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ARTICLE

MOBILITY PATTERNS IN GRANDE ABRIGO DE SANTANA DO RIACHO, MINAS GERAIS, BRAZIL: INSIGHTS FROM A HISTOMORPHOMETRIC ANALYSIS OF SKELETAL REMAINS

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ABSTRACT

This study analyses skeletal remains from Grande Abrigo de Santana do Riacho to investigate mobility patterns. Previous studies have suggested high mobility based on macroscopic observations of skeletons. This study examined thin sections of femoral Linea aspera of six adult individuals using histomorphometric techniques. The analysed variables include intact and fragmented secondary osteons, osteon area, and the Haversian canal. These data were then compared with other groups to test associations between histological features and mechanical stimuli. The great density of osteons found indicated high levels of physical activity. An attempt to compare sexes was also undertaken but remained inconclusive due to the limitations of the sample. Furthermore, a statistically significant ($p < 0.05$) positive relation occurred between the area of the Haversian canal and the density of secondary osteons, suggesting relatively short events of bone remodeling. This study also highlights the potential of bone paleohistology to investigate Brazilian contexts.

Keywords: histology; paleohistology; Central Brazil; bioarchaeology; biological anthropology.

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PADRÕES DE MOBILIDADE EM GRANDE ABRIGO DE SANTANA DO RIACHO, MINAS GERAIS, BRASIL: PERCEPÇÕES DE UMA ANÁLISE HISTOMORFOMÉTRICA DE SEPULTAMENTOS

ARTIGO

O presente estudo analisa sepultamentos de Grande Abrigo de Santana do Riacho, com objetivo de investigar os padrões de mobilidade. Estudos anteriores, baseados em análises macroscópicas, sugerem alta mobilidade. Neste estudo, foram examinadas lâminas microscópicas da região da linha áspera do fêmur de seis indivíduos adultos, utilizando técnicas histomorfométricas. As variáveis analisadas incluem ósteons secundários intactos e fragmentados, área do ósteon e do canal de Havers. Esses dados foram comparados com outros grupos para testar associações entre características histológicas e estímulos mecânicos. Alta densidade de ósteons foram encontrados, indicando elevados níveis de atividade física. Também foi realizada uma tentativa de comparação entre os sexos, que se mostrou inconclusiva devido às limitações da amostra. Ao mesmo tempo, foi observada uma relação positiva estatisticamente significativa ($p < 0,05$) entre a área do canal de Havers e a densidade dos ósteons secundários, sugerindo eventos relativamente curtos de remodelação óssea. O estudo também destaca o potencial da paleohistologia para investigações de contextos brasileiros.

Palavras-chave: histologia; pelehistologia; Brasil Central; bioarqueologia; antropologia biológica.

PATRONES DE MOVILIDAD EN GRANDE ABRIGO DE SANTANA DO RIACHO, MINAS GERAIS, BRASIL: PERSPECTIVAS A PARTIR DE UN ANÁLISIS HISTOMORFOMÉTRICO DE ENTIERROS

RESUMEN

El presente estudio analiza entierros en el Grande Abrigo de Santana do Riacho, con el objetivo de investigar patrones de movilidad. Estudios previos sugieren alta movilidad basada en observaciones macroscópicas de esqueletos. En este estudio se examinaron láminas microscópicas de la región de la línea áspera del fémur de seis individuos adultos, utilizando técnicas histomorfométricas. Las variables analizadas incluyen osteonas secundarias intactas y fragmentadas, área de osteonas y canal Haversiano. Estos datos se compararon con otros grupos para probar las asociaciones entre las características histológicas y los estímulos mecánicos. Se encontró una alta densidad de osteonas, lo que indica altos niveles de actividad física. También se realizó un intento de comparación entre sexos, pero no concluyente debido a las limitaciones de la muestra. Al mismo tiempo, se observó una relación positiva estadísticamente significativa ($p < 0,05$) entre el área del canal de Havers y la densidad de osteonas secundarias, lo que sugiere eventos de remodelación ósea relativamente cortos. El estudio también destaca el potencial de la paleohistología para investigaciones de contextos brasileños.

Palabras clave: histología; pelehistología; Brasil Central; bioarqueología; antropología biológica.

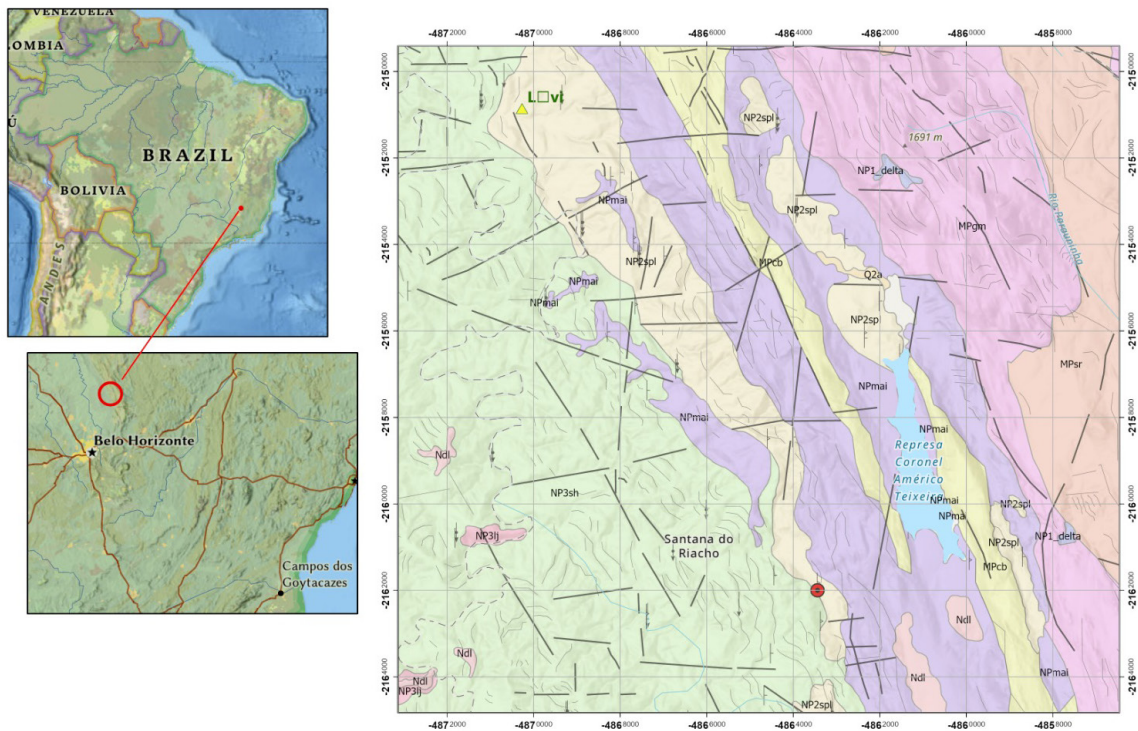
INTRODUCTION

Microscopic analyses constitute only a small portion of bioarchaeological studies, although some previous successful reconstructions of ancient human bone function from histology have been published (e.g., Miskiewicz; Mahoney, 2016, 2017; Miskiewicz *et al.*, 2021; Mulhern, 2000; Mulhern; Van Gerven 1997; Robling; Stout, 2003). This recognizes histology as a helpful tool to understand bone responses to lifestyle in archaeological contexts. As adult bone remodelling reflects bone metabolic processes, it suffers the influence of several factors, including age (Paine; Brenton, 2006; Richman; Ortner; Schuler-Ellis, 1979), health and disease (Aaron; Roger; Kanis, 1992), hormones (Manolagas, 2002), sex (Mulhern; Van Gerven, 1997), genetics (Thompson; Gunness-Hey, 1981), and physical activity (Robling; Castillo; Turner, 2006). While paleohistology has been applied to multiple archaeological contexts from across North America, Africa, Europe, and Asia-Pacific (Miskiewicz; Mahoney, 2016; Miskiewicz *et al.*, 2021), no study has yet analysed bone histology in an archaeological human Brazilian sample. Only a few studies applied histological methods to South American collections (Barrientos; Sarmiento; Galligani, 2016; Brachetta Aporta *et al.*, 2016; Desántolo; Inda, 2016; Suzuki; Tiesler, 2015; Vazquez *et al.*, 2021), with only one study aiming to discuss behaviour (Robling; Stout, 2003) This study described the first data for archaeological Brazilian human remains and used it to test a hypothesis that these individuals experienced high mobility.

