Ancient Human Bone Microstructure in Medieval England: Comparisons between Two Socio-Economic Groups

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ABSTRACT

Understanding the links between bone microstructure and human lifestyle is critical for clinical and anthropological research into skeletal growth and adaptation. The present study is the first to report correspondence between socio-economic status and variation in bone microstructure in ancient humans. Products of femoral cortical remodeling were assessed using histological methods in a large human medieval sample (N = 450) which represented two distinct socio-economic groups. Osteonal parameters were recorded in posterior midshaft femoral sections from adult males (N = 233) and females (N = 217). Using univariate and multivariate statistics, intact, fragmentary, and osteon population densities, Haversian canal area and diameter, and osteon area were compared between the two groups, accounting for sex, age, and estimated femoral robusticity. The size of osteons and their Haversian canals, as well as osteon density, varied significantly between the socio-economic groups, although minor inconsistencies were observed in females. Variation in microstructure was consistent with historical textual evidence that describes differences in mechanical loading and nutrition between the two groups. Results demonstrate that aspects of ancient human lifestyle can be inferred from bone microstructure. Anat Rec, 00:000–000, 2015. © 2015 Wiley Periodicals, Inc.

Key words: histology; femur; socio-economic status; bone health

Anthropological and clinical investigations of human skeletal adaptation and growth often infer or reconstruct aspects of lifestyle from bone (e.g., Nguyen et al., 1994; Agarwal and Grynpas, 1996; Larsen, 2002; Robling and Stout, 2003). While studies utilizing forensic and cadaver hard tissue have provided important insights into bone microstructural growth (e.g., Britz et al., 2009; Schlecht et al., 2012), obtaining large sample sizes can prove

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difficult. In contrast to this, by adhering carefully to the ethical guidelines[1] provided for sampling archaeological collections, a much larger number of individuals can sometimes be sampled and analyzed for studies of bone histology. Although an experimental approach is not possible in such cases, the array of internal and external mechanical, dietary, or pathological factors that affect bone homeostasis throughout life (Robling et al., 2006) can still be inferred as evidence of these can be preserved in archaeological samples of bone (Stout, 1978; Stout and Simmons, 1979).

STUDY AIM

Several histology studies have previously reconstructed aspects of human lifestyle from archaeological samples of bone (Table 1; Fig. 1). However, it has not yet been demonstrated whether socio-economic status corresponds with variation in ancient human bone microstructure. The present study accesses and compares femoral bone histology between two socio-economic human groups (higher and lower status) from medieval England (11th to 16th centuries AD), recovered from an archaeological site in Canterbury (Hicks and Hicks, 2001). Microstructural variation from each group is explored against historical textual accounts of medieval lifestyle. Sex, age, and estimated femoral robusticity are accounted for.

POTENTIAL EFFECTS OF HUMAN SOCIO-ECONOMIC STATUS ON BONE MICROSTRUCTURE

Different Growth and Remodeling Factors Make Bone a Living Tissue

Bone is a living tissue that changes its shape and structure through the processes of modeling and remodeling, in response to several external and internal factors and/or stimuli (Robling et al., 2006). A secondary osteon, composed of a central Haversian canal surrounded by lamellae, represents a microscopic functional unit of cortical bone, formed by osteoclasts and osteoblasts (Bone Multicellular Units) that execute remodeling (Heřt et al., 1994; Martin et al., 1998). Bone is supported by a network of osteocytes that reside within lacunae interconnected by canaliculi that exchange nutrients, oxygen, and communicate mechanical signaling. This metabolic activity, as well as targeted remodeling (accounting for an estimated 30% of all remodeling activity), maintains bone health and strength, and repairs micro-damage that accumulates from mechanical stress (Burr et al., 1985; Jee, 2000; Martin, 2000, 2003; Burr, 2002; Robling et al., 2006).

Research performed on human and non-human animals has demonstrated that: (1) age (e.g. Jowsey, 1960; Macho et al., 2005; Cambra-Moo et al., 2015), (2) sex (e.g. Seeman, 2002; Zanchetta et al., 2003; Tommasini et al., 2007; Vicente et al., 2013; Jepsen et al., 2015), (3) mechanical stimuli (e.g. Raab et al., 1991; Frost, 1997; Pearson and Lieberman, 2004; Ruff et al., 2006), (4) diet (e.g. Dämmrich, 1991; Paine and Brenton, 2006; Alexy et al., 2005; Krivosíková et al., 2010; Gerbaix et al., 2012), (5) disease (e.g. Storm et al., 1993; Lill et al., 2002; Kearns et al., 2008), and (6) genetics (e.g. Cho et al., 2006; Bassett et al., 2012; Wallace et al., 2012) influence and/or determine bone biology. Therefore, when studying the effect of human lifestyle (which can be a choice but sometimes is determined by socio-economic status) on bone health it is important to control for these variables. However, access to large samples of ancient humans with a known personal recorded history for each of these variables is rare. Instead, some of these variables (1, 2) can be reconstructed from the skeletal remains, while the effect of others (3, 4, 5) can be inferred (for example) by comparing variation in bone biology to historical textual evidence. This methodological approach differs from clinical studies of socio-economic status which usually access these variables, and others aspects of bone biology, such as bone mass, mineral content, and density (e.g., Arabi et al., 2004; Zingmond et al., 2006; Brennan et al., 2011), from medical records.

PREVIOUS STUDIES OF ANCIENT HUMAN BONE MICROSTRUCTURE AND LIFESTYLES

The effect of socio-economic status on the skeleton in ancient humans has been studied previously through a range of macroscopic methodologies (Jankauskas, 2003; Porčić and Stefanović, 2009; Miszkiewicz, 2012; Dawson and Robson Brown, 2013; Woo and Sciulli, 2013). Generally, as habitual behavior is a key component of past lifestyle inferences, it has been frequently studied (see Meyer et al., 2011) from entheseal changes (e.g., Hawkey and Merbs, 1995; Eshed et al., 2004; Molnar, 2006; Weiss, 2007; Porčić and Stefanović, 2009) or whole bone morphometrics (e.g., Ruff, 2005; Pomeroy and Zakrzewski, 2009). However, macroscopic approaches provide limited information about bone remodeling, which can give insights into bone health in adulthood (Schlecht, 2012). Gross morphology is likely to indicate adaptations to physical activity undertaken during the first two decades of life when bone shape and size are modeled (Frost, 1994; Pearson and Lieberman, 2004).

The relationship between bone microstructure and socio-economic status in ancient humans has not been reported previously. While histology has been used to study extinct vertebrates (Enlow and Brown, 1956; Chinsamy, 1997), and to infer human paleophysiology (Enlow, 1966; Stout, 1978), few studies have assessed archaeological samples of human tissue (see Table 1) and/or fossil hominins (e.g., Schultz, 1999). Generally, studies of human archaeological collections have either been able to relate variation in bone microstructure to documented lifestyles (e.g., Robling and Stout, 2003; Richman et al., 1979; Stout and Lueck, 1995) or they have highlighted the difficulties with this methodological approach (e.g., Pfeiffer et al., 2006) due to, for example, variability in

[1]As the human remains are not recent (i.e., deriving from a forensic or clinical context), the undertaken examination followed the official anthropological codes of ethics and practice:

- British Association for Biological Anthropology and Osteoarchaeology Code of Ethics. 2008. http://www.babao.org.uk/index/ethics-and-standards
- British Association for Biological Anthropology and Osteoarchaeology Code of Practice. 2010. http://www.babao.org.uk/index/ethics-and-standards
- Code of Ethics of the American Anthropological Association. 2012. http://ethics.aaanet.org/ethics-statement-0-preamble/
- Code of Ethics of the American Association of Physical Anthropologists. 2003. http://physanth.org/documents/3/ethics.pdf

TABLE 1. Key studies where histology has been used to examine bone biology in archaeological (Holocene) humans (also see Fig. 1).

