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METRIC DIFFERENCES IN ADULT SECOND METACARPAL BONES RELATED TO AGE-AT-DEATH AND THEIR COMPARISON BETWEEN RECENT AND HISTORICAL POPULATIONS

ABSTRACT: Hand bones can reflect external factors during ontogenesis and individual life history. The goal of this study was to examine age-related changes in the 2nd metacarpal in adulthood. Metacarpal bones of archaeologically excavated adult skeletons from three different medieval burial sites—Cedynia (Poland), Brno–Videňská Street (Czech Republic), and Dětkovice–Za zahradama (Czech Republic)—were collected, described, and scanned by means of a flatbed scanner in standardized dorsal and radial positions into two-dimensional images. On them, four measurements (one length and three widths) were taken on each view and subsequently subjected to statistical regression methods in order to quantify their relationship with age at death. These trends were compared with those in a documented sample of the Athens Human Skeletal Reference Collection of the recent Greek population. In females, no significant relationship between the length of the 2nd metacarpal and age at death (AAD) was observed. In females, mostly positive relationships between width measurements and AAD were observed, ranging typically between 3 to 7% over 30 years, with maximum of ca. 9% in midshaft width in the right hand in dorsal view. These relationships were more statistically significant for the recent than for the medieval sample which might be attributed to differences in sample size, and the nature of AAD (documented vs. estimated). In males, relationships between width measurements and AAD (i.e., an increase with age) were also prevalently positive but much lower than in females and mostly not statistically significant. The systematic increase of the width measurements in females and the differences from males of the same samples suggest certain specificities of women's life histories in adulthood that would be worth further investigation in terms of the influence of external factors. Potential methodological biases due to the cross-sectional nature of the samples and sampling selectivity are further discussed.

KEY WORDS: Metacarpal bones - Age-related changes - Life history - Age at death - Osteometry

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INTRODUCTION

The human hand represents a morphological and functional complex which is sensitive to variations in ontogenetic factors (Poznanski 1984) and suitable as an "indicator" structure in retrospective analysis of biological processes of human biology according to the skeleton after death. Even though small bony elements of the hand are frequently lost in skeletal finds in archaeology, metacarpals are relatively compact and usually well preserved which predisposes them to be potentially applicable in bioarchaeology (yet not utilized to sufficient extent) (Lewis 2001). The shape and size of the hand is controlled by genes and hormones. The differentiation of the fingers (as well as the urogenital system) is controlled by HOX genes (Kondo *et al.* 1997). The development of the hand is also controlled by sex hormones. It is known that the 2D:4D ratio negatively correlates with prenatal testosterone levels and positively with prenatal estrogen levels (Manning 2002). Higher levels of maternal distress are associated with higher fetal testosterone levels and lower offspring 2D:4D ratios (Cecchi, Duchoslav 2018).

Bone growth and development is also influenced by ethnicity (Ruff 2002), environmental factors (Holz *et al.* 2007), socioeconomic status (Silva *et al.* 2012) or diet (Prentice *et al.* 2006). Studies of the shape of the long bones of the hand usually use indices of bone length to bone width to describe bone proportions or bone tubulation. Various combinations of these ratios served to identify common hand shape variants (LaVelle Moerman 1981).

Our preliminary (unpublished) study on the recent osteological sample from The University of Athens Human Skeletal Reference Collection (Eliopoulos *et al.* 2007) revealed biologically important age effect during adulthood. The most sexually dimorphic measurements reflecting bone width of the 2nd metacarpal (in fact robustness) significantly increased between 40 and 50 years of age (at death) which was recorded in females but not in males. An important question arose whether this is a general trend of women's individual life history relating to cessation of HPG regulatory axis and forthcoming menopause (which is theoretically possible since hormonal changes in this age period are true biological changes with real physical consequences at an individual level) or it is specific only to samples of 20th century women (undergoing a secular change at a population level) and are given, at least partially, by cohort differences between older—earlier born (more robust) and younger—later born (more gracile)

generation of females (whatever proximate mechanism in fact involved in it—cohort inborn differences or cohort differences in physical load), or any other effect non-related with true age changes, e.g. hidden heterogeneity of frailty known within the frame of the so called osteological paradox. If the age difference resulted from true individual changes, we should explain the mechanism why the width of the bones increased with age. If it was caused by cohort effect, we should explain why it was limited only to women. The answer to these questions requires the acquisition of metacarpal bone samples from (a) other historical periods and (b) other populations. We are including skeletal samples from Early to High Middle Ages found in burial sites in the region of Moravia: Brno–Videňská ulice, Dětkovice–Za zahradama and one sample from Poland–Cedynia locality. It should be made clear that this study is not an attempt to create a new method for estimating age from metacarpal dimensions. The purpose of our study is to determine whether the trend in age changes of bone measurements for the two groups (modern vs. medieval) is similar and if it is different in sexes and, hence, could be attributed to hormonal changes in menopause in females.

MATERIAL AND METHODS

Reference sample

An archived database of scanned 2nd metacarpal bones from individuals of The University of Athens Human Skeletal Reference Collection was used as a reference sample. This collection consists of 225 skeletons of individuals who lived mainly in the second half of the twentieth century. These remains come from cemeteries in the area of Athens (Greece). The sex, age, cause of death and occupation was known for most of the individuals (Eliopoulos *et al.* 2007). Metacarpal bones from this collection show significant sexual dimorphism in their dimensions (Manolis *et al.* 2009).

In total, 432 images of 2nd metacarpal bones were used for this study. These images were scanned by one of the coauthors (M.K.) along with other bones (Králík *et al.* 2014). The 2nd metacarpal was chosen for this study because of its well specified sex differences (Králík *et al.* 2017) and also its documented progressive bone loss with age (Plato, Norris 1980). At the same time, it is the largest bone of the hand and is usually more morphologically uniform than the other bones of the hand (Plato, Norris 1980) which increases the chance of its preservation in archaeologically excavated skeletal samples.

Archaeological samples

Brno–Videňská Street site

The burial ground is placed in Brno (Czech Republic) on the western side of Videňská Street. It is situated on a steep eastern slope, at an altitude of 206–207 meters above sea level. The research took place here in the years 2009, 2011 and 2012–2013. It was a rescue archaeological research under the auspices of Archaia Brno, led by Lenka Sedláčková. In total, 440 graves from the second half of the 11th century were discovered. The locality was most intensively populated in the early Middle Ages, resp. in the Late Hillfort Period (Sedláčková 2012). The previous stages of this research were followed by another in 2014. During this research, additional graves were found, so the total number is 545 funerals. However, due to bone accumulations, the total number of buried individuals will be higher. Most of the dead were buried in coffins, although these have not been directly preserved (Černá *et al.* 2015). In total, 111 images of 2nd metacarpal bones of individuals from this locality were used for this study.

