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Cortical bone loss in a sample of human skeletons from the Muge Shell Middens

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Cortical bone loss in a sample of human skeletons from the Muge Shell Middens

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ABSTRACT

The Muge shell middens of Cabeço da Arruda, Cabeço da Amoreira and Moita do Sebastião (central Portugal) have been key sites of archeological research for 150 years, possibly working as residential sites occupied by semi-sedentary communities during the final Mesolithic. The purposes of this article include the biocultural assessment of metacarpal cortical bone fragility and its associations with age at death, sex, osteoporotic fractures in the Portuguese Mesolithic, as well as a diachronic comparison of cortical bone health in Mesolithic (N=34) and modern reference (N=219) samples. Cortical bone at the Muge shell middens displays age and sex-specific trajectories of periosteal apposition and endosteal bone loss, most likely associated with hormonal and behavioural/cultural influences. Metacarpal endocortical bone loss seems to increase with age at death in females, with a simultaneous expansion of the diaphysis. The overall pattern of cortical bone health is similar to the pattern observed in a reference skeletal collection but elderly women from Muge seem to lose less cortical bone than late 20th century counterparts from Coimbra. Two older males exhibited vertebral compression fractures, but only one is possibly related with bone fragility.

Keywords: metacarpal radiogrammetry, medullary width, diaphysis total width, osteoporotic fractures, Mesolithic

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INTRODUCTION

Muge shell middens: chronological, historical and geographical setting

The importance of the Muge shell middens of Cabeço da Arruda, Cabeço da Amoreira and Moita do Sebastião, in central Portugal, is undeniable due to the large number of human skeletons recovered, representing more than 300 individuals belonging to the final Mesolithic. With chronological boundaries between 8400 and 5080 cal BP (Bicho et al. 2010) they represent the last hunter-gatherer communities of central and southern Portugal (Bicho et al. 2013). From the several radiocarbon dates already performed it seems that Cabeço da Arruda is the oldest site, with a date obtained from a human skeleton (Skeleton 6, Beta-127451) consistent with an interval between 8400 and 8030 cal BP (Bicho et al. 2010), followed by Moita do Sebastião and Cabeço da Amoreira. Nevertheless, it is worth mentioning that besides this skeleton dated before 8000 cal BP, it looks like burial practices in Cabeço da Arruda still may have occurred after those in Moita do Sebastião and Cabeço da Amoreira (Bicho et al. 2013).

From the thirteen shell middens identified in the Tagus valley, Cabeço da Arruda was the first shell mound to be discover in 1863, by Carlos Ribeiro, while conducting a geological survey for the drawing of the Portuguese geological map, followed by Cabeço da Amoreira and Moita do Sebastião, one year after (Ribeiro 1884; Cardoso and Rolão 1999-2000; Rolão 1999). In the following 100 years, several archaeological campaigns were carried out at these sites, directed by Francisco Paula de Oliveira (1884-1885), Mendes Corrêa (1930, 1931 and 1933), Jean Roche and Octávio da Veiga Ferreira (1952-1954, 1962-1965), with the support of other archaeologists, such as Francisco Pereira da Costa, Nery Delgado, Alfredo Ataíde, Joaquim R. dos Santos Júnior and Rui de Serpa Pinto (Paula e Oliveira 1888-1892; Mendes Corrêa 1933, 1934; Cardoso and Rolão 1999-2000; Umbelino 2006). In the first years of the 21st century the excavations at Cabeço da Arruda and Cabeço da Amoreira were resumed by a team leaded by the archaeologist João Rolão (Ferreira et al. 2015). In 2008, a multidisciplinary team directed by Nuno Bicho assumed the long-term excavations at Cabeço da Amoreira.

Geographically these three shell middens are located in quaternary terraces not very distant from the left Tagus river bank, at the margins of the Muge river, one of the Tagus tributary, approximately 60 to 70 km far from the seashores of the mouth of the Tagus (Ribeiro 1884). Cabeço da Arruda is placed on the right slope of the Muge river valley, in a 15 m high terrace, 2 km away from the confluence of the Muge river with the river Tagus, while Cabeço da Amoreira and Moita do Sebastião are on the left shore of the Muge river (Figure 1), respectively, at 1.9 and 1.2 km far from the confluence of the Tagus river (Gonçalves 2014). Cabeço da Amoreira is located at the extremity of a spur, nearly 22 m above the flood river bed and Moita do Sebastião about 15 m high (Arnaud 1987; Rolão 1999).

The Muge shell middens are artificial deposits with an elliptical shape forming a quite conspicuous mound. At the time of their discovery, Cabeço da Arruda had about 100 m long and 60 m wide, with a maximum thickness of around 7 m (Ribeiro 1884). Cabeço da Amoreira was 90 m long and 50 m wide, with a maximum thickness of about 3.3 m (Roche and Veiga Ferreira 1967) and Moita do Sebastião stretched over an area of 300 m^2 with a maximum height of 2.5 m (Paula e Oliveira 1888-1892). Moita do Sebastião was almost completely destroyed in 1952, as a result of the use of land for agriculture (Arnaud, 1987). Nowadays, a building covers part of the excavated features (Rolão 1999). Muge shell middens have partially functioned as large residential sites attending to their size, period of occupation, diversity of material culture, and the occurrence of numerous human burials (Arnaud 1987; Rolão 1999; Bicho et al. 2010, 2013), occupied by semi-sedentary communities based on the exploitation of aquatic and terrestrial resources obtainable from the estuarine ecosystem.

The human skeletal material collected in the Muge shell middens is housed in three different Museums, *Museu de História Natural da Faculdade de Ciências da Universidade do Porto*, in Oporto, *Museu do Instituto Geológico e Mineiro*, in Lisbon, and the former *Museu Antropológico da Universidade de Coimbra*, currently belonging to the Department of Life Sciences from the University of Coimbra. The institutional dispersion of the skeletal remains – and also its state of preservation – frustrates an accurate determination of the number of individual skeletons recovered from the three sites. According to Rolão (1999), 145 skeletons were retrieved from Cabeço da Arruda, 139 from Moita do Sebastião and 26 from Cabeço da Amoreira. The 21st archaeological campaigns resulted in the recovery of seven individuals from Cabeço da Amoreira and two from Cabeço da Arruda.

Figure 1 Geographical location of the Mesolithic shell middens in Tagus Valley. The Muge shell middens of Cabeço da Arruda, Cabeço da Amoreira and Moita do Sebastião are surrounded by a green circle

Osteoporosis: a silent disease

Osteoporosis, defined as a metabolic condition of skeletal fragility, attributed to the decrease in bone mass and to the deterioration of bone microarchitecture, resulting in the increase in the risk of fracture (Consensus Development Conference 1993), also belongs to the «history of suffering» (in the faultless expression of Jacques Le Goff [1985: 7]), a tragic narrative where individual horror merges with communal consciousness. Nevertheless, the immersion of bone fragility in history was, until recently, only experienced when connected with major unbearable events such as fractures: in the proximal femur, proximal humerus, distal radius and vertebrae (Curate 2014a). The late clinical awareness of osteoporosis and its ominous consequences does not turn it into a disease without biography or history, but into a disease whose past is a tomb of forgotten bodies, a derelict building expecting redemption.

Anthropology considers the interactions between people and the world; paleopathology investigates the interactions between people and disease in an elapsed world. Usually, paleopathology questions how external forces acted upon human bodies in the past and how the bodies and societies responded to these pressures (Ortner 2003; Sofaer 2004). Bone fragility has been widely studied in historical/archaeological skeletal samples, supplementing diachronic depth to the clinical knowledge about bone modifications related to age, menopausal status or lifestyle (Mays 2000; Agarwal 2008; Curate 2014a).

The purpose of this study includes the exploratory assessment and biocultural interpretation of the overall patterns of sex-specific and age-related cortical bone loss in the second metacarpal in an adult skeletal sample from the Muge shell middens (Portugal) – with an important focus on endocortical bone loss and periosteal apposition. This paper also aims to compare the magnitude of cortical bone loss in the Mesolithic with a modern (late 19th – early 20th centuries) reference skeletal sample. The presence of pathognomonic osteoporotic fractures (hip, distal radius and vertebral compression fractures) in the Muge shell middens sample is also investigated.

MATERIALS AND METHODS

All adult individuals from the Muge shell middens (henceforth also Muge) collections housed at the *Museu dos Serviços Geológicos* (Lisbon, Portugal) and the Department of Life Sciences from the University of Coimbra with an intact second metacarpal were co-opted into the study sample (N=71). Notwithstanding, only those individuals for which sex and age at death could be estimated were included in the final analysis. As such, the Mesolithic sample included 34 adult individuals (♀: 14; ♂: 20) from the Cabeço da Amoreira (N=1), Cabeço da Arruda (N=18) and Moita do Sebastião (N=15). Hereafter also designated as Amoreira, Arruda, Moita and, collectively, as Muge.

Biological sex (i.e., skeletal sex, and not emic sex or gender) was estimated from morphological features of the skull and pelvis (Buikstra and Ubelaker 1994) and postcranial metric techniques (Silva 1995; Spradley and Jantz 2011; Curate et al. 2016a). Age at death assessment relied in standard anthropological aging indicators, including degenerative joint surface changes at the auricular surface (Buckberry and Chamberlain 2002) and pubic symphysis (Brooks and Suchey 1990). Age categories were divided as follow: young adult (20 – 29 years), middle adult (30 – 49 years), and old adult (50+ years).

The Coimbra Identified Skeletal Collection (CISC) includes 505 individual skeletons mainly recovered in the *Cemitério Municipal da Conchada* (Coimbra, Portugal). Biographical details for each individual are available, e.g., name, place of birth, sex, age at death and occupation, among others (Cunha and Wasterlain 2007). The studied sample comprised 219 Portuguese citizens (♀: 105; ♂: 114), with ages at death ranging from 20 to 96 years old. All individuals were born between 1827 and 1914; and died between 1910 and 1936 (i.e., before the systematic biomedical management of bone loss). Individuals were mostly manual workers with low socioeconomic status.