Publication	Sample(s)	Bone(s)	Main finding
Martin and Armelagos, 1979, 1985	Sudanese Nubia N = 74 (1979), N = 185 (1985)	Femora	Remodeling affected by reproduction related nutritional stress (childbearing, childrearing), and age in females.
Richman et al., 1979	Alaskan Inuit N = 51, Arikara N = 57, Pueblo N = 65	Femora	Increased remodeling associated with high-protein diet consumption.
Ericksen, 1980	Alaskan Inuit N = 53, Pueblo N = 68, Arikara N = 68	Femora	Remodeling differences associated with sex (pregnancy, lactation in females), age, some physical activity components, and genetic factors.
Thompson and Gunness-Hey, 1981; Thompson et al., 1981	St. Lawrence Island N = 53, Kodiak Island N = 92, Baffin Island N = 44, Southampton Island N = 69	Femora	Physical activity (strenuous Arctic and sub-Arctic lifestyles), age, dietary, and genetic factors linked to differences in remodeling.
Burr et al., 1990	Pecos N = 55	Femora	Increased remodeling associated with active lifestyle.
Lazenby and Pfeiffer, 1993	Historical amputee N = 1	Femora	Differences in remodeling between left and right femur associated with biomechanics.
Stout and Lueck, 1995	Windover N = 38, Gibson N = 25, Ledders N = 18	Ribs	Bone formation rate variation due to skeletal maturity (effective ages of adult compacts) differences rather than subsistence strategies.
Mulhern and Van Gerven, 1997	Kulubnarti N = 43	Femora	Differences in remodeling and microstructural parameters associated with sex and mechanical strain.
Mulhern, 2000	Kulubnarti N = 80	Ribs	Rib remodeling data linked with age, genetic factors, and some high strains.
Robling and Stout, 2003	Peruvian Paloma N = 48	Femora and ribs	Decreased remodeling corresponded with decreased levels of physical activity.
Pfeiffer et al., 2006	Holocene foragers N = 44, Spitalfields = 20, St. Thomas N = 20	Femora and ribs	Osteon and Haversian canal size variation did not match evidence for behavior.
Cho and Stout, 2011	Imperial Romans N = 149	Femora and ribs	Increased bone loss and remodeling in females associated with reproduction (menopause, lactation) and gender-specific cultural practices.
Present study	Medieval Canterbury N = 450 (St. Gregory's Priory N = 40, Cemetery N = 410)	Femora	Small osteons and Haversian canals, and low remodeling in a physically active, but poor and malnourished group.

bone microstructure (Pfeiffer, 1998). This lack of consistency in the bone histology studies may be due in part to the complexity of bone growth processes (Pfeiffer and Pinto, 2011), but it also highlights the need for methodological improvements, especially the examination of larger sample sizes.

MEDIEVAL SOCIO-ECONOMIC STATUS AND LIFESTYLES

In the present study, bone microstructure was accessed in a human group dating to the medieval period, for which there was historical textual and archaeological evidence

Fig. 1. World map indicating geographical locations of adult human archaeological (Holocene) groups whose bone biology has been studied using histology (**A**–**H**: North and South America, **I**–**J**: Europe, **K**–**L**: Africa). A: Alaskan Inuit 18th–20th centuries AD (Richman et al., 1979; Ericksen, 1980), Yupik 19th century and AD 700 BC–1700 AD (Thompson and Gunness-Hey, 1981); (**B**) Arikara, South Dakota 1550–1845 AD (Richman et al., 1979; Ericksen, 1980); (**C**) Pueblo 919–1600 AD (Richman et al., 1979; Ericksen, 1980), and Pecos Indians 14th–19th centuries (Burr et al., 1990), New Mexico; (**D**) Windover, Florida 6900–8120 BP (Stout and Lueck, 1995); (**E**) Gibson 50 BC–400 AD, Ledders AD 1000, Lower Illinois River Valley (Stout and Lueck, 1995); (**F**) St. Thomas 1827–1873 AD, Belleville, Ontario, Canada (Pfeiffer, 1998; Pfeiffer et al., 2006); London, Ontario 19th century AD, Canada (Lazenby and Pfeiffer, 1993); (**G**) Peruvian Paloma, Chilca Valley 6500 BP–4700 BP (Robling and Stout, 2003); (**H**) Inupiaq 19th century (Thompson and Gunness-Hey, 1981; Thompson et al., 1981); (**I**) Spitalfields 1729–1857 AD, London (UK) (Pfeiffer, 1998; Pfeiffer et al., 2006); St. Gregory's Priory and Cemetery 11th–16th centuries AD, Canterbury (UK) (Miszkiewicz, 2014; present study); (**J**) Imperial Romans, Isola Sacra 100–300 AD, Italy (Cho and Stout, 2011); (**K**) South African foragers 6000–800 BP (Pfeiffer et al., 2006); (**L**) Sudanese Nubia 350 BC–1450 AD (Martin and Armelagos, 1979, 1985; Mulhern and Van Gerven, 1997; Mulhern, 2000).

for socio-economic stratification. This evidence included information on physical activity/occupation (behavior), nutrition, and general well-being.

Fedualism defined social structure in Britain during the Middle Ages (Clark, 1982; Rigby, 1995; Bartlett, 2000). Wealthy lay noblemen enjoyed comfortable lifestyles (Wilkinson, 1969; Dyer, 2002), that could include an organized education (Macray, 1886; Gransden, 1972). Typically, noblemen managed their lands, participating in political discussions and land inspections (Holt, 1972; Mate, 2006), as well as various forms of leisure (e.g., hunting, dancing, and feasting) (Robertson and Sheppard, 1876; Dyer 2000). Knights were involved in years of training that included preparation for warfare (Stubbs, 1872; Leyser, 1995; Bennett and Hollister, 2006). Clergy performed religious services, took part in pilgrimages (Theilmann, 1987; Webb, 2000), and tended the sick who could not afford a physician (Clegg and Reed, 1994). A peasant's lifestyle was based around manual labor (Jordan, 2001; Dunn, 2004), the nature of which differed depending on whether it was town- or farm-based (Dyer, 1989; Graham, 1997). For example, towns mainly offered employment to servants, craftsmen, stewards, smiths, leatherworkers, carpenters, and millers (Jordan, 2001; Dyer, 2002). Occupations in building and cloth industries, mill work, and mining were also common (Wilkinson, 1969; Dyer, 2000). Whereas peasants that lived in villages had an agrarian lifestyle that included sowing cereals and crops (e.g., wheat, barley, oats), ploughing, hay mowing, and rearing domesticated animals (Wilkinson, 1969; Power, 1975; Zvi, 1981; Jordan, 2001; Bennett and Hollister, 2006). Many activities undertaken by the peasantry required carrying heavy loads, and often involved walking for prolonged periods of time (Judd and Roberts, 1999).

Social status dictated diet during the Middle Ages. High status individuals consumed a variety of meats (e.g., capons, chickens, ducks, pigeons, swan) and fish, wines and ales (Gasquet, 1922; Harvey, 1993; Dyer, 2000; Rogers and Waldron, 2001), which often resulted in an average calorie intake almost three times higher than the current daily recommendation (Harvey, 1993; WHO, 2015). Diet for peasants was primarily cereal based, but also included eggs and cheese (Dyer, 1983; Dunn, 2004). A typical peasant meal would have been pottage (thick stew made of oats, peas, and beans) (Bhote, 2004; Dunn, 2004), and was most commonly served to harvest workers (Dyer, 2000). Peasants had little access to meat and freshwater fish (Dyer, 1983, 2002; Van der Veen, 2003).

General well-being and disease susceptibility also differed between social groups. Those of lower status were often affected by infectious diseases, such as tuberculosis and leprosy (Manchester and Roberts, 1989; Roberts and Manchester, 2007), which could lead to an associated negative socio-cultural reaction, such as exclusion from communal events (e.g., attending church) (Covey, 2001). Other common health problems for peasants were famine-related conditions, osteoporosis, Paget's disease, neoplasms, and

TABLE 2. Variables examined in the present study.

Variable (abbreviation[a])	Definition
Midshaft circumference (**Circ**)	Circumference measured at the femoral diaphyseal midpoint (e.g., Stock and Shaw, 2007; Buikstra and Ubelaker, 1994; Moore-Jansen et al., 1994)
Cortical width (**Ct.Wi**)	Thickness of the cortex measured between the most inner endosteal and most outer periosteal surface point (Vajda and Bloebaum, 1999).
Maximum length	Length measured between the femoral head and the condyles (e.g., Stock and Shaw, 2007; Buikstra and Ubelaker, 1994; Moore-Jansen et al., 1994).
Robusticity index (**RI**) a. Circ/Length × 100 b. Ct.Wi/Length × 100	Femoral robusticity (strength in relation to size and shape) quantification (e.g., Stock and Shaw, 2007).
Intact osteon density (**N.On**)	Total number of osteons with intact cement lines and complete Haversian canals per area of sampled bone (in mm^2), here counted at ×10 magnification (e.g., Parfitt, 1983, Stout and Crowder, 2011).
Fragmentary osteon density (**N.On.Fg**)	Total number of fragmentary osteons with Haversian canals and/or osteon surfaces of > 10% resorption per area of sampled bone (in mm^2). Interstitial lamellae are included if clearly identifiable, here counted at ×10 magnification (e.g., Parfitt, 1983, Stout and Crowder, 2011).
Osteon population density (**OPD**)	N.On + N.On.Fg per area of sampled bone (e.g., Stout and Crowder, 2011)
(Mean) osteon area (**On.Ar**)	Area (in μm^2) of osteons with intact cement lines per number of osteons measured, examined at ×20 magnification (e.g. Skedros et al., 2013).
(Mean) Haversian canal area (**H.Ar**)	Area (in μm^2) of complete Haversian canals (with no indication of resorption) per number of canals measured, examined at ×40 magnification (e.g., Skedros et al., 2013).
(Mean) Haversian canal diameter (**H.Dm**)	Minimum[b] diameter (in μm) in complete Haversian canals taken at canal midpoint per number of canals measured, examined at ×40 (e.g., Bell et al., 1999; Jordan et al., 2000) magnification.