Dětkovice

Skeletal burial ground in Dětkovice, Prostějov district (Czech Republic), in the locality Za zahradama, has been known since the end of the 19th century, when archaeologist I. L. Červinka discovered graves dating to the 11th century. These graves were dated thanks to the discovery of a silver denarius from the period of King Andrew I of Hungary (Červinka 1902). The first of a series of rescue research on this necropolis took place in 2000 and was carried out by the Prostějov department of Institute for Archaeological Heritage (IAH, Czech: ÚAPP Brno) (Fojtík, Šmíd 2008). The fiber optic furrow then broke almost a dozen graves; two skeletal burials, also equipped with King Andrew I of Hungary coins, were examined by archaeological research. In 2009, the western edge of the burial ground with 27 graves was discovered in the area affected by the landscaping of the eroded road notch. Until 2013, several other archaeological excavations took place in this locality. During the research, 134 graves with skeletal remains of 131 individuals were discovered. The ratio of men and women at the burial ground was balanced. Children most often died between the first and third year of life, women in younger adulthood and men in older adulthood (Jungerová *et al.* 2016). In total, 77 images of 2nd metacarpal bones of individuals from this locality were used for this study.

Cedynia

The cemetery in Cedynia (Poland) was located on a hill, 200 m northwest of the stronghold, at the turn of

the 10th century (Porzeziński 2012). It was used till the end of the 11th century. Then, a second cemetery was established nearby, which functioned until the first half of the 14th century. The cemeteries served for socially diverse, predominantly medium-to-low status, inhabitants of both the stronghold and the surrounding settlement (Malinowska-Lazarczyk 1982, Porzeziński 2012). Archaeological excavations at both cemeteries recovered approximately 1600 well preserved skeletons, including approx. 16% of individuals under 15 years of age (Porzeziński 2012). In total, 299 images of 2nd metacarpal bones of individuals from this locality were used for this study.

Selecting and scanning of the bones

As the research focuses on changes in adulthood, only bones with completed postnatal growth were recorded, i.e. for the 2nd metacarpal bone, the head of the bone had to be fully fused to the body of the bone. Significantly damaged bones were excluded from further analyses.

We used a simple desktop scanner recording method, designed and tested in a previous study (Urbanová *et al.* 2006). The bones were placed one by one on the surface of Canon CanoScan 4400F scanner and scanned at a scale of 1:1 from the radial and dorsal side. Resulting 2D images were saved in TIFF format with a resolution of 600 dpi, with a color depth of 24 RGB. The bones from the site at Brno–Videňská Street and Dětkovice–Za zahradama were scanned during 2021 in Laboratory of Morphology and Forensic Anthropology (LaMorFa) at Faculty of Science, Masaryk University, Brno, Czech Republic. The bones from the Cedynia site were scanned at laboratory of osteological collections at the Institute of Human Biology and Evolution, Adam Mickiewicz University in Poznań, Poland during June and July 2021.

Measurements

Scanned images of the left 2nd metacarpals were flipped to pseudo-right. The purpose of this adjustment is to avoid systematic differences in the measurement of right and left side bones due to cognitive biases of the human eye. The tpsDig2 software was used for placing landmarks on the images (Rohlf 2006). Nine landmarks were placed on each of the images, eight of these landmarks were used to measure bone dimensions. One was used as an auxiliary point to place landmarks on the contour of the bone in the middle of its body. The location of each landmark is shown in *Figure 1*.

Definition of the landmarks in the images of the 2nd metacarpals from the dorsal view:

- 1 - the most distal point on the contour of the metacarpal head
- 2 - the most distal point on the concave contour in the area of the carpometacarpal junction
- 3 - radial contour of the bone body at half the distance between points 1 and 2
- 4 - ulnar contour of the bone body at half the distance between points 1 and 2
- 5 - the most radial point on the distal radial tubercle, most often at the maximum of the curvature
- 6 - the most ulnar point on the distal ulnar tubercle, most often at the maximum of the curvature
- 7 - point on the radial contour of the base where the body of the bone ends and the ligament tendon begins
- 8 - the most ulnar point on the ulnar contour of the base, near the end of the body of the bone
- 9 - auxiliary point halfway between points 1 and 2

Definition of the landmarks in the images of the 2nd metacarpals from the radial view:

- 1 - the most distal point on the contour of the metacarpal head
- 2 - the most proximal point on the contour of the proximal articular surface
- 3 - palmar contour of the body of the bone at half the distance between points 1 and 2
- 4 - dorsal contour of the body of the bone at half the distance between points 1 and 2
- 5 - the most palmar point on the palmar contour of the metacarpal head
- 6 - the most dorsal point on the dorsal contour of the metacarpal head
- 7 - the most palmar point on the palmar contour of the base, not on the articular surface
- 8 - the most dorsal point on the dorsal contour of the base
- 9 - auxiliary point halfway between points 1 and 2

From Cartesian coordinates of the landmarks, their inter-landmark distances were calculated using the PAST software (Hammer *et al.* 2001) and calibrated to millimeters. Four of these distances on each view were used for this study:

Length of the bone (L) - the total length of the bone computed as the distance between landmarks 1 and 2.

Midshaft Width of the bone (MW) - this dimension was obtained by placing an additional landmark 9 in the middle of the line between landmarks 1 and 2 and placing landmarks in the same proximo-distal position but at the edges of the bone (landmarks 3 and 4)

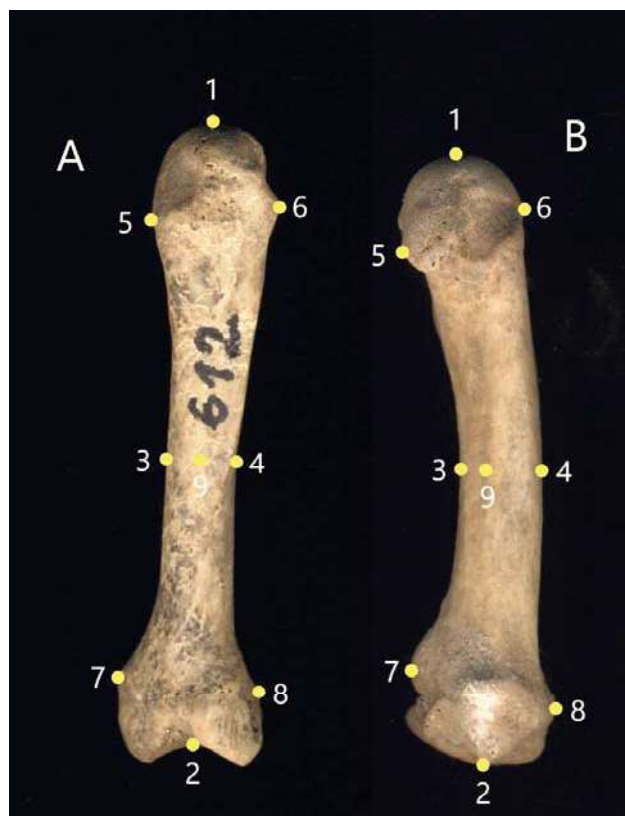


FIGURE 1: Definition of landmarks on the images of the 2nd metacarpal bones from dorsal view (A) and radial view (B).

Head width (HW) - the largest distance between ulnar and radial side of head of the bone for dorsal view or the largest distance between palmar and dorsal side of head of the bone for radial view (landmarks 5 and 6)

Base width (BW) - the largest distance between ulnar and radial side of base of the bone for dorsal view or the largest distance between palmar and dorsal side of base of the bone for radial view (landmarks 7 and 8).

Age at death and biological sex

In the recent Athens sample, age at death (Age at Death, AAD) and biological sex (Sex) were documented. In archaeological samples, AAD and sex had to be estimated.

