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Age-associated bone loss and intraskeletal variability in the Imperial Romans

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Summary - *An Imperial Roman sample from the Isola Sacra necropolis (100 ~ 300 A.D.) offered an opportunity to histologically examine bone loss and intraskeletal variability in an urban archaeological population. Rib and femur samples were analyzed for static indices of bone remodeling and measures of bone mass. The Imperial Romans experienced normal age-associated bone loss via increased intracortical porosity and endosteal expansion, with females exhibiting greater bone loss and bone turnover rates than in males. Life events such as menopause and lactation coupled with cultural attitudes and practices regarding gender and food may have led to increased bone loss in females. Remodeling dynamics differ between the rib and femur and the higher remodeling rates in the rib may be attributed to different effective age of the adult compacta or loading environment. This study demonstrates that combining multiple methodologies to examine bone loss is necessary to shed light on the biocultural factors that influence bone mass and bone loss.*

Keywords - *Bone remodeling, Histomorphology, Osteoporosis, Age-related bone loss, Imperial Roman.*

Introduction

Age-associated bone loss is universal and begins as early as the fourth decade of life, afflicting all regardless of sex, biological ancestry, and socioeconomic class. Osteoporosis, a severe manifestation of bone loss and mechanical incompetence of the skeleton, produces extreme bone fragility and susceptibility to spontaneous fractures from normal activities or minor falls. It is a common metabolic bone disease in contemporary societies (Cole *et al.*, 2008) with 44 million Americans currently at risk for fragility fractures (NIAMS, 2009).

Risk for osteoporosis is due to a combination of factors: age, sex, genetics, nutrition, physical activity level, parity, etc. The aging process is idiosyncratic and variable, with increasing variability in physiology and pathology with age (Kulminski *et al.*, 2007; Brant & Pearson, 1994). Genetic, physiological, and lifestyle factors contribute to the high variability in the incidence and prevalence of osteoporosis among individuals, between sexes, and among populations. The epidemiology of osteoporosis identifies modern, urban Asian and western European groups as especially high-risk populations. Moreover, a clear disparity is observed between ancient and contemporary populations. Although age-related bone loss has been observed in the past, the prevalence of bone fractures, pathognostic of osteoporosis, is fairly low (Agarwal, 2008; Mays *et al.*, 2006; 1998; Nelson & Weiss, 1999; Agarwal & Grynpas, 1996), which is generally attributed to shorter life spans and higher physical activity levels. Such conclusions are based primarily on non-western pre-industrial groups, and the bone density and radiogrammetric studies conducted on European archaeological samples report ambiguous and inconsistent results regarding bone fragility (Bajon *et al.*, 2006; Mays, 2006, 1998, 1996; Mays *et al.*, 2006, 1998; Agarwal & Grynpas, 1996; Kneissel *et al.*, 1994).

Another research area that remains to be further addressed is intraskeletal variability in age-related bone loss. A correlation in bone loss among different skeletal elements of the same individual has been noted (Hernandez *et al.*, 1991), but a lack of consensus exists as to the relative equivalence of bone loss among commonly affected skeletal elements such as the vertebrae and femur (Eckstein *et al.*, 2007; Blake *et al.*, 2006; Thomsen *et al.*, 2002; Kiel *et al.*, 2001; Hordon *et al.*, 2000; Nordin *et al.*, 1996). The inconsistent rates of trabecular and cortical bone loss lead to distinct fracture patterns among skeletal elements with differing proportions of trabecular and cortical bone. Additionally, cortical bone remodeling, or bone turnover, is related to the habitual loading environment, and skeletal elements that receive more load are expected to have higher turnover rates due to accumulating microdamage that triggers remodeling than the ones that are loaded intermittently. Bone remodeling is a lifelong process necessary for fracture repair and maintaining calcium homeostasis, but it contributes to the normal mechanism of bone loss in adults by increasing intracortical porosity through creation of resorptive cavities and basic structural units called osteons with a Haversian canal in each.

The present study is a unique opportunity to explore age-associated bone loss in an ancient Roman skeletal sample by measuring their bone mass and estimating static indices of cortical bone remodeling. The results expand our understanding of the mechanism of bone loss, and the biology and skeletal health of ancient Romans.

Materials

The Isola Sacra necropolis (100 ~ 300 A.D.), approximately 23 km west of Rome, is located on an island between the ancient cities of Ostia and Portus Romae. The people of Isola Sacra were inhabitants of Portus, an urban center of a major harbor receiving foods and supplies for Imperial Rome. Portus was atypical of the Classical Period with a relatively egalitarian society that appeared to lack the aristocratic and political class present in other Roman cities. The inhabitants were mainly middleclass traders and merchants descended from freedmen, whose livelihoods were strictly dependent on the commerce and trade related to the harbor (Garnsey, 1999a; Meiggs, 1973).

This skeletal study sample is distinct for several reasons: 1) the individuals were European and urban, expanding the temporal range of bone loss studies of archaeological populations, 2) a large collection of approximately 2000 individuals was available for research, 3) multiple skeletal sites from the same individuals were available to address intraskeletal variability, and 4) the biology and health of ancient Romans is largely unknown compared to their abundant material culture (Bondioli & Macchiarelli, 1999). Skeletons of individuals older than 17 years with good preservation of sampling sites and without evidence of metabolic diseases were sampled. Subadults (<17 years old) were not chosen for this study as they undergo significant skeletal development and transverse cortical drift, which results in the underestimation of bone microstructures that are used to estimate bone remodeling rates.

