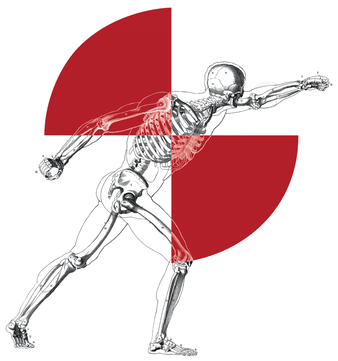

Nonuniform Osteocytic Lacunae Distribution across the Femoral Cortex

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INTRODUCTION

- Osteocytes, the most prolific bone cell, have increasingly been the focus of clinically based studies into bone health over the past few decades. As a major regulator of metabolic activity, these cells are fundamentally linked to the structural integrity of bone. It is widely accepted that osteocytes are crucial in initiating a metabolic response to mechanical loading and microdamage as well as maintaining mineral homeostasis mediated by effects on osteoblastic and osteoclastic activity (Bonewald 2007, 2011; Marotti et al. 1995; Martin 2000; Mullender et al. 2005). The mechanosensing role of osteocytes through its extensive communication network, or osteocyte synctium, allows these cells to direct targeted remodeling in a strain dependent environment (Colopy et al. 2004, Mullender et al. 2005). Osteocyte density and distribution should be considered as an additional tool for assessing bone quality in past and present populations (Ma et al. 2008).

- Osteocytic lacunae density (Ot.Lc.N/B.Ar) is used as a proxy for osteocyte density. Previous studies examining age and disease related changes of Ot.Lc.N/B.Ar have reported conflicting results (Power et al. 2002, Skedros et al. 2005, Qui et al. 2002). Contradictory results in density and distribution changes with age are likely due to variation in sample locations which experience drastically different loading environments between studies. Ot.Lc.N/B.Ar values are likely site specific dependent on the mechanical environment, remodeling rate, type of osseous tissue, and nutrient delivery (Mullender et al. 2005, Skedros et al. 2005). In order to implement osteocytic density as an assessment of bone quality in human populations, a more complete picture of normal variation and age related changes is needed (Carter et al. 2013).

- The objective of this project is to explore the age related changes in osteocytic lacunar total density across the entire midshaft femoral cortex as well as between anatomically determined regions of interest (ROI) to demonstrate density distribution. As an already often employed sampling region for other histological analyses, the femoral midshaft was chosen to investigate interindividual variation in Ot.Lc.N/B.Ar.

MATERIALS AND METHODS

- Femoral midshaft cross sections were obtained from 20 male cadavers ranging from 29 to 79 years of age with no known systemic issues affecting skeletal metabolism . Undecalcified transverse thin sections were prepared using standard histological procedures and subsequently imaged under bright field light.

- cellSens Dimension® was used to differentially enhance the contrast of each femoral cross-section to allow for easier identification of lacunae. Using a small subsection of a femoral cross section, 200 lacunae were manually and subsequently automatically counted to verify the efficacy of the program. In this region, cellSens Dimention ® accurately detected all 200 lacunae. Using specific thresholding and size parameters that optimize detection, each sample was automatically counted (see Figure 1A, B, and C). Any missed or misidentified lacunae were manually added or removed as necessary. Osteocytic lacunar counts were then divided by total cortical area (B.Ar) to obtain density values (Ot.Lc.N/B.Ar).

- To assess age related changes for the entire sample, an ANOVA was performed on Ot.Lc.N/B.Ar for four age categories (n=5 per category): <50 years old, 51-59 years old, 60-69 years old, 70-79 years old. Samples were then combined into two age categories (n=10 per category): 29 to 59 years old and 60 to 79 years old and compared using independent samples t-test. A correlation was performed between age and Ot.Lc.N/B.Ar.

- For ROI comparisons, lacunae were automatically counted using the same method for determining total density counts (see Figure 1D). Independent sample t-tests were run to determine age related changes in individual ROI density distributions between the younger (29 to 59 years old) and older (60 to 79 years old) age groups. Additionally, an ANOVA was performed between means of each ROI across the sample regardless of age.