Sex and age at death are very important factors for this study. These variables were known for the samples from Brno and Dětkovice in advance, because they had already been examined for purpose of other studies. The Cedynia sample was examined by A. Š. in 2021 for the purpose of this study.

Brno-Videňská Street

Two methods for sex assessment on pelvis were used for this sample, specifically the *Diagnose sexuelle probabiliste* program (DSP) (Murail *et al.* 2005) and the Brůžek method (Bruzek 2002). For the estimation of the age at death, methods focusing on the estimation of age according to age changes on the *facies symphysialis*, *facies auricularis*, the joint socket of the hip and changes in the internal structure of the heads of long bones were chosen (Todd 1920, Schranz 1959, Lovejoy *et al.* 1985, Brooks, Suchey 1990, Szilvássy, Kritscher 1990, Buckberry, Chamberlain 2002, Rissech *et al.* 2012). These assessment were made by Kopecký for the purpose of his thesis (Kopecký 2015). The age at death estimation was also made based on the relative pulp size of canines in X-ray images (Cameriere *et al.* 2007) and carried out by Morávek for the purpose of his thesis (Morávek 2017).

Dětkovice-Za zahradama

Several morphoscopic (Steele 1976, Černý, Komenda 1980, Novotný 1985, Murail *et al.* 2005, Ousley, Jantz 2013) and morphometric (Bruzek 2002, Walrath *et al.* 2004) methods were used for sex determination of this sample. Age at death was estimated on the basis of the degree of abrasion of permanent dentition (Lovejoy 1985) and age degenerative changes in the relief of the articular surfaces of the *facies symphysialis* (Todd 1920, Brooks, Suchey 1990) and *facies auricularis* (Lovejoy *et al.* 1985, Buckberry and Chamberlain 2002) on pelvis (Jungerová *et al.* 2016).

Cedynia

For the age at death estimation, several morphometric and morphoscopic methods on pelvis, sacrum, ribs and clavicle were used (Todd 1920, Lovejoy *et al.* 1985, Boldsen *et al.* 2002, DiGangi *et al.* 2009, Passalacqua 2009, Langley-Shirley, Jantz 2010, Calce 2012). Three morphometric methods on pelvis and humerus were used for the sex estimation (Černý, Komenda 1980, Novotný 1986, Murail *et al.* 2005).

Statistical analyses

All data analyses were performed in *R*-software (R Core Team 2021) and supplementary packages *psych* (Revelle 2021), *ggplot2* (Wickham 2016), and others mentioned below.

The radial and dorsal views on the bones were analyzed separately. If a method of estimating age at death provided a point estimate, we used this one. If a range of ages was an estimate (an interval), we used the midpoint of this interval for statistical purposes.

Population statistical parameters of AAD differed significantly between the *recent* sample and the archaeological samples. The mean AAD of the archaeological samples was significantly lower than mean AAD in the recent sample, and, as a consequence, the variance of AAD in the archaeological samples was lower than the variance of AAD in the recent sample (see *Appendix 1*). It cannot be clearly stated to what extent this difference reflects real differences and to what extent it is a consequence in the methodological origin of the AAD, a combination of both mechanisms cannot be ruled out; in the recent sample AAD was represented by exactly documented values, whereas in the archaeological samples it was represented by estimations based on various osteological methods, their discriminating power is low especially at older ages. However, if we were to include both samples in a single comparison, this discrepancy would affect the analysis of the relationships of the measurements on AAD. Therefore, we analyzed the recent sample and the archaeological samples separately.

On the other hand, the sizes (numbers of cases) of archaeological samples were smaller and it was appropriate to merge them. Using Kruskal-Wallis ANOVA, we did not reject the agreement of AAD means in the females ($n=61$, K.-W. statistics=0.382, $df=2$, p -value=0.826). We found a difference in AAD in the male samples ($n=110$, K.-W. statistics=11.7, $df=2$, p -value=0.003), with males from the Dětkovice site systematically dying at a higher age than males from the other two archaeological samples (Cedynia and Brno-Videňská Street), see *Appendix 1*. As our study was primarily focused on the effect of ageing and women's life histories, for the purpose of statistical testing the association of measurements with AAD, we combined all archaeological samples into one, which we labelled *medieval*.

Each bone measurement was then statistically described in each assessed group by means of descriptive statistical parameters. Effects of sex and sample, including their interactions, were tested by means of Art ANOVA (Analysis of Variance of Aligned Rank Transformed Data) using R-package *ARTool* (Kay *et al.* 2021).

Since the nature of the AAD values distribution cannot be taken as perfectly continuous in the archaeological samples, strength of this relationship (measurements vs. AAD) was expressed through the Spearman rank order correlation coefficient. Direction of the linear relationships between measurements and AAD were expressed and tested by Siegel version (Siegel

1982) of nonparametric Kendall–Theil regression using R-package *mblm* (Komsta 2019). This is fully nonparametric method to linear regression which for each point computes $n-1$ lines connecting it with all other points, computes median slope of these lines, repeated it for all points, and the slope of the final model represents median value of all these medians. Analysis of Variance (ANOVA) of the regression model was used for testing statistical significance of the linear relationship.

In addition to assessing the regression model according to the direction of possible change and the statistical significance of the variance extracted by the regression model, we also calculated the percentage change in a given bone dimension as the ratio of the slope of the regression line to the mean value of the dimension in a given group (in %). In addition to assessing the regression according to the direction of possible change and the statistical significance of the variance extracted by the regression model, we also calculated the average *percentage change* in a given bone dimension as the ratio of the slope of the regression line to the mean value of the dimension in a given group (in %). To make this change more readily interpretable, we multiplied this "relative slope" by thirty, which then represents the percentage change in bone dimension over 30 years. This allows us to compare changes not only between groups but also between dimensions.

The relationship between size and AAD was also expressed non-linearly using smoothing splines, and we visually compared any differences in the pattern of this relationship between age categories: up to 35 years, 35–50 years, more than 50 years.

RESULTS

Descriptive statistics

Descriptive statistics of the AAD in each sample is available in *Appendix 1* and *Figure 2*. Descriptive statistics for each measurement in both views are given in *Tables 1–8* (numerical descriptive statistics for each archaeological sample separately are given in *Appendix 2*), and boxplots for these measurements are visualized in *Appendix 3*. In all measurements, males have on average higher values of measurements than females.