Mobility at Santana do Riacho

Human skeletal remains excavated from a rock shelter in Santana do Riacho in Minas Gerais, Southeast Brazil (Figure 1), were reported in the early 1990s (Karfunkel *et al.*, 1992). The dates obtained for this site range from $8,280 \pm 40$ – $9,460 \pm 110$ BP, representing one of the earliest archaeological sites with human remains on the South American continent (Neves *et al.*, 2003). Grande Abrigo de Santana do Riacho was excavated twice during the 1970s, unearthing 40 individuals (Prous *et al.*, 1992). The site is considered part of the Lagoa Santa region, in which one of the earliest human skeletons in America was found (Neves *et al.*, 1999). Therefore, the site shows evidence of the very early human occupation of the continent.

Genetic research showed connections between the individuals representing this early South American population and the Clovis contexts (Posth *et al.*, 2018). One of the central debates brought by genetic evidence refers to differences in the subsistence strategies between the North and South American contexts. While the Clovis population have been primarily described as hunters, showing indications of a physically demanding lifestyle and reliance on big game animals (Martin, 1973), the Lagoa Santa population is associated with a different subsistence strategy, relying mostly on plants (Resende; Prous, 1992) and presenting a lithic industry more compatible with the hunting of small to medium-sized animals (Araujo *et al.*, 2018). The paleopathological analysis of human remains (Gloria, 2012) also indicates a less mobile lifestyle, more compatible with gathering activities than with hunting. These differences in archaeological records are important because, if proven correct, it can be inferred that the Lagoa Santa population migrated to South America and adopted a very different lifestyle in a relatively short period, showing a very high degree of environmental adaptability (Gloria; Hubbe; Neves, 2018).

Figure 1. Site context and location.

Source: Authors.

Several prior studies have been conducted on the sample (Cornero, 2005; Cornero; Neves; Prous, 1999; Gloria, 2012; Melo e Alvim, 1977; Souza, 1992, 1993). The first complete paleopathological assessment of the remains described a high level of sexual dimorphism (Cornero, 2005). In adult individuals, males showed greater stature, 12% taller than females (Cornero, 2005). Additionally, sex differences were noted in some of the skeletal features: male individuals' thoracic vertebra had more osteophytes, while more lesions associated with osteoarthritis were noticed in females (Cornero, 2005). In general, females presented showed a more gracile build (Cornero, 2005). Diseases associated with nutritional deficiencies were moderate when compared with other South American hunter-gatherer populations, with 11% of individuals showing signs of porotic hyperostosis and cribra orbitalia (Cornero, 2005). Additionally, Cornero (2005) argued that the local hygiene and sanitary conditions were sufficient to avoid severe pathological effects. Evidence of this includes the low frequency of prenatal (5%) and early childhood death (7.5 %) (Cornero, 2005). Occupational fractures and functional stress, such as compression fractures, osteophytes, and Schmörl nodes, have also been found suggesting occupations related to lifting and transport of weight, although insufficient to provoke mobility issues to individuals. Traumatic lesions occurred in 33% of individuals, indicating fracture remodelling and survivorship (Cornero, 2005). The number of caries was higher (10.5%) than expected for a hunter-gatherer population (Steckel; Sciulli; Rose, 2002), indicating relatively high ingestion of sugar at Santana do Riacho (Cornero, 2005).

Dental analysis is particularly significant because one of the primary interests of early researchers in the region was related to the hunting activities of the earlier Americans and its connection to the megafauna. The assessment of human remains and archaeological assemblage in Santana do Riacho has indicated that, although megafauna and humans had co-existed, such interactions showed no indication of this early population relying on hunting as a primary source of their diet. The archaeological assemblage shows reliance on roots, fruits, and tubers, suggested

by the recovered materials (Resende; Prous, 1992) and the incidence of caries (Cornero, 2005; Cornero; Neves; Prous, 1999). The materials obtained point to a low impact on the environment, with appropriate conditions to undertake a relatively healthy lifestyle (Cornero, 2005).

The most recent paleopathological analysis of the site included Santana do Riacho and other sites in the Lagoa Santa region (Gloria, 2012). In general, the analysis for Santana do Riacho matched the descriptions by Cornero (2005), and the difference observed between sexes has been interpreted as behavioural variance, such as a possible sexual division of the labour (Gloria, 2014).

Researchers assessed the mobility patterns of the population using two approaches: analysis of femoral midshaft diaphyseal shape (FMS) (Gloria, 2012) and recently by the analysis of strontium ($^{86}\text{Sr}/^{87}\text{Sr}$) isotopic signatures sampled from teeth enamel (Gomes, 2021).

Regarding femoral midshaft diaphyseal shape (FMS) at Lagoa Santa, analysis showed low values when compared to other hunter-gatherer populations, being interpreted as a population with low mobility. However, when considering only the data for Santana do Riacho, values suggest a highly mobile population. The comparisons between sexes show that the female individuals from Santana do Riacho show data that better agrees with the other sites at the Lagoa Santa region, whereas male individuals show higher values (Gloria, 2012). The sexual dimorphism in the FMS of the individuals from Santana do Riacho can also be interpreted as an indication of the high mobility of the population (Wescott, 2006).

Regarding strontium isotopic analyses ($^{86}\text{Sr}/^{87}\text{Sr}$), Gomes (2021) compared Lagoa Santa with Santana do Riacho, showing substantial differences. Santana do Riacho had higher strontium levels, indicating diverse subsistence strategies despite both groups being hunter-gatherers. Furthermore, the data from Santana do Riacho showed no significant differences in isotopic signatures in comparisons between sexes (Gomes, 2021). The author also used the mobility models developed by Binford to describe the mobility of both populations (Gomes, 2021). Binford distinguished between residential (involving the relocation of all members of a residential base from one locality to another) and logistical mobility, which pertains to the movement of specifically organized task groups on temporary excursions from a residential base (Binford, 1980). Gomes (2021) describes Santana do Riacho as a population with a high logistic mobility and low residential mobility, whereas Lagoa Santa evinced the opposite pattern.

Concerning the insertion of Santana do Riacho in the Lagoa Santa region, it is important to consider the different geology of the two locations; while Lagoa Santa is in the karst, Santana do Riacho is a quartzite rock shelter in the Espinhaço Mountain Range. The topography of the Lagoa Santa Karst includes *lapiés*, poljes, dolines, canyons, and caves. Isotopic analysis also showed that the individuals from Santana do Riacho would not primarily focus their foraging strategies in the areas southwest of the shelter in the geological context of the Bambuí Group of Neoproterozoic age. Similarly, individuals from Lapa do Santo would not obtain food resources beyond the limits of the karst to the northeast, specifically in the Espinhaço Supergroup with Mesoproterozoic age (Gomes, 2021).

Bone structure, modelling and remodelling

Bones are categorized into two types: woven and lamellar. Woven bone, deposited during foetal development, has a fibrous appearance, and is quickly replaced by mature bone. It occurs in embryonic skeletons, fracture repair sites and bone tumours (White; Folkens, 2005). Lamellar bone, a more organized structure, forms by the ordered deposition of layers (lamellae) during appositional growth, gradually replacing primary bone at a slower pace (White; Folkens, 2005).

At the microstructural level (Figure 2), cortical bone lamellae are concentrically organized around Haversian canals, blood vessels that facilitate bone nourishment. A network of these canals, along with Volkmann's Canals interconnecting them with the periosteum, contributes

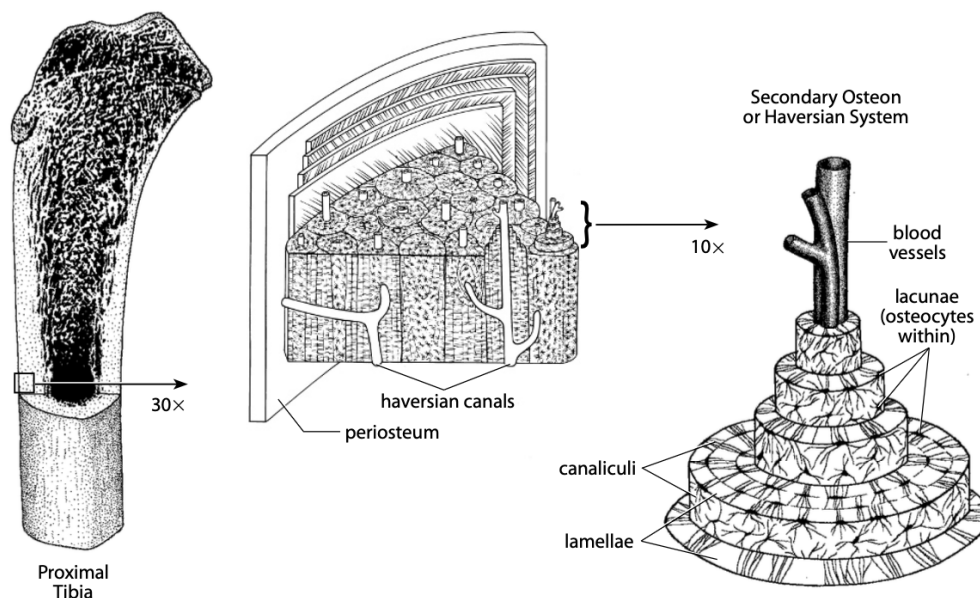
to the high vascularization of bones. Osteocyte lacunae, small cavities within lamellae, house bone maintenance cells called osteocytes. Nutrient exchange occurs by canaliculi connecting lacunae with Haversian canals and other lacunae (White; Folkens, 2005). Apart from concentric lamellae, the skeletal system shows interstitial (between osteons, remnants of older bone) and circumferential lamellae (parallel to the outer edge of bone, beneath the periosteum).

