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Histomorphometry specific to anthropological studies concerning the human condition

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Summary - *Bone histomorphometry refers to the study of the structure and microscopic features of bone tissue. It involves the measurement and assessment of bone microanatomy, and it provides valuable information on bone properties. Through the application of histomorphometry, researchers can acquire information on bone metabolism and on remodeling dynamics, which is useful to the study of bone health. During the last 50 years, biological anthropologists have adopted the use of histomorphometry while examining issues specific to human health and evolutionary trends from prehistoric remains. Scientists coming from the medical field have applied histomorphometry in their research as it allows the study of bone changes, useful to describe pathological conditions among these ancient human remains. This paper reflects on some of the research that involves histomorphometric analysis specific to diet and health, forensic anthropology, taphonomic assessment of bone, non-human primate research and biomechanics. The purpose of the paper is to consider past and future applications of bone histomorphometry to enable a discussion which might direct research towards under-explored areas of bone biology. For example, looking at renewed interest in clavicular histology and stimulating investigations that focus on osteocyte density. Additionally, a discussion is offered concerning OPD values used to correlate chronological age to biological age estimations.*

Keywords - *Forensic, Evolution, Taphonomy, Osteons.*

Introduction

Bone histomorphometry is the application of measured data towards the quantitative evaluation of skeletal microanatomy; traditionally, scientists that are in the forefront in this research come primarily from the medical field. Their research tended to focus on changes in bone properties due to systemic skeletal diseases such as osteoporosis (Bassan et al. 1963; Frost 1973; Parfitt 1979) and other metabolic disorders, for example, hyperparathyroidism's effect on bone remodeling, that is an increase in cortical bone loss (Wilde et al. 1973). Early medical research results of the 1960's -1980's offered by Frost and his many co-authors (for example, Epker and Frost 1964; Frost 1966, 1985, 1986) set the stage for how other disciplines, like biological anthropology, also evaluate bone microanatomy. The purpose of this paper is to visit past anthropological literature specific to how cortical histomorphometry has been employed to examine the human skeletal condition. Several directions for future histomorphometric research are also shared. Reflection is a critical part of the growth of a discipline, and understanding how anthropologists have employed cortical bone histomorphometrics is clearly worth examining. Towards this end, the sub-topic bias presented in this paper reflects personal observations and research that have matured during 40 years of histological work. Hence, some of the older references listed here are from my education in bone histology and represent the foundation of previous research (RRP).

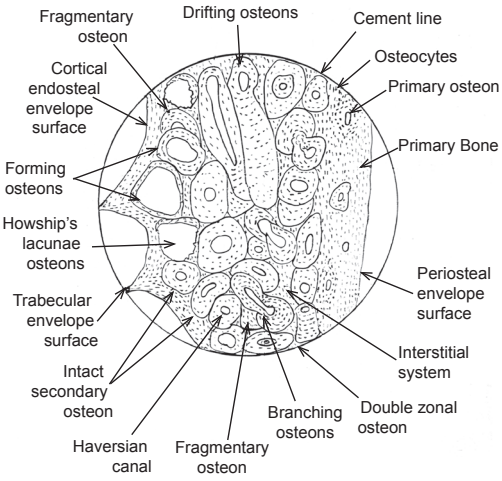

Fig. 1 - Drawing and labeling of basic bone micro-anatomical features (RR Paine).

Skeletal histomorphometry is the assessment of bone micro-features through the combination of measurements and statistics to discover anatomical patterns. This of course differs from descriptive histological assessment that simply reports the presence or absence of features and their frequency as seen in bone cross-section. Early on as scientists examined bone for specific features, descriptive assessment was common enough. The reporting of the presence of Howship's lacunae, secondary and primary osteons along with other basic bone features was in itself a critical goal. A goal that began the conversion concerning why bone differs between animal species and within the skeletal structure of human samples (see Currey 1964; Enlow and Brown 1956, 1957, 1958; Jowsey 1966, 1968).

Stemming from this work a very fundamental question is asked: why collect measured data from biological samples? A simple yet critical answer to this question is that scientists seek to discover predictable patterns in nature. It is assumed that patterns seen in the human anatomy might lead to a better understanding of how our bodies work under normal circumstances. Through science we seek to understand normality so that we can better understand abnormal human states (diseased conditions), see Frost (1973, 1985) for an early conversation about this. Measured data (bone histomorphometry) tends to lend itself to making predictions and to the potential discovery of patterns that are normal in bone cross-section so that we can analyze what the result of biological stressors might be (that is abnormal bone formation).

This research goal assumes that nature actually has patterns that can be determined and that scientists have the tools to reveal these patterns. After 50 plus years of research what patterns in skeletal anatomical features have been revealed and agreed upon by anthropologists? How has finding these patterns aided in our understanding of the human skeletal condition? These are basic questions that seldom appear obvious in anthropological publications focusing on bone histomorphometry. Yet, they should be clearly stated as such. As part of the purpose of this article, a discussion of several lesser examined biological variables will be offered including osteocytes numbers and patterns.

During the late 1980's Dr. George Armelagos and I had a brief conversation concerning the future of histology research among anthropologists. My impression was that he considered this work to be critical, but he was not sure whether there would be enough students interested in the methodology and science to continue this approach. At the time, there were very few anthropological programs working on histological studies: Pfeiffer, Stout, Weaver and Armelagos come to mind. The 4 of them were working in anthropological programs producing PhD students; others were working in Museums or non-anthropological programs (Burr, Ubelaker, Owsley, and Ortner). His concern was well founded. Yet, 40 years later it appears that bone histology is now well seated among the anthropological methods/approaches used for understanding the human condition. This can be attested to by the work done in many US anthropological programs and by research groups in Greece, Australia, UK, Korea and Japan that are focused on collecting histomorphometric data.

Histomorphometric parameters

It is often a good idea to see how specific research goals began so we can determine how far science has moved. For many older students of bone histology, the adventure started with Wu et al. (1970) and the application of remodeling assessment in human, for health reasons, and archaeological bone from a mastodon. This is true for most of Dr. Sam Stout's students. Wu et al. (1970) purpose was to show how counts of secondary osteon density could determine bone formation rates; a valuable goal still looked for by anthropologists examining bone today. Figure 1 shows basic bone micro-anatomical features that are often recorded by anthropologists.

Early on typical data recorded by histologists included:
AI: Number of sites of bone formation per mm².
Ar: Number of sites of bone resorption per mm².
C/T: Proportion of cortical and total bone section area.

Today there are many more features that can be counted and measured (see Parfitt et al. 1987; Robling and Stout 2008). However, it is not uncommon for many of these features not to be reported due to several considerations: the nature of dry bone samples and the tendency to focus on commonly discussed variables that have been examined in previous studies. OPD for example is a ratio variable that is often collected while many other variables are not (Table 1). Osteon population density (OPD) is calculated by using the following variables (see Table 1. For an example of how the formula is used see example Kranioti et al. 2020).