[a]Dempster et al., 2013.
[b]Accounting for shape variation (also see Hennig et al., 2015).

general joint disease (Roberts and Cox, 2003). Whilst poorer life quality certainly impacted health, it is not to say that noblemen were free from disease. For example, the Black Death pandemic affected all members of the society (Daniell, 1998). Higher status and monastic groups also suffered from diffuse idiopathic skeletal hyperostosis (DISH) (Rogers and Waldron, 2001) in association with diabetes and obesity. It is however speculated that healthier fats from fish and offal in aristocratic diets would have minimized the risk of diabetes (Harvey, 1993; Dyer, 2002).

PREDICTION

Given the association between physical activity, nutrition, biological homeostasis, and bone growth, it was predicted that bone histology would differ between the two socio-economic groups from medieval Canterbury. It was expected that variation in the osteonal parameters listed here, which are products of bone remodeling, (see Table 2 for definitions), would correspond with these two different lifestyles. The osteonal parameters are:

a. Density of intact (N.On), fragmentary (N.On.Fg) osteons, and total osteon population (OPD) may be informative of bone changes in response to mechanical stimuli and bone structural properties (e.g., Young et al., 1986; Frost, 1994; Britz et al., 2009; Schlecht et al., 2012), dietary patterns (Richman et al., 1979; Pfeiffer and Lazenby, 1994), and general health (Martin and Armelagos, 1979).
b. The size of osteons (area: On.Ar) and Haversian canals (area: H.Ar, diameter: H.Dm) known to be affected by type of mechanical load (e.g., tension vs. compression) (Skedros et al., 1994; Martin et al., 1998; Smit et al., 2002; van Oers et al., 2008) can provide insights into behavior, and general bone metabolism.

MATERIALS AND METHODS

The human remains[1] were from a medieval (early 11th and 16th centuries AD) archaeological site in Canterbury (Hicks and Hicks, 2001). They were recovered from two burial locations: the medieval Priory (Pr N = 40) and an associated cemetery outside of the Priory (Cem N = 410). The total sample in the present study consisted of 450 adults (233 males and 217 females) (Table 3). The two burial locations at the archaeological site correlate with status. Higher status individuals were buried within the Priory, which was a popular way of displaying status during the medieval period (Ottaway, 1992; Daniell, 1998). Fewer individuals were recovered from the Priory graves, as these were reserved for the clergy, as well as wealthy families that could pay for the burial location (Anderson, 1989). Historical textual records indicate that the cemetery served poorer families (see below). The remains were excavated between 1988 and 1991, and a portion of them is now curated by the Skeletal Biology Research Centre, School of Anthropology and Conservation, University of Kent (UK).

St Gregory's Priory and Cemetery Historical and Archaeological Context

Until Henry VIII's Dissolution of the Monasteries (1537 AD), St. Gregory's Priory was one of three main priories in medieval Canterbury, the others being St. Augustine's and Christ Church (Tatton-Brown, 1995). It was established in 1084 AD by Lanfranc, the first Norman Archbishop of Canterbury (Duncombe, 1785; Lyle, 2002; Méar-Coulstock, 2010). Canons from the Priory assisted the sick and poor in Canterbury (Somner, 1703; Brent, 1897; Tatton-Brown, 1987; Lyle, 2002), served St. John's hospital located across the road (Duncombe, 1785;

TABLE 3. Present sample sub-divided by sex and age, with abbreviations used in Tables (4–7).

Group	Priory N	Cemetery N	Total N
Entire dataset (ES)	40	410	450
Young adults (YAs)	8	118	126
Middle-aged adults (MAs)	32	287	319
Old adults (OAs)	0	5	5
Males (Ms)	30	203	233
Females (Fs)	10	207	217
Young females (YAFs)	3	74	77
Young males (YAMs)	5	44	49
Middle-aged females (MAFs)	7	132	139
Middle-aged males (MAMs)	25	155	180
Old females (OAFs)	0	1	1
Old males (OAMs)	0	4	4

Brent, 1879; McKilliam 1913; Woodcock, 1956), and provided free burial in the neighboring cemetery to the poorest residents (Somner, 1703). Priest and cannons enjoyed a wealthy life with meals that consisted of meat dishes, beer, and wine (Bishop, 1983). Toward the end of the 15th century AD, St. Gregory's Priory became an obscure religious center (Tatton-Brown, 1989) with a reduced number of canons (Woodcock, 1956). The clergy started gambling with locals and engaged in heavy drinking (Bickley, 1901). Following Henry VIII's Dissolution of the Monasteries, the Priory was dissolved (Tatton-Brown, 1989; Lyle, 2002), and later became a private house that underwent demolition in 1848 (Tatton-Brown, 1989).

Archaeological finds and an initial paleodemographic analysis support the historical textual evidence for socioeconomic divisions at the Priory (Hicks and Hicks, 2001). For example, a male with a chalice and a gold-embroided monastic-like garment, speculated to be Prior Alured, was excavated in the Priory (Hicks and Hicks, 2001). Hicks and Hicks (2001) also reported 21 subadults and a number of female burials in the Priory. Although the present study only considers adults, the juvenile remains suggest that secular families were also buried within the high status area. The cemetery was established just before the Priory (Sparks, 1988), and a total of 1,342 skeletons were recovered during excavation. Historical textual records indicate that the cemetery served poorer families from local parishes, people who could not afford burial fees, and patients from nearby St. John's hospital (Brent, 1879; Somner, 1703). It was in constant use until a few years after the Priory was dissolved (Sparks, 1988). Miszkiewicz (2012) reported high prevalence of linear enamel hypoplastic (LEH) lines in the cemetery, inferring the lower social status of this group. Evidence for numerous corn, meat, and cattle markets in and around Canterbury (Brent, 1879; Lincoln, 1955; Utting, 1997; Sweetinburgh, 2010) suggests the presence of peasant farmers.

Osteological Assessment

Sex and age-at-death (AAD) were determined following standard osteological methods summarized by Buikstra and Ubelaker (1994) and Brickley and McKinley (2004). Multiple techniques were applied to each skeleton to increase the accuracy of the estimates. Sex was determined following a gross anatomical examination of the human skull, pelvis, and from post-cranial joint surface measurements. Since the true biological age of each individual was not known, AAD was estimated from anthropologically established macroscopic changes that affect skeletal morphology as humans age. Following the examination of cranial suture closure, dental wear on the permanent mandibular and maxillary dentition, and age-specific morphology of the pubic symphysis and auricular surface on the pelvis, individuals were placed into classic anthropological age categories (Buikstra and Ubelaker, 1994): young adults: 25–34 years; middle-aged adults: 35–49 years; older adults: 50+ years.

Femoral Measurements and Sectioning

As the femur is a major weight-bearing bone that receives variable biomechanical loads in different individuals (unlike ribs exposed to a regular respiration-related muscle contraction, Skedros et al., 2013), it is often studied in a biomechanical (locomotion and physical activity) context (Drapeau and Streeter, 2006). Therefore, it serves as a suitable bone in the present study because its cortical remodeling may indicate both biomechanical and other variations in bone metabolism. The right (with no obvious pathological lesions) femur was consistently selected, but if it was fragmented or missing, the left femur was examined per individual. Each femur had midshaft diaphyseal circumference (Circ) and maximum length measured (mm) using a standard tape measure and an osteometric board, respectively (Table 2). Raw bone length data were obtained from 305 intact femora, whereas estimates were determined for 118 fragmented femora [F1 $N = 64$, F2 $N = 33$, F3 $N = 11$, F4 $N = 10$, see Jacobs (1992) for method], giving a total of 423 bone length data points. However, all 450 femora were sectioned at midshaft for histological analysis, increasing sample size. Robusticity indices (RI) were estimated using Circ data standardized by bone length (RI = Circ/bone length $\times$ 100) (Stock and Shaw, 2007).