Correlations and linear models

Correlograms of the relationships of the measurements of the 2nd metacarpals with AAD and among themselves are presented in *Appendix 4*. The linear models, both parametric (simple linear regression) and non-

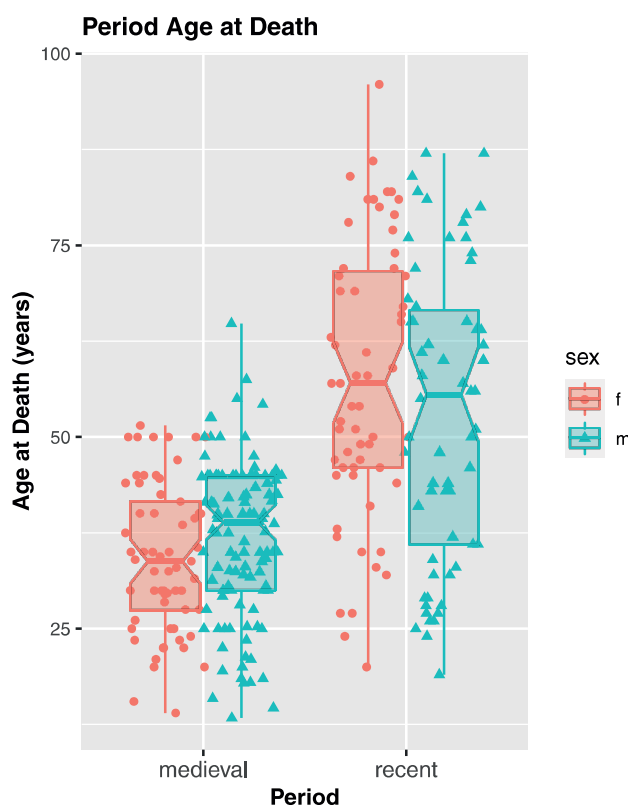


FIGURE 2: Boxplots augmented with scatter plots of Age at Death in the medieval and recent sample, separately for males (m, blue triangles) and females (f, red dots), thick horizontal – median, notch – 95%CI for median, box – interquartile range, whiskers – non-outlier range.

parametric (Kendall–Theil regression), are shown in scatterplots in *Appendix 5*. While the course of the parametric and nonparametric models is not exactly the same, the sense of the resulting trend is. Values of the coefficients of non-parametric Kendall–Theil regression can be seen in *Figure 3*, and the significance of the linear trends is presented in *Table 9* and *10*, containing results of analysis of variance for the non-parametric linear models.

Neither by correlation nor by linear model did we observe any relationship between bone length and AAD in females. This was true for both recent and medieval sample, left and right side, and both views of the bone. However, the situation was different for all of the width measurements of the bone.

In females of the *recent* sample, we observed a positive relationship with AAD for all width measurements (MW, HW and BW) and in all groups tested (on both sides

TABLE 1: Descriptive statistics of the length of the second metacarpal from the dorsal view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Length – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medieval	dex	f	43	63.69	3.43	63.94	63.71	56.01	71.46	-0.16	-0.42	0.52
recent	dex	f	50	61.99	3.03	61.86	62.05	54.11	68.54	-0.18	-0.12	0.43
medieval	sin	f	40	63.25	3.17	62.74	63.23	55.73	69.61	0.01	-0.43	0.50
recent	sin	f	54	61.21	3.15	60.88	61.19	53.81	67.96	0.07	-0.62	0.43
medieval	dex	m	89	68.19	3.13	68.25	68.20	61.05	76.13	-0.03	-0.15	0.33
recent	dex	m	48	66.64	3.65	66.66	66.56	57.34	75.81	0.11	0.21	0.53
medieval	sin	m	78	68.28	3.07	68.13	68.23	60.58	76.76	0.15	0.14	0.35
recent	sin	m	58	66.49	3.40	66.45	66.38	59.74	76.11	0.37	0.12	0.45

TABLE 2: Descriptive statistics of the length of the second metacarpal from the radial view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Length – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medieval	dex	f	42	66.40	3.51	66.88	66.47	58.89	73.71	-0.29	-0.41	0.54
recent	dex	f	43	64.99	2.66	64.43	64.92	60.20	71.23	0.28	-0.51	0.40
medieval	sin	f	36	65.78	3.33	65.38	65.77	57.84	72.53	-0.07	-0.41	0.56
recent	sin	f	47	63.49	3.09	63.13	63.45	56.26	69.78	0.07	-0.63	0.45
medieval	dex	m	85	71.24	3.22	71.40	71.26	64.18	78.38	-0.10	-0.45	0.35
recent	dex	m	40	69.68	3.50	69.50	69.50	63.71	79.57	0.47	0.31	0.55
medieval	sin	m	74	71.29	3.25	71.40	71.27	63.68	79.75	0.02	-0.21	0.38
recent	sin	m	55	69.36	3.62	69.36	69.18	62.59	79.87	0.47	0.20	0.49

TABLE 3: Descriptive statistics of the midshaft width of the second metacarpal from the dorsal view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Midshaft Width – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medieval	dex	f	43	7.68	0.66	7.87	7.69	6.31	9.06	-0.14	-0.90	0.10
recent	dex	f	50	7.81	0.58	7.75	7.81	6.73	9.23	0.15	-0.32	0.08
medieval	sin	f	40	7.46	0.58	7.49	7.48	6.01	8.51	-0.29	-0.58	0.09
recent	sin	f	54	7.48	0.64	7.47	7.45	6.31	9.23	0.46	-0.27	0.09
medieval	dex	m	89	8.76	0.80	8.76	8.75	6.69	10.58	0.13	-0.29	0.09
recent	dex	m	48	8.87	0.67	8.81	8.85	7.45	10.46	0.33	-0.61	0.10
medieval	sin	m	78	8.47	0.77	8.38	8.43	6.69	10.54	0.39	-0.22	0.09
recent	sin	m	58	8.64	0.62	8.66	8.66	7.11	10.20	-0.20	-0.29	0.08

TABLE 4: Descriptive statistics of the midshaft width of the second metacarpal from the radial view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Midshaft Width – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medieval	dex	f	42	7.96	0.72	7.83	7.92	6.39	10.03	0.57	0.43	0.11
recent	dex	f	43	7.92	0.59	8.04	7.92	6.82	9.14	-0.01	-0.92	0.09
medieval	sin	f	36	7.87	0.66	7.75	7.82	6.48	9.78	0.71	0.65	0.11
recent	sin	f	47	7.67	0.57	7.62	7.65	6.56	9.06	0.19	-0.62	0.08
medieval	dex	m	85	9.24	0.74	9.27	9.23	7.62	11.35	0.20	0.05	0.08
recent	dex	m	40	8.94	0.72	8.89	8.95	7.45	10.50	-0.03	-0.5	0.11
medieval	sin	m	74	8.97	0.67	8.95	8.97	7.58	11.13	0.18	0.14	0.08
recent	sin	m	55	8.78	0.73	8.72	8.78	7.24	10.50	0.15	-0.59	0.10

TABLE 5: Descriptive statistics of the head width of the second metacarpal from the dorsal view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Head Width – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medieval	dex	f	43	13.75	1.14	13.54	13.66	11.97	16.87	0.72	0.21	0.17
recent	dex	f	50	13.69	1.02	13.53	13.67	10.98	16.00	0.13	-0.11	0.14
medieval	sin	f	40	13.70	1.12	13.67	13.70	11.05	16.17	0.01	-0.05	0.18
recent	sin	f	54	13.40	0.98	13.45	13.43	10.75	15.41	-0.36	0.29	0.13
medieval	dex	m	89	15.75	1.13	15.63	15.68	13.56	20.07	0.93	1.64	0.12
recent	dex	m	48	15.37	1.06	15.41	15.40	12.32	17.92	-0.37	0.51	0.15
medieval	sin	m	78	15.20	1.03	15.28	15.19	13.00	17.95	0.10	-0.58	0.12
recent	sin	m	58	15.24	0.96	15.33	15.24	13.34	17.25	-0.02	-0.79	0.13