The defining criteria for intact secondary osteons and fragmentary secondary osteons are well discussed in Robling and Stout (2008). So, what are the features that do not get measured? Primary osteons and their canal dimensions are rarely included in histomorphometric data, same goes for osteocyte numbers/densities (total number of lacunae (N.Lc) and lacunar volume (Lc.V) their measures (Carter et al. 2013; Bromage et al. 2016), Howship's lacuna numbers or dimensions, and Volkman's canal area/size. The lack of data on these features leads to missed opportunities for understanding or realizing biological patterns. Some early work by anthropologists/researchers did record the presence of these features and sometimes offered them as ratio variables (for example Kerley 1965, non-Haversian canals: Walter et al. 2004, frequency of osteocytes per secondary osteon feature) or metrics (Jowsey 1966). It seems that we should revisit the possibility of recording metric data on these features. In fact, it appears that many students follow a dogma pattern of collecting histological data without thinking about why they are collecting the data in the first place, nor do they seem to think about what else might be observed.

***Tab. 1** - Typical histological counts and ratio values currently used.*

VARIABLES	
Total cortical area	Ct.Ar
Total number of intact secondary osteons counted	N.On
Total number of fragmentary secondary osteons counted	N.On.Fg.
N.On. + N.On.Fg./ Ct.Ar	OPD, osteons population density

Disease and diet

As biological anthropologists looked for predictable patterns in bone microanatomy, it has been suggested that there might be population specific patterns that can be identified and put to practical use as in the forensic assessment of dry bone (Cho et al. 2002). Population-specific patterns in bone microanatomy may exist but are unlikely to be the result of genetic factors.

Previously, population-specific patterns in bone remodeling and their rates have been investigated and anthropologists have attempted to link these factors to diet, not genetics. Richman et al. (1979) made a connection between a high-protein diet and the frequency of intra-cortical

bone remodeling. They argued that this type of diet leads to the formation of numerous type II secondary osteons. A more recent study suggests a link between bone remodeling rates, diet and behavioral patterns: a greater consumption of protein supports more stable processes for bone remodeling rates (Pfeiffer et al. 2019).

Brenton and Paine (2007, 2006a,b) and Paine and Brenton (2019) have also argued that differential remodeling patterns that appear to be population specific may be linked to human behavior such as habitual activity patterns and/or diet (Tab. 1). Specifically, they have found that food-security issues are linked to low bone turnover rates (larger than expected secondary osteons are formed and very low OPD values specific to age are seen) and that a lack of an adequate diet may play a key role in how patterns of rib remodeling occur (Paine and Brenton 2006b).

In an attempt to better understand the relationship between chronological age and biological age a comparison among the original rib data from Stout and Paine (1992) , along with Dart samples (Paine and Brenton 2006a) and archaeological samples from Ledders and Gibson sites (Stout 1976) was done (Table 2). The histological results from the Dart collection were then used to provide a foundation for assessing rib OPD values at the age-of-death for the skeletal assemblages from several archaeological sites. Our focus was on rib samples from burials located in the lower Illinois Valley region, the Middle Woodland (ca.350 AD) Gibson Site (n = 25) and the terminal Late Woodland (ca. 900AD) Ledders site (n = 19) (Cook 1976).

When nutritional deficiencies appear to be life-long, chronic, and perhaps seasonally exacerbated, they may produce extreme differences in expected verses observed OPD values as one ages.

There have been several inconsistent interpretations for low remodeling rates observed in archaeological/modern samples. Dubar et al. (1993) attributes it to inactivity; Mulhern (2000) links it to a high level of physical activity; Stout and Lueck (1995) talk about it in terms of a slower skeletal maturity rate in past populations. We have provided a model and context for interpreting the phenomena of lower remodeling rates in humans across several populations as a problem associated with metabolic stress (food insecurity). The factors causing low remodeling rates in some archaeological contexts is most likely linked to malnutrition. In the end, we suggest that OPD rates must be understood in the context of synergistic effect of infection coupled with malnutrition.

Along with dealing with the issue of how diet may affect bone turn-over rates, researchers can rely on new methods for collecting critical histomorphometric data. An example consists in the application of 2D images to examine bone microanatomy, as histologists assess health conditions. Dr. Sam Stout and his students are working with this approach (Cole and Stout 2018, Cole et al. 2022). A recently published pilot study outlined how intra-cortical porosity can be measured using semi-automated method. The study illustrates the use of a free online program, "Pore Extractor 2D", to collect 2D data (Cole et al. 2022). This approach for collecting data can be employed in several studies specific to the human condition (age estimation, evolutionary issues, health status and biomechanics).

In addition to 2D, a group of histologists have been working on three dimensional studies (Maggiano et al. 2016) for some time and their work is very promising. Through these studies, they are providing improved metric assessment of femoral bone cross-sections that offers insights to both health and biomechanical issues. Specifically, they employ synchrotron radiation-based micro-CT to mapout the formation of Haversian systems, both longitudinal and branching formations (Maggiano et al. 2016). Continuing this line of research, (Maggiano et al. 2021) they have used these methods to examine secondary osteon circularity (On.Cr.) variation as seen in the transverse cross-sections of 3 human femora. Research assessing secondary osteons circularity provides the potential for understanding the shape nature and variability of osteons longitudinally (Maggiano et al. 2021) which then offers insights as to how osteons may change shape over tissue space.

Table 2. A Summary of OPD values specific to influencing factors.

COLLECTION	PHYSICAL ACTIVITY	OPD VALUES EXP	OPD VALUES OBS	DIETARY HISTORY	OPD VALUES EXP	OPD VALUES OBS	METABOLIC/ INFECTION	GENETIC / BIOGEO
Ledders and Gibson	high	high	low	Food insecurity	low	low	high	different
Dart autopsies	high	high	low	Food insecurity	low	low	high	different
RRP autopsies	Medium/low	medium	high	No food insecurity	Medium high	high	Medium/low	different

Ledders and Gibson (Stout 1976)

The presence or absence of type II and drifting osteons in cortical bone signifies a critical question about what they might mean in terms of health and human behavior. Robling and Stout (1999) offered their insight on drifting osteons seen in ribs suggesting they are one of the most common features seen in juvenile bone. Cooke et al. 2022 offer a very good summary of these anatomical features and provide a current view as to their significance in cortical bone. Maybe there is no specific reason for their presence other than they are naturally forming micro-anatomical features (Cooke et al. 2022). Good results from this work, if not a bit disappointing for some that have speculated on their meaning. This conclusion resulted from collected data that included histomorphometric measurements: OPD, On.Ar, H.Ar, H.Dm, and the ratio of H. Ar:On.Ar. Their work also marked the presence and frequency of drifting osteons and type II osteons (Cooke et al. 2022). The question about the presence of these features and what they might mean has been examined a number of times over the years Stout and Simmons (1979); Robling and Stout (2008) both offer a historic overview on this topic including the presence of double zonal osteons. Most references cited in their book chapter suggest an association between a higher frequency of type II osteons and age. Yet no clear statistical assessment was available to support this position. It may be that Cooke et al. (2022) have it right that they are simply natural features that may change in frequency of time.