The Haversian system, or osteon, constitutes the foundational functional unit of compact bone, with primary osteons forming without previous resorption and secondary osteons resulting from remodelling.

Bone cellular structure involves four main cell types: osteoblasts, osteoclasts, bone lining cells, and osteocytes. Osteoblasts, responsible for bone production, also contribute to collagen synthesis, hematopoiesis, immune functions and osteoclast regulation (Lynnnerup; Klaus, 2019). They secrete an unmineralized bone matrix (osteoid) consisting of structured layers of collagen type I fibrils. Hydroxyapatite crystals are then deposited between the collagen fibres, leading to mineralization and the transformation of mature osteoblasts into osteocytes or bone lining cells (Gartner; Hiatt, 2006).

Bone histology analysis include two fundamental processes: bone modelling and remodelling. Bone modelling involves forming bone by osteoblasts without prior bone resorption. It occurs by appositional processes, with some resorption on the endosteal surface. This process is particularly active during skeletal development, spanning from prenatal stages to early adulthood, facilitating changes in bone size and shape (Seeman, 2008). Bone remodelling is defined as “The mechanism by which older bone is replaced by the coordinated (tethered) action of bone-resorbing osteoclasts and bone-forming osteoblasts, collectively referred to as a basic multicellular unit (BMU) of remodelling or simply bone remodelling units” (Stout; Crowder, 2011, p. 2). Bone remodelling is essential for the maintenance of mineral homeostasis, the adaptation to mechanical loads, and the repair of microdamage (Burr, 2002).

Figure 2. Gross and microscopic bone structure.



Source: White and Folkens (2005, p. 45).

MATERIALS AND METHODS

Archaeological Context

When alive, bone is a dynamic tissue, adapting and responding to external and internal stimuli by modelling and remodelling (Robling *et al.*, 2006). While the process of modelling is vigorous during skeletal growth, remodelling occurs throughout life.

In biological anthropological approaches, bone histology analysis applied to human remains has been used to address a variety of research questions, such as differentiation between human and non-humans (Mulhern; Ubelaker, 2001), the identification of taphonomy processes (Stout, 1978; Turner-Walker; Jans, 2008); age-at-death estimation (Stout; Crowder, 2011), paleopathology (Aaron; Rogers; Kanis, 1992; Wakely; Manchester; Roberts, 1991; Weston, 2009) and responses to biomechanical stress and behaviour (Miszkievicz; Mahoney, 2016; Mulhern, 2000; Robling; Stout, 2003). The studies aiming to assess behaviour in past contexts using bone histology investigated a range of variables (PEARSON; BUIKSTRA, 2006) such as mobility patterns (Robling; Stout, 2003), the sexual division of labour (Mulhern; Van Gerven, 1997), socio-economic status (Miszkievicz; MAHONEY, 2016), nutritional stress (Martin; Armelagos, 1985) and diet (Richman; Ortner; Schuller-Ellis, 1979). In South America, the only study investigating the relationships between bone histomorphometry and behaviour was carried out in a Peruvian context, dated from 6,500 to 4,500 B.P (Robling; Stout, 2003). The authors studied the changes in bone histology across time in a Peruvian population and confirmed that bone microstructure alterations followed changes in economic strategies. The economic shift from strict hunter-gatherer subsistence to a maritime economy changed the bone microstructure, showing less evidence of responses to mechanical stimuli (Robling; Stout, 2003).

The literature has a gap concerning bone microanalysis in South American archaeological assemblages, and analysis of bone histology in such contexts can potentially bring a deeper and more complete understanding of past contexts that is unattainable by other types of analysis. Paleohistological analyses offer a unique opportunity to observe the responses of the body to the environment and cultural change from an observed perspective rarely.

Histological variation will confirm whether the early individuals in Santana do Riacho evince high mobility at the microscopic level. Mobility patterns in archaeological contexts have been investigated by different lens. Therefore, it is important to establish that this study consider mobility “a proxy to lower limb activity, implying not only the linear distance crossed everyday but also the amount of force applied to the lower limb” (Gloria, 2012, p. 111).

This study aims to 1) use bone histology to establish the mobility of the Santana do Riacho population and 2) describe bone remodelling in the Santana do Riacho population.

1. We hypothesize that individuals with a lifestyle associated with a high mobility would show higher values of osteon densities in association with smaller areas of secondary osteons and Haversian canals, whereas the opposite characteristics occur in individuals with a more sedentary lifestyle.
2. We hypothesize the relationship between Haversian canal and osteon areas in the analysed samples. Bone remodelling can be understood as a self-organising process of adaptation to mechanical loads (Smit; Burger; Huyghe, 2002), and such correlation may offer insights into bone remodelling events and their effects on the geometric properties of microstructure features.

The Samples

The analysed samples represent a pre-colonial population dating to $8,280 \pm 40 - 9,460 \pm 110$ BP (Neves *et al.*, 2003). The human remains were recovered from Santana do Riacho, southeastern Brazil. The samples belong to a larger collection of 40 individuals and 28 burials

(Cornero, 2005; Gloria, 2012). This study relies on the osteological assessment conducted by Pedro da Gloria (2012), which resulted in two males and three females, and one individual with indeterminate biological sex.

The following paragraphs summarize the individuals sampled for this study, describing their sex, age at death, preservation conditions, and details about the health assessment undertaken. It is important to note that the Museu de História Natural e Jardim Botânico da UFMG (MHNJB-UFMG), the institution responsible for safe keeping the collection for more than 40 years, was partially devastated by a fire in 2020, which destroyed the Santana do Riacho Collection.

Individual II: The individual showed excellent preservation. The individual was estimated as a 35-50-year-old male (Gloria, 2014). Concerning oral health, the individual presented caries, antemortem tooth loss, and an abscess on tooth 27 (Gloria, 2014). The infection on tooth 27 likely affected the sinus bone as the bone showed signs of said infection (Gloria, 2014). Trauma was identified on the arms, hands, and nasal bone. A degenerative joint disease was present on the shoulders, elbows, hips, knees, wrists, and osteophytes were observed on two cervical, three thoracic, and two lumbar vertebrae (Gloria, 2014). Additionally, the individual showed *criba orbitalia*, porotic hyperostosis, and an infection on one of the ribs, but due to the fragmentary state of the bone, it was not possible to establish its possible causes (Gloria, 2014).

Individual Va: The individual was estimated as a 15-25-year-old female (Gloria, 2014). Concerning oral health, caries, abscess, antemortem tooth loss, and linear enamel hypoplasia was observed (Gloria, 2014). The presence of *criba orbitalia* and porotic hyperostosis was observed, as well as trauma on one of the arms and the vault (Gloria, 2014).

Individual IX: The individual was estimated as a 50+ year-old female (Gloria, 2014). Osteophytes were present on one of the cervical, one thoracic, and one lumbar vertebra (Gloria, 2014).

Individual XII: The individual was estimated as a 25-30-year-old female (Gloria, 2014). One of the legs and hand had trauma and one tibia showed signs of infection (Gloria, 2014). Moreover, signs of a degenerative joint disease were present at the shoulder, elbow, hip, knee and wrist (Gloria, 2014).