Radiogrammetry quantifies the amplitude or geometry of cortical bone in tubular bones. It is ineffectual to diagnose osteoporosis at the individual level, but perseveres as a useful tool to evaluate cortical bone loss in epidemiological studies (Yasaku et al. 2009; Curate 2014a). Conventional radiogrammetry was used to assess cortical parameters (diaphysis total width [DTW], medullary width [MW] and cortical index [MCI]) at the second metacarpal midpoint (Ives and Brickley 2004). MCI is defined as:

$$\mathrm{MCI} = \frac{\mathrm{DTW} - \mathrm{MW}}{\mathrm{DTW}} \times 100.$$

Radiographs were obtained in a digital radiographic system (Senographe DS, GE Healthcare) at the Coimbra University Hospitals (focal distance 50cm, Kv 27–30 and mAseg 14–20, in compliance with the characteristics of each bone) and measurements were performed with Centricity DICOM Viewer 3.1.1. Osteoporotic fractures (proximal humerus, distal radius, proximal femur and vertebral compression fractures) were assessed macroscopically by the same observer (FC) with the aid of clinical and palaeopathological protocols (Mays 2006a; Curate 2011, 2014a; Curate et al. 2016b). Supplementary medical imaging was performed when required, *i.e.*, all suspected fractures were radiographed.

Descriptive statistics for MW, DTW and MCI, including group means, standard deviation (SD) and 95% confidence intervals (95% CI) for the mean were estimated. Normal distribution for these variables in both samples and sexes was evaluated through skewness and kurtosis (Kline 2010). As such, with values of $|Ku|<7$ and $|Sk|<3$ it was assumed that a violation of normality was not an issue. Homoscedasticity was assessed with a Levene's test. A student's t-test (independent samples) was employed to consider the null hypothesis that the means of two groups were equal. Analysis of variance (ANOVA) was used for comparing multiple groups.

Thirty metacarpals were measured in two different days for repeatability assessment of the cortical parameters (DTW and MW). Measurement error was evaluated with the technical error of measurement (TEM), the relative technical error of measurement (rTEM; Ulijaszek and Kerr 1999) and the reliability coefficient (Rc, Ward and Jamison 1991).

Statistical and graphical analyses were performed with R programming language (R Development Core Team 2016; Chang and Wickham 2016) and IBM SPSS 20.0.

RESULTS

Radiogrammetric measurements are procedurally demanding, particularly MW, as the identification of the endosteal margin is occasionally difficult (Schäfer et al. 2008). Nonetheless, cortical measurements (DTW and MW) were accomplished within suitable levels of measurement error (Table 1).

Table 1: Measurement error for DTW and MW.

Measurement	N	TEM	rTEM	R_c
DTW (mm)	30	0.15	1.93	0.98
MW (mm)	30	0.18	3.78	0.97

Descriptive statistics for both the Muge and CISC samples are summarized in Tables 2, 3 and 4. In the Mesolithic sample, diaphysis total width is significantly higher in men (Student's t: -2.502; df=32; p<0.018). Medullary width and MCI are also greater in men but the differences are not significant (MW Student's t: -0.946; df=32; p=0.351 / MCI Student's t: -0.036; df=32; p=0.971).

In the Muge females' group, DTW increases through the age at death categories but the differences between groups do not reach statistical significance (ANOVA F=0.924; df=2; p=0.426). MW also increases across the age at death categories without reaching statistical significance (ANOVA F=0.926; df=2; p=0.425). Between the first years of adulthood (20 – 29 years) and the 50+ age category, periosteal apposition (DTW as proxy) increases by 10.5%, and endosteal bone loss (MW as proxy) increases by 23.6%. The net loss of cortical bone is 13.1%. Concurrently, MCI decreases through the age categories but the differences between the groups are not significant (ANOVA F=0.517; df=2; p=0.610). In the males' assemblage, no clear patterns were observed. DTW and MW increase from the first to the second age category, decreasing in the older group (DTW ANOVA F=0.267; df=2; p=0.769 / MW ANOVA F=0.008; df=2; p=0.992). Metacarpal cortical index increases across the age categories but not significantly (ANOVA F=0.062; df=2; p=0.940). From the younger age group to the older, periosteal bone apposition increases 3.5% and endocortical bone loss decreases 1.1%, resulting in a net cortical bone gain of 4.6%.

Differences in the cortical parameters (DTW, MW and MCI) between the Muge and the modern reference sample (CISC) are not significant amongst females (DTW Student's t: 0.608; df=117; p=0.544 / MW Student's t: 0.948; df=117; p=0.345 / MCI Student's t: -0.910; df=117; p=0.364). The absolute values for metacarpal cortical index in the 20 – 29 years age category are very similar between samples (Muge: mean=54.14, SD=9.85 / CISC: mean=55.19, SD=10.05). In the older age category the difference, albeit non-significant, increases (Muge: mean=48.33, SD=3.62 / CISC: mean=42.96, SD=10.46). What is noteworthy is the variation in the «rate» of cortical bone loss: in the Muge females, MCI decreases 10.7% from the decade of peak bone mass (20 – 29 years) to the presumed post-menopausal years (50+ years), whereas in the CISC women, MCI declines 20.2%. Amongst males, DTW is significantly higher in the identified skeletal sample from Coimbra (Student's t: 2.347; df=132; $p<0.05$), while differences in both MW and MCI are not significant (MW Student's t: 0.050; df=132; p=0.960 / MCI Student's t: 0.745; df=132; p=0.458). In the males of Muge, MCI increased by 4.1% from the early adulthood to the older age category, while decreasing by 4.3% in the CISC.

A factorial ANOVA design was employed to account for the age structure of each sample. As such, after considering the effects of age structure (i.e., the number of individuals in each age category) it is possible to sustain that the variable «sample» still significantly influenced DTW in men (ANOVA F=4.173; df=1; $p<0.05$; $\eta^2_p=0.032$; observed power=0.527). All other analyses confirmed that the differences between samples are not significant. The density distributions of MCI in both sexes and DTW in males are depicted in Figures 2, 3 and 4.

Figure 2 Density distribution of MCI in the CISC and Mesolithic samples (females)

Figure 3 Density distribution of MCI in the CISC and Mesolithic samples (males)

Figure 4 Density distribution of DTW in the CISC and Mesolithic samples (males)

The crude prevalence of fragility fractures in the Muge overall sample is 5.9% (2/34). The two individuals with fracture were older men (10.0%; 2/20) from Arruda and presented vertebral compression fractures. The first individual (Arruda XV) displayed fractures in the T9 (collapse, no measurements were possible), T11 (grade 1, possibly cuneiform), T12 (grade 1, possibly cuneiform) and L1 (grade 1, cuneiform). From T7 to T12 vertebrae were fused together (Figures 5 and 6). This individual's MCI was 55.3 (Z-Score=0.28), higher than the average MCI for his age group and sex. The second individual (Arruda N) presented vertebral collapse in the T10, and collapse and fusion of the L1 and L2. As a result of taphonomic destruction, it was not possible to measure the affected vertebrae. The MCI was 51.0 (Z-Score=-0.316), lower than age- and sex-matched individual. Osteoporotic vertebral collapse of the Arruda N individual was documented previously by Jackes and Lubell (1999).

Figure 5 Vertebral column (T7 to L1) from the individual Arruda XV presenting compression fractures

Figure 6 Plain radiograph of the vertebral column (T7 to L1) from the individual Arruda XV presenting compression fractures

DISCUSSION

Observed sexual differences in metacarpal cortical features in the Muge sample, especially diaphysis total width (and, likewise – but without a significant statistical support –, medullary width and metacarpal cortical index), arise from gendered disparities in the degree and pattern of bone loss, and bone dimensions (Samuel et al. 2009). As a rule, men have bigger skeletons, with bone dimensions determined early in the life cycle, conceivably still *in utero*, but more so during puberty (Seeman 2003, 2008; Samuel et al. 2009). After sexual maturation, oestrogen production in women constrains periosteal bone formation, confining bone diameter; whereas, in males, pubertal androgen increases bone growth in the periosteal envelope and stimulates the expansion of bone diameter. In addition, men experience a long-term period of bone gain during growth (Seeman 2003, 2008). Around and after menopause, bone loss accelerates in women – oestrogen curtailment boosts bone remodelling rates, and less bone is formed and more is resorbed at the basic multicellular units (Seeman 2008) – this is especially relevant since older women from Muge have larger MW when compared to younger women and men from all age categories.