Age-at-death and sex estimates based on multiple standard osteological methods were provided for 149 individuals by the collection curators; age estimation involved the analyses of cranial sutures, dental wear, pubic symphysis and auricular surface morphologies, and proximal femur trabecular pattern, while sex estimation was based on long bone metric traits and morphologies of the crania and os coxae (Prowse *et al.,* 2004). The samples were chosen (male N = 74, female N = 75) so that each of four age categories and both sexes were equally represented (19 male, 20 female 20~29 year old cohort; 18 male, 22 female 30~39 years; 22 male, 22 female 49~40 years; 15 male, 11 female ≥50 years). Due to poor preservation, a smaller sample was included in the oldest age category. Samples from two different skeletal elements were also collected from a subsample of 114 individuals: the midshaft femur (weight-bearing cortical bone) and the midshaft rib (non-weight-bearing cortical bone and biomechanically homogeneous).

Sampling and sample preparation

Rib

The midshaft sixth rib has been used in a number of bone remodeling studies (Crowder & Rosella, 2007; Qiu *et al*., 2003; Cho, 2002; Stout & Lueck, 1995; Stout & Paine, 1994). It is often a preferred site for bone histology, the study of skeletal tissue at the microscopic level, due to its accessibility during autopsies, and in bioarchaeology and paleoanthropology since sectioning the rib is less invasive than sectioning a larger skeletal element. A 2~3 cm section per individual was cut from the midshaft third of any one of the fourth to eighth ribs. A recent study by Crowder & Rosella (2007) affirms that the histologic variables do not differ significantly among the third to eighth ribs; this is clearly an advantage when archaeological remains are fragmentary and distinguishing the sixth rib is not possible. Each rib sample was embedded in EpoThin (Buehler Ltd., Lake Bluff, IL) and placed inside a vacuum impregnation equipment to infuse the bone with plastic resin. After the resin cured, several ~100 μm thick transverse sections were cut using an Isomet low-speed gravity-feed petrographic saw (Buehler Ltd., Lake Bluff, IL) and manually ground to a final thickness of ~50 μm on carbide paper. Two sections per individual exhibiting adequate microstructural preservation were necessary for histology. For cortical area measurements, one cross-section per individual with an intact cortex was scanned on a flatbed scanner with a ruler for scale.

Femur

Preservation of the entire femur was a necessary condition, and a 2~3 cm midshaft cross-section per individual was cut perpendicular to the diaphysis. Each embedded bone sample was cut exactly at the mechanical length midpoint as described by Ruff *et al*. (1997) with an Isomet saw. One half was used to prepare a thin section as per rib histology, and the other half was scanned with a ruler for cross-sectional area measurements. During sample collection, femoral head diameters were measured to estimate body mass from the formulae below for later use as a covariate in statistical analyses (Ruff *et al*., 1997, 1991).

Male body mass = (2.741 X FHD) – 54.9;
SEE = 13.7
Female body mass = (2.426 X FHD) – 35.1;
SEE = 17.5

FHD = femoral head diameter
SEE = Standard Error of Estimate

Methods

Cortical bone histomorphometry

Bone loss is most effectively quantifiable through histology, particularly histomorphometry to derive bone remodeling rates. Bone histomorphometry is the quantitative study of bone in which discrete features in the skeletal tissue and their characteristics are quantified. These variables are used in an algorithm to derive static indices of bone remodeling (Cho *et al*., 2006; Cho, 2002; Stout & Paine, 1994; Frost, 1987). One of the assumptions in histomorphometry is that remodeling is at a steady state, or that the activation and remodeling of basic multicellular units are constant in rate, time, and space. Archaeological bone cannot be labeled *in vivo* to observe transient changes in the remodeling process, thus in two-dimensional histomorphometry, remodeling in a cross-section is assumed to be representative of the three-dimensional region of bone (Martin *et al*., 1998).

The histomorphometric variables were measured directly as described by Stout & Paine (1994), using an Olympus BX-50F light microscope with 20x objective and 10x widefield oculars (Olympus Optical Co., Ltd., Tokyo, Japan) fitted with a Zeiss Integrationsplatte II eyepiece reticule (Georgia Instruments, Atlanta, GA). Approximately 50% of the rib cortex was examined using a checkerboard pattern in which every other microscope field in each row was sampled. Two rib sections per individual were examined and the results averaged to avoid sampling bias. The derived indices of bone remodeling included the following variables: 1) osteon population density (OPD) or the total number of intact and fragmentary osteons per mm^2 of bone, 2) osteon area (On.Ar) or the average area of structurally complete osteons for each rib section, 3) mean annual activation frequency (Ac.f) or the mean number of osteons created annually per mm^2 of bone, and 4) mean annual bone formation rate (BFR) or bone formation rate of an individual averaged over the effective age of their adult compacta. Ac.f and BFR are estimates of the rate at which an individual remodels their cortical bone, and estimation of Ac.f requires the effective birth of adult compacta variable that occurs around 12.5 years of age in the sixth rib. In turn, Ac.f is a variable necessary to derive BFR. Effective age of adult compacta refers to the chronological age when cortical drift ceases, a bone modeling process of skeletal development by which skeletal elements achieve adult size and shape and different tissue ages are produced throughout a cross-section.