Adhering to the English Heritage guidelines for invasive sampling (Mays et al., 2013), sections were removed from the posterior linea aspera aspect (Chan et al., 2007; Miszkiewicz, 2015b) where the most within- and between-bone histological variation had been observed in a preliminary study (Miszkiewicz and Mahoney, 2012). Linea aspera is an insertion site for lower limb muscles from the adductor and hamstring family (Moore et al., 2014), although it is stressed that the present study does not ascertain or test a direct muscle-tendon-bone growth relationship. Femora were stabilized using a hand- (Irwin Quick-Grip Mini®) or table-mounted (Dremel Muli-Vise®) holder. A standard hand saw (Irwin BiMetal®) and an electronic drill (Dremel Rotary Tool®) were used to extract 1 cm $\pm$ 0.2 cm sized midshaft sections. In order to account for continued sub-periosteal deposition associated with aging (Ruff and Hayes, 1988), cortical width (2D) (Ct.Wi) data (mm) were collected using a standard digital caliper (Vajda and Bloebaum, 1999), and also used in calculating robusticity indices (Ct.Wi_RI) (Table 2).

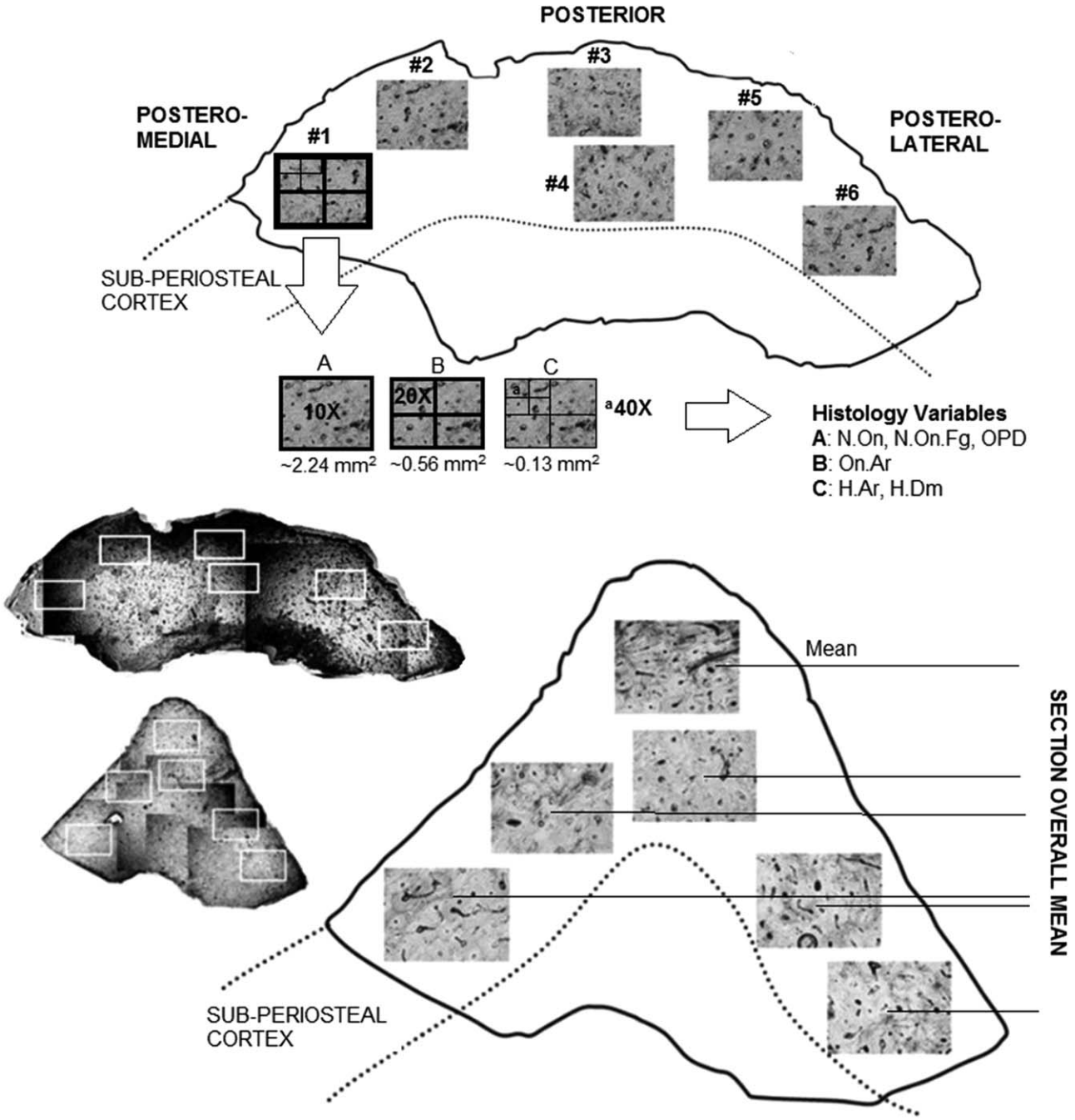

Fig. 2. Schematic (neither ROI boxes nor section montages are to scale) diagram illustrating histological evaluation procedures, with sample montages showing differences in posterior cortical wall width (modified after Miszkiewicz, 2015b).

Histological Preparation and Analysis

Thin sections were prepared following standard methods (e.g., Bancroft and Gamble, 2002; also see Miszkiewicz, 2015a, 2015b). Samples were embedded in epoxy resin (Buehler EpoxiCure ®) and sectioned in half from the medial toward the lateral end on a Buehler Isomet 1000 precision saw. Sections were attached to glass slides, reduced on a Buehler Eco-Met 300 Grinder-Polisher, polished, and cleaned in an ultrasonic bath. This was followed by dehydration in 95% and 99–100% ethanol, clearing in Histoclear, and covering with glass slips.

Images of each slide were taken with an Olympus DP25 Camera mounted on a Olympus BX51 high-powered microscope and analyzed using CELL® Live Biology Imaging software. Images were captured from a maximum of six regions of interest (ROIs) (Fig. 2). The selection of ROIs

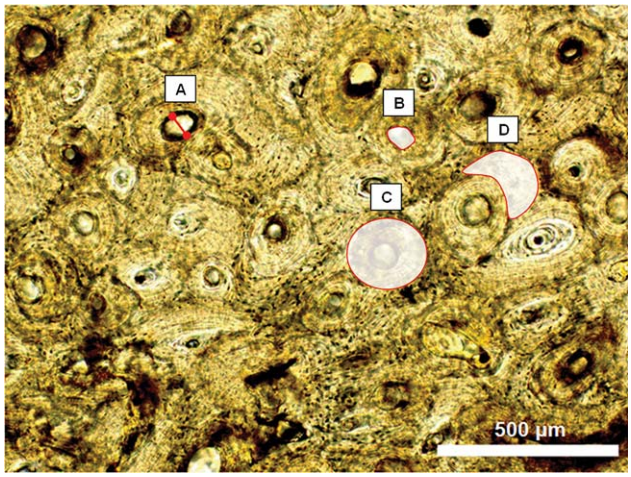

Fig. 3. Micrograph showing a transverse view of adult (secondary) cortical bone indicating variables evaluated in the present study. **A**: Haversian canal diameter, (**B**) osteon area, (**C**) intact osteon, (**D**) fragmentary osteon.

remains unstandardized in bone histomorphometry (Villa and Lynnerup, 2010), with researchers reporting the use of an eyepiece grid micrometer (e.g., Stout and Lueck, 1995), examining countable osteons (e.g., Pfeiffer, 1998), or specific section regions (e.g., Robling and Stout, 2003). However, it has been shown that different techniques do not affect data variation within a section (Iwaniec et al., 1998; Villa and Lynnerup, 2010), but it is recommended that 25–50 osteons should be examined per section (Stout and Crowder, 2011). Here, cortical bone adjacent to the periosteum was selected for a static histomorphometry examination throughout the study (Fig. 2, Miszkiewicz, 2015b), accounting for (potentially) mechanically induced subperiosteal bone deposition. One 2×/4× image served as a reference point throughout the recording procedure. Six 10× (~2.24 mm^2 each) images were taken within the subperiosteum. Four 20× (~0.56 mm^2 each), and 10–16 40× (~0.13 mm^2 each) images were captured within the aforementioned six ROIs. A variety of magnifications were applied depending on the type of examined variable (Fig. 2) to aid identification of microstructure and its substructural parameters. It was not possible to consistently select the same ROIs within a section, because their location was determined by the visibility of osteons, in some cases affected by diagenesis and taphonomy, often reported when examining ancient bone (e.g., Ericksen, 1980; Hanson and Buikstra, 1987; Pfeiffer et al., 2006; Booth, 2015). However, it was consistently ensured that (if suitably preserved) medial, lateral, most posterior (i.e., underneath linea aspera), postero-medial, and postero-lateral regions were evaluated (Fig. 2), which resulted in 60–120 osteons measured per section. Histology variables were counted and measured (in µm or $µm^2$) (Dempster et al., 2013; Table 2; Fig. 3). All complete secondary osteons with intact cement lines were examined regardless of their deviations from circularity as it has been recently suggested that excluding irregularly shaped osteons may overlook vital biomechanical information (Skedros et al., 2014). Not all sections were suitable for recording all histology variables, hence minor sample size differences by variable are reported in the results below.

