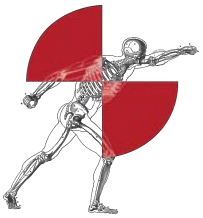

Potential Influences on Rib Osteon Area

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INTRODUCTION

• The size of secondary osteons varies considerably between individuals, though the factors acting to define osteon size remain uncertain

• Understanding the biological mechanisms that act in remodeling and determine osteon size is essential for accurately addressing and interpreting histological findings

•The aim of this study is to explore multiple factors currently thought to influence osteon size in an attempt to better understand mechanisms influencing the remodeling process. Specifically, we test the premise that sex, age, cortical area, porosity, and local loading environment have a significant effect on osteon area in the human rib.

MATERIALS AND METHODS

• The sample is composed of 80 individuals, 49 male and 31 female, between 6–94 years of age (mean = 59.9 years, SD = 23.5 years). Complete cross-sections were taken at the left or right, midshaft of the 6th rib and slides were prepared following standard histological protocols. All slides were photographed at 40X magnification and all data were collected using ImageJ.

• Ribs were photographed and then digitally bisected into pleural and cutaneous regions for data collection (defined by Imin). Variables collected are listed in Table 1. All variables were manually collected using scaled images and a digitizing tablet (Fig. 1). Only intact osteons, defined by an intact reversal line, were measured to establish mean On.Ar. All pores were measured to calculate Po.Ar, excluding the medullary cavity and osteocytic lacunae.

•Natural log transformation was applied to Pl.On.Ar, %Cu.Ct.Ar, %Po.Ar, %Cu.Po.Ar, and %Pl.Po.Ar and the normalized values were used for all analyses. Independent sample *t*-tests were used to compare On.Ar between sexes. Paired sample *t*-tests were used to compare On.Ar, %Ct.Ar, and %Po.Ar between the cutaneous and pleural cortices

Table 1. Collected Variables[a]

Variable	Definition
On.Ar	Area of an intact osteon, including the Haversian canal
Tt.Ar	Total area within the periosteum, including medullary cavity
Ct.Ar	Total area between the periosteal and endosteal borders
Po.Ar	Total area of pores within the cortex
%Ct.Ar	(Ct.Ar/Tt.Ar)*100
%Po.Ar	(Po.Ar/Ct.Ar)*100

[a]Each variable was also collected and analyzed for the cutaneous and pleural halves of the rib specifically.

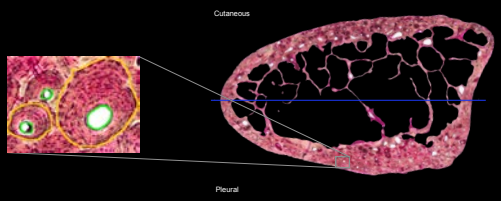

Figure 1. Blue line denotes Imin, used to define the cutaneous and pleural cortices during data collection. Inset is a close-up demonstrating measurements of collected variables. Orange lines = Intact secondary osteons, Green lines = Cortical porosity

• Hierarchical multiple regression (HMR) was chosen to analyze the data, because age is known to have a significant relationship with both %Ct.Ar and %Po.Ar. HMR allows age to be treated as a covariate, accounting for the effects of age on the other tested variables in the analysis. HMR was run for the total cortex and for the cutaneous and pleural cortices, respectively.

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RESULTS AND DISCUSSION

• Descriptive statistics for the histomorphometric variables by cortex are given in Tables 2a-c.

• Independent sample *t*-tests indicated no significant differences in On.Ar between males and females. Samples were pooled for all further analyses.

• Paired sample *t*-tests showed that there were significant differences between cortices in On.Ar, %Ct.Ar, and %Po.Ar

• HMR revealed that age and %Ct.Ar have a significant influence on On.Ar, while %Po.Ar does not (Table 3). These results held true whether looking at the total cortex or at the cutaneous and pleural cortices independently.

Figure 2. A) Rib cross-section from an 18 year old female. B) Rib cross-section from a 91 year old female. The younger sample shows a robust cortex, with a high density of remodeling on the cutaneous cortex compared to the sparse remodeling and larger osteons visible in the pleural cortex. The older sample shows reduced cortex throughout, though bone loss is particularly apparent on the cutaneous cortex.

• Increasing age and loss of cortical bone, even accounting for age-related bone loss, result in a reduction of osteon size (Fig. 2). The mechanism by which increased age influences osteon size is uncertain, however, physiologic changes such as diminished osteoclastic activity may play a role (Martin et al, 1980). The contribution of %Ct.Ar to osteon size is much smaller than that of age, however, the amount of cortical bone present, regardless of age, appears to limit the size of forming osteons.

• %Po.Ar does not appear to significantly influence On.Ar, though there is a trend of decreasing osteon size with increasing porosity. This variable may be confounded by issues inherent in the accurate assessment of trabecularized cortex (Zebaze and Seeman, 2015).

Table 2a. Total Rib

Variable	Mean	SD	Min	Max
On.Ar	0.036	0.008	0.021	0.065
%Ct.Ar	34.74	11.31	10.96	63.69
%Po.Ar	7.83	5.68	2.48	42.04

Table 2b. Cutaneous Cortex

Variable	Mean	SD	Min	Max
On.Ar	0.035	0.009	0.016	0.063
%Ct.Ar	30.48	13.70	8.66	99.89
%Po.Ar	9.22	6.78	2.54	47.19

Table 2c. Pleural Cortex

Variable	Mean	SD	Min	Max
On.Ar	0.037	0.009	0.023	0.066
%Ct.Ar	40.85	12.24	14.02	80.60
%Po.Ar	6.54	5.15	1.83	36.92

Table 3. Hierarchical Multiple Regression Results[a]

Total Rib	R	R^2	ΔR^2	β
Step 1	0.553	**0.306**		
Age				**-0.553**
Step 2	0.600	*0.359*	*0.054*	
Age				**-0.374**
%Ct.Ar				*0.27*
%Ct.Po				-0.085
Cutaneous	**R**	**R^2**	**ΔR^2**	**β**
Step 1	0.509	**0.259**		
Age				**-0.509**
Step 2	0.616	*0.380*	*0.121*	
Age				*-0.294*
%Cu.Ct.Ar				**0.398**
%Cu.Ct.Po				-0.103
Pleural	**R**	**R^2**	**ΔR^2**	**β**
Step 1	0.542	**0.294**		
Age				**-0.542**
Step 2	0.605	*0.366*	*0.072*	
Age				**-0.373**
%Pl.Ct.Ar				**0.288**
%Pl.Ct.Po				-0.098

[a] Statistical significance: *p<0.05*, **p<0.01**, p<0.001

CONCLUSIONS

• Osteon size in the rib is largely influenced by chronological age and to a lesser extent by available Ct.Ar, while sex and %Po.Ar do not appear to play a significant role.

• While the factors outlined here as influencing On.Ar are important considerations, recent evidence suggests that genetic effects may be the primary force defining osteon size. Recent studies suggest that up to 62% of remodeling differences may be due to genetic factors (Bjørnerem et al. 2015). As such, further research is warranted.

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