Bone development and size is also affected by mechanical loading and nutrition, among others, and it is plausible that the resulting influence on bone dimensions might be sex-specific (Gilsanz et al. 1997; Nieves et al. 2005). Jackes and Lubell (1999) observed significant differences in the percent cortical area at the femur midshaft between the Moita and Arruda samples suggesting that lower activity levels and diet may have played a role – in addition to a different demographic structure – in the development of thinner cortical bone in the Arruda individuals. Dietary dissimilarities are hinted by isotopical data, and differential dental attrition (Meiklejohn et al. 2009; Jackes forthcoming). Umbelino (2006) and Umbelino et al. (2015) also found dietary differences between the sites of Amoreira, Moita and Arruda. From the comparison of trace elements and carbon and nitrogen stable isotope analysis it looks like the individuals from Amoreira had a slight predominance of marine resources, followed by Moita and lastly by Arruda (Umbelino 2006). For Amoreira the $\delta^{13}C$ values vary between -16.5 to -14.8‰ (n=7), which corresponds to a percentage of marine resources from 50 to 69%, while for Moita the $\delta^{13}C$ values obtained for three individuals of -16.7‰, -16.6‰ and -16.2‰ reflect marine protein in their diet of 48%, 49% and 53%, respectively (Umbelino 2006; Umbelino et al. 2015). For Arruda the $\delta^{13}C$ values are quite different, of -15.7‰ for a female and -17.2‰ for a male, representing percentages of protein of marine diet of 59% and 42% (Umbelino 2006, Umbelino et al. 2015). The relationship between osteoporosis and nutrition has been known for long, and nutrition is recognized as a key risk factor for bone loss, disturbing bone health in different ways (Curate 2014b) but dietary influences on bone strength must be interpreted with caution (Agarwal 2008; Ruff et al. 2015) – especially because data on calcium consumption is scarce, if not inexistent, for this period. Vitamin D is also a contributing factor for bone health but skeletal indicators of vitamin D deficiency in non-adults and adults (Brickley et al. 2007; Mays et al. 2008) were not present in individuals from Muge. Increased sedentism – with concomitant declining mobility and physical activity – at the Arruda site may have driven changes in femoral cortex (Jackes forthcoming). Physical activity during growth, particularly vigorous activities, influence peak bone mass and physical exercise also promotes bone health in postmenopausal women and elderly individuals from both sexes (Curate 2014a). Due to small sample sizes, the individuals from Arruda and Moita were pooled together and not compared with each other. It is clear, however, that there are subsamples within the burials from the two sites (and also from Amoreira), with sex determining one of the major subsamples. Differential activity levels between sexes are also inferred by the cnemic index of the tibia in Arruda (Jackes and Lubell 1999). In the wider European Mesolithic context, femoral and tibial strength is reduced in females when compared to males (Ruff et al. 2015). Taken together, these data support at least to some extent that sex-mediated physical activity could have influenced cortical bone health in women and men from Muge.

Sexual dissimilarity in cortical bone loss has been described both in modern (e.g., Virtamä and Helelä 1969; Ginsburg et al. 2001) and archaeological/historical populations (e.g., Dewey et al. 1969; Carlson et al. 1976; Thompson & Guness-Hey 1981; Drusini et al. 2000; Mays et al. 1998; Ives 2007; Curate et al. 2009; Agarwal et al. 2011; Cho and Stout 2011; Glencross and Agarwal 2011). Hormonal dynamics were the primary source of these differences, but physical activity and nutrition, especially during growth, certainly played an important role in cortical bone health in the past.

Cortical bone changes with age are not significant in the females and males from Muge – sample size may have constrained statistical power. Nonetheless, a familiar epidemiological pattern emerges in the females' sample: DTW and MW increase, and MCI declines, through the age categories. In men, similar average values for the cortical parameters are the rule across the age groups.

Diaphysis total width is a proxy for periosteal apposition, and enduring apposition of bone in the periosteum throughout aging has been interpreted as an adaptive reaction to uphold resistance to bending (Mays 2001; Szulc et al. 2006; Seeman 2008; Peck and Stout 2009). As such, the potential enlargement of the external dimensions of the second metacarpal in the Muge females (>10% from the younger to the older age category; in men, the increase was only 3.5%) may function as a compensation for endocortical bone loss. The causes of periosteal apposition are manifold, and include physical activity, but it is likely that the magnitude of mechanical compensation is governed by initial bone dimensions, with larger bones – men in the Muge sample are usually larger than women – exhibiting less periosteal apposition (Jepsen and Andarawis-Puri 2012). In a prehistoric Mississippian skeletal sample, periosteal diameter also increased with age in women, while declining in men (van Gerven et al. 1969). Epidemiological and cross-sectional studies are inconsistent, with some showing greater periosteal apposition in men (e.g., Virtamä and Helelä 1969; Feik et al. 2000), others in women (e.g., Garn et al. 1972; Kaptoge et al. 2003; Curate et al. 2009) and others in both sexes (Mays, 2000, 2001).

Metacarpal central cavity widens during the life course as a consequence of the discrepancy between endosteal bone resorption and formation that cause bone loss at the endocortical envelope (Jergas 2008). Even though remodelling at the endosteal surface increases slightly in aged men, it increases substantially in perimenopausal and early postmenopausal women, stemming from oestrogen withdrawal, and decelerating later in life (Clarke 2008). Menopause partially explains why the «rate» of endocortical bone loss is faster in women during aging. Indeed, in the Muge sample medullary width – functioning as a surrogate for endosteal bone loss – increases a whopping 23.6% in women from early adulthood (presumably a pre-menopausal phase) to the older age category (probably a peri- or post-menopausal phase), while remaining stable in men during the same period of life. The unchanging average values of MW with age in the males' sample reflects the nonexistence of a physiological event like menopause in men, and possibly sexual-specific activity patterns that could oppose bone loss in the Mesolithic men from Muge. As pointed before, gendered activity patterns were documented in the Muge shell middens – at least in Cabeço de Arruda (Jackes and Lubell 1999). The European Mesolithic has long been perceived as a period of lower sexual dimorphism (Frayer 1977, 1980; Meiklejohn et al. 1984; Borgognini and Repetto 1986), in which the patterns of activity of both sexes may have been more similar than during any other chronological era (Frayer 1980; Schmidt 2005). Notwithstanding, sexual division of labour – especially in behaviours related with hunting – is still manifest in Mesolithic samples (Villotte et al. 2010). Also, sexual differences in relative bone strength are of the same magnitude in Mesolithic and later samples (Ruff et al. 2015).

The general patterns of cortical bone loss with age observed in the Muge sex groups closely emulate the ones observed in the femur (Curate et al. 2009) and the second metacarpal (Curate et al. 2015) in the skeletal reference collection from Coimbra. The sex-specific pattern of bone loss and maintenance with increasing age is well documented in historical populations (e.g., Mays et al. 1996; Rewekant 2001; Ives 2007; Agarwal et al. 2011; Cho and Stout 2011).

A direct comparison between samples reveals significant differences only in the males' DTW – even after adjusting for the age structure of each sample. Diaphysis total width is highly correlated with bone size, and there is a significant difference in skeletal size between the Mesolithic and the modern reference sample. In Portugal, a secular increase of stature from the Mesolithic to the 20th century was also observed (Cardoso and Gomes 2009; Lubell and Jackes 1985).

Peak metacarpal cortical bone mass (MCI as proxy) appears very similar in females from both samples. Peak bone mass – the maximum amount of bone acquired during growth – is affected by genetic and environmental / behavioural factors. Of the latter, nutrition and physical activity are possibly the most significant.

The intensity of strain and effort associated with a pre-historic hunter-gatherer economy – even with the emergence of semi-permanent settlements, declining mobility and constancy of regional occupation – was far greater than the observed in recent historical times (Cunha and Cardoso 2001; Ruff et al. 2015; Villotte et al. 2010; Tarli and Reppetto 1986). Nonetheless, it is important to note that most of the CISC women were involved in physically demanding types of work (Cunha and Umbelino 1995). Malnutrition throughout growth can hamper peak bone mass and bone mass in later stages of life. The Muge shell middens were at an interface of marine, freshwater and terrestrial environments with an extraordinary suite of nutritional resources, from shellfish to edible plants (Jackes and Meiklejohn 2008). Carbon and nitrogen stable isotope analysis substantiate a mixed diet, based on a large spectrum of food resources that characterizes the estuarines ecosystems, of marine and terrestrial origin, animal and vegetable (Umbelino 2006; Umbelino et al. 2015). The higher strontium levels observed on Muge human bones when compared to those of Sado shell middens is interpreted as a stronger reliance on marine food in Muge, which is corroborated by the $\delta^{13}C$ data that points to a proportion of marine resources of 50% in Muge and nearly 30% in Sado (Umbelino 2006).

Calcium consumption for Muge is not known but calcium intake in the past was generally adequate (Curate 2014b). In the early 20th century Coimbra, calcium deficiency was also unlikely: crop fields, fruit orchards and animal farms surrounded – and infiltrated – the city (Santos 1995). Other factors, like age at menarche and fertility, may influence peak bone mass in women (Curate 2014a). Relative fertility was perhaps slightly higher in Muge (Jackes and Meiklejohn 2008) when compared to Coimbra in the early 20th century (Curate 2011): the mean number of offspring per woman was roughly four to six in Muge (Jackes et al. 1997; Jackes and Meiklejohn 2008; Jackes forthcoming) and 3.3 in Coimbra (Morais 1983).

Interestingly, MCI declines in both female groups but at a faster «rate» in the modern reference sample (10.7% vs. 20.2%), a trend observed in other archaeological samples (Lees at al. 1993; Cho and Stout 2003). On the contrary, Mays (2006b) observed that the magnitude of cortical bone loss in a 3rd–4th century AD sample (Ancaster, England) was greater than that documented for a modern reference population. LOESS regression suggests that MCI decrease in the CISC females occurs only after the 50th decade, with a precipitous decline after the presumed age at menopause (Figure 7). The term «menopause» was introduced in the medical literature by Charles de Gardanne in 1812 but textual references to this physiological event are known since biblical times (Pavelka and Fedigan 1991). It is noteworthy that the average age of menopause in historical populations – from classical Greece and Rome to Medieval Europe – most likely occurred around 45–50 years (Amundsen and Dyers 1970; Post 1971). Thus, it is plausible that mean age at menopause in the Mesolithic was also in the vicinity of the fifth decade of life. Long-term physical activity and reproductive factors possibly counterbalanced – at least partially – the menopausal effects on bone turnover in the Muge sample. As stated before, the women from Muge had a higher relative fertility (Jackes and Meiklejohn 2008), and it has been suggested that, later in life, parity (i.e., the total number of births) protects bone health (Streeten et al. 2005).

