events represented by yellow points.

MATERIALS AND METHODS

- Thirty complete cross-sections from the femoral midshaft of modern cadaveric donors were used for this study: 15 males and 15 females, ranging in age from 21-97 years (mean = 58.9; SD = 22.1 years). Age distribution was similar for both sexes (Mann-Whitney *U* test, p=0.486). No individuals exhibiting severe osteoporosis were included in the sample.

- Each cross-section was photographed under polarized light and micrographs were compiled into seamless images (Figure 1a). These images were imported into ArcGIS v10.1 (ESRI®), and polygon features were created to overlay the cortical, medullary, trabecular, and resorption areas (Figure 1b). To assess remodeling around the circumference and across the depth of the cortex, cortical area for each sample was digitally divided into anterior, posterior, medial, and lateral quadrants and eighths, as well as divided into periosteal, middle, and endosteal thirds (Figure 1c). Point features were used to mark all remodeling events (Figure 1c).

- A total of 230, 870 remodeling events were manually notated from all 30 samples. The total number of remodeling events contained within the area of each segmented cortical region was used to calculate its osteon population density. Density maps were also generated for each sample to visualize the clustering of remodeling events throughout the entirety of the cortex; these density maps depict locally defined OPD values at a fine scale that is not dependent on any particular sampling method (Figures 2-6). Scatter plots of OPD and age were also generated (Figure 7).