Evolutionary studies

Understanding how human bone has changed or stayed the same over our evolutionary history has also been a keen scientific goal and anthropologists have applied the study of cortical bone microanatomy to this purpose (Thompson and Trinkaus 1981; Trinkaus and Thompson 1987; Abbott et al. 1996). Questions specific to the nature of secondary osteons and other micro-anatomical features have included the presence and patterning of these features (Abbott et al. 1996; Streeter et al. 2001; Pfeiffer 2016). The implication is that the assessment of histological features seen in hominid remains may provide information specific to our ancestor's life history, their biomechanical behavior and health status. Most of this work involves the assessment of secondary osteons number frequencies and OPD values, as well as cross-sectional geometry, for example by Ruff (1987). The goal of this research is to find critical evolutionary-base insights as we examine bone features in modern humans; behaviors associated with diet, infection and other influences that may affect our health status. Recently, Micarelli et al. (2024) used histological features of a fossil femur shaft from the Italian site of Venosa-Notarchirico. They did so for two purposes: to reassess the age-at –death for the remains and to re-examine the periosteal lesion seen on this fossil. They concluded that the individual was most likely an adolescent with a non-specific infection of the periosteum envelope of the shaft.

Another critical question examined by anthropologists attempts to determine if the cortical bone features of Pleistocene *Homo sapiens,* and that of Neanderthals, represent a modern human condition or an earlier more ape-like pattern? Streeter et al. (2010) re-evaluated (Abbott et al. 1996) and implied that based on micro-anatomical feature densities, Pleistocene *Homo* did not differ in remodeling rates from modern humans. Still, their conclusions stated that additional assessment is required to support this position (Streeter et al. 2010). Sawada et al. (2004) used bone histomorphometry (percent osteonal bone, osteon population density, non-Haversian canal population density, secondary osteon area, and Haversian canal area) while comparing samples of modern children's bones with that of a femoral mid-shaft cross-section sample from that Dederiyeh 1 Neanderthal child. They found that the cortical bone formation in Dederiyeh 1 differed from modern children; the reason for this was not specifically identified (Sawada et al. 2004).

Most of the studies that have been conducted have been specific to identifying the fragmented bones while placing them in an evolutionary context; for example, analyzing features (secondary osteon size) to decide the taxonomic grouping assigned to the fragment (Pfeiffer and Zehr 1996). Additionally, histology (OPD values) is sometimes used to create age-at-death estimation for hominid remains (Pfeiffer et al. 2016). This is a common finding, in part due to the small size of the samples used in this research. Thin sectioning of hominid skeletal remains has also been problematic due to the destructive nature of side preparation. Very few researchers are willing to subject rare fossil bones to the destructive process of bone preparation for histological assessment.

On the other side of the equation, a number of researchers have looked at the general primate condition to clarify how remodeling may differ or follow similar patterns among members of this taxonomic Order (Burr 1992; Paine and Godfrey 1997; McFarlin et al. 2016). For example, Burr et al. (1989) found that intracortical remodeling of femoral bone seen in growing macaques was slow and that it was more similar to that of older humans than that of children. Paine and Godfrey (1997) used secondary osteon cross-sectional patterns to suggest that biomechanics may play a role in how secondary osteons are distributed within the cross-section of humeral and femoral bones. They did so by comparing percentages of osteonal bone to that of primary bone with the cross-sections. Mcfarlin and colleagues (2008) followed Paine and Godfrey's methods for assessment of osteonal bone by further examining the relationship between percent secondary osteon area (% Hav.) in long cortical cross-sections and locomotor behavior in non-human primates. They suggested that local cortical strains may influence patterns of secondary osteon bone distribution as seen in the femoral mid-shaft. Mulhern and Ubelaker (2009) provided a critical research look at this topic offering insight suggesting that bone microstructure (OPD values) may have been consistent among primates over considerable periods of time. The results of these publications look promising but the size of some of the samples remains small. Hence, further observations are required.

Forensic applications, age estimation

One of the areas of considerable work and research that has applied histology to the understanding of human bone biology is forensic anthropology. In fact, for many students of forensic anthropology, learning about skeletal histomorphometry begins with reading Kerley (1965). Certainly, this holds true for me (Paine 1985; Stout and Paine 1992). Kerley's work examined micro-anatomical features including secondary osteon counts from long bones (femur, tibia and fibula). Specifically, he used secondary osteon counts as a predictive feature in assessing age-at-death. This counting method was improved as it was adjusted for creating more accurate counts (Kerley and Ubelaker 1978) to account for potential errors specific to the femora cortical bone area that was considered. As

some might attest, the articles were easy enough to read and understand but the method was difficult to apply. Over the years, most of his work has been set aside except for femoral osteon counts. Research on the histology of the fibula has all but ended.

Robling and Stout (2008) wrote a critical book chapter on the histomorphometric assessment of human bone specific to age-at-death determination. This is a critical publication in which they focus on several key concepts that are necessary for understanding how to apply results of bone microanatomy to this end. They review the literature on basic yet critical mechanisms for the formation of secondary osteons during the remodeling process, BMU Basic Multi-cellular unit formation (Parfitt 1979) and the anatomical terms specific to this process. Secondly, they discuss the methods by which age estimation via secondary osteons and other micro-anatomical features have been used by anthropologists in both the forensic/archaeological context and lastly, they review the several factors (biology of sex, activity patterns and population specific biology) that could potentially influence remodeling rates relative to skeletal age. This assessment was further updated by Crowder and Stout's (2012) edited book on anthropological histomorphometry.

Kerley's (1965) publication on long bone histology and Sam Stout's mentoring set the stage for my own research: the assessment of rib and clavicular secondary osteons and their use for predicting age-at-death (Stout and Paine 1992). At the time, (during the 1980's and early 1990's) using bone histomorphometry towards an "Applied approach" for evaluation of human remains was considered unworthy of traditional anthropology research. Clearly, opinions have changed, and the teaching of forensic anthropology is now the reason why some anthropology programs have grown during the last few decades. In particular, rib histology has contributed to growth in teaching, research and publications.

Several studies have been made since the 1990's to test for population-based usefulness of equations that estimate the age-at-death for unidentified skeletal remains (Dudar et al. 1993; Cho et al. 2010; Crowder and Rosella 2007; Garcia-Donas et al. 2016; Garcia-Donas et al. 2021). Much of this has been an attempt to modify, test the accuracy of or determine the unsuccessful nature of OPD values from the left 6th rib in age estimation (Stout and Paine 1992). A few additional variables have been employed in developing other age predicting equations (for example, Cho et al. 2002, 2006) but none have been shown to have additionally improved predicting powers. Recent work by Katydid and co-workers (2022) suggest that Cho's equation is more accurate than others. Unfortunately, other than the region and the time period for the Katydid's samples (14 individuals recovered from St. Peter's burial ground in Blackburn, who died between 1839 and 1857) no other cultural context is provided (Katydid et al. 2022). As Paine and Brenton (2006a,b) have suggested a poor diet could account for the under-age estimation by some of the equations.

It has become clear to us that the prediction of age based on bone features like OPD is problematic due to a number of factors (Karydi et al. 2022; Garcia-Donas et al. 2021, 2022; Thompson and Trinkaus 1981; Paine and Brenton 2006a). We suggest that the confounding aspect of this issue is tied to an understanding of the relation between chronological age and biological age (Couoh 2017). Histologists see this specifically when it comes to working with fossil material or archaeological remains (Karydi et al. 2022; Thompson and Trinkaus 1981) and sometime with forensic case work (Paine and Brenton 2006a). What is at the base of this discussion is that the two do not always match; often factors like diet, environment, lifestyle, and behavior will affect this difference either increasing or decreasing the biological age relative to the chronological age (Couoh 2017). In addition, why does gross age assessments differ from histological assessment? For archeological remains in which age estimations (gross and histological) are both biological ages they too also do not always match. So, what variables in nature, behavior or culture are we missing that might account for

this? This is a critical question. Then there is the lack of matching of chronological age with biological age (both by gross and histological methods) in forensic cases. Yet sometimes they do match. Again, the question remains; what influences these results?