TABLE 6: Descriptive statistics of the head width of the second metacarpal from the radial view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Head Width – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medieval	dex	f	42	13.26	0.95	13.09	13.20	11.11	15.66	0.49	0.18	0.15
recent	dex	f	43	13.19	0.83	13.27	13.18	11.63	15.32	0.12	-0.21	0.13
medieval	sin	f	36	13.07	0.83	12.96	13.05	11.39	15.33	0.38	0.20	0.14
recent	sin	f	47	12.96	0.99	12.99	12.92	11.05	15.44	0.37	-0.17	0.14
medieval	dex	m	85	14.96	1.14	14.78	14.94	12.38	18.35	0.26	-0.17	0.12
recent	dex	m	40	14.81	0.95	14.71	14.79	13.13	17.14	0.31	-0.52	0.15
medieval	sin	m	74	14.60	1.01	14.54	14.59	11.30	17.61	-0.04	1.10	0.12
recent	sin	m	55	14.64	1.04	14.65	14.59	12.77	17.81	0.58	0.09	0.14

TABLE 7: Descriptive statistics of the base width of the second metacarpal from the dorsal view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Base Width – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medieval	dex	f	43	14.38	1.09	14.24	14.35	11.93	16.99	0.30	-0.17	0.17
recent	dex	f	50	14.22	0.87	14.25	14.23	12.04	16.12	-0.13	-0.42	0.12
medieval	sin	f	40	14.31	1.01	14.25	14.37	11.51	15.85	-0.45	-0.17	0.16
recent	sin	f	54	13.99	1.04	14.02	13.99	11.46	16.45	-0.06	-0.30	0.14
medieval	dex	m	89	16.64	1.29	16.84	16.68	13.65	19.18	-0.28	-0.76	0.14
recent	dex	m	48	16.29	1.14	16.08	16.28	13.08	19.70	0.19	0.92	0.16
medieval	sin	m	78	16.48	1.27	16.56	16.52	13.33	18.95	-0.31	-0.51	0.14
recent	sin	m	58	16.04	1.08	15.93	16.08	13.50	17.91	-0.25	-0.51	0.14

TABLE 8: Descriptive statistics of the base width of the second metacarpal from the radial view; dex – right, sin – left, f – female, m – male, n – number of cases, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Base Width – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
medievall	dex	f	42	14.80	1.10	14.65	14.73	12.94	17.10	0.49	-0.51	0.17
recent	dex	f	43	14.72	0.91	14.62	14.68	12.81	17.47	0.57	0.53	0.14
medieval	sin	f	36	14.44	0.76	14.40	14.41	13.06	16.68	0.43	0.47	0.13
recent	sin	f	47	14.25	0.97	14.20	14.21	12.54	17.36	0.58	0.59	0.14
medieval	dex	m	85	16.41	0.92	16.40	16.43	14.21	18.55	-0.18	-0.28	0.10
recent	dex	m	40	16.30	1.16	16.25	16.31	13.98	18.42	-0.01	-0.78	0.18
medieval	sin	m	74	16.09	1.00	16.21	16.12	13.54	18.23	-0.38	-0.35	0.12
recent	sin	m	55	16.04	1.18	15.83	16.03	13.45	18.31	0.12	-0.77	0.16

and from both views), and there is an agreement between Spearman rank order correlation coefficient and linear models. Variances extracted by the linear models were almost always statistically significant, except for BW on the left hand from the dorsal view, where the significance of the test was borderline ($p=0.056$) (Table 10). As the models show, the slope of the regression line varies by absolute size of each measurement (Figure 3) and they are generally positive in direction, i.e., increase. The slope of regression lines (i.e., change per 1 year) seems generally small if expressed in millimeters per year (e.g., for HW slope=0.031mm/year) but when expressed as a percentage change over the period of 30 years (Figure 4), the change is noticeable. In recent females, the increase varies between ca. 3 and 7% per 30 years with the highest change in the right HW measurement.

In females of the *medieval* sample, there was also an overall positive relationship between the width measurements and AAD, except for HW in dorsal view on the right side and MW in radial view on the left side. However, the positive trend was statistically significant only for MW in dorsal view on the right hand and HW in radial view on the left hand, although the result was borderline for the other three tests (Table 10). Although in the medieval sample the relationship between the width measurements and AAD did not reach the statistical significance that we observed in the women of the recent sample, the percentage change (increase) in bone width measurements with AAD over 30 years is similar to that of the recent sample. Although in some measurements and groups the increase was smaller (MW in the radial view of the left hand, HW in the

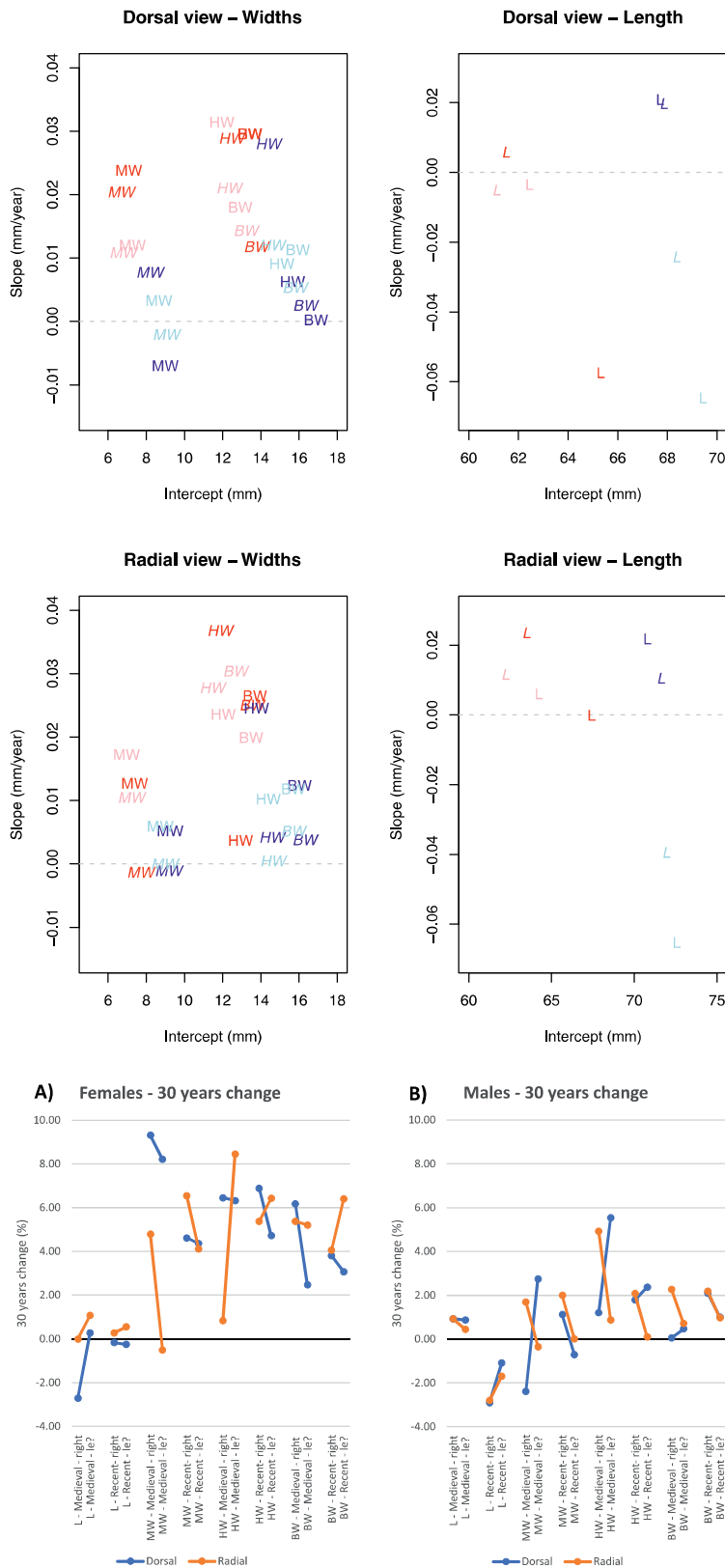


FIGURE 3: Values of regression coefficients (x-axis – intercept, y-axis – slope) of the nonparametric Kendall–Theil regression for the relationship between 2nd metacarpal measurements and age at death (AAD); measurements – expressed by their abbreviations, blue color – males, red color – females, saturated color – medieval, light color – recent, normal font – right hand, italics – left hand.

