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Forensic Anthropology Population Data

Variation in osteon histomorphometrics and their impact on age-at-death estimation in older individuals

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ABSTRACT

Histomorphometric studies have reported relations between osteon size and age; however, data focused on the shape of osteons is sparse. The purpose of this study was to determine how osteon circularity (On.Cr) varies with age in different skeletal elements. Regression analysis was used to evaluate the relationship between age and osteon shape and size. We hypothesized that age would be negatively related to osteon size (area, On.Ar) and positively related to osteon shape (On.Cr). On.Cr and On.Ar were determined for the ribs and femora of 27 cadaveric specimens with known age-at-death. As predicted, age was significantly related to osteon size and shape for both the femur and rib. With age, there was a decrease in size and an increase in circularity. No relationship between sex and On.Cr was detected. An age predicting model, including On.Cr, On.Ar and OPD, is proposed to improve our ability to estimate age-at-death, especially for older individuals.

1. Introduction

Histology is a valuable methodology to estimate age-at-death for both recent and ancient skeletal remains. Typical of most osteological age estimation methods, age estimations for the remains of elderly individuals (50+ years) have proven to be problematic [1–3]. Most current histological age estimating methods rely primarily upon the well-established increase in the number of osteons and their fragments, known as the osteon population density (OPD), with age [e.g., 4–6]. It has also been shown that osteon size and osteon shape decrease and increase with age respectively [7,8]. In this paper we explore possible underlying causes of the link between osteon shape and size with age, and offer an age predicting model that incorporates osteon size and shape (i.e., circularity) in addition to OPD to predict age-at-death, and that is more applicable to older ages.

A number of histological methods have been developed for age estimation of archeological and forensic skeletal remains [5,6,9–16]. Because these methods rely upon well-established increases in the number of intact and fragmentary osteons within defined fields or per measured unit area with age, it is important to take the dimensions of osteons into consideration when estimating age, especially for older individuals exhibiting high osteon densities. Various dimensions and aspects of osteons have been studied in recent years [7,14,16–22], and the dimensions of osteons have been reported to vary with age. An age-dependent decrease in osteon size has been repeatedly observed in humans [12,23–30]. This decrease has also been found in nonhuman primates such as macaques [31,32].

However, despite the focus on the relationship between age and osteon size, few studies have examined the relationship between age and shape of osteons. Moreover, when aspects of On.Cr are examined the results are frequently limited to qualitative associations in regards to strain effect [33] and location within the cortex [29]. Currey [8] and Britz and colleagues [7] are notable exceptions. Currey [8] reported that the osteons of older individuals are nearly circular whereas younger individuals have more irregularly shaped osteons. Britz and colleagues [7] found that circularity increased with age in the femur. Moreover, circularity has been used as a variable in assessing species identification, since human osteons have been reported to be less circular than their non-human counterparts [19,20].

The effect of osteon circularity on histological age-at-death estimation is still not well understood. The purpose of this study is

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to evaluate the impact that osteon shape (i.e., circularity) has on age-at-death estimations for these skeletal elements. Because secondary osteons serve as the primary variable used to estimate histological age-at-death, variables such as their shape and size are important factors that can potentially affect osteon population density when it is based upon sampling a limited number and size of fields of view. Furthermore, because osteon population density increases and osteon area decreases with age, we predict that smaller more circular osteons will be more prevalent as age increases and OPD asymptote is reached. This may be due to a decrease in the size of osteons created during remodeling in such individuals, or merely the greater likelihood of smaller, more circular osteons surviving intact for measurement. The femur and rib were chosen as sampling sites because they represent bones that are commonly studied histomorphologically, that experience different biomechanical loading histories and remodeling rates [34], and the age at which an OPD asymptote is reached for these two bones should also differ. A predicting model based upon osteon circularity (On.Cr), size (On.Ar), and OPD derived from both the femur and rib, therefore, should expand the age range over which age estimation is applicable.

2. Materials and methods

The study sample includes 11 males and 16 females of European ancestry with known age and cause of death (Table 1). Ages range between 39 and 82 years with an average age of 62. These 27 individuals are a subset of a dissecting room cadaver collection obtained from the Departments of Anatomy of Washington University, St. Louis, Missouri, and the University of Missouri, Columbia by SDS. Undecalcified thin cross-sections (~100 μm thick) of rib and femur samples analyzed in the present study had already been prepared using standard histological procedures [31–33] for earlier research on intraskeletal variability for cortical bone histomorphometry [e.g., 6, 34, 35]. Bone samples were taken from the middle-third of the rib and mid-shaft of the femur of each of the 27 individuals.