The mismatching of age (chronological and biological) also appears to be an issue in living individuals specifically when they reach older ages (Mitnitski et al. 2002). That is, the biological age can appear to be much older than the chronological age reflecting lifestyle and diet. Too much sun exposure (environmental factor) and smoking/vaping (behavioral factor) tends to increase biological age in modern individuals.

As it happens often with archeological material, there seems to be a disconnect between chronological age (assumed by gross age predicting methods) and biological age that has been assessed histologically. The histological age estimation tends to be younger relatively to the estimated age based on gross assessment methods (for example, Pfeiffer and Zehr 1996; Cho et al. 2002; Crowder 2005). The fact that bone histologists do not always obtain similar results in age prediction as the ones obtained through gross morphology methods provides us with a unique opportunity to ask the question concerning why histological changes in bone and the gross appearance of bone reflect a different timing to structural bone changes relative to chronological age. Does it slow it down or speed it up relatively to chronological age?? Seemingly, whatever is happening to archaeological burials (for example, higher activity levels, food insecurity issues) tends to lower the biological age estimation obtained by OPD values. Specifically, the question is how does diet, environment, lifestyle and behavior actually affect bone biology? This has been the cornerstone of our work with dietary issues and bone biology (Paine and Brenton 2006). In previous research, we observed that poor health and food insecurity may slow down the biological age of bone (OPD production) compared to chronological age. It appears that this observation should be a critical perspective for discussing why sometimes age prediction equations provide accurate age estimations while at other times it does not.

Taphonomic applications

Research into bone diagenesis usually begins with a review of Hackett's (1981) study that describes fungal involvement in microanatomy bone loss. Recent use of examining micro-anatomical features of archaeological bone related to bone loss has been to determine how funeral practices were employed by prehistoric communities. As Hackett (1981) first pointed out, microbial bioerosion brings about diagenetic change in archaeological bone. Since then, others like Booth (2016) and Lemmers et al. (2020) have based their research on these findings and have focused on understanding how past mortuary practices occurred. This has been done by assuming that diagenetic changes to bone is related to the length of time that the body is exposed to above ground influences (bacteria and fungus) in decomposition (Booth 2016). De Cataldo et al. (2020) applied this assumption to burials from the site of Arabic RH5 in Oman, a Neolithic fishing community. The percentage of bioerosion rib samples was documented (area of involvement; CBAD/Ct.Ar.). Ninety-five percent of the rib cross-sections showed diagenetic alteration due to fungal invasion. The authors concluded that variability of bioerosion in rib cross section indicates that different lengths of time of surface decomposition were involved during funeral practices at Arabic RH5.

Taphonomic processes can make identifying bone fragments difficult when it comes to figuring out species, or at the very least human versus animal origin of the remains. This is a problem for both forensic scientists and archaeologists. The assessment of bone micro-anatomical features between animal and human remains has long been attempted with a range of successes (Hillier et al. 2007). Much of this work begins with Enlow and Brown (1958) and Jowsey (1966). Enlow and Brown (1956, 1957, 1958) offer a range of species-specific histological features in their description-based research. They point out the presence of primary osteonal bone, secondary osteonal bone and of other features such as plexiform patterns of bone. Jowsey

(1966) examines a number of mammalian species and offers a series of measurements for microanatomical features. For example, she provides diameter and perimeter measurements for secondary osteons and Haversian canals.

Currently, determining specific species identity among mammals still eludes anthropologists. The best we can affirm is that if the remains show plexiform bone, it is most likely not human (Hillier et al. 2007). Mulhern and Ubelaker (2001) suggest that smaller mammals including rat, cat, dog, hare, badger, and racoon dog, and the larger mammal deer can be separated from human remains. This can be done by carefully measuring Haversian system diameter, Haversian canal diameter, and Haversian system density. These animals tend to have smaller Haversian canal areas for example. The presence of banding osteons also tends to be found in non-human bone (Mulhern and Ubelaker 2001, 2009). Recently, Gigante et al. (2021) attempted to resolve an archaeological mystery using these criteria and assumptions by sorting out very fragmentary bone samples from cremated remains found in Tomb 168 (The Nestor burial) from the Greek site of Pithekoussai, located on the Italian Island of Ischia. For over fifty years, classical archaeologists have debated about the nature of these remains with little satisfaction. While attempting to resolve this discussion, Gigante et al. (2021) have been the first researchers to successfully state that the remains actually represent both animal and human fragmentary bone. They obtained these results by comparing osteon area and Haversian canal area (animal fragments exhibited smaller Haversian canal area). Furthermore, the human remains may represent at least 3 individuals exhibiting 3 different OPD values (the 3 OPD non-overlapping cluster ranges are: (5.96-7.9, n = 6 specimen) (10.14-14.68, n = 8 specimens) (19.28-19.65, n = 3) (Gigante et al. 2021). These findings reopen the discussion concerning human remains and their connection to the Nestor cup artifact.

Additional work on forensic taphonomic specific to bone integrity includes the work by Mavroudas et al. (2023). Their work suggests that short period of taphonomic influences have little effect on bone. The authors offer a good summary of how researchers are finding various results of bacteria influences depending on the burial condition of the remains. Their own results are also highly influenced by the Texas environment that they held their own experiments in (Mavroudas et al. 2023).

Osteocytes and their application for understanding bone changes

Very few, if any, research attempts by anthropologists have focused on osteocyte formation and density (lacunar volume, Lc.V) and its implication in remodeling processes. Some of the work by Skedros (2012) looks into this as it is related to long bone biomechanics. Bromage and colleagues (2016) examined 12 human midshaft femurs of Bantu origin with known life history which included age, sex, height and weight. It was found that osteocyte density is positively related to body height in taller humans: we grew quickly producing bone micro-anatomy features fast and therefore creating a positive relationship between osteocyte density and height. Sex specific examination of osteocytes has also been worked on by Carter et al. (2013). They began with the idea that osteocytes are the agents of mechanosensing in bone which stimulate bone growth over time and tissue space. Basically, the morphology of osteocyte is linked to physiological mechanical loading in bone (Dong et al. 2014). Low osteocyte density has been linked to bone loss in older women (Carter et al. 2013). We think this work has considerable potential. Still, most of the literature on this topic comes from the medical field and not from anthropological studies.