Figure 7 LOESS smoothing for MCI and age at death in females from CISC

Fragility fractures are not the most frequent type of fracture in archaeological samples but evidences of such fractures in past populations – including prehistoric communities – are multiplying (Mays 2006b; Curate et al. 2011; Curate 2014a). In this study, the individuals with fragility fractures – compression fractures of the vertebrae – were both older males. Compression fractures are the hallmark of osteoporosis, being the most prevalent fracture in postmenopausal women (Johnell & Kanis 2006). Older men (> 65 years) are also at increased risk of vertebral compression fractures (Felsenberg et al. 2002).

Osteoporosis and bone loss are major risk factors for vertebral compression fractures (Johansson et al. 2009) but, while Arruda N displayed a lower for age MCI, Arruda XV had an MCI slightly higher than age-matched individuals. This suggests that, at least in Arruda N, the fracture can be a consequence of bone loss. In the Arruda XV individual other factors must have been involved, including activity-related trauma, infection (e.g., tuberculosis) or neoplastic diseases (Curate and Tavares 2012). The age-standardized prevalence of vertebral fractures is similar for both men and women, but before age 65, men presented a higher prevalence (O'Neill et al. 1996). The incidence of vertebral fractures in men, however, is approximately half the rate of women (Felsenberg et al. 2002).

A significant proportion of osteoporotic fractures in younger men stems from occupational hazards (Zebaze and Seeman 2003) – and it is possible that the compression fractures observed in the Arruda XV individual are the result of high energy-trauma and not bone fragility. It is also probable that these individuals faced increased morbidity related with the fractures, including lower energy, poorer sleep, pain, immobility and functional impairment (Burger et al. 1997).

CONCLUSIONS

Metacarpal cortical bone at the Muge shell middens shows sex-specific configurations of periosteal apposition and endosteal bone loss, which are probably related to hormonal and behavioural / cultural factors. Endocortical bone loss increases with age in women, with a concomitant enlargement of the metacarpal diaphysis. In men, periosteal and medullary expansion at the second metacarpal is essentially absent, with bone strength conserved during the course of life. The general patterns of cortical bone maintenance and loss mimic those from a modern reference skeletal collection. Notwithstanding, older women from Muge appear to lose less cortical bone than late 20th century elderly women from Coimbra – with long-term physical activity and reproductive factors possibly playing a pivotal role in bone health.

This study presents some methodological limitations, including the small Mesolithic sample size and the lumping of different Muge sites for analysis. The Muge shell middens of Arruda, Amoreira and Moita show social diversity (Bicho et al. 2013) with indications of dietary, activity and fertility dissimilarities (Jackes forthcoming) and span roughly tree millennia – with broader anthropological implications, viz., secular changes in bone dimensions and bone loss. However, genetic differentiation between sites was probably insignificant, as well as the overall social relations and subsistence economy. In the future, the study of bone health in the Portuguese Mesolithic would benefit from an increment in sample size and inter-site comparisons.

Table 1: Mean values of DTW according to sample, sex and age class.

		Females				Males			
		Mean	SD	95% CI	N	Mean	SD	95% CI	N
Muge	20-29	6.69	0.80	5.85-7.52	6	7.42	0.89	6.31-8.53	5
	30-49	7.01	0.56	6.12-7.90	4	7.70	0.74	7.20-8.20	11
	50+	7.39	1.00	5.79-8.99	4	7.68	0.22	7.33-8.03	4
	Total	6.98	0.80	6.52-7.44	14	7.63	0.69	7.30-7.95	20
CISC	20-29	7.04	0.59	6.71-7.36	15	7.95	0.88	7.44-8.46	14
	30-49	6.99	0.64	6.75-7.23	30	8.02	0.84	7.73-8.32	33
	50+	7.16	0.63	7.00-7.32	60	8.09	0.72	7.92-8.27	67
	Total	7.09	0.62	6.71-7.36	105	8.06	0.73	7.44-8.46	114

Table 2: Mean values of MW according to sample, sex and age class.

		Females				Males			
		Mean	SD	95% CI	N	Mean	SD	95% CI	N
Muge	20-29	3.09	0.88	2.16-4.01	6	3.74	0.96	2.55-4.93	5
	30-49	3.57	1.05	1.90-5.24	4	3.77	1.24	2.93-4.60	11
	50+	3.82	0.55	2.94-4.70	4	3.70	0.18	3.40-3.98	4
	Total	3.44	0.85	2.94-3.93	14	3.75	1.01	3.28-4.22	20
CISC	20-29	3.24	0.83	2.78-3.70	15	3.63	1.07	3.01-4.26	14
	30-49	3.13	0.84	2.81-3.44	30	3.48	1.10	3.09-3.87	33
	50+	4.09	0.89	3.86-4.33	60	3.93	1.16	3.64-4.21	67
	Total	3.70	0.98	3.51-3.89	105	3.76	1.14	3.55-3.97	114

Table 3: Mean values of MCI according to sample, sex and age class.

		Females				Males			
		Mean	SD	95% CI	N	Mean	SD	95% CI	N
	20-29	54.14	9.85	43.80-64.48	6	49.81	10.37	36.93-62.69	5
Muge	30-49	49.55	12.83	29.14-69.96	4	51.76	12.46	43.39-60.13	11
	50+	48.33	3.62	42.57-54.09	4	51.86	2.72	47.53-56.19	4
	Total	51.17	9.23	45.82-56.51	14	51.29	10.31	46.47-56.12	20
	20-29	55.19	10.05	48.63-59.76	15	54.11	13.24	46.48-61.76	14
CISC	30-49	55.47	10.60	51.51-59.42	30	56.79	12.18	52.47-61.11	33
	50+	42.96	10.46	40.26-45.66	60	51.77	12.49	48.72-54.81	67
	Total	48.14	11.97	45.82-50.45	105	53.51	12.58	51.18-55.84	114

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Agarwal S (2008) Light and broken bones: examining and interpreting bone loss and osteoporosis in past populations. In: Katzenberg AK, Saunders S (eds) Biological anthropology of the human skeleton, 2nd ed. Wiley-Liss, New York, pp 387–410

Agarwal S, Glencross B, Beauchesne P (2011) Bone growth, maintenance and loss in the Neolithic Community of Çatalhöyük, Turkey: preliminary results. Archaeological Research Facility Laboratory Reports, UC Berkeley

Arnaud JM (1987) Os concheiros mesolíticos dos vales do Tejo e Sado: semelhanças e diferenças. Arqueologia 15:53-64

Bicho N, Umbelino C, Detry C, et al (2010) The emergence of Muge mesolithic shell middens in central Portugal and the 8200 cal yr BP cold event. J Isl Coast Archaeol 5:86-104.

Bicho N, Cascalheira J, Marreiros J, et al (2013) Chronology of the Mesolithic occupation of the Muge valley, central Portugal: the case of Cabeço da Amoreira. Quatern Int 308-309:130-139

Borgognini SM, Repetto TE (1986) Skeletal indicators of subsistence patterns and activity régime in the Mesolithic sample from Grotta dell'Uzzo (Trapani, Sicily): a case study. Hum Evol 1:331–351. doi: 10.1007/BF02436707

Brickley M, Mays S, Ives R (2007) An investigation of skeletal indicators of Vitamin D Deficiency in adults: effective markers for interpreting past living conditions and pollution levels in 18th and 19th century Birmingham, England. Am J Phys Anthropol 79:67–79. doi: 10.1002/ajpa

Brooks S, Suchey JM (1990) Skeletal age determination based on the *os pubis*: a comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. Hum Evol 5:227–238

Buckberry JL, Chamberlain AT (2002) Age estimation from the auricular surface of the ilium: A revised method. Am J Phys Anthropol 119:231–239. doi: 10.1002/ajpa.10130

Buikstra JE, Ubelaker DH (1994) Standards for data collection from human skeletal remains. Arkansas Archaeological Survey, Arkansas

Burger H, Van Daele PL, Grashuis K, et al (1997) Vertebral deformities and functional impairment in men and women. J Bone Miner Res 12:152–157

Cardoso H, Gomes JEA (2009) Trends in adult stature of peoples who inhabited the modern Portuguese territory from the Mesolithic to the late 20th century. Int J Osteoarchaeol 19:711–725

Cardoso JL, Rolão JM (1999-2000) Prospecções e escavações nos concheiros mesolíticos de Muge e Magos (Salvaterra de Magos): contribuição para a história dos trabalhos arqueológicos efectuados. Estudos Arqueológicos de Oeiras 8:83–240

Carlson DS, Armelagos GJ, van Gerven DP (1976) Patterns of age related cortical bone loss (osteoporosis) within the femoral diaphysis. Hum Biol 48:295–314

Chang W, Wickham H (2016) ggvis: Interactive Grammar of Graphics. http://CRAN.R-project.org/package=ggvis. Accessed 24 May 2016

Cho H, Stout SD (2003) Bone remodeling and age-associated bone loss in the past: an histomorphometric analysis of the Imperial Roman skeletal population of Isola Sacra. In: Agarwal SC, Stout S (eds) Bone loss and osteoporosis: an anthropological perspective. Kluwer Academic/Plenum Publishers ,New York, pp 91–101

Cho H, Stout SD (2011) Age-associated bone loss and intraskeletal variability in the Imperial Romans. J Anthropol Sci 89:109–125. doi: 10.4436/jass.89007

Consensus Development Conference (1993) Diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 94:646–650

Cunha E, Cardoso F (2001) The osteological series from Cabeço da Amoreira (Muge, Portugal). Bull Mem Soc Anthropol Paris 13:323–333

Cunha E, Umbelino C (1995) What can bones tell about labour and occupation: the analysis of skeletal markers of occupational stress in the Identified Skeletal Collection of the Anthropological Museum of the University of Coimbra (preliminary results). Antropol Port 13:49–68

