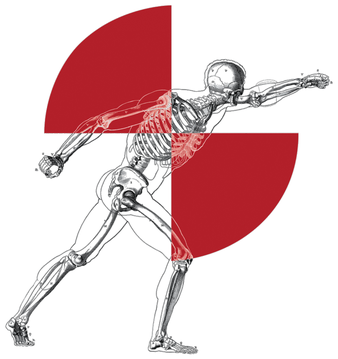

Regional Variation in Osteon Size at the Femoral Midshaft

Timothy P. Gocha Ph.D., Amanda M. Agnew Ph.D.

Skeletal Biology Research Laboratory, The Ohio State University

INTRODUCTION

- Osteon size can help distinguish bone fragments as human or non-human (Dominguez and Crowder 2012), demonstrates a significant relationship with chronological age (Britz *et al*. 2009), and has been suggested useful in interpreting biomechanical strains on bone (Skedros *et al*. 1994).

- Some previous research investigating osteon size at the femoral midshaft has employed sampling strategies that examine only a small amount of the anterior cortex; Britz *et al*. (2009) did this to specifically avoid potential spatial variation in osteon size, while Pfeiffer *et al*. (2006) explicitly assumed there to be no spatial variation in osteon size. Still others have reported measuring osteon size around the cortex, but either averaged measurements from all sampling areas (Mulhern and Van Gerven 1997), or failed to specify which areas of the cortex were sampled (Martiniaková *et al*. 2006).

- As a result, there is little empirical evidence regarding potential spatial variation in osteon size in the human femur. Recent research has demonstrated there is significant spatial patterning in cortical remodeling rates and osteon density at the femoral midshaft (Gocha and Agnew 2014; Figure 1), and therefore we hypothesize spatial variation in osteon size may also exist.

MATERIALS & METHODS

- Ten complete cross-sections from the femoral midshaft of modern cadaveric donors were used for this study. All subjects were male and ranged in age from 21-97 years (mean = 56.7; SD = 24.5 years).

- Each cross-section was photographed under polarized light and micrographs were compiled into seamless images (Figure 2). These images were imported into ArcGIS v10.1 (ESRI®) where the cortex was circumferentially divided into anterolateral (AL), anteromedial (AM), posteromedial (PM), and posterolateral (PL) quadrants, and subdivided radially into periosteal, middle, and endosteal thirds (Figure 2).

- Using polygon overlays in ArcGIS, a total of 100 intact osteons were measured within the periosteal third of each quadrant for each individual, resulting in a total of 4000 intact osteons measured. Osteon area (On.Ar) was measured only on intact osteons where the reversal line was ≥ 90% undisturbed (Figure 3); branching events were excluded from analysis.

- Logarithmic transformation was applied to On.Ar and normalized values were used for all analyses. An analysis of covariance (ANCOVA) was used to examine if mean On.Ar was similar for all quadrants, controlling for age related effects on all ten samples. Jackknife resampling was then applied in conjunction with ANCOVA tests to examine the variance in mean On.Ar by quadrant. Post-hoc pairwise comparisons using a Bonferroni correction were run after all ANCOVA tests to examine potential differences in On.Ar by quadrant.

CONTACT INFORMATION

Gocha.3@osu.edu

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RESULTS AND DISCUSSION

- Results from the ANCOVA analysis run on all ten samples, as well as the subsequent ten Jackknife ANCOVA tests (each run on nine samples) indicates there is a significant difference between mean On.Ar in femoral quadrants (all p-values < 0.001; Table 1).

- Post-hoc pairwise comparisons demonstrated On.Ar in the anterolateral quadrant to be consistently and significantly smaller than all other quadrants (all p-values ≤ 0.001). Mean On.Ar between the anteromedial, posteromedial, or posterolateral quadrants were not found to differ significantly from each other in any pairwise comparison (all p-values > 0.05). The range of variance in mean On.Ar by quadrant, and for the entire cortex, is depicted in Figure 4.