For example, Lanyon (1993) pointed out that the osteocyte network perceives strain throughout the bone matrix, and this is how the remodeling process proceeds. In an attempt to better explain how remodeling in human bone is connected to biomechanics and an adequate diet, Martin (2003) also argues that osteocytes

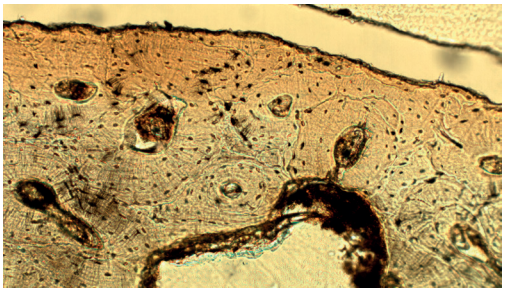

***Fig. 2** - A rib section with extremely thin cortical bone. Rib with a cortical wall is not much thicker than the diameter of a normal size secondary osteon. This image is from a 70-year-old female who died from pellagra. Source: The Raymond Dart Skeletal Collection.*

function as strain sensors and fatigue damage sensors that initiate remodeling activity (osteoclast and osteoblast recruit). Skedros (2012) makes use of this information in his own research. Osteocytes' health is one of the critical factors that influences bone turnover rates. Upon further reflection, osteocyte health is influenced by the immune system (Lanyon 1993) which is tied to micronutrients from our diet. This process is linked to the presence of Cytokines, which are secreted by cells of the immune system (Martin 2003) and appear to be influenced by diet. Diet is one of the critical "agents "described by Frost (1989) that can change the timing of completion of a fully formed secondary osteon. The idea that micro-nutrients "permissive factors" like vitamin D, C and niacin/B_3 (Brenton and Paine 2000, 2007) calcium and phosphorus have a considerable impact on the formation of bone and on the onset of osteoporosis and other skeletal lesions has been well documented (Jaworski et al. 1981). Jaworski (1984) refers to these problems as "extrinsic factors" (modulatory and permissive) that affect the organ level of function and recruitment of cells (osteoblasts and osteoclasts) which influence bone turnover processes.

It has been have argued that dietary factors that affect the metabolic rate which then influences bone turnover rates, and the formation of secondary osteons may well be the critical factor for why some populations (black South Africans and Native Americans) exhibit few secondary osteons specific to age–at-death. It does not appear to be an issue of "race/genetics" but instead it appears to be influenced by the quality of life and the availability of food and medical treatment.

For instance, in the rib samples (Fig. 2) obtained from South Africa, secondary osteons are much larger than expected (Paine and Brenton 2006a, 2019). A lack of micro-nutrients from chronic food insecurity might be enough to counter the normal bone turnover rates specific to mechanics and tissue repair that leads to high numbers of secondary osteons in older individuals. How does this occur? By affecting the immune system that is responsible for the health of bone marrow pre-curser cells that are recruited to form osteoclasts and osteoblast cells which drive the bone turnover process (Jaworski et al. 1981; Jaworski 1984; Frost 1989; Lanyon 1993; Martin 2003). This might be indicated by slight changes in osteocyte recruitment, possibly detected by osteocyte numbers per secondary osteon.

Few skeletal biologists working on archaeological samples attempt to examine dry bone histologically for this line of evidence and those that have (e.g., Stout and Teitelbaum 1976) offer only tentative ideas as to how they have viewed the response of bones to dietary problems. On the other hand, while working out problems using histology to create a demographic profile from burial samples, Weinstein et al. (1981) realized that there was a problem between age assessment and the effects of metabolic disturbances on bone microstructure. They caution that changes due to age and disease (dietary or infectious) may be difficult to separate and that one influence might compound or affect the assessment of the other. All of this makes for a difficult relationship to unravel as we attempt to understand the complex connection of age, diet and bone formation rates as seen in a variety of human samples from different times and geographical regions. It appears that research in this direction might help to fine-tune the discrepancies that occur as researchers from various institutions

come up with different findings specific to the success or failure of age predicting equations as we evaluate the relationship between dry bone microanatomy and age estimation (Crowder and Rosella 2007; Garcia-Donas et al. 2016). There are considerable future research opportunities that will provide anthropologists with the means to contribute to this literature.

Avenues for future research

Traditionally, the ending comments of a review article often consist of statements made about future research along the topics mentioned early on in the article: forensic assessment, evolutionary biology of bone anatomy, and dietary application of histomorphometric research performed by anthropologists. Here we would like to offer a suggestion towards bone assessment. Trying to link what is happening to bone via chronological to biological age seems to be a critical issue. We can begin with the idea that both should be correlated but as it turns out there is plenty of evidence to suggest otherwise. Seems to be related to various environmental factors as well as behavior.

One other age estimation issue concerns the considerable lack of alternative bones assessed. Interestingly, most anthropological researchers tend to focus on rib OPD values and do not consider the clavicle as they evaluate the potential for forensic application of bone histological assessment. I am not sure why this is? Stout and Paine (1992) showed, both bones offer equally promising results. Unfortunately, there have only been a few researchers that have continued exploring the use of clavicular histology in age-at-death assessment since 1992; they include Sobol et al. (2015), Lee et al. (2014), Kranioti et al. (2020). Kranioti et al. (2020) found that only 5 articles had been previously written about the use of clavicular histological features to predict age-at-death. Their work also showed that for the autopsied Albanian remains with known age-death and general health status, it was not an accurate means for age prediction. They suggested that a population specific method would be better suited for the Albanian sample; a histological assessment that takes into account accurate pathological and dietary factors. Once again, work like this highlights the disconnect between chronological and biological ages.

Table 3. Summary of Metacarpal and metatarsal data (Smith et al. 2016).

VARIABLES	METACARPAL MEAN & RANGE	METATARSAL MEAN & RANGE
Intact osteons	406, 165-625	368, 142-502
Fragmentary osteons	50, 23-77	56, 22-83
Cortical area	41.85, 28.8-59.9 mm^2	29.6, 15.3-50.1 mm^2
	Age prediction equation	
OPD	9.92 4.79-16.27	13.6, 4.03-20.9
Equation	LnAge = 3.45 + 0.0375 OPD_{MC}	LnAge= 3.385 + 0.0329 OPD_{MT}

I have also tried to encourage students to work on the OPD values from metatarsal and metacarpal bones with limited success (Table 3). As with the ribs and clavicles, these bones have small diameters which make preparation easy. The small diameter also allows for easy-to-read cross-sections in which the entire section can be read. In general, this should provide us with additional insight to bone formation and OPD production in areas of the body with considerable biomechanical demand, offering more information that can be applied to evolutionary questions concerning the changes in the hand and foot structure across primate species. We presented a poster on this topic of hand and foot bone histology at the AAPA meeting back in 2016. Bone samples for this project were collected from the Lubbock County Medical Examiner's Office from autopsies, and from the Southeast Texas Applied Forensic Science (STAFS) facility in Huntsville, Texas from donated individuals. A total of 31 adults provided samples of which

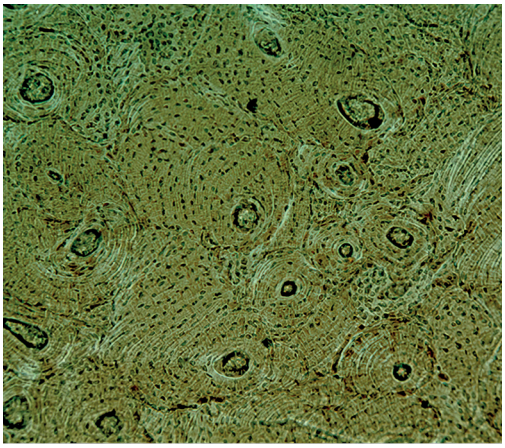

***Fig. 3** - Metatarsal bone microanatomy with secondary intact osteons/small Haversian canals; including drifting osteons and fragmentary osteons.*

there are 31 metacarpals and 31 metacarpals (a total of 62 thin sections). Each autopsied adult was with known age at death and sex. Twenty-five of the adults are male and 6 are female. The age range of the samples is 21-60, with a mean age of 46 (Smith et al. 2016). Clearly our sample size was small and unfortunately none of the age regression equations using OPD with age showed a significant r^2. Hence, the metacarpal and metatarsal OPD's do not accurately predict age. Still this type of data maybe useful in looking at how biomechanics influence secondary osteons formation patterns as well as other micro-anatomical features. We offer a summary of this data for researchers to have to simulate future work in this direction (Tab. 2 and Fig. 3).