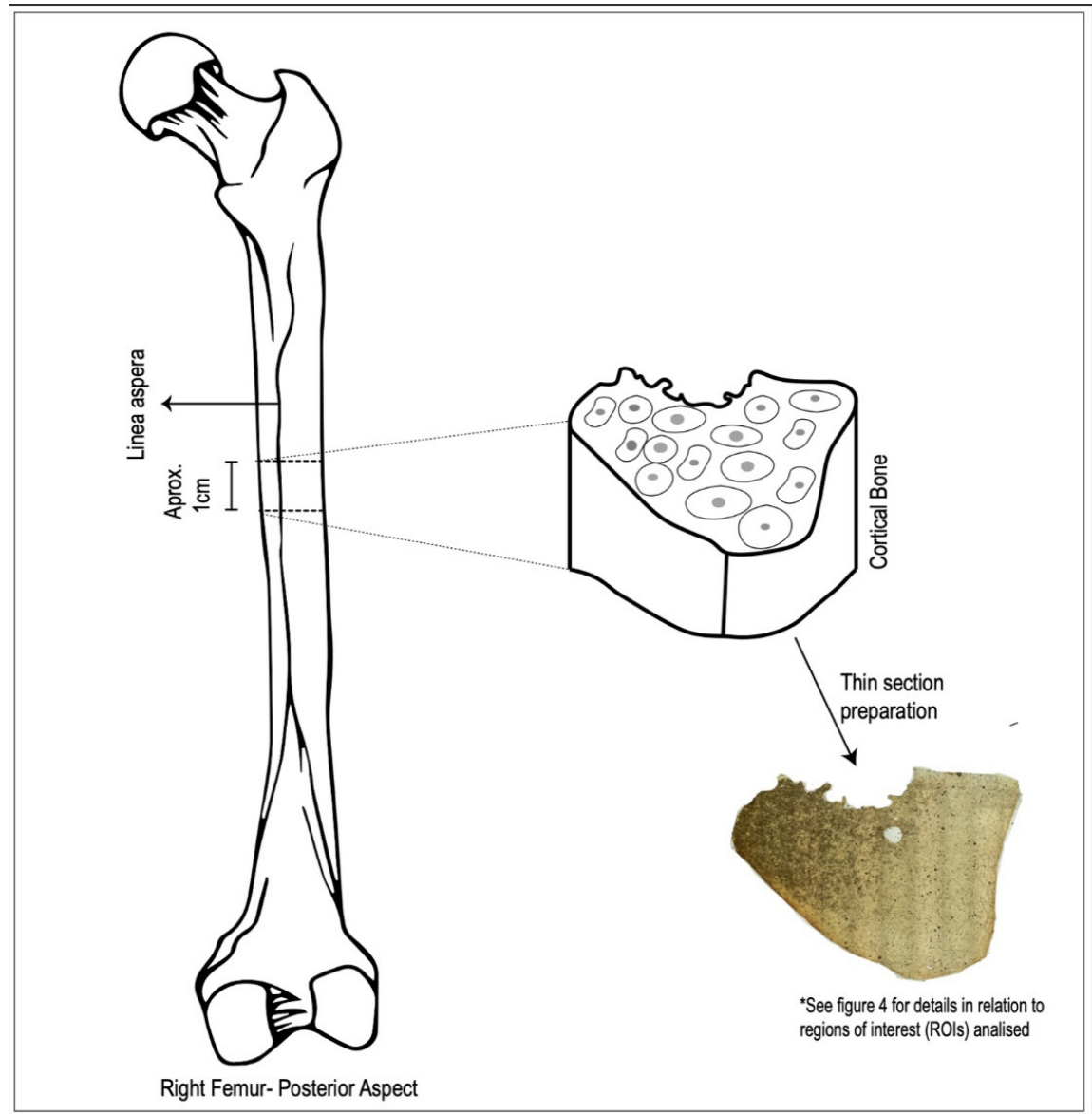
Individual XVIII: The individual was estimated as an adult male (Gloria, 2014). Although Gloria estimated the age as an adult, Cornero (2005) estimated the individual as a young adult, with a mean age of 22 years. Only part of the postcranial was preserved (Cornero, 2005). Cornering the health assessment showed signs of degenerative joint disease on the shoulder, elbow, hip and knee, and signs of trauma on the leg and hand (Gloria, 2014)

Individual XXVII: Only one rib, one femur, and two tibiae were preserved (Cornero, 2005), thus limiting estimations regarding age at death and health assessments. Still, signs of degenerative joint disease were noted on the hips and knees, as trauma on one of the legs, accompanied by infectious disease on the tibiae (Gloria, 2014). Gloria (2014) estimated the sex as undetermined but Cornero deemed the individual as a male (Cornero, 2005). Here, for consistency reasons, the sex will be considered unknown.

Despite the size of the collection, permission to undertake invasive sampling was limited to a small sample size (see the “Statements and Declarations” section of this article). This was further impacted by the fact that femora were largely fragmented, leaving only six individuals for study. In some cases, skeletal elements were well preserved, whereas others showed signs of exposition to fire or evidence of chemical changes due to insect activities (Cornero, 2005). Still, the preservation of the human femora selected for this study was satisfactory enough to enable the removal of bone for histological analysis. Considering that the femur is a major weight-bearing bone, receiving different mechanical loads depending on the individual (Miszkiewicz, 2016; Pfeiffer *et al.*, 2006), this bone was chosen for sampling to address mobility and physical activity. Using standard digital callipers, measurements of the femur were taken where possible, including maximum length, midshaft circumference, midshaft diameter (anterior-posterior, medial-lateral planes) and femoral head

(all in cm). Approximately 1 cm of cortical bone from the posterior aspect of the femur was removed to produce thin sections. Bone sections were removed from the posterior linea aspera (Miszkiewicz; Mahoney, 2016, 2017). A rotary tool (Ozito® RTR-2000) was used to make parallel and longitudinal cuts into the bone to extract the material that became loose.

Figure 3. Posterior aspect of the femur and details of the sampled area in this study.



Source: Authors.

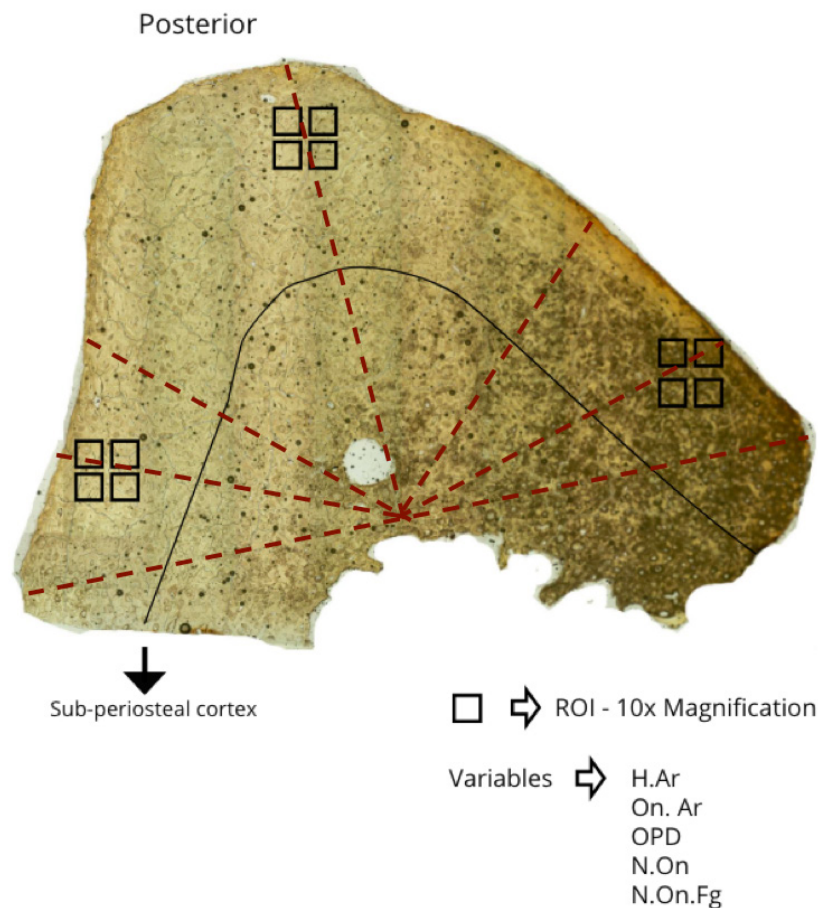
Histological Procedures

Samples were fixed in resin (Buehler Epocixure® resin and hardener). The embedded samples were then divided into two parts by a Kemet 151 precision saw equipped with a diamond blade. The cut samples were later mounted onto microscopic glass slides using epoxy glue. This was followed by grinding on a series of grinding pads placed on a rotating plate of a Buehler Ecomet 300 Grinder-Polisher. The samples were next polished using Buehler Micro polish II 0.3µm powder. Finally, the samples were placed in an ultrasonic bath for debris removal, which was followed by

dehydration in ethanol baths. The final steps in the preparation of the thin sections consisted of cleaning the samples using xylene and then sealing them with a glass coverslip.