- Martin *et al*. (1980) suggest osteon size results from the functional efficacy of basic multicellular units, and Havill *et al*. (2013) argue osteon size is largely under genetic control. While these undoubtedly affect osteon size to some degree, it is unlikely they explain spatial variation in On.Ar.

- van Oers *et al.* (2008) argue that osteons are typically smaller in bone regions experiencing larger strains. Finite element modeling of the human femur suggests that no one region of the cortex is consistently subject to the highest strain magnitudes; rather, strain magnitude varies during different phases of locomotion (Speirs *et al*. 2007, Phillips 2009). Consequently, it is unclear if the pattern of spatial variation in On.Ar found here is a result of strain magnitude or not.

- Takahashi *et al*. (1965) suggest that larger osteons are more likely to be partially remodeled by new remodeling events, and therefore measuring intact osteons to estimate On.Ar results in a sampling bias towards smaller osteons. Considering the higher rate of intracortical remodeling an the anterolateral quadrant, this could partially explain the results here.

- The spatial variation in On.Ar is also likely, at least in part, a result of the modes of biomechanical strain acting at the femoral midshaft, and also an adaptation to them. The higher remodeling rate and osteon density present in the anterolateral cortex, a likely by-product of tensile strains (Gocha and Agnew *in prep*.), result in more cement lines in this region. Since cement lines absorb energy and inhibit microcrack propagation (O'Brien *et al*. 2005), new microfractures should be shorter in the anterolateral cortex, and therefore require smaller osteons to repair them.

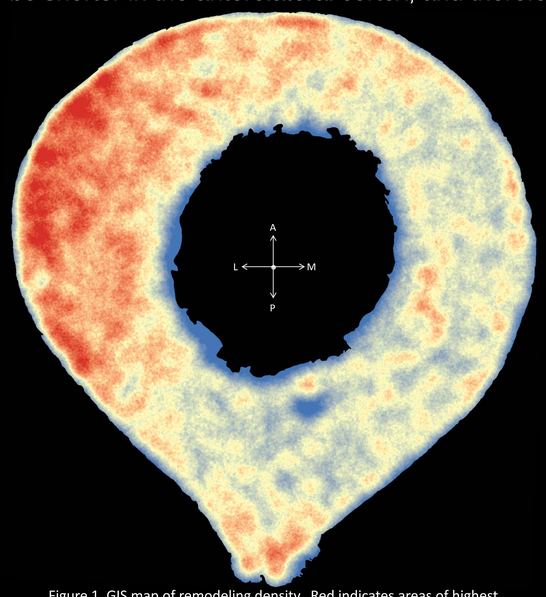

Figure 1. GIS map of remodeling density. Red indicates areas of highest remodeling density, blue indicates lowest remodeling density.

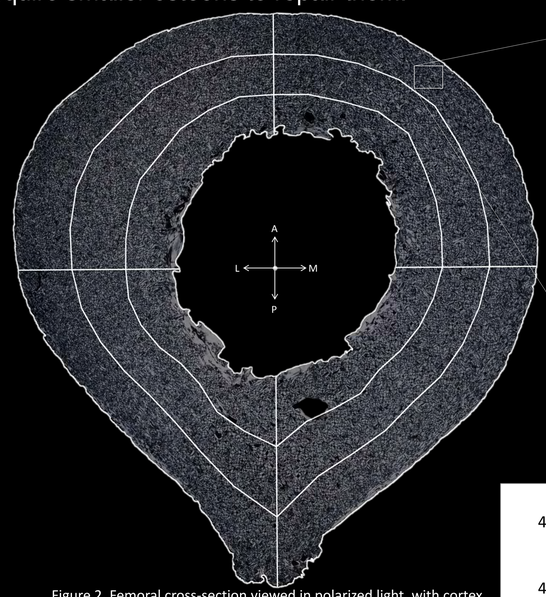

Figure 2. Femoral cross-section viewed in polarized light, with cortex subdivided into circumferential quadrants and radial thirds.