Statistical Procedures

Statistical analyses were performed using IBM SPSS® 22 (2014) and *R* (2.5.0)® (2007) at $P = 0.05$. Data were assessed for normality with a Kolmogorov–Smirnov test, and transformed for parametric inferential testing if data were not normally distributed. Non-parametric tests were applied to all data that were not normally distributed, where sample sizes were smaller than 10, and/or where samples sizes were highly unequal when comparing groups. An intra-observer error test was conducted on 10% (N = 45) of thin sections. Secondary histology data collected on 10% of thin sections were compared against their primary values (Lynnerup et al., 1998) in paired correlations, Bland–Altman graphs (Bland and Altman, 1986), and paired samples *t*-tests. Data agreement between right (N = 367) and left (N = 83) femora was checked using an independent samples *t*-test. A *K*-means cluster analysis was performed on the entire dataset to create five "robusticity" categories, two of which had sample sizes that were large enough to compare the two social groups (i.e., Pr vs. Cem) using an inferential analysis. Due to unequal sample sizes, a Mann–Whitney *U* Test was used to compare the Priory and Cemetery data. A discriminant function analysis (DFA) (a brief description follows, and for more details see Tabachnick and Fidell, 2013) was also performed to determine which series of variables best discriminated between the two groups. Only significant variables identified in univariate tests were inserted into the DFAs using the "Enter" method. In cases where both OPD and one of the other osteon density variables were significant, only OPD was selected as it is formed of N.On and N.On.Fg. Results with the highest classification outputs are reported in this article.

A DFA combines independent variables linearly and creates a new variable referred to as a function. A coefficient value is assigned to each variable and each individual is scored from the function. The value of this score is used to assign group memberships. If a significant function is obtained, cases are classified into groups more accurately. However, a significant function may not necessarily indicate a good discrimination. Therefore the Eigen (*E*) and canonical correlation (*U*) values, indicating the degree of discriminating power, were noted. The equality or inequality of means across groups was tested using a Wilks' lambda Chi-Square value.

A DFA has a number of assumptions. Data normality, absence of univariate and multivariate outliers, linearity, multicollinearity or singularity, and absence of heterogeneity of variance–covariance matrices, can all affect the accuracy of a DFA. This was addressed prior to performing the analysis. Data normality, univariate outliers, and transformations had been previously tested, but multivariate outliers were screened using the Mahalanobis measure of distance and eliminated. Linearity was tested by examining scattergrams and multicollinearity was assessed from correlation matrices. Homogeneity of variance–covariance matrices was checked using Box's *M* Test. If heterogeneity was observed, cases were classified upon separate covariance matrices, as opposed to pooled covariance matrices.

RESULTS

All data ($P = 0.000$), except for Ct.Wi ($P > 0.05$) were not normally distributed and were transformed, although

TABLE 4. Mann–Whitney *U* test results for histology and robusticity differences between the Priory and the Cemetery.

Histology						
Variable	Sub-group	N	Priory N	Cemetery N	*U*	*P*
$N.On.Fg_{(sqrt)}$	ES	413	40	373	3,960.500	0.000
$OPD_{(sqrt)}$		413	40	373	5,300.500	0.003
$H.Dm_{(Lg10)}$		450	40	410	6,032.500	0.006
$H.Dm_{(Lg10)}$	YAs	126	8	118	250.500	0.027
$N.On.Fg_{(sqrt)}$	MAs	292	32	260	2,129.500	0.000
$OPD_{(sqrt)}$		292	32	260	2,842.500	0.003
$N.On.Fg_{(sqrt)}$	Ms	220	30	190	1,219.500	0.000
$OPD_{(sqrt)}$		220	30	190	1,938.000	0.005
$H.Ar_{(Lg10)}$		233	30	203	2,257.000	0.022
$H.Dm_{(Lg10)}$		233	30	203	1,884.000	0.001
$On.Ar_{(sqrt)}$		211	30	181	2,041.000	0.030
$On.Ar_{(sqrt)}$	MAFs	121	7	114	598.000	0.027
$N.On.Fg_{(sqrt)}$	MAMs	170	25	145	699.000	0.000
$OPD_{(sqrt)}$		170	25	145	1,187.500	0.006
$H.Dm_{(Lg10)}$		80	25	155	1,884.000	0.006
$On.Ar_{(sqrt)}$		165	25	140	2,041.000	0.037
Robusticity measures						
$Circ_{(sqrt)}$	ES	449[a]	40	409	5,973.500	**0.005**
$Circ_{(sqrt)}$	YAs	125	8	117	272.000	**0.048**
Ct.Wi	ES	450	40	410	7,136.500	0.176
Circ_RI	ES	422	37	385	7,474.000	0.620
Circ_RI	MAMs	168	23	145	2,107.000	**0.043**
Ct.Wi _RI	ES	423	37	386	6.654.500	0.493
Histology and robusticity measures						
$N.On.Fg_{(sqrt)}$	Circ #1	140	17	123	663.000	0.015
$N.On.Fg_{(sqrt)}$	Circ #2	90	13	77	139.000	0.000
$OPD_{(sqrt)}$		90	13	77	271.000	0.008
$H.Ar_{(Lg10)}$		95	13	82	309.000	0.015
$H.Dm_{(Lg10)}$		95	13	82	269.000	0.004
$N.On.Fg_{(sqrt)}$	$Circ\ \#1_{Ms}$	94	14	80	331.500	0.015
$N.On.Fg_{(sqrt)}$	$Circ\ \#2_{Ms}$	86	13	73	130.000	0.000
$OPD_{(sqrt)}$		86	13	73	256.000	0.008
$H.Ar_{(Lg10)}$		90	13	77	283.000	0.013
$H.Dm_{(Lg10)}$		90	13	77	248.000	0.004
$N.On.Fg_{(sqrt)}$	$Circ\ \#1_{MAMs}$	79	12	67	230.000	0.019
$N.On.Fg_{(sqrt)}$	$Circ\ \#2_{MAMs}$	65	11	54	53.000	0.000
$OPD_{(sqrt)}$		65	11	54	123.500	0.002
$H.Dm_{(Lg10)}$		69	11	58	188.000	0.032
$N.On.Fg_{(sqrt)}$	$Circ\_RI\ \#1_{MAMs}$	33	7	26	34.500	0.010
$H.Dm_{(Lg10)}$		37	7	30	52.000	0.040
$N.On.Fg_{(sqrt)}$	$Circ\_RI\ \#2_{MAMs}$	125	16	109	271.500	0.000
$OPD_{(sqrt)}$		125	16	109	438.500	0.001

[a]One fragmented midshaft where Circ value could not be obtained.

data in some sub-groups still remained not-normal following the transformations (Supporting Information Table 1). No observer bias was identified (Supporting Information Table 2). Variables from the left femora did not differ significantly when compared to the right femora (Supporting Information Table 3) and were pooled for all analyses.

Univariate Analysis

Significant results from the univariate tests are given in Table 4. Corresponding descriptive statistics are provided in Table 5 for the Priory and Table 6 for the Cemetery. As there were only five old adults, all from the Cemetery (Table 3), they were excluded from age-controlled analyses due to the small sample size and the OPD asymptote effect (Robling and Stout, 2003).

There was an overall trend for the Priory samples to display significantly more and larger osteons and Haversian canals when compared to the Cemetery (Table 4). The finding was consistent when analyzing the entire dataset (N.On.Fg, OPD, H.Dm), young individuals (H.Dm), middle-aged adults (N.On.Fg, OPD), and males (all histology variables except for N.On), and middle-aged males (N.On.Fg, OPD, H.Dm, On.Ar). One inconsistent finding with these results was noted for middle-aged females from the Cemetery whose osteons (On.Ar) were significantly larger when compared to the Priory.