Cunha E, Wasterlain S (2007) The Coimbra identified osteological collections. In: Grupe G, Peters J (eds) Skeletal series and their socio- economic context. Marie Leidorf, GmbH, Rahden/Westf, Germany, pp 23–33

Curate F (2011) O perímetro do declínio. Osteoporose e fracturas de fragilidade em três amostras osteológicas identificadas Portuguesas – sécs. XIX & XX. Dissertation, University of Coimbra

Curate F (2014a) Osteoporosis and paleopathology: A review. J Anthropol Sci 92:119–146. doi: 10.4436/JASS.92003

Curate F (2014b) Osteoporosis and nutrition – A paleopathological insight. Antropol Port 30:29–51. doi: 10.14195/2182-7982_31_2

Curate F, Tavares A, Piombino-Mascali D, et al (2009) Assottigliamento corticale del femore e fratture da fragilità ossea: uno studio della Collezione Scheletrica Identificata di Coimbra (Portogallo). Arch per l'Antropologia e la Etnol CXXXIX:129–146

Curate F, Assis S, Lopes C, et al (2011) Hip fractures in the Portuguese archaeological record. Anthropol Sci 119:87–93. doi: 10.1537/ase.100211

Curate F, Tavares A (2011) Cifosis vertebral en la pintura de Francisco Goya (1764-1824): un ejercicio de diagnóstico diferencial. In: González Martín A, Cambra-Moo O, Rascón Pérez J, et al (eds) Paleopatología: ciencia multidisciplinar. Sociedad de Ciencias Aranzadi, Donostia-San Sébastian, pp 611–616

Curate F, Cunha E, Matos V, et al (2015) Cortical bone loss and osteoporotic fractures in the Coimbra Identified Skeletal Collection. Am J Phys Anthropol 156:114

Curate F, Coelho J, Gonçalves D, et al (2016a) A method for sex estimation using the proximal femur. Forensic Sci Int 266: 579.e1–579.e7. doi:10.1016/j.forsciint.2016.06.011

Curate F, Silva TF, Cunha E (2016b) Vertebral compression fractures: towards a standard scoring methodology in Paleopathology. Int J Osteoarchaeol 26:366–372. doi: 10.1002/oa.2418

Dewey J, Armelagos G, Bartley M (1969) Femoral cortical involution in three Nubian archaeological populations. Hum Biol 41:13–28

Drusini A, Bredariol S, Carrara N, et al (2000) Cortical bone dynamics and age-related osteopenia in a Longobard archaeological sample from three graveyards of the Veneto Region (Northeast Italy). Int J Osteoarch 10:268–279

Felsenberg D, Silman AJ, Lunt M, et al (2002) Incidence of vertebral fracture in Europe: results from the European Prospective Osteoporosis Study (EPOS). J Bone Miner Res. 17:716–24. doi: 10.1359/jbmr.2002.17.4.716

Ferreira MT, Umbelino C, Cunha E (2015) The Mesolithic skeletons from Muge: the 21st century excavations. In: Bicho N, Detry C, Price TD, Cunha E (eds) Proceedings of the Muge 150th: the 150th anniversary of the discovery of Mesolithic shellmiddens – Volume 1. Chapter Fifteen. Cambridge Scholars Publishing, Cambridge, pp 199- 208

Frayer DW (1977) Dental sexual dimorphism in the European Upper Paleolithic and Mesolithic. J Dent Res 56:871

Frayer DW (1980) Sexual dimorphism and cultural evolution in the later Pleistocene and Holocene of Europe. J Hum Evol 9:399–415

Gilsanz V, Kovanlikaya A, Costin G, et al (1997) Differential effect of gender on the sizes of the bones in the axial and appendicular skeletons. J Clin Endocrinol Metab 82:1603–1607. doi: 10.1210/jcem.82.5.3942

Ginsburg E, Skaric-Juric T, Kobyliansky E, et al (2001) Evidence on major gene control of cortical index in pedigree data from Middle Dalmatia, Croatia. Am J Hum Biol 13:398–408

Glencross B, Agarwal SC (2011) An investigation of cortical bone loss and fracture patterns in the neolithic community of Çatalhöyük, Turkey using metacarpal radiogrammetry. J Archaeol Sci 38:513–521. doi: 10.1016/j.jas.2010.10.004

Gonçalves C (2014) Modelos preditivos de ocupação do território no Mesolítico entre os vales do Tejo e do Sado. Dissertation, University of Algarve

Ives R (2007) An investigation of vitamin d deficiency osteomalacia and age-related osteoporosis in six post-medieval urban collections. Dissertation, University of Birmingham

Ives R, Brickley MB (2004) A Procedural Guide to Metacarpal Radiogrammetry in Archaeology. Int J Osteoarchaeol 17:7–17. doi: 10.1002/oa.709

Jackes M (forthcoming) Muge Mesolithic heterogeneity: comparing Moita do Sebastião and Cabeço da Arruda. In: Proceedings of MESO 2010, Santander

Jackes M, Lubell D, Meiklejohn C (1997) Healthy but mortal: human biology and the first farmers of Western Europe. Antiquity 71:639–658

Jackes M, Lubell D (1999) Human biological variability in the Portuguese Mesolithic. Arqueol (Porto) 24:25–42

Jackes M, Meiklejohn C (2008) The paleodemography of Central Portugal and Mesolithic-Neolithic transition. In: Bocquet-Appel J-P (ed) Recent advances in paleodemography: data, techniques, patterns. Springer, Berlin, pp. 209-258

Jepsen K, Andarawis-Puri N (2012) The amount of periosteal apposition required to maintain bone strength during aging depends on adult bone morphology and tissue-modulus degradation rate. J Bone Miner Res 27:1916–1926. doi: 10.1002/jbmr.1643

Johansson H, Kanis J, Oden, et al (2009) BMD, clinical risk factors and their combination for hip fracture prevention. Osteoporos Int 20:1675–1682. doi: 10.1007/s00198-009-0845-x

Johnell O, Kanis J (2006) An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 17:1726–33. doi: 10.1007/s00198-006-0172-4

Kline RB (2010) Principles and practice of structural equation modeling. The Guildford Press, New York

Lees B, Molleson T, Arnett T, Stevenson J (1993) Differences in proximal femur bone density over two centuries. Lancet 341:673–675

Le Goff J (1985) As doenças têm história. Terramar, Lisboa

Lubell D, Jackes M (1985) Mesolithic-Neolithic continuity: evidence from chronology and human biology. In: Ramos M (ed) Actas da I Reunião do Quaternário Ibérico, pp 113–133

Mays S (1996) Age-dependent bone loss in a medieval population. Int J Osteoarch 6:144–154. doi: 10.1002/(SICI)1099-1212(199603)6:2<144::AID-OA261>3.0.CO;2-G

Mays S (2000) Age-dependent cortical bone loss in women from 18th and early 19th century London. Am J Phys Anthropol 112:349–61. doi: 10.1002/1096-8644(200007)112:3<349::AID-AJPA6>3.0.CO;2-0

Mays S (2001) Effects of age and occupation on cortical bone in a group of 18th-19th century British men. Am J Phys Anthropol 116:34–44. doi: 10.1002/ajpa.1099

Mays S (2006a) A palaeopathological study of Colles' fracture. Int J Osteoarchaeol 16:415–428. doi: 10.1002/oa.845

Mays S (2006b) Age-related cortical bone loss in women from a 3rd – 4th century AD population from England. 528:518–528. doi: 10.1002/ajpa

Mays S, Lees B, Stevenson J (1998) Age-dependent bone loss in the femur in a medieval population. Int J Osteoarchaeol 8:97–106. doi: 10.1002/(SICI)1099-1212(199803/04)8:2<97::AID-OA412>3.0.CO;2-U

Mays S, Brickley M, Ives R (2009) Growth and Vitamin D deficiency in a population from 19th century Birmingham, England. Am J Phys Anthropol 415:406–415. doi: 10.1002/oa

Meiklejohn C, Schentag C, Venema A, et al (1984) Socioeconomic change and patterns of pathology in the Mesolithic and Neolithic of Western Europe: some suggestions. In: Cohen MN, Armelagos GJ (eds) Paleopathology at the origins of agriculture, Academic Press, San Diego, pp 75-100

Meiklejohn C, Roksandic M, Jackes M, et al (2009) Radiocarbon dating of Mesolithic human remains in Portugal. Mesolithic Miscellany 20:4–16

Mendes Corrêa AA (1933) Les nouvelles fouilles à Muge (Portugal). XVe Congrès International d'Anthropologie et d'Archéologie Préhistorique, Paris 1931. Librairie E. Nourry, Paris, pp 1-16

Mendes Corrêa AA (1934). Questions du Mésolithique Portugais. Proceedings of the First International Congress of Prehistoric and Protohistoric Sciences, London 1932. Oxford University Press, London, pp 89-91

Morais MG (1983) A substituição das gerações em Portugal: análise regional (1930-75). Anal Soc 19:79–99

Nieves JW, Formica C, Ruffing J, et al (2005) Males have larger skeletal size and bone mass than females, despite comparable body size. J Bone Miner Res 20:529–535. doi: 10.1359/JBMR.041005

Ortner D (2003) Identification of pathological conditions in human skeletal remains. Academic Press, San Diego

Paula e Oliveira F (1888-1892a) Nouvelles fouilles faites dans les Kjoekkenmoeddings de la vallée du Tage. Comunicações da Comissão dos Trabalhos Geológicos de Portugal II:57–81

Peck J, Stout SD (2007) Intraskeletal variability in bone mass. Am J Phys Anthropol 132:89–97

R Development Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/. Accessed 24 May 2016

Ribeiro MC (1884) Les kioekkenmoeddings de la Vallée du Tage. Compte Rendu de la IXème session du Congrès International d'Anthropologie et d'Archéologie Préhistoriques, Lisbonne 1880. Typographie de l'Académie des Sciences, Lisboa, pp 279-290