One section per individual was examined for femur histomorphometry and the same variables as the rib were derived. The effective age of adult compacta for the midshaft femur is unknown and thus, 12.5 years was also applied to the femur; this is addressed below under limitations of the present study. The anterior cortex was examined following the method described by Iwaniec (1997) and Iwaniec *et al.* (1998). Four centrally located 0.47 mm wide columns separated by two columns of bone were sampled from periosteum to endosteum. This method accounts for over 90% of the entire anterior cortex variability. It is particularly suitable for this study due to loss of structural integrity during the grinding process. However, it is unknown if the anterior section is representative of the entire femur cross-section, and if each quadrant receives different mechanical loads. There is limited evidence regarding the remodeling variance throughout the femur diaphysis. Goldman *et al.* (2005, 2003) found variability among the 48 segments within a single femur mid-diaphyseal cross-section, which suggests remodeling variation within a single skeletal element of a single individual. Pfeiffer *et al.* (1995) caution that sampling locations within the femoral midshaft cross-sections demonstrate remodeling variability, while Ural & Vashishth (2006) report variation in the microstructural indices of remodeling in proximal and distal regions of the tibia mid-diaphysis. Recently, Villa and Lynnerup (2010) report that histomorphometric variables that include OPD are not significantly variable between anterior, lateral, and medial regions of the femoral midshaft. Therefore, if the cross-section is intact, it is recommended that the same method be applied to all four quadrants as demonstrated by Robling (1998). Lastly, the packing factor (*k*) in the algorithm necessary to derive the indices differs in the femur from that in the rib, and the value of 2.0 (Robling, 1998) was used to calculate the femur histomorphometric variables.

Cross-sectional area measurements

Diaphyseal cross-sectional analysis of cortical bone is used to quantify the bone area and to observe the progression of bone loss with age and by sex, but do not factor in intracortical porosity produced by resorptive cavities and Haversian canals. Thus, combining microscopic and macroscopic analyses to describe bone loss is more informative than employing a single method. The rib and femur cross-sections removed for histological analyses were used to measure cross-sectional areas: 1) total subperiosteal area (Tt.Ar) is the area under the subperiosteum including the endosteal area, 2) cortical area (Ct.Ar) is the amount of cortical bone in a cross-section of bone excluding the endosteal area, 3) endosteal area (Es.Ar) is the area of the marrow cavity obtained by subtracting cortical area from total area, and 4) relative cortical area (Ct.Ar/Tt.Ar) is the relative amount of cortical bone in a cross-section and is a measure of bone mass. Using Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA), the cortex of scanned images was painted black to distinguish it from the white background, and NIH Image was used to compute Ct.Ar and Tt.Ar, while Es.Ar and Ct.Ar/Tt.Ar were derived from the former two.

Statistical analysis

The statistical goals of this study are 1) to describe the effects of age and sex on the variables measured for Isola Sacra samples, and 2) to compare the same variables in the rib and femur for intraskeletal differences. Statistical analyses were accomplished using STATISTICA (StatSoft, Inc., Tulsa, OK) and SAS (SAS Institute, Inc., Cary, NC) software.

Two-way analysis of variance (ANOVA) with age, sex, and age-sex interaction terms as predictor variables was carried out for Ct.Ar/Tt.Ar, OPD, On.Ar, Ac.f, and BFR for the rib and femur. Analysis of covariance (ANCOVA) was used for the rib and femur Tt.Ar, Ct.Ar, and Es.Ar with body mass as a covariate because males are expected to yield larger values than females due to sexual dimorphism. If the age-sex interaction coefficient differed significantly from zero, then the effect of age on the predictor variable differed between males and females. If the age-sex interaction effect was not significant but age and/or sex main effects were significant, age differed significantly between the four age groups and/or males and females differed significantly in their mean values.

For the second goal of the statistical analysis, rib and femur histomorphometrics and cortical area measurements were compared to determine if the means differed significantly and if so, whether the differences between the two elements are the same across age. ANOVA was used to determine the significance of the age, sex, and age-sex interaction effects for the differences between the rib and femur. When significant age-sex interaction or main effects were observed, paired t-tests for correlated samples were used to test for significant differences. If the interaction term was significant, t-tests on eight possible age-sex combinations were carried out. If only age had a significant effect, four t-tests were carried out by age category. If only sex had a significant effect, two t-tests were carried out by sex.

In a two-way ANOVA or ANCOVA, an age-sex interaction effect was considered significant at $p<0.05$ and age and sex main effects at the $p<0.01$ levels. The significance level was less stringent for the interaction effect because it was necessary to be more conservative in interpreting the effects of age and sex on the variables of interest. Although one or both of the main effects were likely to be significant at the $p<0.01$ level when the interaction effect was significant, it was more informative to look at the age-related changes by sex since the rate of change was expected to differ between males and females. The significance level for the main effects was more stringent because of the large number of comparisons that were made.

Results

Cortical bone histomorphometry: Rib

A two-way ANOVA was carried out on the histomorphometrics with age and sex as independent variables (see Tab. s1, supplementary online material at www.isita-org.com/jass/Contents.htm). The age-sex interaction effect is not significant for OPD, but age and sex effects are significant. Mean OPD values increase with age and females have a larger mean (23.216/mm^2) than males (21.102/mm^2), especially after the fifth decade of life (Tab. 1, Fig. 1). A comparison across age groups reveals that OPDs for 20~40 year olds differ significantly from 40~50+ year olds. For On.Ar sex and age effects are significant. Osteons decrease in size with age and the means differ between the <30 year and ≥ 40 year age groups, and the mean is significantly larger in males (0.029 mm^2) than in females (0.026 mm^2) (see Tab. s2, Fig. s1). A significant age effect exists for Ac.f; activation frequencies decrease with age and their means differ between all age groups except between 40~50+ year groups (Tab. 2, Fig. 2). Similarly, bone formation rates decrease with age and mean BFRs differ between all age groups except between 40~50+ year olds (see Tab. s3, Fig. s2).