Figure 3. Close-up example of GIS polygon features overlaid on intact osteons selected for On.Ar measurement.

Table 1. Mean On.Ar (backtransformed into μm²) for each quadrant and the entire cortex from all ANCOVA tests.

Statistical Test	Mean On.Ar (μm²)				
	AL Quadrant	AM Quadrant	PM Quadrant	PL Quadrant	Entire Cortex
ANCOVA (all samples)	37,067	43,289	41,940	41, 376	40,918
Jackknife ANCOVA (no 21 year old)	34,527	40,646	39,427	39,978	38,645
Jackknife ANCOVA (no 29 year old)	36,936	42,510	40,938	41,538	40,480
Jackknife ANCOVA (no 32 year old)	36,515	43,088	41,969	41,104	40,669
Jackknife ANCOVA (no 42 year old)	37,621	43,571	42,701	41,299	41,298
Jackknife ANCOVA (no 52 year old)	36,419	42,423	40,664	40,322	39,957
Jackknife ANCOVA (no 60 year old)	37,642	43,301	42,068	41,622	41,158
Jackknife ANCOVA (no 70 year old)	38,015	44,228	42,714	41,526	41,620
Jackknife ANCOVA (no 80 year old)	37,280	44,110	42,245	42,372	41,501
Jackknife ANCOVA (no 84 year old)	37,687	44,269	43,165	41,801	41,730
Jackknife ANCOVA (no 97 year old)	38,029	44,749	43,505	42,200	42,121

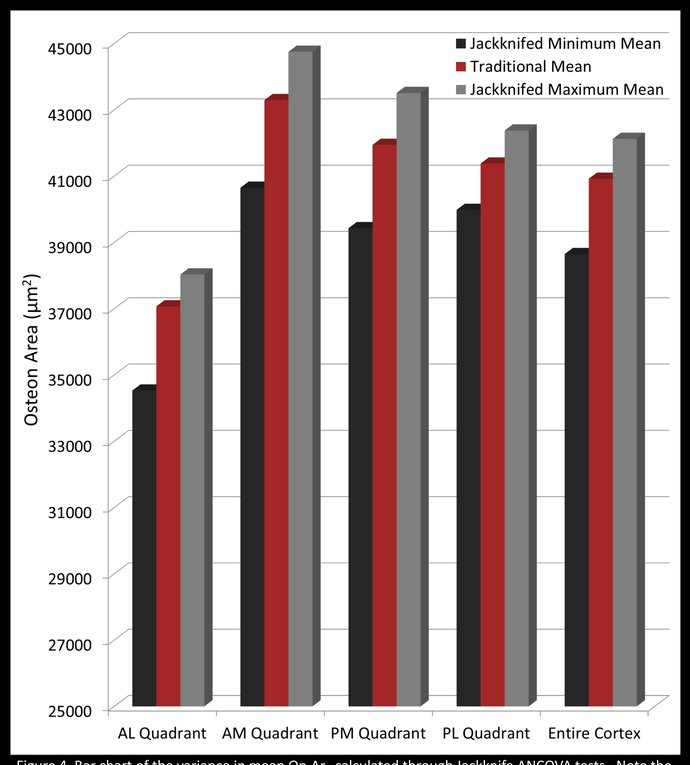

Figure 4. Bar chart of the variance in mean On.Ar, calculated through Jackknife ANCOVA tests. Note the maximum mean for the AL quadrant is less than the minimum mean for any other quadrant.

CONCLUSIONS

- On.Ar demonstrates significant spatial variation around the circumference of the femoral midshaft, with osteons in the anterolateral cortex being ~10-15% smaller than other quadrants.

- In light of the spatial variation found here, it is recommended that other researchers examining On.Ar should employ sampling strategies that account for regional variation in osteon size.

- Ongoing research in our lab is working to expand research on On.Ar in a larger sample that investigates the full depth and circumference of the femoral cortex.

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