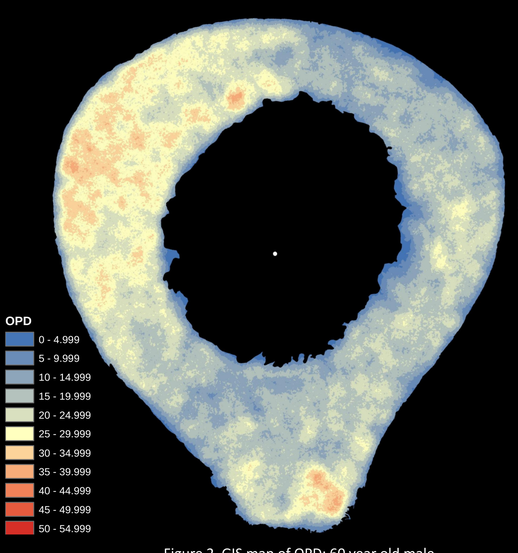

Figure 2. GIS map of OPD; 60 year old male

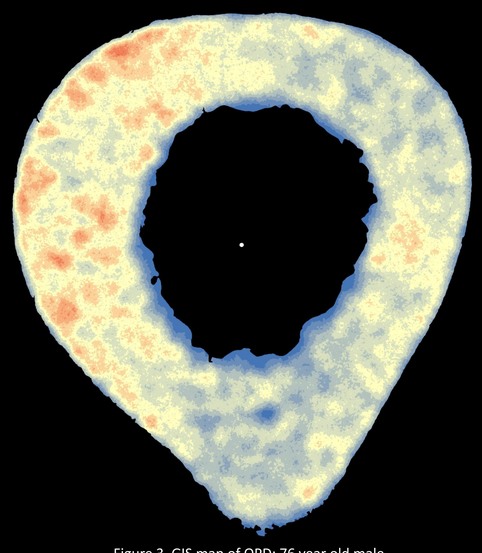

Figure 3. GIS map of OPD; 76 year old male

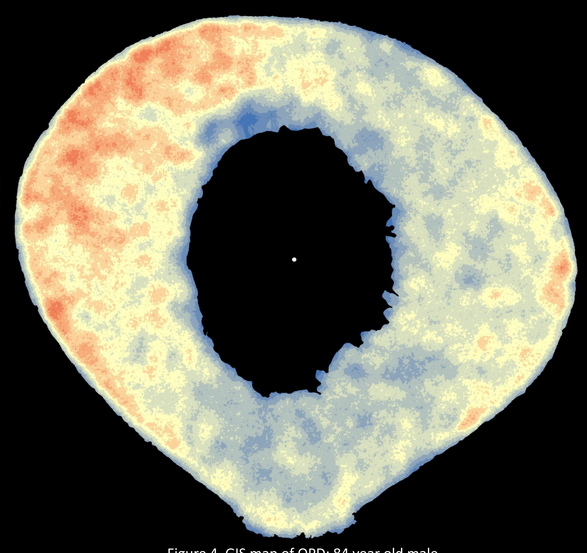

Figure 4. GIS map of OPD; 84 year old male

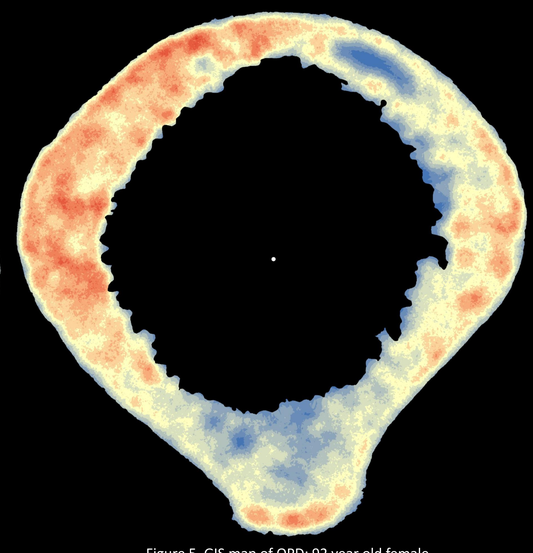

Figure 5. GIS map of OPD; 92 year old female

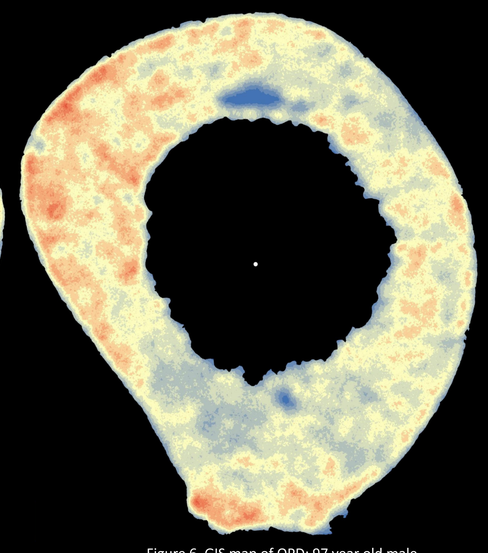

Figure 6. GIS map of OPD; 97 year old male

RESULTS AND DISCUSSION

- In skeletal elements smaller than the femur, the entire cortex can become saturated with remodeling events and reach the asymptote. For example, OPD in the rib is reported to occur at approximately $30/mm^2$ and as early as 50 years of age (Stout and Paine 1994). From the density maps in Figures 2-6 it is clear that remodeling density across the femoral cortex exhibits a great amount of spatial variation, with the areas of highest density concentrated in the lateral and anterolateral regions. When OPD was calculated for entire cross-sections, no individual in this study had a value higher than $31/mm^2$, indicating that individuals of normal bone health will likely fail to reach the OPD asymptote during a normal human life span.

- The highest local OPD value observed in this study was $55/mm^2$, suggesting this to be the upper limit, and therefore the asymptotic value past which OPD at the femoral midshaft can no longer increase. This OPD value, however, was achieved in only small regions of the lateral and anterolateral cortex. Local OPD values above $50/mm^2$ were not observed in our data until the 8th decade of life, suggesting that histological age estimation from the femur is particularly useful for older individuals that may not be accurately assessed by more traditional macroscopic or other histological methods.

- When small regions of interest are used to quantify remodeling they will fail to adequately address the spatial variation across the cortex (Frost 1969). When larger sampling areas are employed (Figure 7) enough variation in remodeling density is accounted for such that OPD continues to increase in a linear fashion even through the 10th decade of life.

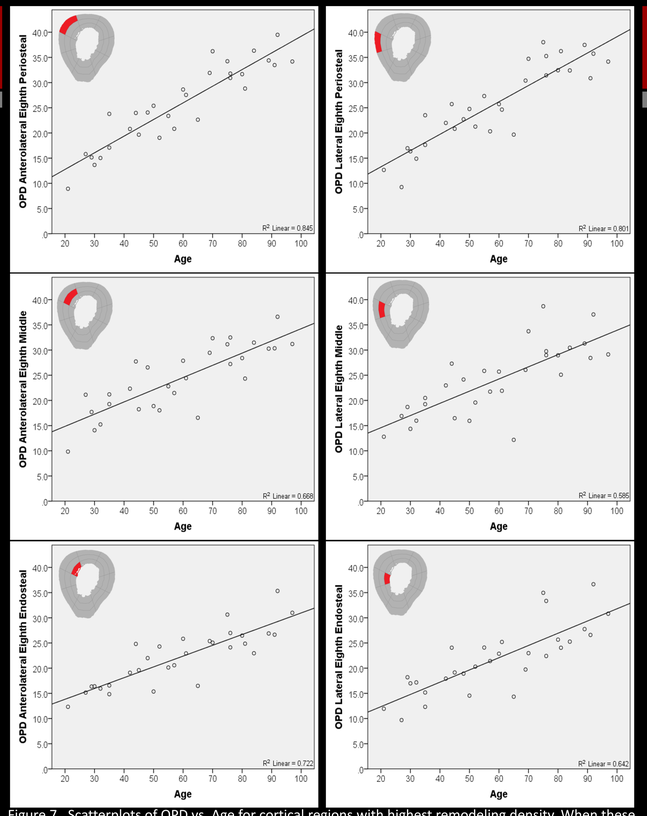

Figure 7. Scatterplots of OPD vs. Age for cortical regions with highest remodeling density. When these larger sized cortical ROIs are used enough variability in remodeling is encompassed such that there is no apparent asymptote, but rather a linear increase in OPD, even through the 10th decade of life.

CONCLUSIONS

- Despite being the most commonly employed skeletal site for developing histological aging methods, the femoral midshaft has hitherto not been thoroughly investigated regarding the OPD asymptote. Our data indicate that Frost's (1987) hypothesized value of $50/mm^2$ (based on data from Kerley 1965) was not far off, and we suggest a minor upward revision to $55/mm^2$.

- Unlike smaller skeletal elements such as the rib, an entire femoral cross-section will fail to hit the OPD asymptote due to its greater cortical area and spatial variation in remodeling density, so long as an individual does not suffer from severe osteoporosis.

- Regions of interest and sampling areas used for developing methods for age estimation from the femoral midshaft should be 1) precisely defined, 2) extend deeper into the cortex than the periosteal third, and 3) be large enough to encompass sufficient variation in remodeling density so as to avoid small, regional asymptotic areas of the cortex that begin to appear during the 8th decade of life.

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