Tab. 1 - ANOVA summary and age effect results for rib osteon population density (OPD).

EFFECT	DF EFFECT	MEAN SQUARE EFFECT	DF ERROR	MEAN SQUARE ERROR	F	P>F
Age	3	405.316	128	18.640	21.744	0.000
Sex	1	144.557	128	18.640	7.755	0.006
Age-Sex	3	46.382	128	18.640	2.488	0.063

MEAN RIB OPD BY AGE GROUP (#/MM²)				
AGE	**{1} 17.787**	**{2} 20.889**	**{3} 23.624**	**{4} 26.338**
20' {1}s		0.011	0.000*	0.000*
30's {2}	0.011		0.029	0.000*
40's {3}	0.000*	0.029		0.075
50+ {4}	0.000*	0.000*	0.075	

* Significance at p<0.01

Tab. 2- ANOVA summary and age effect results for rib activation frequency (Ac.f)

EFFECT	DF EFFECT	MEAN SQUARE EFFECT	DF ERROR	MEAN SQUARE ERROR	F	P>F
Age	3	4.267	128	0.080	53.090	0.000
Sex	1	0.449	128	0.080	5.580	0.020
Age-Sex	3	0.098	128	0.080	1.220	0.305

MEAN RIB AC.F BY AGE GROUP (#/MM²/YR)				
AGE	**{1} 1.505**	**{2} 1.025**	**{3} 0.790**	**{4} 0.702**
20's {1}		0.000*	0.000*	0.000*
30's {2}	0.000*		0.002*	0.000*
40's {3}	0.000*	0.002*		0.632
50+ {4}	0.000*	0.000*	0.632	

* Significance at p<0.01

Cortical bone histomorphometry: Femur

There are no age-sex interaction or sex effects for femur OPD (see Tabs. s4 and s5). The means increase with age and the two youngest age cohorts differ significantly from the 50+ year group (see Fig. s3). There are no interaction, age, or sex effects on On.Ar (see Tab. s6). Age has a significant effect on Ac.f; mean Ac.f decreases with age and differs between the 20~30 year and the older groups (see Tabs. s7, Fig. s4). Similarly, the mean BFRs decrease with age and differ between the 20~30 year olds and others (Tab. 3, Fig. 3).

Cross-sectional area measurements: Rib

There is a positive linear relationship between Tt.Ar, Ct.Ar, and Es.Ar and body mass. Pearson correlation coefficients are 0.544, 0.299, and 0.525, respectively ($p<0.001$ for all), and ANCOVA was carried out for these variables but not for the derived variable, Ct.Ar/Tt.Ar (see Tab. s8).

Only the sex effec t is significant for Tt.Ar (see Tab. s9, Fig. s5). Males have a significantly larger mean (68.305 mm^2) than females (49.689 mm^2). There is a significant interaction effect for Ct.Ar (see Tab. s10, Fig. s6). Sex differences are observed only in the 20~40 age groups with larger means in males, and the overall mean for males is 23.766 mm^2 and 17.652 mm^2 for females. In females, mean Ct.Ar decreases with age, but in males, mean Ct.Ar generally decreases and then increases in the 50+ year group. There is a significant sex effect for Es.Ar, with males having a larger mean (44.540 mm^2) than females (32.037 mm^2) (see Tab. s11, Fig. s7). Only the age effect is significant for Ct.Ar/Tt.Ar; the means decrease with age and the 20~30 year olds differ significantly from the 40~50+ year group (see Tab. s12, Fig. s8).

Cross-sectional area measurements: Femur

There is a positive linear relationship between Tt.Ar, Ct.Ar, and Es.Ar and body mass; Pearson correlation coefficients are 0.679 ($p<0.001$), 0.594 ($p<0.001$), and 0.277 ($p=0.002$), respectively, and ANCOVA was employed (see Tab. s13).

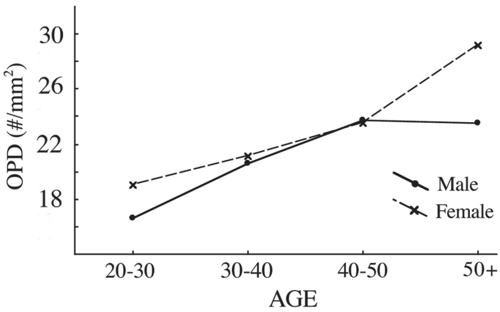

Fig. 1 - Mean plot of rib osteon population density (OPD) by age and sex.

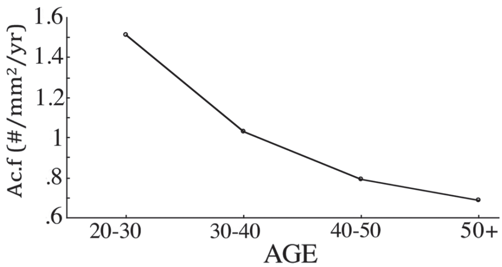

Fig. 2 - Mean plot of rib activation frequency (Ac.f) by age.