Another area of interest for age specific histological research involves assessing the cortical bone during early human growth stages (Pitfield et al. 2017). In particular, bone of individuals from birth to 18 years of age was examined. The findings showed that some variables had a weak yet positive connection to age at death, including osteon size. While primary osteons were the main bone feature for individuals under the age of 7, they were almost completely replaced by secondary osteons by 14 years of age (Pitfield et al. 2017). These results may have critical implications for assessing age at death for subadults examined in forensic cases.

Finding patterns specific to factors that influence bone remodeling has been and continues to be the primary research goal among human osteologists. What do we seem to know towards this goal? Biomechanics, state of health, lack of nutrients and the formation of systemic cancer cells affect bone remodeling rates. We can go back to Frost (1966; 1985) to realize that this was well predicted. Still, what these specific patterns might be has yet to be fully realized. What we can say is that an influence by gender and sex does not seem to matter. Arguably, some would take issue with this position, but I counter with the following point: there is not a single accurate age predicting formula specific to sex or gender, none. Cho et al. (2006, 2007) offered sex specific equations, but they tend not to be used by histologists in current publications.

How do we improve our ability to contribute to this research? As already mentioned, state-of-the-art technology is already being employed to improve data collection (Cole and Stout 2018). This should increase the volume of data needed to make better predictions concerning bone microanatomy and the influences that affect it. This is critical since histological patterns need to first be understood in sample groups large enough to show potential trends specific to remodeling and the factors we examine for. Having re-read Robling and Stout (2008), it appears that my suggestion is a reiteration of what was first offered in their conclusion specific to age-at-death assessment. Unfortunately, years later their point remains true. Modern human samples with a known bio-history are required for this work. In Europe, there is the option of using cemetery samples with known age and sex specific information (for example in Crete and Cyprus). Using these samples helps to provide a better understanding of population specific patterns of histological features and this is being done by Garcia-Donas and her colleagues (Garcia-Donas et al. 2016, 2021; Kranioti et al. 2020). This work is offering additional samples from regions that were previously not examined

histologically while also testing the accuracy/usefulness of established age-at-death methods as suggested by Robling and Stout (2008). In general, working with forensic pathologists and obtaining autopsied samples with accurate bioprofile seems to be a critical solution towards understanding how factors influence why chronological age does not match our understanding of biological age estimations via OPD values.

Returning to an early concerned shared by Dr. George Armelagos, he was wondering if bone histology research by anthropology students would increase in scope and interest over time. It seems clear now that work on bone histological conducted by anthropologists has grown and well published in both medical and anthropological journals. Still there is much to do; fortunately we have a wealth of well-educated individuals to do this. The future in bone histological research done by anthropologists looks very promising.

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References

Abbott S, Trinkaus E, Burr, DB (1996) Dynamic bone remodelling in later Pleistocene fossil hominids. Am J Phys Anthropol 99:585-601. https://doi.org/10.1002/(SICI)1096-8644(199604)99:4<585::AID-AJPA5>3.0.CO;2-T

Bassan J, Frame B, Frost HM (1963) Osteoporosis: A review of pathogenesis and treatment. Internal Med 58:539-550.

Booth TJ (2016) An investigation into the relationship between funerary treatment and bacterial bioerosion in European archaeological human bone. Archaeometry 58:484–499. https://doi.org/10.1111/arcm.12190

Bromage TG, Juwayeyi YM, Katris JA (2016) The scaling of human osteocyte lacuna density with body size and metabolism. Comptes Rendus Palevol 15:32-39. https://doi.org/10.1016/j.crpv.2015.09.001

Brenton BP, Paine RR (2000) Pellagra and paleonutrition: Assessing the diet and health of maize horticulturists through skeletal biology. NutrAnthropol 23:2-9. https://doi.org/10.1525/nua.2000.23.1.2

Brenton BP, Paine RR (2007) Reevaluating the health and nutritional status of maize dependent populations: Evidence from the impact of pellagra on human skeletons. Eco Food Nutr 46:345-360. https://doi.org/10.1080/03670240701486545

Burr DB (1992) Estimated intracortical bone turnover in the femur of growing macaques: Implications for their use as models in skeletal pathology. Anat Rec 232:180-189. https://doi.org/10.1002/ar.1092320203

Burr DB, Nishikawa RY, Van Gerven D (1989) Bone growth and remodeling in Cayo Santiago-derived *Macaca mulatta*. Puerto Rico Health Sci J 8:191-196. PMID: 2780963

Carter Y, Thomas CDL, Clement J et al (2013) Femoral osteocyte lacunar density, volume and morphology in women across the lifespan. J Struct 183:519-526. https://doi.org/10.1016/j.jsb.2013.07.004

Cho H, Stout SD, Madsen RW et al (2002) Population-specific histological age-estimating

method: A model for known African-American and European-American skeletal remains. For Sci 47:12-18. https://doi.org/10.1520/JFS15199J

Cho H, Stout SD, Bishop TA (2006) Cortical bone remodeling rates in a sample of African American and European American descent groups from the American midwest: Comparisons of age and sex in ribs. Am J Phys Anthropol 130: 214–226. https://doi.org/10.1002/ajpa.20312

Cole ME, Stout SD (2018) Automated extraction of two-dimensional cortical porosity descriptors from histological and micro-computed tomography serial sections. Am J Phys Anthropol 165:51–52. https://doi.org/10.1002/ajpa.20312

Cole ME, Stout SD, Dominguez VM et al (2022) Pore Extractor 2D: An ImageJ toolkit for quantifying cortical pore morphometry on histological bone images, with application to intraskeletal and regional patterning. Am J Phys Anthropol 179:365-385. https://doi.org/10.1002/ajpa.24618

Cook DC (1976) Pathologic states and disease process in Illinois woodland populations: An epidemiologic approach. Ph.D. dissertation, University of Chicago, IL.

Cooke KM, Mahoney P, Miszkiewicz JJ (2022) Secondary osteon variants and remodeling in human bone. Anat Rec (Hoboken) 305:1299-1315. https://doi.org/10.1002/ar.24646

Crowder C (2005) Evaluating the use of quantitative bone histology to estimate adult age at death. Ph.D Dissertation, University of Toronto, NR 07569.

Crowder C, and Rosella L (2007) Assessment of intra-and intercostal variation in rib histomorphometry: Its impact on evidentiary examination. For Sci 52:271-276. https://doi.org/10.1111/j.1556-4029.2007.00388.x

Crowder C and Stout SD (2011) Bone histology: an anthropological perspective. Editors CRC Press.

Currey JD (1964) Some Effects of Aging in Human Haversian Systems. J Anat Lond 1:69-75.