FIGURE 4: Values of regression slopes of the nonparametric Kendall–Theil regression for the relationship between 2nd metacarpal measurements and age at death (AAD) in females (A) and males (B); slopes are expressed as relative change for 30 years in percentage (%) of mean value of respective measurement (for a given group), blue dots and lines – dorsal views, red dots and lines – radial views.

TABLE 9 Results of Analysis of Variance of the nonparametric Kendall–Theil regression models of relationship between of the right second metacarpal measurements (in radial and dorsal view) and age at death (AAD) for each sample separately in *males*.

Males									
		Right hand				Left hand			
		Dorsal view		Radial view		Dorsal view		Radial view	
		F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Recent	Length	3.32	0.08	4.50	0.04	0.81	0.37	1.57	0.22
	Midshaft width	0.84	0.36	0.76	0.39	0.04	0.85	0.0001	0.99
	Head width	1.85	0.18	1.28	0.26	3.58	0.06	0.44	0.51
	Base width	2.05	0.16	1.12	0.30	0.34	0.56	0.54	0.47
Medieval	Length	0.39	0.53	0.40	0.53	0.34	0.60	0.08	0.80
	Midshaft width	0.66	0.42	0.42	0.52	0.85	0.36	0.02	0.89
	Head width	0.27	0.60	4.01	0.049	5.79	0.02	0.13	0.72
	Base width	0.0004	0.98	1.58	0.21	0.03	0.90	0.10	0.80

TABLE 10 Results of Analysis of Variance of the nonparametric Kendall–Theil regression models of relationship between of the right second metacarpal measurements (in radial and dorsal view) and age at death (AAD) for each sample separately in *females*.

Females									
		Right hand				Left hand			
		Dorsal view		Radial view		Dorsal view		Radial view	
		F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Recent	Length	0.02	0.88	0.06	0.80	0.05	0.83	0.21	0.65
	Midshaft width	6.90	0.01	12.32	0.001	5.20	0.03	4.92	0.03
	Head width	21.35	0.00003	12.34	0.001	9.95	0.003	14.13	0.0005
	Base width	6.87	0.01	7.50	0.01	3.82	0.06	16.60	0.0002
Medieval	Length	1.01	0.32	0.00	1.00	0.01	0.93	0.12	0.73
	Midshaft width	4.67	0.04	1.16	0.29	3.82	0.06	0.01	0.92
	Head width	0.0004	0.98	0.06	0.82	2.10	0.16	7.08	0.01
	Base width	2.92	0.10	2.11	0.15	0.45	0.51	3.09	0.09

radial view of the right hand), in others it was greater than in the females of the recent sample. The largest increase was observed in MW in dorsal view on the right hand, where it reached more than 9% over 30 years.

In the males of the *recent* sample, only one statistically significant relationship was found, namely a negative association between length and AAD in radial view on the right hand, and the relationship between AAD and length in dorsal view on the right hand was of borderline statistical significance. None of the width measurements

correlated statistically significantly with AAD in any of the views on either side of the body (*Table 9*). The change in the bone length (L) with AAD was negative and although statistically significant in radial view, the percentage was at most up to 3% of the length over 30 years. The changes in the width measurements were mostly positive on average, but very weak, with values for individual models between -1% and 3%.

In the males of the *medieval* sample, most of the observed relationships were weak and statistically

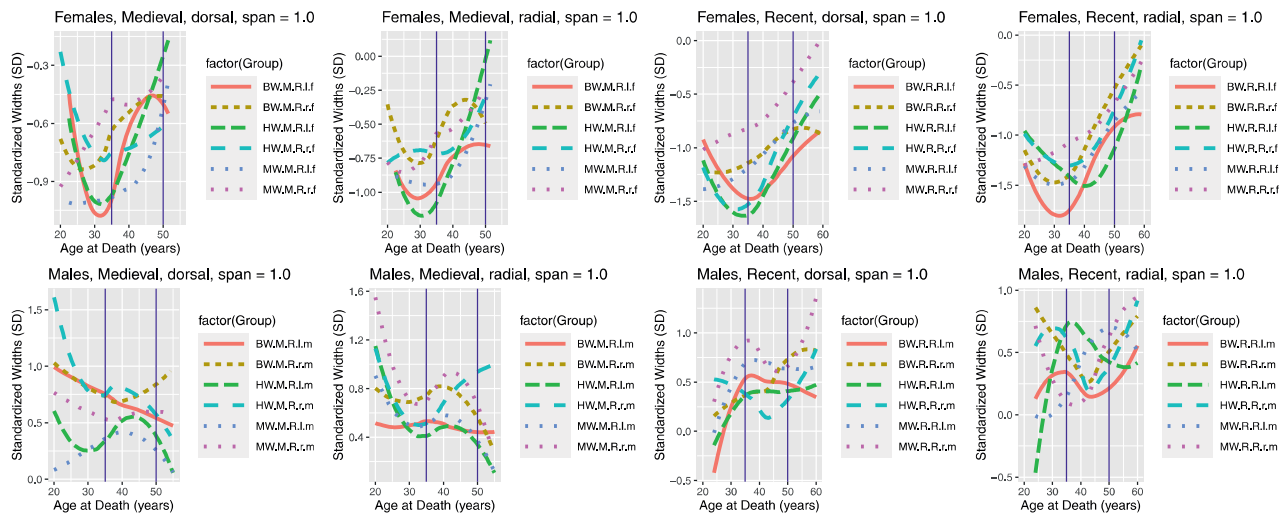


FIGURE 5: Smoothing splines (span=1.0) representing nonlinear relationships between standardized width measurements of the 2nd metacarpal (y-axis) and age at death (AAD, x-axis); in each combination of sex, sample and view all three width measurements of both sides are superimposed; abbreviations for each spline are: measurements (MW, HW, BW) followed with sample (M – Medieval, R – Recent), then view (D – dorsal, R – radial), followed with body side (r – right, l – left), and finished with sex (m – males, f – females); blue verticals represent borders of 35 and 50 years.

insignificant, except for the relationship of AAD with HW in radial view on the right hand and HW in dorsal view on the left hand, for which the change was about 5% over 30 years. Overall, the correlations of AAD with 2nd metacarpal measurements in the males of the medieval sample are similar to the situation in males of the recent sample, but there was no negative relationship between AAD and the bone length.