Rewekant A (2001) Do environmental disturbances of an individual’s growth and development influence the later bone involution processes? A study of two mediaeval populations. Int J Osteoarchaeol 11:433–443. doi: 10.1002/oa.584

Roche J, Veiga Ferreira O (1967) Les fouilles récentes dans les amas coquilliers mésolithiques de Muge (1952-1965). O Arquéologo Português I:19-41

Rolão JMF (1999) Del Würm final al Holocénico en el Bajo Valle del Tajo (Complejo Arqueológico Mesolítico de Muge). Dissertation, University of Salamanca

Ruff CB, Holt B, Niskanen M, et al (2015) Gradual decline in mobility with the adoption of food production in Europe. Proc Natl Acad Sci 112:7147–7152. doi: 10.1073/pnas.1502932112

Samuel SP, Baran GR, Wei Y, et al (2009) Biomechanics – Part II. In: Khurana JS (ed) Bone Pathology. Humana Press, Totowa, pp 69–77

Santos AL (1995) Death, sex and nutrition: analysis of the cause of death in the Coimbra human skeletal collection. Antropol Port 13:81–91

Schäfer M-L, Pfeil A, Renz DM, et al (2008) Effects of long-term immobilisation on cortical bone mass after traumatic amputation of the phalanges estimated by digital X-ray radiogrammetry. Osteoporos Int 19:1291–1299. doi: 10.1007/s00198-008-0570-x

Schmidt RA (2005) The contribution of gender to personal identity in the Southern Scandinavian Mesolithic. In: Casella EC, Fowler C (eds) The archaeology of plural and changing identities – beyond identification. Kluwer Academic / Plenum Publishers, New York, pp 79–108

Seeman E (2003) Invited Review: Pathogenesis of osteoporosis. J Appl Physiol 95:2142–2151. doi: 10.1152/japplphysiol.00564.2003

Seeman E (2008) Structural basis of growth-related gain and age-related loss of bone strength. Rheumathology 47: iv2-iv8. doi: 10.1093/rheumatology/ken177

Silva AM (1995) Sex assesment using the calcaneus and talus. Antropol Port 13:107–119

Spradley MK, Jantz RL (2011) Sex estimation in forensic anthropology: Skull versus postcranial elements. J Forensic Sci 56:289–296. doi: 10.1111/j.1556-4029.2010.01635.x

Streeten E, Ryan K, McBride DJ, et al (2005) The relationship between parity and bone mineral density in women characterized by a homogeneous lifestyle and high parity. J Clin Endocrinol Metab 90:4536–41. doi: 10.1210/jc.2004-1924

Szulc P, Seeman E, Duboeuf F, et al (2006) Bone fragility: failure of periosteal apposition to compensate for increased endocortical resorption in postmenopausal women. J Bone Mineral Res 21:1856-1863. doi: 10.1359/jbmr.060904

Thompson D, Guness-Hey M (1981) Bone mineral-osteon analysis of Yupik-Inupiaq skel- etons. Am J Phys Anthropol 55:1–7

Ulijaszek SJ, Kerr DA (1999) Anthropometric measurement error and the assessment of nutritional status. Br J Nutr 82:165–177

Umbelino C (2006) Outros sabores do passado: as análises de oligoelementos e de isótopos estáveis na reconstituição da dieta das comunidades humanas do Mesolítico final e do Neolítico final/Calcolítico do território português. Dissertation, University of Coimbra

Umbelino C, Gonçalves C, Figueiredo O, et al (2015) Life in the Muge shellmiddens: inferences from the new skeletons recovered from Cabeço da Amoreira. In: Bicho N, Detry C, Price TD, Cunha E (eds) Proceedings of the Muge 150th: the 150th anniversary of the discovery of Mesolithic shellmiddens – Volume 1. Chapter Sixteen. Cambridge Scholars Publishing, Cambridge, pp 209-224

Van Gerven D, Armelagos G, Bartley M (1969) Roentgenographic and direct measurement of femoral cortical involution in a prehistoric Mississippian population. Am J Phys Anthropol 31:23–38

Virtamä P, Helelä T (1969) Radiographic measurements of cortical bone. Variation in a normal population between 1 and 90 years of age. Acta Radiol (Supplementum) 293:1–268

Ward R, Jamison P (1991) Measurement precision and reliability in craniofacial anthropometry: implications and suggestions for clinical applications. J Craniofac Genet Dev Biol 11:156–164

Yasaku K, Ishikawa-Takata K, Koitaya N, et al (2009) One-year change in the second metacarpal bone mass associated with menopause nutrition and physical activity. J Nutr Heal Aging 13:545–549. doi: 10.1007/s12603-009-0105-y

Zebaze R, Seeman E (2003) Epidemiology of hip and wrist fractures in Cameroon, Africa. Osteoporos Int 14:301–305

Figure 1

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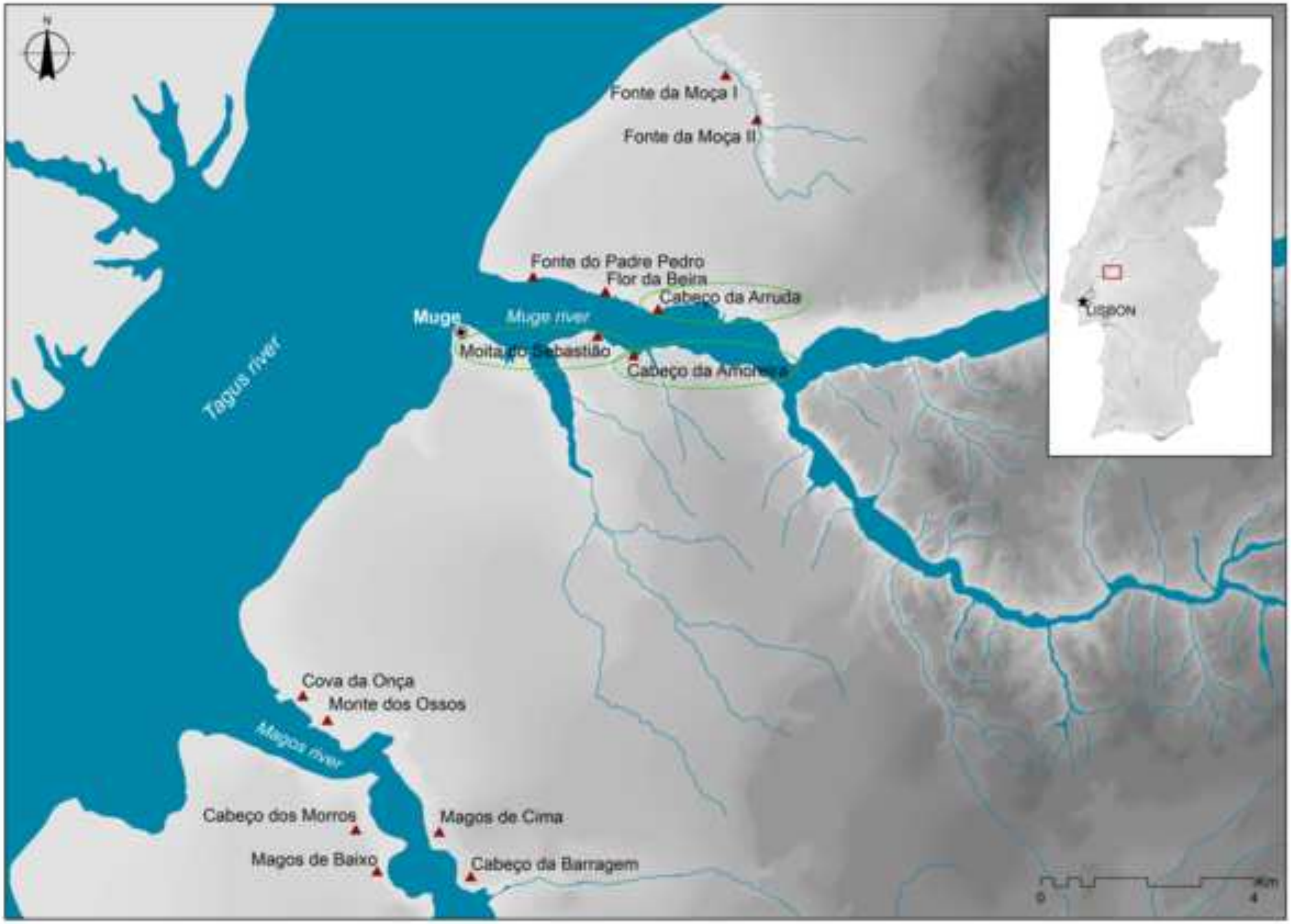