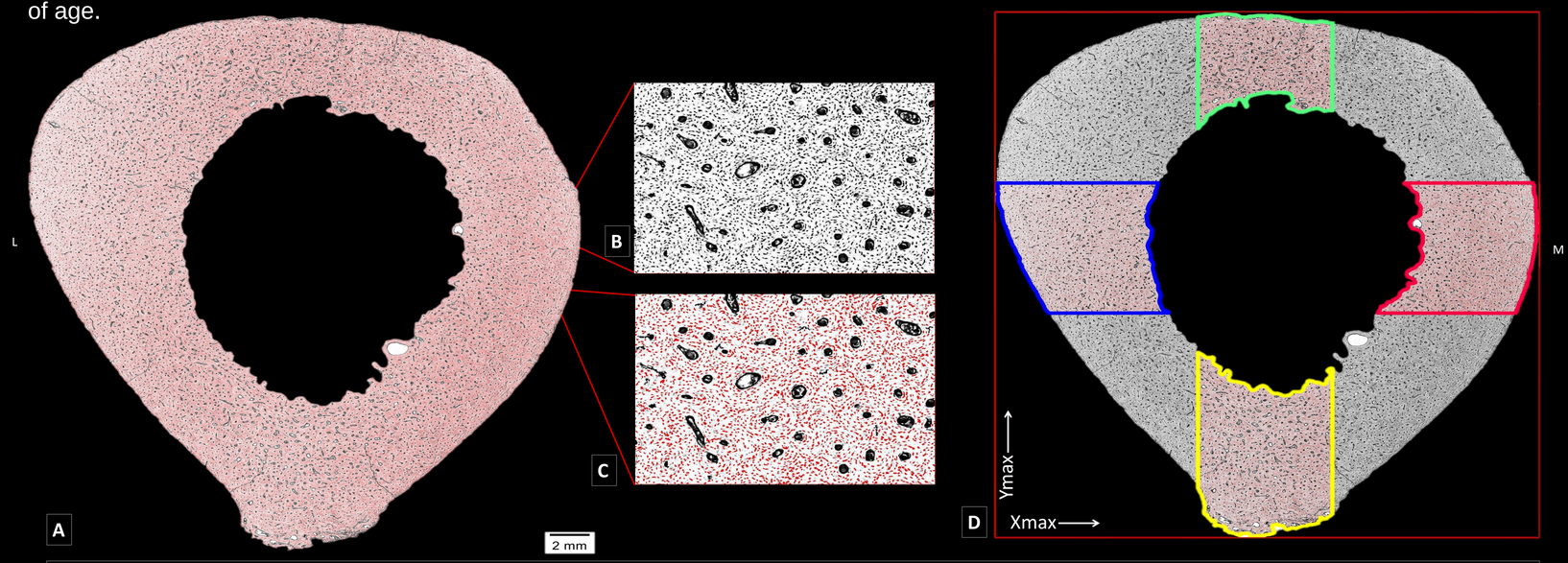

Figure 1: Femoral cross section from same individual depicting cellSens Dimension ® annotation of osteocytic lacunae. A) total lacunar density across cortex. B and C) subsample of contrast enhanced bone prior to automatic annotation (B) and after annotation (C). D) ROI calculated using 25% of Ymax (medial and lateral) and Xmax (anterior and posterior) denoted by the red extent box. Osteocytic lacunar density counted for individual ROI and divided by ROI area to obtain density (Ot.Lc.N/B.Ar) distributions across the cortex

RESULTS AND DISCUSSION

- ANOVA results for total femoral osteocytic lacunae density did not reveal significant differences between four age categories (p= 0.071). However, a significant difference was found after combining the sample into only two age categories (Table 1, Figure 2A). Figure 3 shows a significant negative correlation between age and Ot.Lc.N/B.Ar (Pearson correlation= -0.541, p= 0.014) indicating osteocyte density does decrease with age in the midshaft femoral cortex.