The values of Ct.Wi and associated robusticity indices remained equal when compared between the two sites (Table 4). However, the size of femoral midshaft was significantly greater in the Priory and young Priory males. The robusticity index values computed using Circ values only differed significantly in the middle-aged male subgroup. In order to account for these significant differences, histology was compared in each of the following four categories of similar femoral size (Circ data only)

TABLE 5. Descriptive (non-transformed) data for the Priory.

		Histology				
Variable	Sub-set	N	Min.	Max.	Mean	SD
N.On.Fg (#/mm^2)	ES	40	4.02	11.61	6.82	2.28
OPD (#/mm^2)		40	13.66	28.57	20.49	3.87
H.Dm (μm)		40	21.44	130.15	57.76	25.01
H.Dm	YAs	8	34.87	96.06	60.83	19.51
N.On (#/mm^2)	MAs	32	7.95	19.64	13.73	3.01
OPD		32	13.66	28.57	21.04	3.95
N.On.Fg	Ms	30	4.13	11.61	7.47	2.27
OPD		30	13.66	28.57	21.32	4.05
H.Ar (μm^2)		30	396.45	12894.90	3364.15	2966.58
H.Dm		30	21.44	130.15	59.84	26.01
On.Ar (μm^2)		30	9321.63	80120.16	33509.47	19803.07
On.Ar	MAFs	7	3061.36	43530.20	16439.18	13916.92
N.On.Fg	MAMs	25	4.13	11.61	7.87	2.25
OPD		25	13.66	28.57	21.69	4.25
H.Dm		25	21.44	130.15	61.29	27.88
On.Ar		25	9321.63	80120.16	35739.27	20525.19
Robusticity measures						
Circ (mm)	ES	40	73.00	106.00	90.73	7.67
Circ	YAs	8	73.00	106.00	90.75	10.36
Circ_RI	ES	37	17.51	23.25	19.80	1.13
Circ _RI #1	MAMs	7	17.51	19.51	18.67	.62
Circ _RI #2	MAMs	16	19.68	21.54	20.38	.62
Ct.Wi (mm)	ES	40	5.10	14.89	9.64	2.51
Ct.Wi _RI	ES	37	1.12	3.11	2.12	.50
Histology and robusticity measures						
N.On.Fg	Circ #1	17	4.13	10.71	6.58	2.11
N.On.Fg	Circ #2	13	4.46	11.61	8.30	2.43
OPD		13	14.73	28.57	22.47	4.67
H.Ar		13	429.72	8758.53	3079.76	2229.56
H.Dm		13	25.41	130.15	59.27	26.18
N.On.Fg	Circ #1$_{Ms}$	14	4.13	10.71	6.97	2.12
N.On.Fg	Circ #2$_{Ms}$	13	4.46	11.61	8.30	2.431
OPD		13	14.73	28.57	22.47	4.67
H.Ar		13	429.72	8758.53	3079.76	2229.56
H.Dm		13	25.41	130.15	59.27	26.18
N.On.Fg	Circ #1$_{MAMs}$	12	4.13	10.71	7.25	2.12
N.On.Fg	Circ #2$_{MAMs}$	11	5.02	11.61	8.83	2.22
OPD		11	14.73	28.57	23.15	4.67
H.Dm		11	25.41	130.15	59.12	28.30
N.On.Fg	Circ_RI #1$_{MAMs}$	7	4.69	10.71	7.43	2.05
H.Dm		7	33.86	121.69	73.77	27.74
N.On.Fg	Circ_RI #2$_{MAMs}$	16	4.13	11.61	8.17	2.36
OPD		16	14.73	28.57	22.73	4.17

and robusticity index (Circ data standardized by femoral length), where sample sizes were sufficiently large within each social status group (see Tables 5 and 6 for social status group sample size information):

1. Circ #1: midshaft size 86–93 cm (total N = 151, mean- = 89.27, SD = 2.32)
2. Circ #2: midshaft size 94–101 cm (total N = 95, mean- = 96.76, SD = 2.36)
3. Circ_RI #1: femoral RI 15.76–19.53 (total N = 166, mean = 18.52, SD = .78)
4. Circ_RI #2: femoral RI 19.55–24.94 (total N = 254, mean = 20.82, SD = 1.05)

Histology remained consistently greater in the Priory when compared to the Cemetery, even though the size of femoral midshaft was on average the same when controlling the groups using the entire dataset (Circ #2: H.Ar, H.Dm), in males (Circ #1: N.On.Fg; Circ #2: N.On.Fg, OPD, H.Dm) and middle-aged adults (Circ #1: N.On.Fg; Circ #2: N.On.Fg, OPD); and when the overall femoral robusticity index was similar when comparing middle-aged males (Circ_RI #1: N.On.Fg, H.Dm, Circ_RI #2: N.On.Fg., OPD).

Multivariate Analysis

Five DFAs were performed with the social status group (Pr vs. Cem) as the dependent variable (DV) (Table 7, Fig. 4). The assumptions of linearity, normality, multicollinearity, or singularity were met for each DFA. Firstly, OPD and H.Dm were inserted as predictor variables for the entire sample (N = 408, 7 outliers eliminated). Homogeneity of variance–covariance matrices were observed ($P = 0.106$). One significant discriminant function was calculated (Fig. 4a), with a successful classification of cases in 71.8%. Secondly, OPD, H.Ar, H.Dm, and On.Ar were inserted as predictor variables for males (N = 205, 6 outliers eliminated). Homogeneity of variance–covariance

TABLE 6. Descriptive (non-transformed) data for the Cemetery.

		Histology				
Variable	Sub-set	N	Min.	Max.	Mean	SD
N.On.Fg (#/mm^2)		373	1.93	12.50	5.08	1.50
OPD (#/mm^2)		373	10.95	30.36	18.58	3.16
H.Dm (μm)		410	20.14	136.55	46.93	17.69
H.Dm	YAs	118	24.15	102.55	46.09	15.74
N.On (#/mm^2)	MAs	282	7.89	20.91	13.69	2.34
OPD		260	11.77	30.36	18.99	2.99
N.On.Fg	Ms	190	2.23	12.50	5.28	1.59
OPD		190	10.95	30.36	19.19	3.10
H.Ar (μm^2)		203	355.15	14789.47	2256.81	2459.58
H.Dm		203	20.14	134.68	45.07	19.26
On.Ar (μm^2)		181	4671.52	96666.91	25041.33	15484.23
On.Ar	MAFs	114	2101.39	94452.20	28378.84	16190.97
N.On.Fg	MAMs	145	2.57	12.50	5.49	1.67
OPD		145	12.50	30.36	19.44	2.98
H.Dm		155	20.14	134.68	46.65	20.58
On.Ar		140	6751.83	96666.91	26518.48	16382.42
Robusticity measures						
Circ (mm)	ES	409	71.00	113.00	87.31	8.04
Circ	YAs	117	71.00	106.00	83.88	7.35
Circ _RI	ES	385	15.76	30.94	19.98	1.67
Circ _RI #1	MAMs	30	16.90	19.53	18.48	.67
Circ _RI #2	MAMs	114	19.57	24.94	21.00	1.11
Ct.Wi (mm)	ES	410	4.83	15.73	8.92	1.70
Ct.Wi _RI	ES	386	1.10	3.89	2.05	.39
Histology and robusticity measures						
N.On.Fg	Circ #1	123	2.23	12.50	5.3138	1.80
N.On.Fg	Circ #2	77	2.46	8.93	5.1557	1.20
OPD		77	12.50	25.30	19.08	2.62
H.Ar		82	367.32	6885.67	1734.16	1456.82
H.Dm		82	20.14	134.68	40.91	16.88
N.On.Fg	Circ #1$_{Ms}$	80	2.23	12.50	5.55	2.04
N.On.Fg	Circ #2$_{Ms}$	73	2.46	8.93	5.15	1.22
OPD		73	12.50	25.30	19.02	2.58
H.Ar		77	367.32	6885.67	1703.45	1460.87
H.Dm		77	20.14	134.68	40.67	17.00
N.On.Fg	Circ #1$_{MAMs}$	67	2.98	12.50	5.79	2.11
N.On.Fg	Circ #2$_{MAMs}$	54	2.57	8.93	5.35	1.21
OPD		54	12.50	23.81	19.05	2.34
H.Dm		58	20.14	134.68	42.28	18.80
N.On.Fg	Circ_RI#1$_{MAMs}$	26	2.98	11.16	5.53	2.11
H.Dm		30	24.59	134.68	53.96	26.34
N.On.Fg	Circ_RI#2$_{MAMs}$	109	2.57	9.82	5.28	1.17
OPD		109	12.50	26.19	19.30	2.60

TABLE 7. Discriminant function analysis results (DV = Priory vs. Cemetery).