Figure2

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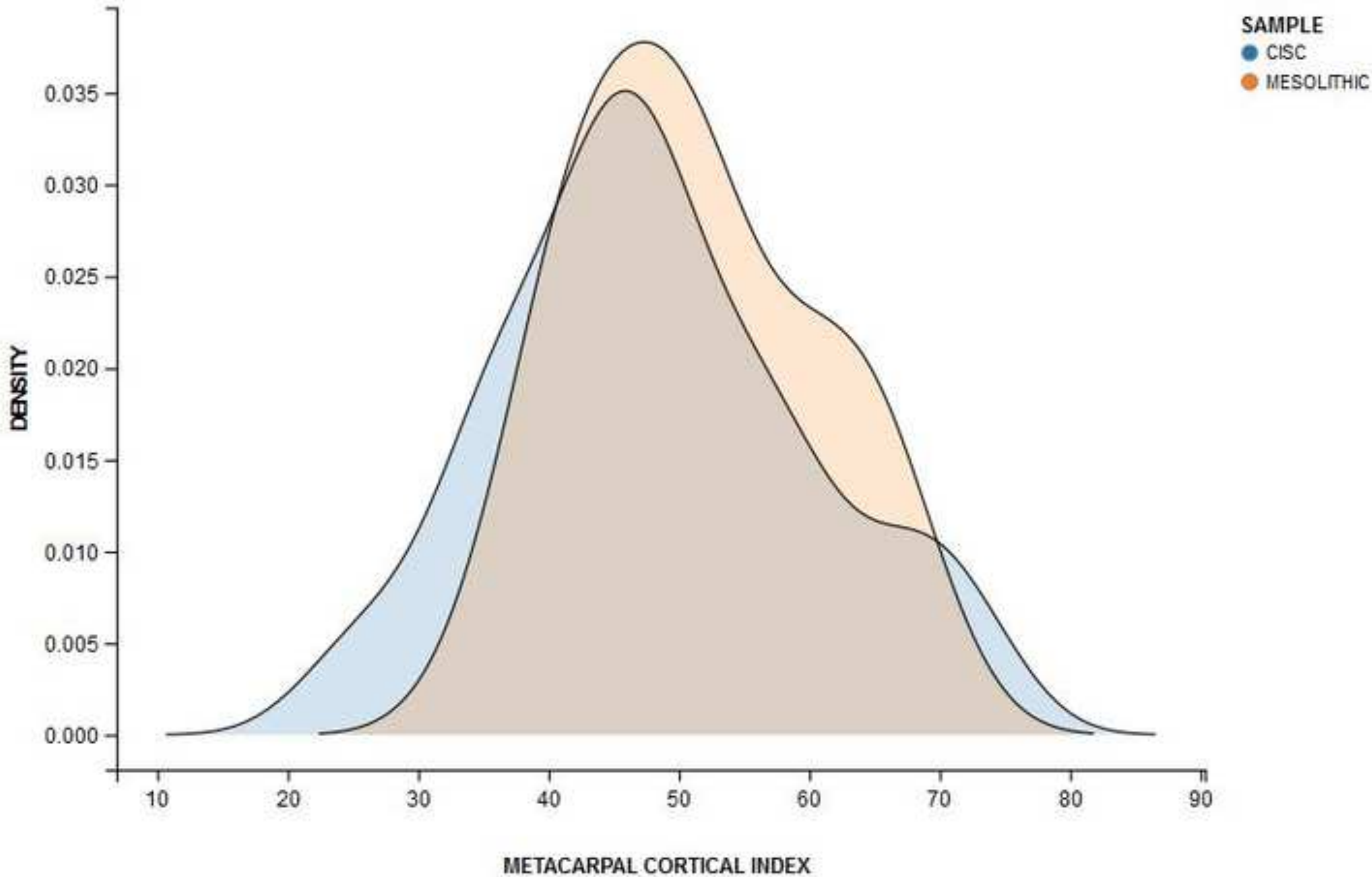