- For ROI comparisons, as there were no significant differences between age categories for individual ROIs (see Table 2), all individuals were pooled to compare differences between regions. Medial and lateral means are slightly higher than anterior and posterior, consistent with previous work in femoral cortex (Carter et al. 2013). However, ANOVA results indicate there are no significant differences between regions for the entire sample (p= 0.19) suggesting the anatomical ROIs do not adequately capture the variation in osteocyte density.

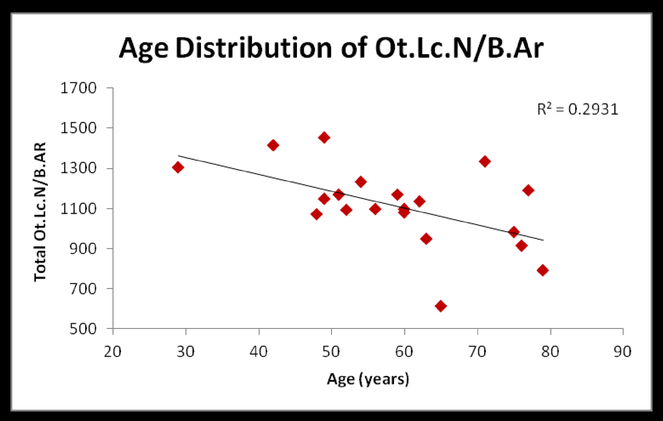

Figure 3: Negative correlation between total osteocyte density and age

ROI	Age	N	Mean	Std. Dev	df	t	p
Medial	1	10	1102.547	229.409	19	0.431	0.672
	2	10	1140.263	154.766			
Lateral	1	10	1027.254	219.108	19	1.471	0.159
	2	10	1236.679	393.321			
Anterior	1	10	907.104	218.170	19	1.513	0.148
	2	10	1177.338	520.943			
Posterior	1	10	930.648	222.283	19	0.502	0.622
	2	10	973.920	157.846			

Table 2: ANOVA results comparing ROI densities between age group 1 (29-59 years) and age group 2 (69 to 79 years)

Age Cat (years)	N	Mean	Std. Dev	df	t	p
1 (29-59)	10	1214.813	134.678	18	2.643	0.017
2 (60-79)	10	1008.673	206.629			

Table 1: ANOVA results for total Ot.Lc.N/B.Ar (alpha = 0.05)

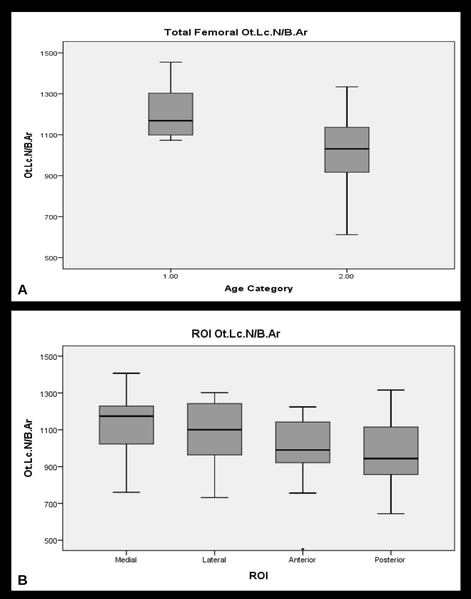

Figure 2: A) Age category 2 demonstrating significantly lower average total osteocytic density than younger age group (p=0.017). B) Pooling all 20 individuals, nonsignificant differences in densities per region

CONCLUSIONS

- Total osteocyte density across the midshaft femoral cortex decreased with age similar to patterns obtained by other researchers at varying skeletal sampling sites (Power et al. 2002, Mullender et al. 2005). However, interpretations of Ot.Lc.N/B.Ar likely differ due to the complex mechanical forces experienced between skeletal elements.

- Standardized anatomical ROIs were not effective at capturing the variation in Ot.Lc.N/B.Ar due to the cell's strain dependent response.

- Future research will include intersite variation with respect to age and disease processes; mechanically relevant ROIs within the femoral cortex; comparisons with remodeling event distribution and intracortical porosity. Due to its metabolic roles and mechanical influences, quantifying the density and distribution of the most prolific bone cell will be an important factor in assessing bone quality in past and present human populations.

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