Images of each slide were taken using an Olympus DP74 camera mounted on a BX53 high-powered microscope and the Olympus software cellSens®. The images were analysed using ImageJ®. In each sample, 12 regions of interest (ROIs) (Figure 3) were identified and imaged using a 10× objective lens (100x total magnification). As different criteria for selecting regions of interest have been reported in the literature (ROIs) (Villa; Lynnerup, 2010), ROIs were selected for the guiding question of this study. For this study, ROIs lied in the sub-periosteal region of the bone, defined as the most immediate area to the most outer periosteal layers. These ROIs were chosen as the periosteum, the most affected region by remodelling as a response to high mechanical stimuli (Miskiewicz; Mahoney, 2016; Robling; Castillo; Turner, 2006). Overall, four images were taken at the midpoint of each sample immediately below the linea aspera and four additional images were taken from each side of the sample (lateral and medial).

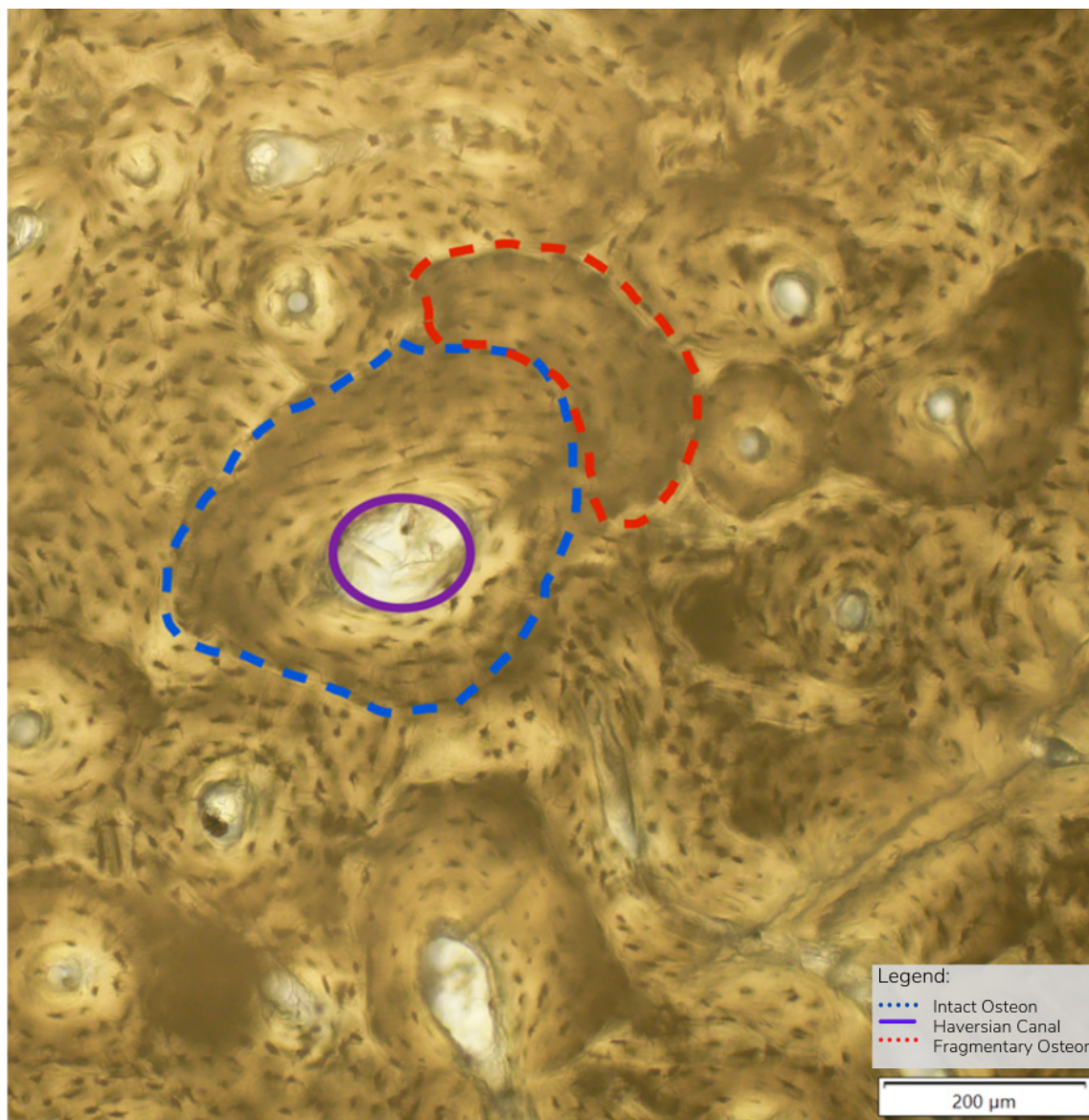
Figure 4. Analysed Regions of Interest.



Source: Authors.

Using these images, five histology variables were measured (Figure 4). These variables were chosen to describe the geometric properties of products of bone remodelling — secondary osteons and Haversian canals. Haversian canal area (H.Ar), osteon area (On.Ar), osteon population density (OPD), intact osteon density (N.On) and fragmentary osteon density (N.On.Fg) were collected.

Figure 5. Analysed structures, such as intact osteon (blue), fragmentary osteon (red) and Harversian canal (purple).



Source: Authors.

Table 1. Variables examined in this study.

VARIABLE AND ABBREVIATION	DEFINITION
Midshaft Circumference (Circ)	The measure of the circumference on the diaphyseal midpoint (e.g., Buikstra; Ubelaker, 1994; Miskiewicz; Mahoney, 2016).
Intact Osteon Density (N.On)	The number of osteons with complete Haversian canals and intact cement lines per area of sampled bone, measured in mm ² . This variable was counted at 10× magnification (e.g., Miskiewicz; Mahoney, 2016; Stout; Crowder, 2011).

continues...

Table 1. Continuation

Fragmentary Osteon Density (N.On.Fg)	The number of fragmentary osteons with surfaces and/or Haversian canals of >10% resorption per area of sampled bone, measured in mm ² . This variable was counted at 10× magnification (e.g., Miskiewicz; Mahoney, 2016; Stout; Crowder, 2011).
Osteon Population Density (OPD)	N.On.Fg+N.On per area of sampled bone, measured in mm ² . This variable was counted at 10× magnification (e.g., Miskiewicz; Mahoney, 2016; Stout; Crowder, 2011).
Haversian Canal Area (Mean) (H.Ar)	Area of Haversian canals reported in μm. The magnification used to take the measurements was 10× (e.g., Pfeiffer; Crowder; Harrington, 2006).
Osteon Area (Mean) (On.Ar)	Area of osteons with intact cement lines and complete Haversian Canals, reported in μm. The magnification used to take the measurements was 10× (e.g., Pfeiffer; Crowder; Harrington, 2006).

Source: Authors.

Due to the small sample size, the presented analysis was limited to descriptive statistics (minimum, maximum, mean and median). A comparison with histology data published for other ancient populations and one inferential statistical (Pearson's *r* correlation) approach to test relationships between some characteristics of the bone responses to physical activity and weight-bearing were made.

Comparisons

Population comparisons with the same type of paleohistology data available for other archaeological samples used data extracted from five studies (nine contexts) published from 1964 to 2016:

- The Late Stone Age foragers dated from 6,000 to 2,000 BP in South Africa (Pfeiffer; Crowder; Harrington, 2006). The sample size included 44 individuals: 19 males and 25 females, most of whom with an age-at-death around 30 years old. Up to 2,000 BP, the population included exclusively foragers (Pfeiffer; Crowder; Harrington, 2006). Analysis of the relative strength of adult long bones, combined with archaeological evidence, indicates a reliance on overland trekking (Stock; Pfeiffer, 2004).
- Christ Church Spitalfields, East London, dated from AD 1729 to 1857 (Pfeiffer; Crowder; Harrington, 2006). In total, 20 young adults aged from 25 to 50 years were included in this sample, totalling 11 males and nine females (Pfeiffer; Crowder; Harrington, 2006). Regarding context, the sample includes individuals that lived during the industrialization period, with considerable coal-generated air pollution (Pfeiffer; Crowder; Harrington, 2006). Some sampled individuals are believed to be of French origin and involved in textile industries (Pfeiffer; Crowder; Harrington, 2006). Individuals came from the lower middle class, and some were relatively wealthy (Pfeiffer; Crowder; Harrington, 2006).
- St Thomas Anglican Church in Ontario (Pfeiffer; Crowder; Harrington, 2006), consisting of 21 sampled individuals who died from AD 1827 to 1873: 14 males and seven females. Age-at-death ranged from 17 to 81 years (Pfeiffer; Crowder; Harrington, 2006).
- St Gregory's Priory in Canterbury, with 40 individuals from the Middle Ages (Miskiewicz; Mahoney, 2016). The sampled individuals included young and middle-aged adults. Overall,

10 females and 30 males were analysed (Miszkievicz; Mahoney, 2016). Archaeological and historical evidence shows that wealthy individuals were buried at the location, with individuals believed to be less subject to biological and physical stress and with a high protein intake (Miszkievicz; Mahoney, 2016).