Table 1
Summary of sample data.

Individual	Age	Sex	Cause of death
1	39	F	Hemorrhage metastic breast cancer
2	47	F	Suicide (drug overdose)
3	52	M	Carcinomatosis
4	53	M	Carcinosis
5	53	M	Carcinomatosis
6	54	F	Intracerebral hemorrhage
7	55	F	Metastic colon cancer
8	57	F	Liver failure
9	59	F	Ovarian cancer
10	59	F	Breast cancer
11	60	M	Cardiac arrest
12	60	F	Bladder cancer
13	61	F	Cerebral hemorrhage
14	62	F	Cardiac arrest
15	65	F	Metastic breast cancer
16	66	M	Lung cancer
17	66	F	Carcinomatosis
18	67	M	Heart attack
19	68	M	Pneumonia
20	68	M	Chronic congestive heart failure
21	70	M	Lung cancer
22	72	F	Natural causes
23	72	F	Metastic breast cancer
24	75	M	Gastric carcinoma
25	77	F	Cerebrovascular accident
26	81	F	Cerebral hemorrage
27	82	M	Ventricular fibulation

The following standard osteon-based variables [38–41] were identified and quantified for each rib and femur cross-section using a transmitted light Olympus® BX51 research microscope (Fig. 1):

Intact Osteon Density (N.On): the number per unit area of intact osteons, defined as any osteon that is circumscribed by a complete reversal line and contains an intact Haversian canal.

Fragmentary Osteon Density (N.On.Fg): the number per unit area of partially remodeled osteons with incomplete reversal lines for which their Haversian canal, if present, is ≥10% incomplete.

Osteon Population Density (OPD): the sum of the intact and fragmentary osteon densities (osteons/mm^2); i.e., N.On + N.On.Fg.

Osteon Area (On.Ar): the area of bone, including Haversian canal, contained within the reversal line of an intact osteon. Mean osteonal area (On.Ar, mm^2) was calculated based upon the average area of 30–35 osteons for each bone for each individual.

Osteon Circularity (On.Cr): is defined by a circularity index (4π(area/perimeter^2)) that indicates to what extent a measured object is similar in shape to a true circle. A value of one (1) represents a true circle and values approaching zero (0) represent increasingly elongated shapes [42]. A minimum of 30 and maximum of 35 osteons were measured for each of the femur and rib thin sections. As osteon size tends to vary in different areas of the bone cortex, possibly relating to regional differences in strain levels [43,44], osteons were sampled from different locations throughout the thin section to obtain representation from multiple regions (Fig. 2).

On.Cr was determined using a digital camera (Spot Insight® QE Color 4.2.1) mounted on the microscope. Polarized and semi-polarized images of the cross-section were captured from four areas of the bone (Fig. 1). Using *SPOT Basic 3.5.9.1* software (Diagnostic Instrument Inc.) and *ImageJ* software platform (v 1.42; National Institutes of Health), the images were calibrated at 100× magnification (10× objective and 10× eyepiece) and the micrometer scale was converted to pixel counts in *ImageJ*, allowing for the assessment of histomorphometric variables. An outline of each osteon was manually drawn using a drawing pen tablet (Intuos3, Wacom Co. Ltd., Japan).

Circularity index (On.Cr) and osteon area (On.Ar) were determined using the area and shape descriptors functions of *ImageJ*. Each individual osteon was outlined separately and served the basis for the calculation of osteon circularity. For determining osteon circularity (On.Cr), only structurally complete intact osteons with complete reversal lines and round Haversian canals were measured to avoid measuring osteons represented by tangential cuts. Five to eight osteons with well-defined boundaries were measured for each quadrant of a cross-section.

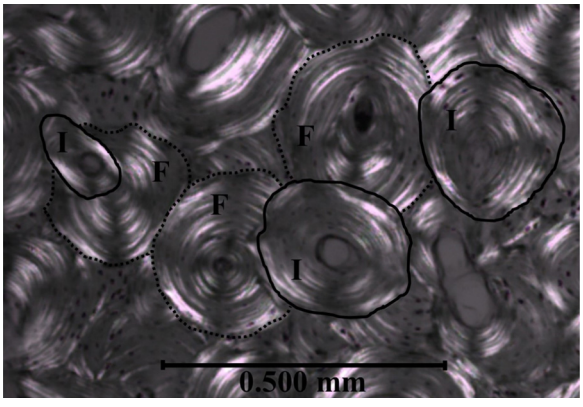

Fig. 1. Example of intact (I) and fragmentary (F) osteons. Photomicrograph of a femoral cross-section under polarized light at 10× magnification.