The age and sex effects are significant for Tt.Ar (see Tab. s14, Fig. s9). Femur Tt.Ar increases with age and the two younger groups differ significantly from the two older groups and males have a significantly larger mean (555.108 mm^2) than females (484.934 mm^2). There is an interaction effect for Ct.Ar and the means differ between males and females in each age cohort and the overall mean for males (406.316 mm^2) is significantly larger than in females (299.241 mm^2) (see Tab. s15, Fig. s10). In males, mean Ct.Ar increases up to 50 years and decreases thereafter, but the age-related changes are statistically insignificant. The mean for the 20~30 year old males differs from the mean for the 40~50 year males. There is no age-related pattern in females, and only the mean in the 20~30 year olds differs significantly from that for the 50+ year olds. Age effect is significant for Es.Ar. and mean Es.Ar increases with age; 50+ year group mean differs from 20~50 year groups

Tab. 3 - ANOVA summary and age effect results for femur bone formation rate (BFR).

EFFECT	DF EFFECT	MEAN SQUARE EFFECT	DF ERROR	MEAN SQUARE ERROR	F	P>F
Age	3	0.0026	95	0.0001	41.151	0.000
Sex	1	0.0003	95	0.0001	3.969	0.049
Age-Sex	3	0.0001	95	0.0001	1.016	0.389

MEAN FEMUR BFR BY AGE GROUP ($MM^2/MM^2/YR$)				
AGE	{1} 0.036	{2} 0.019	{3} 0.015	{4} 0.014
20's {1}		0.000*	0.000*	0.000*
30's {2}	0.000*		0.304	0.099
40's {3}	0.000*	0.304		0.870
50+ {4}	0.000*	0.099	0.870	

* Significance at p<0.01

and the 20~30 year group differs from 40~50 year group (see Tab. s16, Fig. s11). ANOVA was carried out for Ct.Ar/Tt.Ar and the interaction effect is significant (Tab. 4). The means decrease with age in both sexes, but they are not statistically significant. The younger female means are significantly larger than that for 50+ year females but the means do not differ in males. The means of males and females differ significantly after 40 years and males have an overall larger mean (0.715) than females (0.637) (Fig. 4).

Intraskeletal variability

Two-way ANOVA was run on the difference between the rib and femur histomorphometrics across age groups and by sex (see Tab. s17). Paired t-tests were carried out for femur and rib Ct.Ar/Tt.Ar, OPD, On.Ar, Ac.f, and BFR which had significant main effects. Mean femur Ct.Ar/Tt.Ar is larger than that for the rib, while mean rib OPD, Ac.f, and BFR are larger. Only the mean On.Ar does not differ between the femur and rib (see Tab. s18).

Age-sex interaction effect is significant in the difference between femur and rib Ct.Ar/Tt.Ar (see Tab. s19). Paired t-tests were done on eight possible age-sex combinations and the mean differences are significant across all age groups and by sex. In females, Ct.Ar/Tt.Ar decreases with age in both elements. In males, the magnitude of decrease in Ct.Ar/Tt.Ar is greater in the femur than in the rib, which accounts for the inconsistent pattern of decrease in the difference between the femur and rib. There are no significant age, sex, or interaction effects in the difference between femur and rib OPD, Ac.f, and BFR (see Tab. s20 ~ s22).

Discussion

Interskeletal variability: age differences

Rib and femur OPDs increase with age, consequently increasing the intracortical porosity due to a greater number of Haversian canals and resorptive spaces. Although the increases in mean OPDs between decades are not statistically significant, the mean OPDs for older individuals are larger than the younger means in both the rib and femur. Age-related increases in the number of intact and fragmentary osteons are typical of the pattern reported for modern and other archaeological populations (Mulhern, 2000; Mulhern & Van Gerven, 1997; Cho, 1996; Stout & Lueck, 1995), and the resulting net bone loss from porosity is partly responsible for normal age-associated bone loss in Isola Sacra.

While there are no age-related changes in the femur osteon size, for the rib, 40~50+ year old individuals are remodeling smaller packets of bone than 20~30 year olds. Smaller osteons have a greater propensity for osteon debonding or "pull-out" because there are more osteons packed per unit area of bone, but there are more osteons available for energy absorption and limiting the propagations of microcracks by cement lines (Nafaji *et al.,* 2007; O'Brien *et al.,* 2005; Sobelman *et al.,* 2004; Martin *et al.*, 1998; Burr *et al.*, 1990). It is possible that actual osteon size does not change with age; smaller mean osteon size may be due to more densely packed osteons and a higher probability that larger osteons will become partially remodeled through subsequent remodeling activity and, therefore, are not measured. Pfeiffer (1998) found smaller osteons in the rib than the femur, but the effect of osteon size on bone mechanics and the explanation for osteon size variability are indeterminate. Osteon size may reflect the size of the microcracks that were subsequently remodeled, and microcracks in the human rib range in size from 20~200µm with the majority shorter than 150µm (Qiu *et al.,* 2003). An alternate explanation is that osteon wall width and Haversian canal size may be regulated by osteocyte density in order for the osteocytes to receive nutrients to maintain newly formed bone (Metz *et al.,* 2003; Qiu *et al.*, 2003).

In both skeletal elements, older individuals exhibit a pattern of decreased activation of remodeling foci and bone remodeling with age, typical in archaeological and modern populations (Mulhern, 2000; Stout & Lueck, 1995). The rib exhibits significant differences between all age groups except between 40~49 and 50+ year olds. For the femur, consistent with the findings for OPD, differences in activation frequency are significant only between the youngest and the other three age categories. Although the remodeling rate typically increases in females after menopause, grouping all individuals over 50 years old does not allow for such observations. Bone loss studies of bioarchaeological populations are generally constrained due to the difficulties in estimating the ages of older individuals when age-related bone loss and sex differences on bone mass and remodeling rates from menopause become evident.