- A cemetery in medieval Canterbury, England, associated with the aforementioned St. Gregory's Priory but of lower socioeconomic status (Miszkievicz; Mahoney, 2016). For the study, 205 males and 207 females were analysed (Miszkievicz; Mahoney, 2016). The individuals were mainly peasant farmers, with low protein intake and a physically demanding lifestyle (Miszkievicz; Mahoney, 2016).
- Kulubnarti in Sudanese Nubia with late medieval burials (AD 1250-1450) included a total of 43 individuals; 24 females and 19 males, with age-at-death ranging from 20 to 50 years (Mulhern; Van Gerven, 1997). Populations in this region lived in farming villages under harsh environmental conditions (Mulhern; Van Gerven, 1997). Staple crops included sorghum, millet, barley, beans, lentils, peas, dates and wheat. Cattle, sheep and pigs were a source of some protein (Mulhern; Van Gerven, 1997). The terrain also contributed to physical stress, and studies show a high rate of fractures and traumatic lesions on long bones, describing a rate of degenerative joint disease that agrees with populations enduring intense physical stress (Kilgore, 1984).
- The Paco Indians, an agriculturalist Native American community from north-central New Mexico, dated from the 14th to the 19th century, were also included in comparisons (Burr; Ruff; Thompson, 1990). The sample consisted of 55 individuals; 27 women and 28 men, with an age-at-death from 21 to 60 years (Burr et al., 1990). In this egalitarian agricultural community, all had equal access to environmental and cultural resources, without evidence of serious malnutrition or food shortages (Burr; Ruff; Thompson, 1990).
- This modern human sample was studied by Currey (1964). For the study, 19 samples were extracted from the autopsy of individuals with ages ranging from 23 to 89 years (Currey, 1964). The samples represented 11 females and eight males.

We attempted to obtain data for the Peruvian sample (Robling; Stout, 2003), but it was not possible. We acknowledge this South American sample would have otherwise formed a geographically close and suitable comparison to the sample used in this study.

RESULTS

Bone Histology and Mobility

Tables 2 and 3 show the descriptive data. Table 2 shows the data obtained for each individual and the means and medians for the entire sample. Table 3 shows the minimum and maximum values and the standard deviation for each analysed variable.

Table 2. Sampled individuals, corresponding values obtained for the analysed variables, and the mean value for each variable.

Individual	Sex	OPD (mm ³)	N.On (mm ²)	N.On.Fg (/mm ²)	H.Ar (µm)	On.Ar (µm)	Circ (cm)
II	Male	20.51	17.35	3.15	1113.32	20770.8	8.5
Va	Female	15.78	14.43	1.30	1120	22616.02	6.8

continues...

Table 1. Continuation

IX	Female	20.03	15.25	4.77	1255.11	21436.91	7.2
XII	Female	16.85	13.11	3.73	2085.69	31513.04	7.5
XVIII	Male	12.36	11.61	0.74	2235.51	27636.16	8.1
XXVII	Unknown	16.55	14.55	1.99	1582.24	25747.29	8
Mean		17.01	14.38	2.61	1565.31	24953.37	7.68

Source: Authors.

Table 3. Means, minimums, maximums, and standard deviations for each variable analysed in this study.

Variable	Mean	Min.	Max.	Standard deviation
OPD	17.01	12.36	20.51	2.991
N.On	14.38	11.61	17.35	1.943
N.On.Fg	2.61	0.74	4.77	1.536
H.Ar	1565.31	1113.32	2235.51	493.775
On.Ar	24953.37	20770.8	31513.04	4151.614

Source: Authors.

Previous studies have indicated sexual dimorphism, highlighting variations in diets and potentially distinct routines and activities between males and females (Gloria, 2012; Gloria; Larsen, 2014). This study attempted to compare bone microstructures between sexes (Table 4). However, it became evident that the limitations imposed by the collection and the small sample size hindered the reliability of results.

This is evident in the osteon population density (OPD) comparisons. For instance, the individual with the highest OPD value is a male (ind. II), followed by a female (ind. IX) and an elderly individual, which would potentially explain the observed bone microstructure characteristics. Regarding OPD, both the highest and lowest values belong to males (ind. II and XVIII). The difference might be attributed to a possible age gap, but age-at-death estimation for Individual XVIII was not feasible.

Another limitation in comparing sexes is evident in Individual XXVII's data. It is not possible to use histological information to gain any insights into a sex estimation for the individual due to the absence of clear patterns for a match. These instances underscore the challenges and constraints in drawing conclusive insights from the available data regarding sex differences.

Table 4. Comparison between sexes.

	Mean H.Ar	Mean On.ar	OPD	N.On	N.On.Fg
Indeterminate	1582.24	25747.29	16.55	14.55	1.99
Female	1486.93	25188.65	17.55	17.55	3.266
Male	1674.41	24203.48	14.48	14.48	1.945

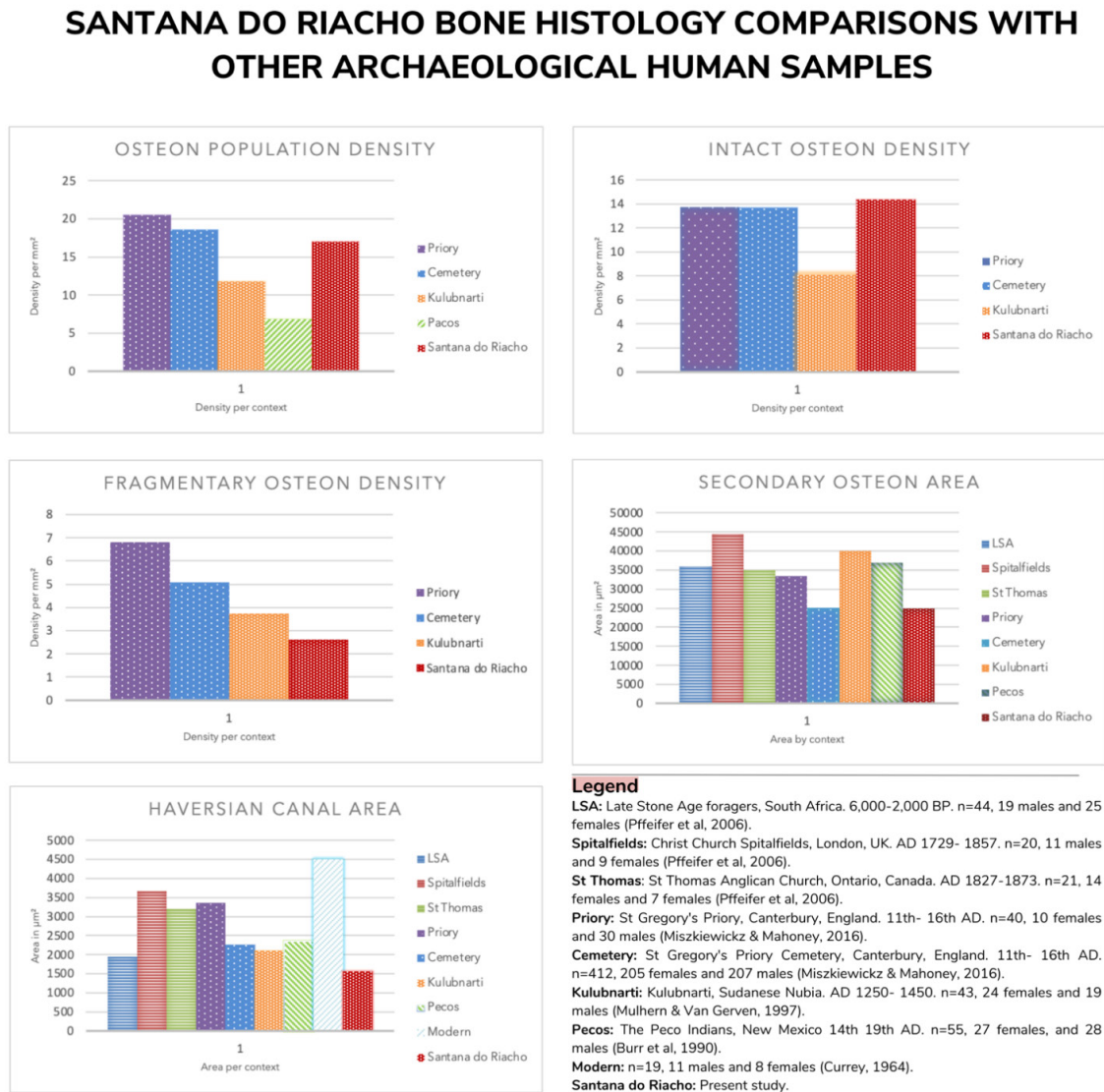
Source: Authors.