De Cataldo F, Paine RR, Coppa A (2020) An assessment of the Arabic RH5 Neolithic fishing community's mortuary practices by examining human rib cross-sections for bioerosion patterns. J Archaeol Sci 33:1-10. https://doi.org/10.1016/j.jasrep.2020.102490

Dong P, Haupert, S Hesse B et al (2014) 3D osteocyte lacunar morphometric properties and distributions in human femoral cortical bone using synchrotron radiation micro-CT images. Bone 60:172-185. https://doi.org/10.1016/j.bone.2013.12.008

Dudar JC, Pfeiffer S, Saunders SR (1993) Evaluation of morphological and histological adult skeletal age-at-death estimation techniques using ribs. For Sci 38:677-685. https://doi.org/10.1520/JFS13455J

Enlow DH, Brown SO (1956). A Comparative Histological Study of Fossil and Recent Bone Tissue. Part I. Tx J Sci 8:405-443.

Enlow DH, Brown SO (1957) A Comparative Histological Study of Fossil and Recent Bone Tissue. Part II. Tx J Sci 9:186-214.

Enlow DH, Brown SO (1958) A Comparative Histological Study of Fossil and Recent Bone Tissue. Part III. Tx J Sci 10:187-230.

Epker BN, Frost HM (1964) The parabolic index: A proposed index of the degree of osteoporosis in ribs. J Geront 19:469-473.

Frost HM (1973) Bone remodeling and its relationship to metabolic bone diseases. Charles C. Thomas, Springfield, Ill.

Frost HM (1966) Morphometry of bone in palaeopathology. In: Jarcho S (ed) Human paleopathology, New Haven University Press, Yale, p. 130-150.

Frost HM (1985) The "New Bone:" Some anthropological potentials. Yrbk Phys Anthropol 28:211-226. https://doi.org/10.1002/ajpa.1330280511

Frost HM (1986) Intermediary organization of the skeleton. Vol. 1, CRC Press, Boca Raton, FL.

Frost HM (1989) Some ABCs of skeletal pathophysiology Ih general mediator mechanism properties. Calcif Tissue Int. 45:68-70. https://doi.org/10.1007/BF02561403

Garcia-Donas JG, Duke J, Paine RR et al (2016) Accuracy and sampling error of two age estimation techniques using rib histomorphometry on a

modern sample. J For Legal Med 38:28-35. https://doi.org/10.1016/j.jflm.2015.11.012

Garcia-Donas JG, Scholl AR, Bonicelli A et al (2021) Rib histomorphometry: A reliability and validation study with a critical review of histological techniques for forensic age estimation. Legal Med 49:101827. https://doi.org/10.1016/j.legalmed.2020.101827

García-Donas JG, Paine RR Bonicelli A et al (2022) Age estimation for two mediterranean populations: rib histomorphometry applied to forensic identification and bone remodelling research. Int J Legal Med 136:1469–1481. https://doi.org/10.1007/s00414-022-02812-2

Gigante M, Nava A, Paine RR et al (2021) Who is buried with the Nestor's cup? Macroscopic and microscopic analyses of the cremated remains from Tomb 168 (mid-8th century BCE, Pithekoussai, Ischia Island, Italy). PLoS One 16:e0257368. https://doi.org/10.1371/journal.pone.0257368

Hackett CJ (1981) Microscopical focal destruction (tunnels) in exhumed human bones. Med Sci Law 21:243–265.

Hanson DB, Buikstra JE (1987) Histomorphological alteration in burial human bone from the Lower Illinois Valley: Implications for paleodietary research. J Archaeol Sci 14:549-563. https://doi.org/10.1016/0305-4403(87)90038-0

Hillier M, Bell LS (2007) Differentiating human bone from animal bone: a review of histological methods. J Forensic Sci 53:249-263. https://doi.org/10.1111/j.1556-4029.2006.00368.x

Jaworski, ZFG (1984) Lamellar Bone Turnover System and Its Effector Organ. Calcif Tissue Int 36:s46-s55.

Jaworski, ZFG (1987) Does the mechanical usage (MU) inhibit bone "remodeling"? Calcif Tissue Int 41:239-248. https://doi.org/10.1007/BF02555223

Jaworski ZFG, Duck B, Sekaly G (1981) Kinetics of osteoclasts and their nuclei in evolving secondary Haversian systems. J Anat 133:397-405. PMID: 7328046

Jowsey J (1966) Studies of Haversian systems in man and some animals. J Ant 100:857-864.

Jowsey J (1968) Age and species differences in bone. Cornell Vet 56 (suppl.):74-94.

Katydid C, García-Donas JG, Tsiminikaki K et al (2022) Estimation of age-at-death using cortical bone histomorphometry of the rib and femur: a validation study on a British population. Biology (Basel) 4:1615. https://doi.org/10.3390/biology11111615

Kerley ER (1965) The microscopic determination of age in human bone. Am J Phys Anthropol 23:149-164.

Kerley ER, Ubelaker DH (1978) Revisions in the Microscopic Method of Estimating Age at Death in Human Cortical Bone. Am J Phys Anthropol 49:545-546.

Kranioti EF, Michopoulou E, Tsiminikaki K, et al (2020) Bone histomorphometry of the clavicle in a forensic sample from Albania. For Sci Int 313:1-9. https://doi.org/10.1016/j.forsciint.2020.110335

Lanyon LE (1993) Osteocytes, strain detection, bone modeling and remodeling. Calcif Tissue Int 53:S102-S107.

Lemmers SAM, Gonçalves D, Cunha E et al (2020) Burned fleshed or dry? the potential of bioerosion to determine the pre-burning condition of human remains. J Archaeol Method Theory 27:972-991. https://doi.org/10.1007/s10816-020-09446-x.

Lee UY, Jung GU, Choi SG et al (2014) Anthropological age estimation with bone histomorphometry from the human clavicle. Anthrop 17:929-936. https://doi.org/10.1080/09720073.2014.11891509

Maggiano IS, Maggiano CM, Clement JG et al (2016) Three-dimensional reconstruction of Haversian systems in human cortical bone using synchrotron radiation-based micro-CT: Morphology and quantification of branching and transverse connections across age. J Anat 228:719–732. https://doi.org/10.1111/joa.12430

Maggiano IS, Maggiano CM, Cooper DM (2021) Osteon circularity and longitudinal morphology: Quantitative and qualitative three-dimensional perspectives on human Haversian systems. Micron 140:102955. https://doi.org/10.1016/j.micron.2020.102955

Martin RB (2003) Fatigue microdamage as an essential element of bone mechanics and

biology. Calcif Tissue Int 73:101–107. https://doi.org/10.1007/s00223-002-1059-9

Micarelli I, Minozzi S, Rodriguez L et al (2024) The oldest fossil hominin from Italy: Reassessment of the femoral diaphysis from Venosa-Notarchirico in its Acheulean context. Quater Sci Rev 334:108709. https://doi.org/10.1016/j.quascirev.2024.108709

McFarlin SC, Carl J, Terranova CJ et al (2008) Regional variability in secondary remodeling within long bone cortices of catarrhine primates: the influence of bone growth history. J Anat 213:308–324. https://doi.org/10.1111/j.1469-7580.2008.00947.x

McFarlin SC, Terranova CJ, Zihlman AL et al (2016) Primary bone microanatomy records developmental aspects of life history in catarrhine primates. J Hum Evol 92:60-79. https://doi.org/10.1016/j.jhevol.2015.12.004