DV sub-set	Predictor variables	*n*	Function Chi-square	df	*P*	Loading matrix	Classification
ES	OPD	408	22.295	2	0.000	0.646	78.1%
	H.Dm					0.611	
Ms	OPD	205	27.305	4	0.000	0.549	77.1%
	H.Ar					0.382	
	H.Dm					0.609	
	On.Ar					0.433	
MAMs	OPD	162	25.438	3	0.000	0.636	80.9%
	H.Dm					0.574	
	On.Ar					0.486	
Circ #2	H.Dm	93	11.061	2	0.004	0.937	76.3%
	H.Ar					0.619	
Circ #2$_{Ms}$	OPD	85	33.851	2	0.000	0.548	87.1%
	H.Dm					0.650	

matrices was observed ($P = 0.531$). One discriminant function was calculated (Fig. 4b) where cases were successfully classified in 77.1%. Thirdly, OPD, H.Dm, and On.Ar were inserted as predictor variables for middle-aged males (N = 162, 4 outliers eliminated). However, heterogeneity of variance–covariance matrices was observed ($P = 0.023$),

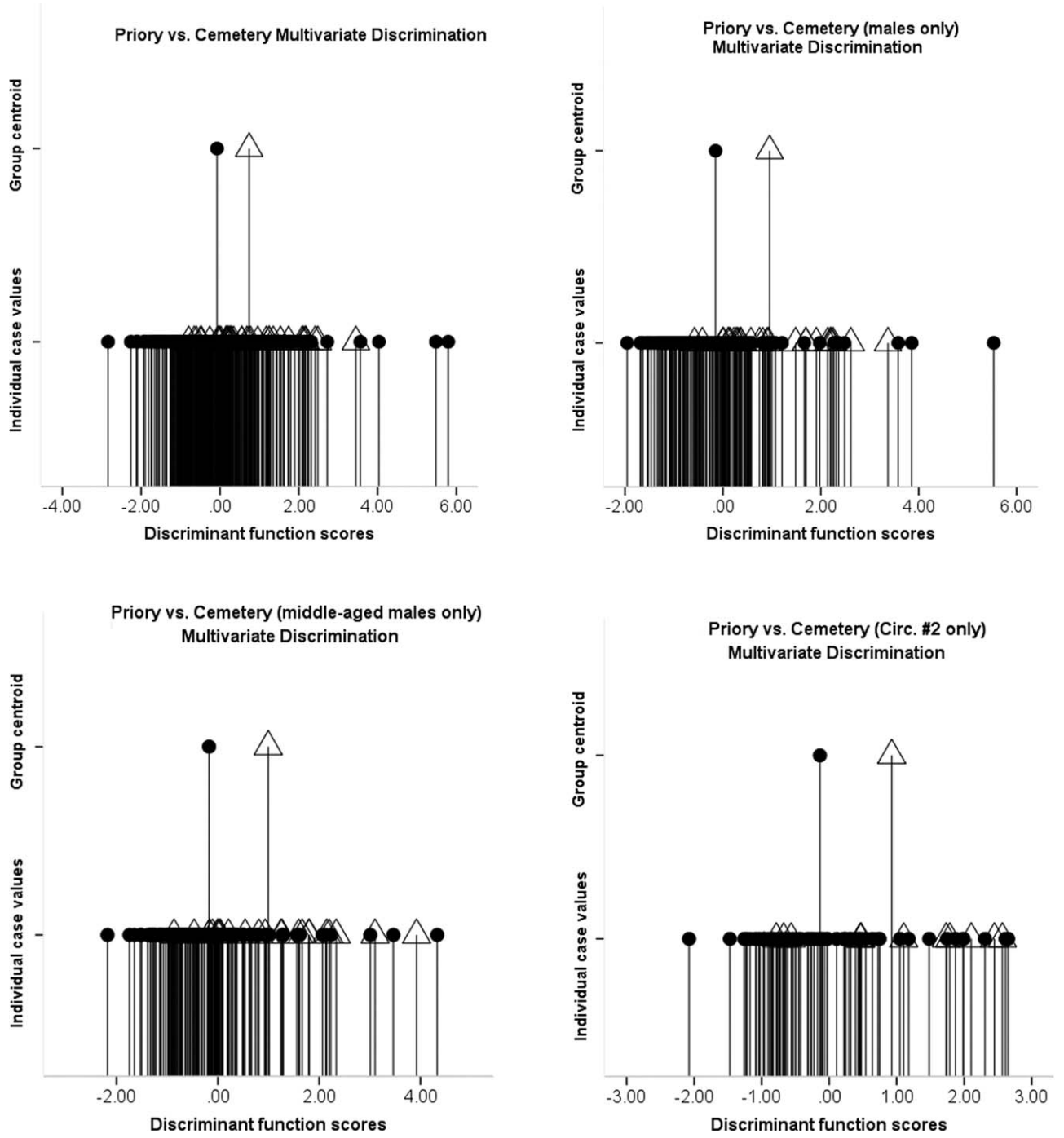

Fig. 4. Discriminant function analysis plots demonstrating how well the calculated functions discriminated (separation is illustrated by group centroids) between the Priory (triangle) and the Cemetery (circle). (**a**) OPD and H.Dm, *E* (Eigen value) = 0.057, *U* (canonical correlation) = 0.231; (**b**) H.Dm, OPD, On.Ar, H.Ar, *E* = 0.146, *U* = 0.356; (**c**) H.Dm, OPD and On.Ar, *E* = 0.174, *U* = 0.385; (**d**) H.Dm and H.Ar, *E* = 0.130, *U* = 0.339; (**e**) H.Dm and OPD, *E* = 0.511, *U* = 0.582.

and it was chosen to classify cases upon separate covariance matrices. One discriminant function was identified (Fig. 4c) with a successful classification of cases in 80.9%. The fourth DFA with H.Dm and H.Ar as predictor variables was run for Circ #2 (N = 93, 2 outliers eliminated). Homogeneity of variance–covariance matrices was observed ($P = 0.601$). One discriminant function was calculated (Fig. 4d) where cases were successfully classified in 76.3%. Finally, OPD and H.Dm were inserted as predictor variables for Circ #2 in males (N = 85, 2 outliers eliminated). However, heterogeneity of variance–covariance matrices was observed ($P = 0.000$), and thus cases were classified

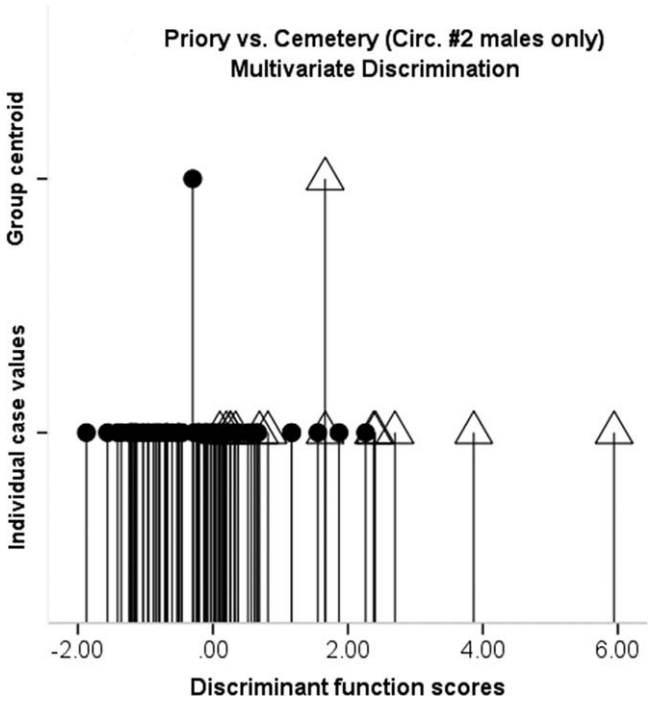

Fig. 4. (Continued).

upon separate covariance matrices. One discriminant function was calculated (Fig. 4e) with a successful classification of 87.1% cases.

DISCUSSION

This study reports significant variation in femoral cortical histology when compared between archaeological samples of humans representing high (Priory) and low (Cemetery) socio-economic groups. Priory femora displayed more and larger osteons and Haversian canals, compared to the Cemetery specimens whose osteons were smaller and of a reduced density. These results indicate differences in bone remodeling between the two groups, which may relate to socio-economic status. It is inferred that the more active lifestyle of those buried within the Cemetery (see background review above) is consistent with their smaller and less frequent osteons. Small and less frequent osteons could indicate higher mechanical loads, but lower remodeling, perhaps linked to insufficient nutrition and general poor health. Given the complexity in bone metabolism processes (i.e., hormonal, genetic, pathological), and the non-experimental setting of the present study, it is difficult to attribute a more specific cause to the histological variation between the two groups.