Smoothing splines

Using smoothing splines, we looked to see if a certain non-linear interleaving of the relationship between size and AAD (Figure 5, Appendix 5) would show any differences between age phases in this relationship (young adulthood to about 35 years vs. middle age to about 35 to 50 years), and whether the recent and middle age samples were similar or different in this. Between the ages of 35 and 50, there are no significant changes in width measurements in dorsal view for either recent or medieval males. In the radial view, there is a noticeable increase in the MW dimension on the right side for the males in the recent sample. The situation is different for the length of the 2nd metacarpal bone (Appendix 5). In the recent males, the spline for this dimension tends to decrease between 35 and 50 years on both the right and left hands in both dorsal and radial views, whereas in the males from the medieval sample, the decrease is evident on the right hand

in both dorsal and radial views. In females of the recent sample aged 35 to 50 years, the spline expresses an increase in all width dimensions on the right and left hand in both dorsal and radial views. In females of the medieval sample, an increase in width dimensions can also be observed. The spline for the length (Appendix 5) of the 2nd metacarpal bone has an increasing trend in females between the ages of 35 and 50, on both the right and left hand in dorsal and radial views in both the recent and medieval samples.

DISCUSSION

The main objective of this study was to determine the relationship between the dimensions of the 2nd metacarpal and age in archaeological samples and to compare it with a similar relationship in a documented recent sample. Our main findings were overall positive associations of the width measurements of the 2nd metacarpal with age, but these relationships were clearly stronger for females than for males. Although the relationships were statistically more conclusive in the females of the recent sample than in the females of the medieval sample, the differences in statistical significance between the samples may be due to differences in the structure of the data, such as the generally smaller range of ages in the medieval sample,

and the fact that the medieval sample estimates of age at death (and by very different methods) rather than precisely documented values were available. Various biases associated with AAD estimation errors can blur the clarity of the observed relationships in the medieval sample. Despite these differences, in the medieval and recent samples, the rate of percent change in bone dimensions per AAD (i.e., the average quantitative change) was similar in both samples, which applied both for women and men. This suggests that the observed trends are real and their interpretation, including possible biological causation, should be addressed.

A limitation of our study is the fact (which is difficult to overcome for skeletal samples of deceased persons) that the samples used, both recent and archaeological, were cross-sectional in nature and not longitudinal. The average trends found, studied and expressed by correlation and regression, do not represent a record of actual age changes at the individual level, but only statistical differences between people of different ages. These people certainly differ in characteristics other than age, and this is undoubtedly reflected in the recorded trends, without us having any control over these differences. As we know from studying living people, results obtained from cross-sectional samples always provide less accurate models of growth change than results from longitudinal samples (Cook, Ware 1983, Caruana *et al.* 2015). If we have this experience from the growth of children and adolescents, where the body size changes are considerable, we must be even more cautious in interpreting skeletal changes in adulthood, when the observed changes are much smaller (although, again, over longer time intervals). The potential biases that the cross-sectional nature of the samples may introduce into the results should therefore be carefully addressed.

To begin with obvious issues, whereas in the documented sample, age at death is directly recorded at the time of death and covers the actual range of calendar ages of the people in question, age estimates from archaeological finds vary in precision at different ages; the range estimates of these methods generally ends somewhere close above 50 years, as systematic manifestations of aging on skeleton beyond that point are already strongly individual and no more reliable estimate is possible (most of the methods for age estimation simply do not provide higher estimates). We see this in our study as well (*Figure 2*); age-at-death values for the recent and medieval samples differ markedly in both mean and variance, and we are unable to quantify how much of this is due to skeletal differences between

the samples (earlier deaths in the medieval period) and how much to differences in age determination (documentation vs. estimation). Consequently, it is not meaningful to consider the documented values and estimates from the bones as equivalent representations of age and to compare their absolute values directly. Moreover, due to the different degree of preservation of the skeletons, it is also unavoidable that the age for each skeleton is estimated by different methods. (To mitigate the effect of this noise, typically multiple methods have been applied to each skeleton and the result is a combination of these methods. We are aware that age change may affect some parts of the skeleton inadequately more or less and a comprehensive assessment of the whole skeleton by multiple methods/features is always needed.) This limitation applies to virtually all studies of skeletal samples using estimates of age at death and cannot be avoided but must be taken into account in methods of comparison and interpretation of results. On the other hand, all age estimation methods are based on the fact that they assess some biological changes in a range from the manifestation in the youngest persons to the manifestation in the oldest persons. If we have a large age range (and in our case we have adults of all ages and the range is widest possible), it is difficult to imagine that even with such imprecise methods, the majority of individuals categorized as young adults would be the oldest seniors and *vice versa*. Certainly, the position of each individual relative to calendar age will be shifted differently and the overall dependence on age will be blurred, but it will never disappear completely or even reverse of the general age trend direction. In terms of the position of the studied age changes relative to calendar age, results based on skeletal estimates may be quite inaccurate, but in terms of the sequence of changes during ageing (if order-based statistical methods are used, e.g., Spearman rank order correlation coefficient), such results may not be incorrect.

The results may be also affected by the internal heterogeneity of each sample. In the case of our archaeological sample, it is a pooled sample from three burial sites. Despite the fact that the males of our three archaeological samples merged into one medieval sample differed in AAD, we observed very similar results for the relationship between measurements and AAD as in the recent sample. Thus, this heterogeneity does not appear to have affected seriously the comparison. In the case of our recent sample, this objection of uncontrolled internal heterogeneity is related to a possible *cohort effect*. A certain secular trend in the size and shape of the long bones of the hand was noted,

for example, in a Chuvashian sample (Kalichman *et al.* 2008). Our documented sample represents inhumations made at the end of the 20th century and is of individuals who died from the late 19th century to the second half of the 20th century (Eliopoulos *et al.* 2007). While people born earlier and who died in the first part of this range had the chance to populate all age categories of the recent sample (both young adults, middle-aged and elderly), people born in the second half of the 20th century could only contribute to the younger age categories of the AAD because they had not yet reached the older age categories at the time of the inhumations. Therefore, it can be assumed that persons with lower AAD values represent some proportion of persons born at various times and dying throughout this range, while persons born in the second half of the 20th century are missing in older AAD categories and persons born earlier predominate. However, if bone size varied among different cohorts born during the 20th century (as would be expected given the secular trend in stature (Papadimitriou *et al.* 2002)), the association of the bone size with AAD in our recent sample could be partly due to the secular trend. Based on the available data, we attempted to test this objection. For a subsample of the recent sample, we had available the *year of birth* in addition to age at death. Given that the earlier an individual was born, the older they could also be at the time of death, AAD and year of birth were strongly negatively correlated both in females (N=44, Spearman rho=-0.89, p-value=2.9e-16) and males (N=32, Spearman rho=-0.91, p-value=7.4e-13). For recent women of this subsample, HW on the right hand was strongly correlated with AAD (N=44, Spearman rho=0.54, p-value=0.00022) but also with *year of birth* (N=44, Spearman rho=-0.40, p-value=0.0073). However, after controlling for *year of birth* using the partial correlation method, HW was still significantly correlated with AAD (Spearman rho=0.45, p-value=0.0028), whereas when we controlled for AAD, the correlation of HW with *year of birth* was no longer statistically significant (Spearman rho=0.21, p-value=0.18). Based on these findings, the secular trend and differential representation of individuals of different age cohorts among individuals of different AAD in the recent sample may partly account for the association of AAD with size, but nevertheless the main effect causing the observed relationships is the effect of AAD.