Cross-sectional area measurements indicate normal age-associated bone loss with age. The most relevant measure of bone mass, relative cortical area (Ct.Ar/Tt.Ar), decreases with age in both elements. In the rib, individuals over 40 years have a significantly smaller mean Ct.Ar/Tt.Ar than those in the 20s. The overall decrease in rib bone mass is due to age-related decreases

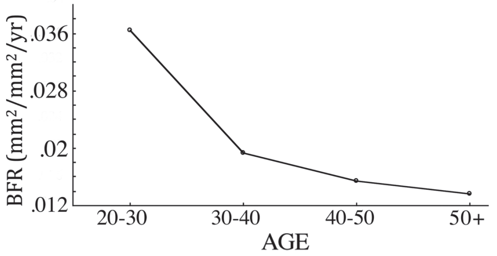

Fig. 3 - Mean plot of femur bone formation rate (BFR) by age.

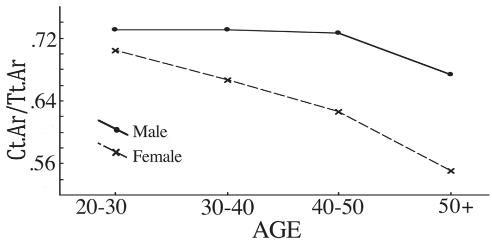

Fig. 4 - Mean plot of femur relative cortical area (Ct.Ar/Tt.Ar) by age and sex.

Tab. 4 - ANOVA summary and age-sex interaction effect results for femur relative cortical area (Ct.Ar/Tt.Ar).

EFFECT	DF EFFECT	MEAN SQUARE EFFECT	DF ERROR	MEAN SQUARE ERROR	F	P>F
Age	3	0.062	136	0.004	14.114	0.000
Sex	1	0.210	136	0.004	47.691	0.000
Age-Sex	3	0.015	136	0.004	3.321	0.022

MEAN FEMUR CT.AR/TT.AR BY AGE AND SEX								
AGE	**{1} 0.705**	**{2} 0.667**	**{3} 0.626**	**{4} 0.551**	**{5} 0.731**	**{6} 0.731**	**{7} 0.726**	**{8} 0.673**
Female 20's {1}		0.599	0.005*	0.000*	0.924	0.939	0.974	0.860
30's {2}	0.599		0.481	0.000*	0.040	0.056	0.074	1.000
40's {3}	0.005*	0.481		0.047	0.000*	0.000*	0.000*	0.429
50+ {4}	0.000*	0.000*	0.047		0.000*	0.000*	0.000*	0.000*
Male 20's {5}	0.924	0.040	0.000*	0.000*		1.000	1.000	0.176
30's {6}	0.939	0.056	0.000*	0.000*	1.000		1.000	0.209
40's {7}	0.974	0.074	0.000*	0.267	1.000	1.000		0.267
50+ {8}	0.860	1.000	0.429	0.000*	0.176	0.209	0.267	

* Significance at p<0.01

in cortical area and endosteal expansion. Ct.Ar/Tt.Ar in the midshaft rib samples from various North and South American archaeological populations and contemporary Americans also decreases with age, with archaeological populations possessing greater bone mass than the modern counterparts (Hayen, 2001). Similarly in the femur, the successive decreases in bone mass between age groups are not statistically significant, and a decrease in bone mass is possibly due to age-related increases in marrow cavity area, which is significantly different between the younger and older cohorts. Although total subperiosteal area in the femur also increases with age, it may be insufficient to compensate for endosteal expansion.

Interskeletal variability: sex differences

Based on the rib histomorphometrics, Isola Sacra females experience more Haversian bone remodeling than males. Indicated by their smaller mean osteon size, females are turning over smaller packets of bone than males, and in conjunction with similar relative cortical areas, females are able to accumulate larger numbers of osteons and Haversian canals per unit area and consequently exhibit greater intracortical porosity. Microstructurally, smaller osteon size and a larger OPD improve the fatigue properties of their bone since a larger number of small osteons increases energy absorption capacity (Nafaji *et al.*, 2007; Sobelman *et al.*, 2004; Burr *et al.*, 1990). Perhaps this is a compensatory mechanism for females who are more vulnerable to fragility fractures due to smaller subperiosteal and cortical areas. Rib Ac.f and BFR do not differ between the sexes, even though females exhibit larger mean OPDs. This apparent contradiction, given that Ac.f and BFR are a function of OPD, may be due to females turning over smaller packets of bone on average per remodeling unit, in addition to accumulated errors produced by the algorithm. Conversely, rib histomorphometry studies of a 19th century Swiss cemetery, archaeological populations from North America and the Sudan, and modern Americans found no sex differences (Mulhern, 2000; Stout *et al.*, 1996; Stout & Lueck, 1995; Stout & Paine, 1992).

There are no sex differences in the femur histomorphometrics for the Isola Sacra skeletal sample. Alternatively, Martin & Armelagos (1985, 1979) report significantly greater intracortical porosity and higher bone turnover rates in ancient Nubian females compared to males. They attribute the sex differences to nutritional and reproductive stress in females, especially in 20-29 year olds. Sex differences in the rib but not the femur may be attributed to a caveat in the present study that only the anterior femur was examined, or the non-weight-bearing and weight-bearing elements may have undergone different loading environments.