The next step was to compare the sample to other contexts for which bone histology had been analysed by other authors (Figure 5). Only data obtained from femoral sections were used in the comparisons, obtained from nine archaeological contexts. Santana do Riacho shows the third highest osteon population density, with a value closer to that of the two groups with the highest scores. Santana do Riacho also shows the highest number of intact osteons, associated with the lowest number of fragmentary osteons. Regarding geometric properties, Santana do Riacho shows the smallest osteon area, associated with the smaller Haversian canal area. Interestingly, for two variables, osteon area and population density, Santana do Riacho showed values quite similar to those in another population: from Canterbury Cemetery (Miszkiwicz; Mahoney, 2016).

Regarding osteon area, while Santana scored the lowest value, Spitalfields scored the highest, followed by Kulubnarti, the Pecos Indians and Late Stone Age foragers. For the Haversian canal area, while Santana do Riacho shows the smallest canals, modern samples showed the largest, followed by Spitalfields and The Canterbury Priory.

Concerning density properties, fewer contexts were compared, and for the number of intact osteons, Santana do Riacho shows the highest value, slightly higher than the Cemetery and the Priory, whereas Kulubnarti shows a significantly smaller value. For the number of fragmentary osteons, the Priory shows the highest number, followed by the Cemetery and Kulubnarti, whereas Santana do Riacho showed fewer fragmentary osteons. Osteon population density also includes the Pecos Indians. Both groups from England showed values higher than Santana do Riacho, whereas the other two populations (Kulubnarti and the Pecos Indians) showed lower values.

Figure 6. Comparison with other contexts.



Source: Authors.

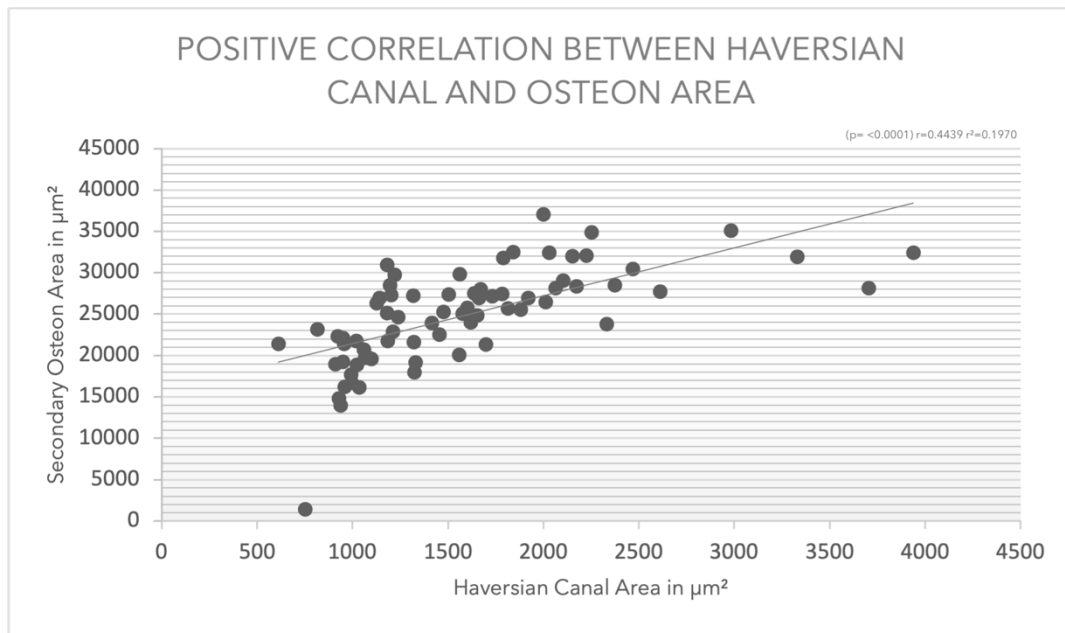
From these basic descriptions, it can be concluded that this sample shows possible indicators of an overall pattern of high mobility. The measurements showed a pattern that combined high osteon population density with small osteons and small Haversian canals, which characterize populations under high levels of physical activity.

Correlations between histological variables

A total of 72 measurements indicated a statistically significant relationship ($p < 0.0001$) with $r = 0.4439$, which obtained $r^2 = 0.1970$. This means that this correlation can explain approximately 20% of the data. This, however, is a weak correlation. This means that the correlation between On.Ar and H.Ar show a positive biological relationship, whereby the size of the Haversian canal increases with an increase in the size of the secondary osteon. Figure 6 shows the distribution of the data, which aligned the data in a positive direction. Osteon and Haversian canal areas show a statistically significant positive relationship, confirming the alternative hypothesis. The observed

positive relationship evinces relatively longer remodelling events, in which osteons would have had enough time to accrue larger surfaces.

Figure 7. Data distribution. Source: Authors.



Source: Authors

DISCUSSION

In comparison with other archaeological samples (Burr; Ruff; Thompson, 1990; Currey, 1964; Miskiewicz; Mahoney, 2016; Mulhern; Van Gerven, 1997; Pfeiffer; Crowder; Harrington, 2006), the analysis of bone histology in the archaeological Santana do Riacho sample showed high values of secondary osteon densities in association with smaller areas of secondary osteons and Haversian canals. Santana do Riacho showed the smallest Haversian canal and osteon areas, associated with an osteon population density higher than the Kulubnarti and the Pecos Indians but lower than medieval England (St. Gregory Priory and the Canterbury cemetery). These characteristics support the hypothesis that Santana do Riacho individuals were highly mobile. They also showed a possible sex difference, whereby the bone histology in male individuals indicated a more mobile lifestyle than that of women.

Bone Histology and Mobility

Bone remodelling is a multifactorial process, in which several variables influence bone maintenance. Among these factors, mechanical load plays an important role in stimulating microstructural bone redistribution and adaptation (Robling *et al.*, 2006). Bone turnover increases in a state of disuse and overuse. Disuse accelerates bone resorption, with consequent bone loss, whereas overuse damages tissues and can stimulate an increase in bone deposition (Robling; Castillo; Turner, 2006). Therefore, periosteal bone formation decreases in disuse and increases in use (Robling; Castillo; Turner, 2006). This increased bone formation is characterized by a high number of osteons (OPD), associated with small osteons and Haversian canals. Osteons and Haversian canals are expected to be smaller under high mechanical load application due to accelerated bone formation, in which more osteons are formed before older osteons increase in size. In comparison

with the other populations, Santana do Riacho indicates patterns compatible with such an increased remodelling, likely linked to a high level of physical activity. However, it is important to note that only one of the compared archaeological contexts includes foraging individuals, namely the Late Stone Age South African foragers (Pfeiffer; Crowder; Harrington, 2006). Considering the geometric properties of the histomorphometric data available alone (On.Ar and H.Ar) indicates that these foragers were less physically active than those from St. Thomas, Priory, Cemetery and Santana do Riacho, which is very unlikely. Pfeiffer, Crowder and Harrington (2006) recognized that measures of the osteon area and Haversian canal area are unable to be solely used to reconstruct behaviour. Among the compared groups, the one that most resembles the patterns observable in Santana do Riacho is the Canterbury cemetery (Miszkiwicz; Mahoney, 2016). High demands of physical activity for a hunter-gatherer population occupying a new and unknown territory for the first time are expected, as previously described by Gloria (2012) and Gomes (2021), which also seems to mirror the laborious lifestyle of British medieval peasants, who configured the backbone of the European workforce at the time (Miszkiwicz; Mahoney, 2016). The limited femur bone exterior data also points to thicker and larger, rather than gracile, femoral shafts.