Mulhern DM (2000) Rib remodeling dynamics in a skeletal population from Kulubnarti, Nubia. Am J Phys Anthropol 111:519-530. https://doi.org/10.1002/(SICI)1096-8644(200004)111:4<519::AID-AJPA7>3.0.CO;2-7

Mulhern DM, Ubelaker DH (2001) Differences in osteon banding between human and nonhuman bone. J For Sci 46:220-222. https://doi.org/10.1520/JFS14952J

Mulhern DM Ubelaker DH (2009) Bone Microstructure in Juvenile Chimpanzees. Am J Phys Anthropol 140:368–375. https://doi.org/10.1002/ajpa.20959

Paine RR, Godfrey LR (1997) The scaling of skeletal microanatomy in non-human primates. J Zoo Lond 241:803-821. https://doi.org/10.1111/j.1469-7998.1997.tb05749.x

Paine RR, Brenton BP (2006a) Dietary health does affect age assessment: An evaluation of the Stout & Paine (1992) age estimation equation using secondary osteons from the rib. J For Sci 51:489-492. https://doi.org/10.1111/j.1556-4029.2006.00118.x

Paine RR, Brenton BP (2006b) The paleopathology of pellagra: investigating the impact of prehistoric and historic dietary transition to maize. J Anthropol Sci 84:125-135.

Paine RR, Brenton BP (2019) Malnutrition. In: Sandra L. López Varela (ed) The SAS Encyclopedia of Archaeological Sciences, John Wiley & Sons, Inc.

Parfitt AM (1979) Quantum concept of bone remodeling and turnover: implications for the pathogenesis of osteoporosis. Calcif Tissue Int 28:1-5.

Parfitt AM, Drezner MK, Glorieux FH et al (1987) Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595–610.

Pfeiffer S, Zehr MK (1996) A morphological and histological study of the human humerus from Border cave. J Hum Evol 31:49-59. https://doi.org/10.1006/jhev.1996.0048

Pfeiffer S, Heinrich J, Beresheim A et al (2016) Cortical bone histomorphology of known-age skeletons from the Kirsten Collection, Stellenbosch University, South Africa. Am J Phys Anthropol 160:137–147. https://doi.org/10.1002/ajpa.22951

Pfeiffer S, Cameron ME, Sealy J et al (2019) Diet and adult age-at-death among mobile foragers: A synthesis of bioarcheological methods. Am J Phys Anthropol 170:131-147. https://doi.org/10.1002/ajpa.23883

Pitfield R. Miszkiewicz JJ, Mahoney P (2017) Cortical histomorphometry of the human humerus during ontogeny. Calcif. Tissue Int. 101:148–158. https://doi.org/10.1007/s00223-017-0268-1

Richman EA, Ornter DJ, Schulter-Ellis FP (1979) Differences in intracortical bone remodeling in three aboriginal American populations: Possible dietary factors. Calcif Tiss Internat 28:209-214.

Robling AG Stout SD (1999) Morphology of the drifting osteons. Cells Tissues Organs 164:192–204. https://doi.org/10.1159/000016659

Robling AG, Stout SD (2008) Histomorphometry of human cortical bone: Application to age estimation. In: Katzenberg MA and Saunders SR (eds) Biological Anthropology of the Human Skeleton, Wiley Blackwell, p. 149-182.

Ruff C (1987) Sexual dimorphism in human lower limb bone structure: relationship to subsistence strategy and sexual division of labor. J Hum Evol 19:391-416. https://doi.org/10.1016/0047-2484(87)90069-8

Sawada J, Kondo O, Nara T et al 2004. Bone histomorphology of the Deheriyeh Neanderthal child. Anthropol Sci 112: 247-256. https://doi.org/10.1537/ase.00094

Skedros JG (2012) Interpreting Load History in Limb-Bone Diaphyses: Important Considerations and Their Biomechanical Foundations. In: Crowder C and Stout SD (eds) Bone histology: an anthropological perspective, CRC Press, p. 153-220

Smith H, Paine RR, Bytheway JA (2016) Histological age determination using the micro-anatomy of 3rd metatarsal and metacarpal bones. Am J Phys Anthrop 159:295.

Sobol J, Ptaszyńska-Sarosiek I, Charuta A et al (2015) Estimation of age at death: Examination of variation in cortical bone histology within the human clavicle, Folia Morphol 74: 378–388. https://doi.org/10.5603/FM.2015.0021

Stout SD (1976) Histomorphometrics analysis of archaeological bone. Ph.D. dissertation, Department of Anthropology, Washington University, St. Louis.

Stout SD (1978) Histological structure and its preservation in ancient bone. Curr Anthropol 19:601-603.

Stout SD, Simmons DJ (1979) Use of histology in ancient bone research. Yrbk Phys Anthropol 22:228–249.

Stout SD, Teitelbaum SL (1976) Histomorphometric determination of formation rates of archaeological bone. Calcif Tiss Internat 21:163-169.

Stout SD Paine RR (1992) Histological age estimation using ribs and clavicles. Am J Phys Anthropol 87:111-115. https://doi.org/10.1002/ajpa.1330870110

Stout SD, Paine RR (1994) Bone remodeling rates: A test of an algorithm for estimating missing osteons. Am J Phys Anthropol 93:123-129. https://doi.org/10.1002/ajpa.1330930109

Stout SD, Lueck R (1995) Bone remodeling rates and skeletal maturation in three archaeological skeletal populations. Am J Phys Anthropol 98:161-171. https://doi.org/10.1002/ajpa.1330980206

Streeter M, Stout SD, Trinkaus E et al (2001) Histomorphological age assessment of the Boxgrove 1 tibia diaphysis. J Hum Evol 40:331-338. https://doi.org/10.1006/jhev.2001.0460

Streeter M, Stout SD, Erik Trinkaus E et al (2010) Brief communication: bone remodeling rates in Pleistocene humans are not slower than the rates observed in modern populations: A reexamination of Abbott et al. (1996). Am J Phys Anthropol 141:315–318. https://doi.org/10.1002/ajpa.21192

Thompson DD, Trinkaus E (1981) Age determination of the Shanidar 3 Neanderthal. Sci 212:575-577. https://doi.org/10:1126/science.6782677

Trinkaus E, Thompson DD (1987) Femoral diaphyseal histomorphometric age determination for the Shanidar 3, 4, 5, and 6 Neandertals and Neandertal longevity. Am J Phys Anthropol 72:123-129. https://doi.org/10.1002/ajpa.1330720115

Walter TL, Paine RR, Horne H (2004) Examination of bone- tempered pottery from mission Espritu Santo (41VT11), Victoria County, Texas. J Archaeol Sci 31:393–398. https://doi.org/10.1016/j.jas.2003.09.006

Weinstein RS, Simmons DJ, Lovejoy CO (1981) Ancient bone disease in a Peruvian mummy revealed by quantitative skeletal histomorphometry. Am J Phys Anthropol 54: 321-326. https://doi.org/10.1002/ajpa.1330540304

Wu K, Schubeck KE, Frost HM et al (1970) Haversian bone formation rates determined by a new method in a mastodon, and in human diabetes mellitus and osteoporosis. Calcif Tissue Res 6:204–219.

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