There are sex differences in cross-sectional area measurements, in particular the femur. Rib and femur total subperiosteal areas are larger in males than females, reflecting sexual dimorphism. Males exhibit larger femoral cortical area means than females, and endosteal expansion occurs similarly in both sexes. Martin & Armelagos (1979) also report sex differences in absolute femoral cortical area measurements for ancient Sudanese Nubians from A.D. 350-550. Nubian females lost 10.9% of cortical area compared to 4.9% in males, and females lost bone endosteally in the third decade of life, possibly attributed to nutritional stress exacerbated by pregnancy and lactation in females. For Isola Sacra femora, however, males and females lose similar relative amounts of bone mass with age, suggesting that the combined effects of endosteal resorption and periosteal expansion with age are similar in both sexes. Although femur relative cortical area decreases with age in both sexes, the amount of decrease is insignificant and sex differences are apparent after the fifth decade. Older females have a significantly reduced bone mass compared to younger females and males, while males maintain bone mass with age.

Endocrinological differences in sex hormones lead to sexual dimorphism in bone mass, geometry, and strength. During puberty, testosterone increases periosteal apposition of bone while estrogen promotes endosteal apposition. While females may have thicker cortices due to narrower medullary cavities, males achieve larger skeletal size, thicker cortices, and increased bone strength with more bone distributed away from the neutral axis in a long bone (Wells, 2007; Kim *et al.*, 2003; Schoenau *et al.*, 2001). Hence, sexual dimorphism in bone mass and strength is attributed to hormonal influences, life events, in addition to sexual division of labor. The sex differences in Isola Sacra are consistent with reports in the clinical literature and bioarchaeological studies. Females typically undergo more demands on the skeletal system due to various life events and accelerated bone loss after menopause, and increased intracortical porosity and the decrease in bone mass in Isola Sacra females after the fifth decade of life supports this trend.

Intraskeletal variability

Rib and femur histomorphometrics were compared for intraskeletal differences in remodeling. The rib receives frequent and continuous biomechanical loading during respiration, while the femur is exposed to more dynamic, variable, and higher loads produced during physical activities. Both the load magnitude and the number of loading cycles are significant factors in influencing the remodeling response (Scott *et al.*, 2008; Ural & Vashishth, 2006; O'Brien *et al.*, 2005; Sobelman *et al.*, 2004; Rubin & Lanyon, 1984; Schaffler & Burr, 1984), and the cortical remodeling values in the rib and femur should reflect their distinct functions and habitual loading environments. Schaffler & Burr (1984) report that different modes of primate locomotion correlate with the number of osteons in the midshaft femur. Peck & Stout (2007) observed intraskeletal variability in the midshaft of six skeletal elements from the same individuals that were examined for measure of bone mass. In particular, differences in bone mass were observed between the elements of the upper and lower limbs and the authors attribute this to differing mechanical loading environments.

Isola Sacra ribs have higher OPDs and remodeling rates than the femora, which is also reported for the medieval Nubians from A.D. 550-1450 (Mulhern, 2000; Mulhern & Van Gerven, 1997). Other than the different functional adaptations of each element, Mulhern suggests that the femur may have a later effective age of adult compacta, with fewer osteons accumulated for a given chronological age of the individual. Whereas cortical drift and modeling would have ceased in the rib around 12 years of age, these activities would continue in the femur, and remodeling would appear to be slower when it is actually similar. That is, osteon evidence would have accumulated over a shorter time in the femoral cortex and the mean tissue age would appear younger in the femur. The differences in OPD and derived parameters for the rib and femur in Isola Sacra and the Nubians may be due to this, rather than a difference in bone remodeling rates.

Secondly, the load magnitude in the rib is considerably less than that for the femur, but the continuous loading cycles in the rib probably creates more microdamage than other cortical bones which activates remodeling and results in higher OPD. Due to the viscoelasticity of cortical bone, it undergoes creep, or gradual deformation that occurs over a long period of time under constant loads of low magnitude. Fatigue occurs under higher magnitude and shorter duration and is closely related to creep. Damage caused by creep is more severe than fatigue because microcracks propagate through osteons and are not arrested by the cement lines. The resistance to crack propagation rather than crack initiation is more important for bone. Cyclical loading allows for strain energy to be diverted elsewhere so that remodeling can be instigated to remove the microdamage. Thus, continuous loading at low strain and high frequency results in less resistance to crack propagation, more microcracks, and increased remodeling (Sobelman *et al.*, 2004; Martin *et al.*, 1998).

Robling & Stout (2003), however report higher remodeling rates in the femur compared to the rib for an ancient Peruvian population, which they interpret to reflect the greater and more dynamic loading of the femur. This discrepancy among studies may be the result of methodological differences, rather than biological. Histomorphometrics for the femora in the current study were derived by sampling only the anterior quadrant of the cross-sections due to the loss of structural integrity during sample preparation. Mulhern & Van Gerven (1997) sampled eight microscopic fields adjacent to either the periosteal or endosteal surfaces along the anterior and posterior axes of the cross-section, while Robling & Stout (2003) sampled two one millimeter columns spanning from periosteum to endosteum at each of four anatomical quadrants.

These different findings emphasize the significant impact of the sampling method on the histomorphometric results and comparability among studies. Intraskeletal variation in the remodeling dynamics in the Isola Sacra rib and femur supports the need to conduct bone loss studies in diverse skeletal sites that undergo different loading environments.