Figure3 Click here to download Figure Figure 3.JPG

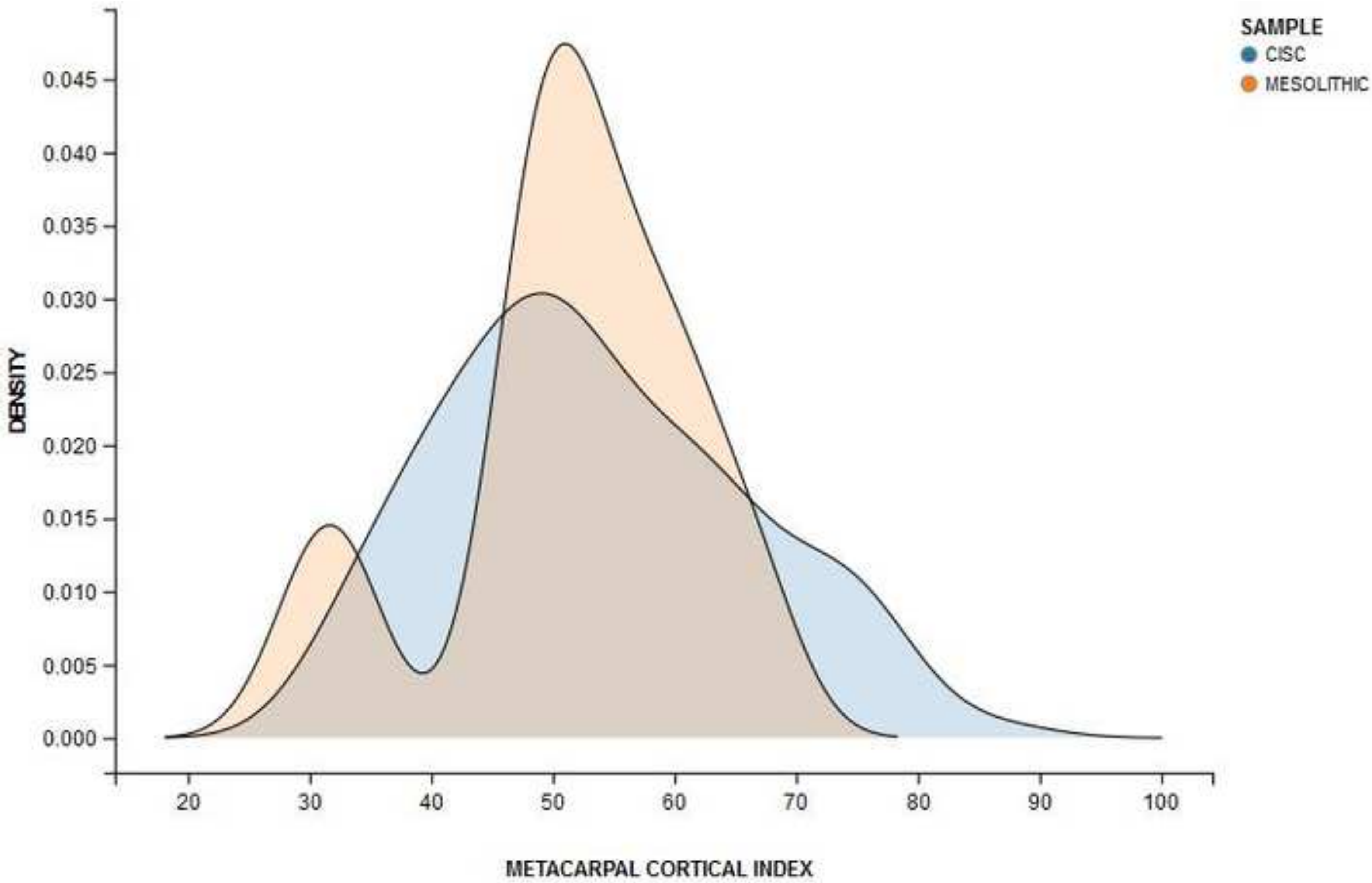

Figure4 Click here to download Figure Figure 4.JPG

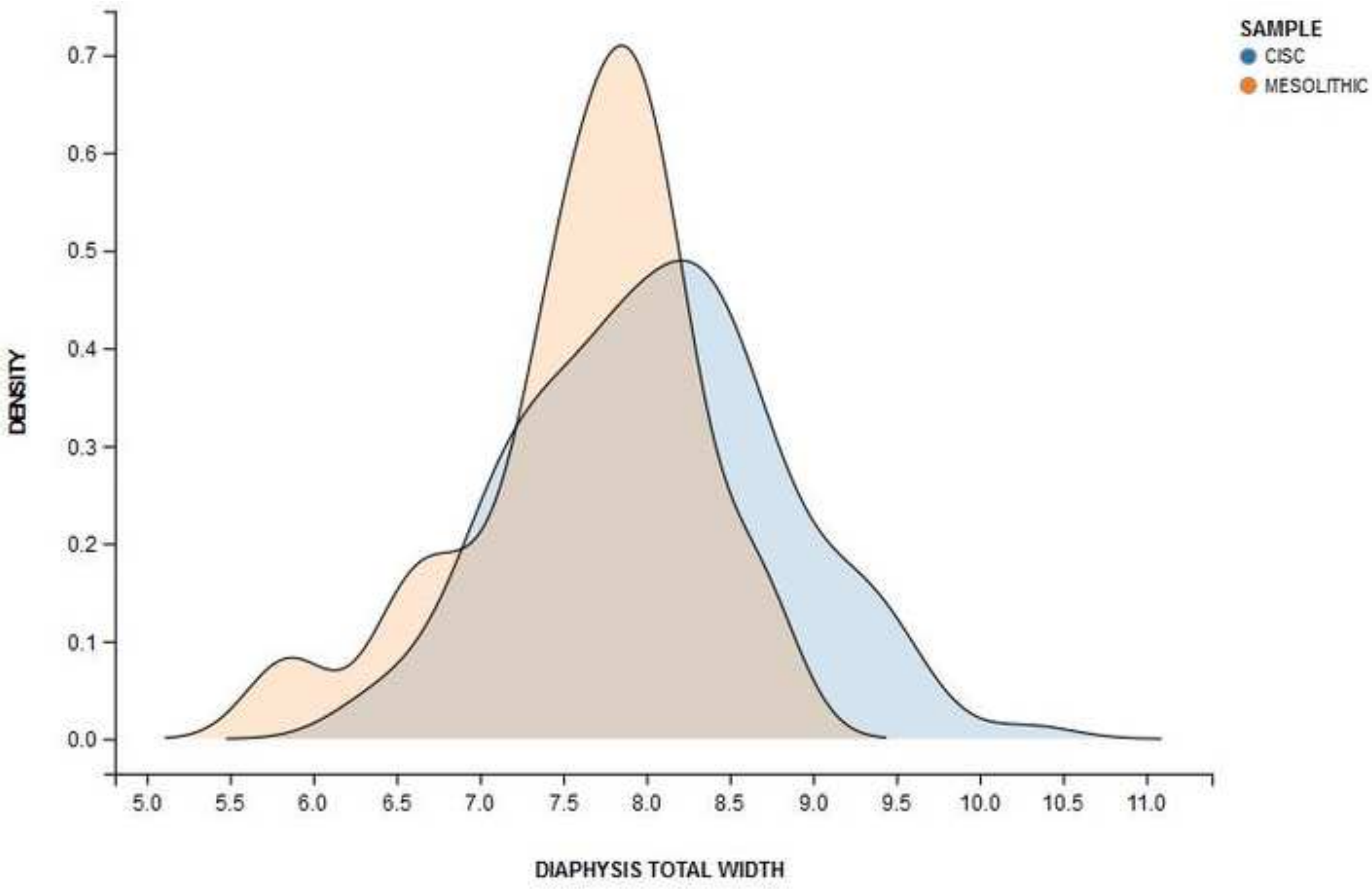

Figure5

Click here to download Figure Figure 5.JPG

Figure6

Click here to download Figure Figure 6.jpg

Figure7

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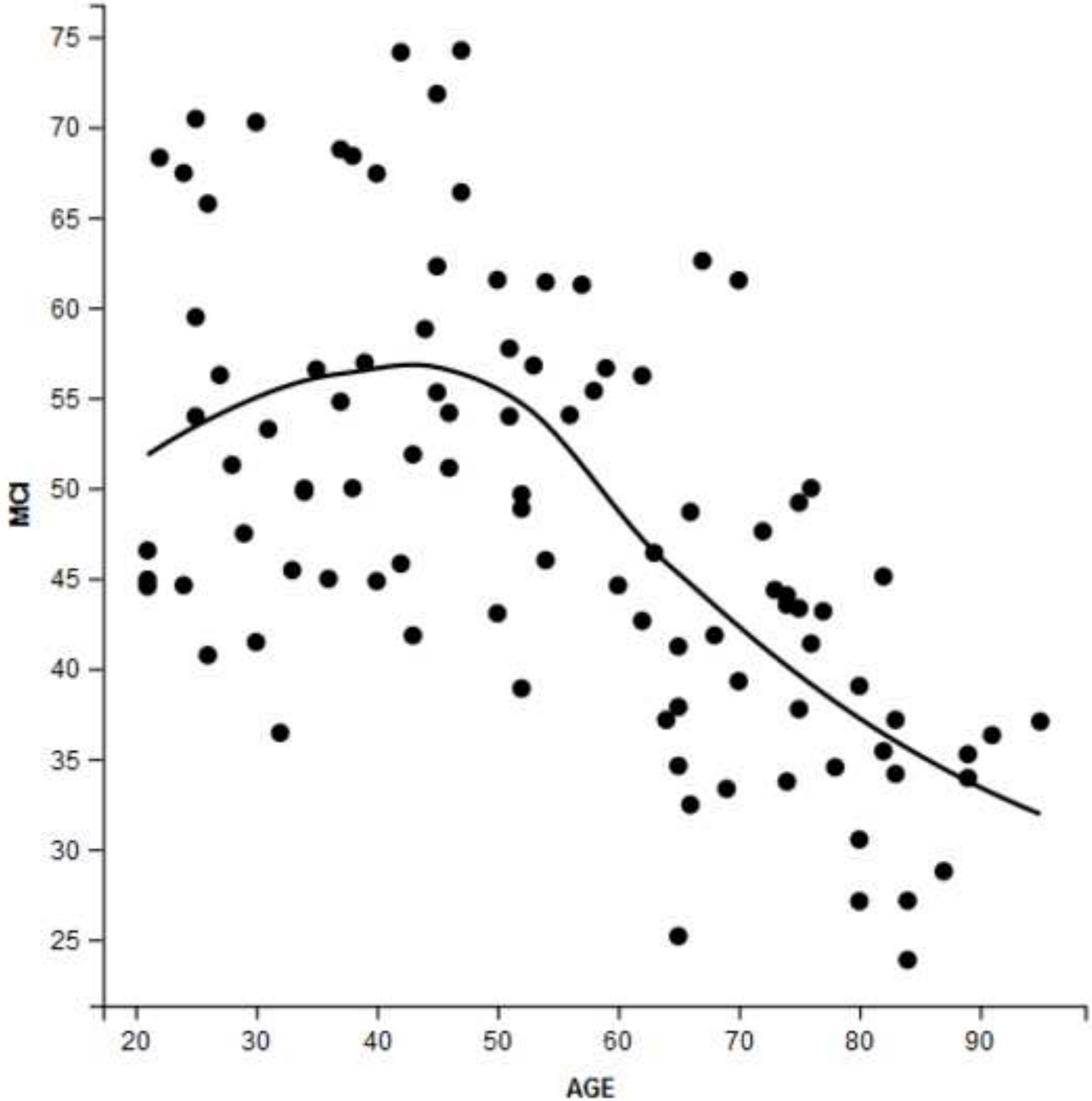

Dear Reviewer #1,

Thank you for the kind remarks and very helpful comments. To best cope with your remarks, a reply to each of your concerns is appended and/or intermingled to the comments.

«In the methods section the authors note 'supplementary medical imaging was performed when required' - please expand this.»

We added a note stating that all suspected fractures were radiographed.

«The small sample size is problematic for a number of reasons (see below), but is probably due to constraints on a) the number of Mesolithic skeletons (with the correct elements present) excavated, which will typically be low and b) access to all of the excavated material. The study would be improved with a larger sample size, incorporating other Mesolithic skeletons from shell middens in the Tagus Valley, but I do not know if more individuals have been excavated. I would like the authors to consider if further data collection is possible, and if it is I would encourage them to gather more data for the paper - but this is not a condition of acceptance, and it is likely to be unfeasible.»

«While the small sample size is discussed in the discussion, I feel it also needs to be noted in the results section. Larger sample sizes would increase the likelihood of the size differences noted in the descriptive statistics being statistically significant (I refer to my comment about sample size above).»

There are more Mesolithic skeletons in Portugal (as stated in the article, the real number of individuals is not known with accuracy), housed at the Natural Museum in Oporto, but, as of this date, we do not have the necessary permissions from the institution that curates the skeletons to study them. Of course, we believe it is only a matter of time to acquire those permissions which will allow us to enlarge sample size. Unfortunately, we do not know if data collection will be possible in the next months or – much more probably – in the next years. As such, new data are not available in a timely manner for inclusion in this special issue of Archaeological and Anthropological Sciences.

«The methods note that tests for normality were performed, but the results are not mentions (one assumes the samples are normally distributed). It would be helpful to indicate how the tests for normality were performed (on which groups of data). Because of size differences in the measurements, the sexes should be tested separately (risk of a bimodal distribution). ANOVA assumes that each of the groups are normally distributed - which would give groups of 6, 4 and 4 (for example) - were all of these normally distributed? This is difficult for the small number of samples used (and why ANOVA could be problematic, although the tests undertaken do meet the minimum case rule for ANOVA). Further discussion of normality testing is required.»

We added the parameters of skewness and kurtosis, as advised by Kline (2010), which configure the assumption of normality. We also tested normality with the Shapiro-Wilk method, which further supported "normality", but did not include the results in the text since many publications are advocating against the use of such tests as Shapiro-Wilk or Kolmogorov-Smirnov. Furthermore, and just to reinforce the statistical interpretations, we have re-run the analysis with a non-parametric test – with the same practical results (we did not include these tests in the text). As for ANOVA, we understand that the small group size can be a problem but ANOVA is considered a robust test against the normality assumption, tolerating violations to its normality assumption rather well (notwithstanding, the variables in these groups seem normally distributed). In any case, we also re-tested with the Kruskal-Wallis H test, achieving the same practical results.

«It is noted that clear patterns with regards to age were not seen for males - was this an expected result? Previous work has indicated that cortical bone loss happens at a slower rate in males and females (see papers by Mays in Am Jl Phys Anthropol, for example).»

We would say – based on both epidemiological and bioarchaeological data – that these results are not unexpected – bone loss is, of course, influenced by multiple factors, such as nutrition or physical activity, but the experience of menopause in older women will partially overwhelm the other etiological factors, something that does not happen in men.

«On p7 prevalence rates are given for fragility fractures - it should be noted if these are true prevalence rates, or crude prevalence rates. I don't think that the 95% CIs are strictly needed for the prevalence rates.»

For the overall sample, it is the crude prevalence. We also removed the 95% CIs.

«As noted above, I really enjoyed the discussion, and found it was very thorough. However, there was one variable I thought the authors should consider - Vitamin D is required to absorb calcium. Although good evidence for adequate calcium in the diet is presented, this section could be improved by also discussing if the samples had adequate access to Vitamin D (almost certainly obtained through sunlight in Portugal, as well as oily fish consumption), and if there was any evidence of rickets or osteomalacia in the populations (including children; which are not the focus of this paper) - I would expect to see little evidence of vitamin D deficiency, but adding this data would enhance the discussion.»

We added a note on the issue of Vitamin D as a contributing factor for bone health, also including the absence in the sample of skeletal indicators of vitamin D deficiency in non-adults and adults, as advised by Mays et al. 2008 and Brickley et al. 2007.

«Finally, I was surprised that none of the papers by Simon Mays on cortical bone loss were cited (Mays 2000, 2001, 2006, Am Jl Phys Anthropol).»

A broader reference to other archaeological samples was incorporated in the text, including these important works by Mays.

We tried to solve the problems with the figures and some parts of the text, as advised.

Our best regards,

The authors

Dear Reviewer #2

Thank you for the useful comments and advises, we expect to carefully cope with the questions identified in our paper. As recommended, we have considered in the discussion of our outcomes the relevant results from histomorphometric studies, namely those from Isola Sacra (Cho and Stout, 2003, 2011). Also, a note on the long time span of the studied Mesolithic sample was added to the limitations of this work.

Our best regards,

The authors