Variation in histology between the Cemetery and Priory was still present when the samples were further subdivided by age and sex. Overall, Haversian canals and osteons remained larger, and there were more osteons in the different age groups from the Priory. Females were the exception to this trend, as their histology remained equal across the two groups. In the middle-aged female category osteons were unpredictably small. This lack of bone microstructure differentiation does not match the written historical evidence for female lifestyle and social stratification. This might relate in part to the highly unequal sample sizes. Also, the significantly smaller osteons in the middle-aged Priory female sub-group were entirely inconsistent with all other results for the Priory males, which may be an indication of sex-specific differences in bone metabolism. This latter observation would be consistent with conclusions drawn in prior studies (e.g., Ruff and Hayes, 1988; Britz et al., 2009; Mulhern, 2000; Vicente et al., 2013; Jepsen et al., 2015).

By controlling for bone size, the present analysis accounted for a potential scaling effect (Tommasini et al., 2007; Nowlan et al., 2011; Macintosh et al., 2013; Goldman et al., 2014), whereby robusticity may explain differences in osteonal geometric properties (Goldman et al., 2014). As discussed by Stock and Shaw (2007), a biomechanical examination of lower limb bones should ideally utilize normalization by some measure of body mass due to the weight-bearing properties of legs in bipedal animals. Here, comparisons undertaken using the robusticity variables demonstrated that the size of femoral midshaft, but not cortical width, was significantly larger in the Priory. Robusticity indices computed from these two variables were significantly different in only some sub-groups, signaling potential bone size variation between the two sites. However, histology comparisons performed when controlling for bone size confirmed the data pattern reported in the preceding analysis. More osteons and larger canals still characterized the Priory relative to the Cemetery, supporting the observed variation in histology. The results confirm that bone growth is a multi-factorial process, and whilst the present study is limited to making inferences only, the present data can be discussed in a broader bone-lifestyle context.

Ancient Bone Microstructure and Documented Lifestyle

Written sources and (bio)archaeological data suggest that the Cemetery population would have undertaken strenuous occupations, and suffered general poor health. The present study revealed consistently smaller osteons and Haversian canals in the femoral cortex of this group, in line with the documented lifestyle. The finding agrees with previous bioarchaeological studies (e.g., small osteons in an active medieval Sudanese Nubian population, Mulhern and Van Gerven, 1997), studies of hominins (e.g., Pfeiffer and Zehr, 1996), and human cadavers (e.g., larger osteons in muscle paralysis, Schlecht et al., 2012), where a decrease in osteonal parameters has been linked with experiences of more rigorous physical activity. In this study, variation in histomorphometric data agrees with the textual historical accounts of larger mechanical loads experienced by those who were buried within the Cemetery, compared to those buried within the Priory.

Osteonal data in the present study also correspond with the historical textual evidence for the general well-being of both groups. Individuals representing the higher status group would have enjoyed wealthier lifestyles with diets rich in protein, allowing for healthier bone growth. The significantly higher osteon density

recorded in the Priory samples may have been a result of substantial food consumption (e.g., Stout and Simmons, 1979; Paine and Brenton, 2006; Metges and Barth, 2000; Brandao-Burch et al., 2005). Individuals holding a higher status in medieval society would have had access to good quality nourishment, which guaranteed a broad range of minerals, vitamins, and proteins, providing a surplus of calories for homeostasis maintenance. Malnutrition and other types of physiological stress are known to affect bone growth in animals and humans (e.g., Lill et al., 2002). Here, lower osteon density in the Cemetery may reflect malnutrition. Also, since intense physical activity would have been common in the Cemetery group, the reduced osteonal parameters may be an indication of bone adapting to loads from a young age, making remodeling less necessary.

The inferred dietary-related effects on bone histology in the present samples are comparable to those reported previously for ancient Alaskan Inuit, Arikara from South Dakota, and the Pueblo (Richman et al., 1979). For example, increased meat and fat consumption and high density of osteons in the Priory is similar to elevated remodeling in a high-protein intake Alaskan Inuit group (Richman et al., 1979). A lowered remodeling and decreased protein ingestion in the Pueblo (Richman et al., 1979) is similar to that which is inferred for the Cemetery. In a different study (Robling and Stout, 2003), conducted on ancient Peruvians from Paloma (6,500–4,700 BP), a decrease in remodeling was noted in individuals undertaking less strenuous physical activity. Here, decreased osteon density was present in the group who would have been part of a physically active community. This discrepancy between the studies may reflect the lower status of the Cemetery adults, which most likely led to a generally poorer quality of life (i.e., health and diet).

While some previous studies successfully inferred behavior (e.g., Burr et al., 1990; Mulhern and Van Gerven, 1997; Mulhern, 2000; Robling and Stout, 2003), others related bone histology variation to diet (Richman et al., 1979), sex (pregnancy and lactation), age, and genetic factors (Ericksen, 1980; Stout and Lueck, 1995), or identified difficulties inherent to this methodology (e.g., Pfeiffer et al., 2006). Perhaps, as demonstrated in the present study (although based upon femoral data only), the measurement of multiple histological variables, evaluation of bone robusticity, and the categorization of lifestyle which included nutrition, physical activity, and well-being, will improve the consistency in future inferences made from ancient histology.

Documented information about past lifestyles characterizing the two studied groups may be teased out more specifically in further analyses. For example, an isotopic examination of bone could provide insights into protein consumption across the site. An evaluation of rib cortical histology in relation to the presented femoral data may also prove informative of biomechanical signals affecting remodeling. Some initial comments about the biomechanics of the lower limb can be made based upon the histology data. Since femora receive a wide range of tensile and compressive forces that originate from upper body weight and physical activity, the localized (i.e., posterior midshaft) cortical histomorphometry differences between the Priory and Cemetery may have been in part a result of documented occupations. Farming, construction work, mining, horse riding, and load carrying, all require lower limb strength and ability to withstand repetitive mechanical loads. As such it seems likely that these activities may have registered in the small osteonal parameters of those buried within the Cemetery, relative to the more sedentary individuals from the Priory. These general inferences may be more specifically explored and validated with additional data strictly associated with biomechanics (strain history) by, for example, estimating cross-sectional geometry (Sylvester et al., 2010), and examining a bone that reflects metabolism with stable mechanical loading, such as the rib.

Differential Interpretation

There are at least four other possibilities that may explain the histological variation reported here. These are as follows:

1. *More strenuous physical activity in the Priory than originally thought*: As much as the documented good health and diet in the Priory might have contributed to bone remodeling, it is also possible that the increased osteon density was caused by strenuous physical activity. Dynamic loads initiate bone formation (Lanyon and Rubin, 1984), thus higher remodeling in the Priory could be related to intermittent dynamic activity, such as prolonged walking by pilgrims, or active jousting by knights. The mid-shaft circumference values in the Priory are indeed larger, which can indicate higher activity during childhood.
2. *Biological age-progressive accumulation of osteons in the Priory*: An age-progressive accumulation of osteons could underlie group differences in histology, meaning that the Priory femora accumulated more osteons over time. Anthropological age ranges are broad, a limitation which can only be overcome if true individual biological age is known.
3. *Effective age of adult compacta differences between the Priory and the Cemetery*: The present findings may reflect differences in the effective age of adult compacta (Cho and Stout, 2011), with the Priory adults having larger bones as a result of a healthy childhood lifestyle that influenced phases of skeletal modeling.
4. *Genetic differences affecting bone formation rate and MES*: Genetic differences that govern bone formation rate and affect the minimum effective strain (MES) thresholds required to suppress remodeling and initiate modeling (Frost, 1983, 1987) may have characterized the two groups. Low MES setpoints in the Priory might have led to higher bone mass.

CONCLUSIONS

This study reported variation in bone microstructure at the femoral midshaft cortex when compared between two different socio-economic groups of ancient humans. The results showed, almost consistently (accounting for age, sex, and femoral size), that higher status adults had more and larger osteons and Haversian canals compared to lower status adults. These statistically significant differences in bone histology between the two groups corresponded with information about medieval lifestyle derived from archaeological and historical

evidence. It is concluded that histological methods can provide insights into ancient human lifestyles. Furthermore, the multivariate analysis revealed that both osteon densities and osteonal geometric properties discriminated between the status groups, suggesting that future studies may benefit from evaluating multiple histological variables (also see Miszkiewicz, 2015b).

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