Skeletal health and age-associated bone loss in Isola Sacra

Based on histomorphometric and cross-sectional area data, the Isola Sacra individuals exhibit normal age-associated bone loss that is consistent with bioarchaeological and clinical literature. Intracortical porosity and reduction in bone mass were more evident in females than in males as expected, most likely due to life events such as lactation and menopause, in addition to lower nutritional status. Other studies on the Isola Sacra necropolis, particularly as they relate to diet, offer further insight into their skeletal health.

The middle class, which made up a large segment of *Portus* and is the group of interest here, had an adequate diet in terms of both quantity and quality (Garnsey, 1998; Waterlow, 1989). Working with the assumption of egalitarianism and the lack of a political class until *Portus* became an independent municipality in the fourth century A.D. (Bunson, 1991), the inhabitants' diets would have been relatively homogeneous and better than that of the plebeians, but not as varied as that of the patricians (Prowse *et al.*, 2004; Cho, 2002). Owing to their location in the port of Rome and surely benefiting from the flow of goods into the harbors, they had better-than-average diets that were dominated by mostly cereals and other terrestrial resources supplemented with a variety of marine resources (Prowse *et al.*, 2005, 2004).

Dietary reconstructions of the Imperial Romans indicate that it was largely cereal-based, predominately wheat and barley (Garnsey, 1999b; Braun 1995; Waterlow, 1989; Rickman, 1980; White, 1976), with differing quantities and qualities of animal protein from pigs, goat, or sheep as they were prestige foods used in sacrifices and religious contexts (Garnsey, 1999b, 1998; Waterlow 1989). Wheat and barley are high in calories, higher in protein than root crops, and sources of vitamin B, vitamin E, calcium, and iron, but low in lysine, threonine, vitamin A, vitamin B_2 (riboflavin), vitamin C, and vitamin D (Garnsey, 1999b, 1998; Bisel, 1988; Rickman, 1980), the latter of which is necessary for calcium absorption in the gut. Romans also consumed broad beans, chickpeas, lentils, peas, and other legumes rich in protein, calcium, and vitamin C (Garnsey, 1999b, 1998), which complemented cereals in nutrient content. Fishing was an important activity at *Portus* and a food resource (Prowse *et al.*, 2005, 2004), and fish, a good source of protein, calcium, iron, vitamins A, B, and D, was also salted and pickled for times of food shortage (Gallant, 1985).

Early nutrition has significant effects on skeletal health later on, including bone mass. Though infants and children in Isola Sacra were nutritionally disadvantaged with compromised health statuses due to cultural attitudes about infant feeding and inadequate weaning foods (Prowse *et al.*, 2008, 2005; FitzGerald *et al.*, 2006), status and diets improved with age (Prowse *et al.*, 2005). Females had less access to marine foods and therefore, lower quantities of calcium and vitamin D, which is evidenced by isotopic analyses of skeletal remains and corroborated by historical records of their lower social status and beliefs and attitudes about gender and food (Prowse *et al.*, 2005). These nutritional factors, in addition to lifetime events, probably contributed to age-related bone loss in the Isola Sacra females.

Limitations of the study

Common clinical tools such as Dual Energy X-ray Absortiometry could not be employed because such methods are standardized for bone surrounded by soft tissue, and the Isola Sacra results could not be compared to clinical data. Second, the most important age category for observing age-related changes in bone mass was very broad and grouped as 50+ years. The effects of menopause and the rapid bone loss after its onset could not be distinguished among older females. Third, due to limited information on the lifestyle variables such as male and female physical activities, we could not address the specific factors that may have contributed to age-associated bone loss which is a highly variable occurrence caused by multiple factors. Fourth, an oxygen isotope analysis study of Isola Sacra teeth suggests that some inhabitants of *Portus* were "outsiders," and had migrated as families from other Imperial provinces such as North Africa, Greece, and northern parts of the Roman Empire (Prowse *et al.*, 2007). The individuals represented in the study may not be derived from a homogeneous, European, and urban population. Fifth, bone mass should be measured longitudinally in the same individuals to observe bone loss with increasing age. Cross-sectional studies such as the present one assume that individuals have the same variability in bone mass and bone loss. Sixth, due to the brittleness of the femur sample, structural integrity of the entire cross-section could not be maintained during sample preparation. The small area of bone sampled may have created the observed differences with the rib variables. In addition, the effective age of the femur adult compacta is unknown, and using the same value as that for the rib may have led to inaccuracies in estimates of femur histomorphometrics. Seventh, intraskeletal variability within a single skeletal element of an individual was not explored in the present study.

Summary and future directions

The pattern of age-associated bone loss in Isola Sacra is similar to other populations. In Isola Sacra, females lost more bone mass and had higher cortical bone turnover than males, probably due to life events such as menopause and lactation and lower status foods. If males had participated in physical activities related to the port, this may have conferred protection from severe bone loss by maintaining, perhaps even increasing their bone mass.

Studying the disparities in the incidence of bone loss and osteoporosis between and among past and modern populations is essential to reveal the biocultural factors that influence bone biology. Especially with osteoporosis, which is caused by a myriad of factors, lifestyle, genetic, and physiological factors must all be considered. Thus, diverse samples across time and space and other skeletal sites that undergo different mechanical functions are needed to address inter- and intraskeletal variability in bone loss. This study has shown that combining the histologic and macroscopic methods can be informative and applied to skeletal samples to explore bone loss in the past. Technological advancements and improved methods for detecting bone loss before fracture thresholds are exceeded should be applied to archaeological samples.

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