Comparisons between sexes examined the histomorphometry variables of two male individuals and three females were examined. The limited sample size significantly challenges drawing conclusive comparisons, rendering the results of the histomorphometry analysis inconclusive. Instead, this analysis serves to indicate the potential for further research to establish whether the results align with findings from previous studies (Cornero, 2005; Cornero; Neves; Prous, 1999; Gloria, 2012; Gloria; Larsen, 2014)

As bone remodelling is a complex process influenced by various factors, a reliable comparison between sexes requires considering factors such as the age and health of individuals. Unfortunately, consistent results were unattainable in this study. The FMS analysis conducted by Gloria (2012) indicated that male individuals showed signs of greater physical activity than females. This opens avenues for additional research, exploring other sites in the region and utilizing bone samples less affected by mechanical stimuli, such as ribs (Mulhern, 2000). Such an approach would enhance the control and understanding of factors influencing bone response and remodelling.

Expanding the sample size could offer a more nuanced understanding of sex-related divisions in population tasks and other factors contributing to differences in bone remodelling, such as diet (Richman; Ortner; Schuler-Ellis, 1979) and hormones (Manolagas, 2002). Previous palaeopathology studies in Santana do Riacho addressed differences between males and females, highlighting a higher incidence of caries in females (Gloria, 2012) in the Lagoa Santa population. This suggests potential variations in diets, with females possibly relying more on carbohydrates than males. Consequently, a plausible division of daily routine activities between sexes may exist, leading to distinct skeletal outcomes, which can be further explored by bone histology.

Relations between bone histomorphometric variables and insights into remodelling

The correlation between H.Ar and On.Ar showed that the two variables are in a statistically significant (although weak) relation. This study deals with archaeological remains dated from 8,200 to 9,500 BP, so a larger sample size would shed more light on these associations. Miszkiewicz (2016) also found this relationship between these two variables by analysing the relationship between several histomorphometric variables in human femoral sections. The author associated the relationship between the two variables with an indication of response to mechanical stimuli, considering that several studies agreed that small osteons and Haversian canal areas lie in an inverse relationship with strain application (Miszkiwicz, 2016). Bones with long-term disuse show lower osteon population density, associated with larger osteons and Haversian canal areas (Schlecht *et al.*, 2012), showing that the relationship between these geometric variables may

provide information over the impact of physical activity on the remodelling of the human skeleton. The positive relationship between Haversian canal and osteon areas on human femurs indicates that remodelling, coordinated by BMUs, occurs in a manner that influences histomorphometrical properties (Miszkiwicz; Mahoney, 2016; Smit; Burger; Huyghe, 2002). In this case, the positive relationship shown by the statistical tests indicates a decreased remodelling rate. Bone remodelling can be understood as a self-organizing process of adaptation to mechanical loads (Smit; Burger; Huyghe, 2002), and the found correlation shows that remodelling likely occurred over a relatively longer length of time, enabling the completion of larger osteons and Haversian canals. This was an unexpected result since descriptive analysis indicated a high impact of physical activity on bone microstructure. This contradiction exemplifies the complexity of bone dynamics and the importance of further research within South American contexts to understand the relationships between the observed phenomena. A broader analysis including other sites of the region could also explain the differences in mobility between Santana do Riacho and other sites located at Lagoa Santa since past analyses have described Santana do Riacho as having a greater sexual dimorphism and more signs of high impact of physical activity on the skeleton (Cornero, 2005; Gloria, 2012).

Further Remarks on Interpretations

This study addresses archaeological human remains from a context without written records, obtaining information about the lifestyle of the studied individuals by bioarchaeology or analysis of the archaeological record. It is important to take into consideration that, although physical activity influences bone remodelling, it remains a natural independent process (Pfeiffer; Crowder; Harrington, 2006). Additionally, human bone remodelling can suffer the influence of a range of other factors (such as sex, diet, disease and genetics). Therefore, it is important to address alternative explanations for the obtained results.

Concerning population comparisons, it is primordial to consider that genetic factors are involved in bone growth and maintenance (Thompson; Gunness-Hey, 1981), which may have influenced results. It is also important to consider that bone modelling and remodelling are multifactorial processes (Miszkiwicz; Mahoney, 2016). Therefore, results cannot be considered as a result only of mechanical load application.

A comparison between North Americans from the same period and Brazilian samples would improve the understanding of what factors determined the observed phenomena in the bone microstructure analysed in this study. Considering the genetic evidence that recently linked the Lagoa Santa population with the Clovis culture in North America (Posth *et al.*, 2018), the comparison between these two groups — which share genetic traits but inhabited very different environments and relied on very distinct sources of food — would bring very interesting results, which could more accurately infer the diet and physical activity effects on bone microstructures.

Among the factors that influence bone remodelling, age can be considered an important factor, since older adults would show osteon accumulation (Kerley, 1965), which would increase osteon population density. Therefore, the age-at-death estimation configures key information when comparing bone histomorphometric variables since comparison between a very varied age group could be biased. The problem is that, age-at-death estimation can be problematic in archaeological remains (especially those without a written record), especially regarding the underestimation of older individuals (Cave; Oxenham 2017). At least three osteological assessments have been conducted in Santana do Riacho (Cornero, 2005; Gloria, 2012; Souza, 1992, 1993), showing very different results concerning the age-at-death of individuals. Cornero (2005), for example, was unable to identify any individuals older than 39 years, whereas Gloria (2012) found a female individual as older than 50 years old.

This example shows that estimation can be complicated, and that the Santana do Riacho collection is complex. Therefore, the high osteon population density at Santana do Riacho can alternatively stem from a higher age-at-death than expected, especially considering that, for two analysed individuals, age-at-death estimation was not possible. Therefore, a greater number of osteons would alternatively indicate osteon accumulation due to age instead as an effect of mechanical stimuli.

CONCLUSIONS

In conclusion, considering the data purely in the light of the influence of mechanical stimuli on human bones, the individuals from Santana do Riacho endured more biomechanical stress than the populations to which they were compared, except for the individuals buried in the Canterbury cemetery. It is also important to acknowledge that most of the compared contexts were agriculturalist, except for the Late Stone Age South African foragers. Moreover, agrarian contexts expectedly configure more sedentary lifestyles. Therefore, it can be concluded that Santana do Riacho was more physically active than other contexts, but a fair comparison would include other South American hunter-gatherer populations to achieve a higher degree of understanding of the observed phenomenon. Unfortunately, more meaningful comparisons are impossible at this stage as this study only constitutes the second research in South America on behaviour by paleohistological analysis.

This first analysis using Brazilian archaeological human remains to investigate the relation between bone microstructure and mechanical stimuli confirms the potential of histological investigations to address past behaviour and lifestyle. The results of this study show that expanding sample sizes, including more samples, and incorporating more sites into the research in the region would offer great possibilities. Santana do Riacho is part of the Lagoa Santa region, one of the largest and earliest collections of human remains in America, and a larger sample size would increase understanding on how physical activity influenced bone microstructures, how genetic factors guided bone adaptation, and the differences between sexes in the population. A deeper understanding of these aspects would further detail how this early population adapted to this new environment.

STATEMENTS AND DECLARATIONS

Authors

Nathalia R. Dias Guimarães: This research is a sub-product of the author's master's thesis.
Justyna J. Miskiewicz: Supervision.

COMPETING INTERESTS

The authors have no competing interest to declare.

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ETHICS

This study used invasive techniques in human remains. Therefore, it is fundamental to acknowledge that the thin sections of ancient human remains were prepared and examined in accordance with codes of ethics and good practice in Biological Anthropology:

- Recommendations made by the National Museum of Australia.
- British Association for Biological Anthropology and Osteoarchaeology Code of Ethics (2008).
- British Association for Biological Anthropology and Osteoarchaeology Code of Practice (2010).
- Code of Ethics of the American Association of Physical Anthropologists (2003).
- Science and the Dead: guidelines for the destructive sampling of archaeological human remains for scientific analysis (MAYS *et al* 2013).

Additionally, the authorization for sampling the evaluated bones was obtained from *Museu de História Natural e Jardim Botânico* at *Universidade Federal de Minas Gerais*, and the transportation of the samples to Australia was granted by the *Instituto do Patrimônio Histórico Artístico e Natural (IPHAN)*- ref no 01514.000056/2019 in accordance with Brazilian legislation *IPHAN - Portaria nº 197/2016*.

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