A different kind of selectivity may be brought about by taphonomic factors in the archaeological sample – only bones with sufficiently resistant tissue may be preserved and measurable and these might be bigger or

more robust. If those with more robust and larger metacarpals were selected among the older women by a mechanism (which would then account for the apparent increase in bone width with age), we should miss the smaller and more gracile women from this burial site in the metacarpal sample. In females, we therefore tested, for example, the difference in mean *vertical diameter of the femoral head* between the group of cases (of both body sides combined) with preserved 2nd metacarpals included in our study (N=15, mean=42.36 mm, SD=1.80 mm) and the group of cases whose 2nd metacarpals were not preserved and therefore not included in our study (N=32, mean=42.42 mm, SD=2.36 mm). The difference between the two mean values tested by Monte Carlo permutation test was not statistically significant (number of permutations=10⁶, p-value=0.91). Similarly, we tested for differences in several other variables (transverse diameter of the femoral head, maximum midshaft diameter of the humerus, upper sagittal diameter of the diaphysis of the femur, tibial circumference at the level of the foramen nutricium) with the same result. We therefore believe that possible taphonomic selection did not affect the results of the archaeological assemblages in our study, although we tested it in the Dětkovice sample only.

Finally, to make the list of possible limitations/objections complete, it should be mentioned that both studied samples, the medieval and the recent, refer to the state at the time of death. We have only skeletons of people who died in a given age category and not a random selection of the entire age category of people (then) alive (the so-called *osteological paradox* (Wood *et al.* 1992)). If people of certain physical characteristics died preferentially at a certain age, the differences between people who died at different ages would then be due to the selectivity of this mortality and not necessary by true age changes. For example, some epidemiological studies suggest that in any given age cohort, mortality rates decrease with height of stature (Waller 1984). Thus, people who live to a higher age should be taller. However, the epidemiologically observed effects are relatively small and not always fully consistent, or the age range studied includes only advanced elderly categories (Brandts, van den Brandt 2019). The results of osteological studies regarding the effect of size-specific selection vary (c.f., Gunnell *et al.* 2001, Hughes-Morey 2016), probably because (A) the possible effect of such selection is attenuated, offset, or even outweighed to varying degrees by degenerative skeletal changes associated with aging, and (B) heterogeneities in skeletal samples caused by other

factors (mentioned above) may be significantly greater than the effect of size selection.

Despite the debated issues regarding the nature of the samples, it is clear from the consistent features of age differences in the medieval and recent samples—which differ significantly both in the genetic origin of the source populations and geographically, temporally (epoch) and culturally – at least part of the observed trend will be related to actual age changes at the individual level. Skeletal ageing is usually associated with bone loss. Bone resorption increases with age in adulthood in humans (Ives, Brickley 2005, Szulc, Delmas 2007, Demontiero *et al.* 2012). Due to decrease in estrogen production, this phenomenon is especially evident in postmenopausal women. Men are 30% less exposed to this change than women. Bone density increases to the age of about 30 years until it gets to the peak bone amount and then the bone resorption exceeds the amount of bone deposition. Bone mass then decreases up to 1% per year. When the bone amount or bone density reduction starts to be clinically significant, the patient might be diagnosed with osteoporosis (Zaki *et al.* 2016). Cortical bone loss in the long bones can be identified by the increasing width of the medullary cavity which is caused by a thinning of the cortical walls. This decreasing cortical bone thickness can be then measured by metacarpal radiogrammetry (Ives, Brickley 2005). Given the relative simplicity and clinical significance, it is no wonder that radiological studies predominate in research of morphology and geometry of metacarpals. Therefore, it might be challenging to find studies about age-related changes in metacarpal bones based on the measurements of the bone itself without the clinical approach.

A different focus comparing to studies describing bone loss bring other studies demonstrating continued bone growth in adulthood, for example in the femur or ribs. (Epker *et al.* 1965, Trotter, Peterson 1967, Feik *et al.* 2000). These changes were explained by the expansion of the periosteal margin (Ahlborg *et al.* 2003). Changes in cortical bone in 2nd metacarpal bone were examined in hand radiological images from anterior-posterior view of a Japanese sample (229 women 41 to 94 years and 505 men aged 40 to 95 years). The width in the middle of the bone was also measured. There was significant increase of bone width from the fifth to sixth decade but no overall significant correlation between age and bone width in women and no significant correlation between age and bone width in men (Iwamoto *et al.* 1998, 2000). Unfortunately, younger age categories were not included in this study, so it is

not clear what the results would look like if these categories were included, especially for women sample. The continuing adult bone growth in the 2nd metacarpal was confirmed in radiographs of women and also men from five different populations (121 adult skeletons from the Terry Collection of Washington University, 677 adult participants in studies of growth and aging at the Fels Research Institute and 2001 adult participants in nutritional surveys in Guatemala, El Salvador and Nicaragua) (Garn *et al.* 1967). Using radiogrammetry of the 2nd metacarpal bone in the Imperial Roman population of Velia (Italy), bone width was found to increase in middle-aged women (30–49 years), but this increase was not statistically significant and did not continue in the older age group (50+). Men remained largely stable in all age groups. This was completely unexpected, and these findings may suggest that women in Velia were belatedly exposed to strenuous physical activity, while men might experience high levels of physical activity much earlier in life and consistently throughout their life (Beauchesne, Agarwal 2017).

The findings of our study seem to be consistent with the study mentioned above (Garn *et al.* 1967), as they show overall higher values of the width of the 2nd metacarpal in males throughout life, while in females they demonstrate an increase in width dimensions during adulthood with a clear increase in middle age (35–50 years). On the other hand, in another study of hand radiographs of men and women in the Croatian population (Kušec *et al.* 1988), it was found that age changes were more evident in women but only the midshaft width of the 2nd, 3rd and 4th metacarpal bone *cavity* correlated positively with age (ranging from 19 to 86 years) while the external midshaft width of the bone did not. However, the graphs in this study (Kušec *et al.* 1988, Fig. 6) show that the relationship of the total width of these metacarpal bones on age has a clear increasing trend in women between 20 and 50 years of age, but there is an opposite trend and a decrease at the older age period (the shape of the inverted letter U). Thus, in our opinion, when linear correlation is used, both trends are nullified and the correlation disappears. Therefore, despite the authors' explicitly stated conclusions, this study does not directly contradict our results, but it does complicate the studied field from one trend to two trends that need to be explained, i.e., the increase in dimension by age 50, but also its decline at high age around 80 years. Of course, it is possible that the two trends are of a completely different nature, including the confounding factors/processes mentioned in the first paragraphs of this Discussion (see above). This example shows that comparing the results

of studies using different methodologies (here, different age estimation/determination and age ranges) requires a careful detailed approach that looks at the substantive comparability of samples and methodologies and not just the headline results.

Despite the generally consistent trend towards increasing width dimensions for women in both the medieval and recent samples, a closer look at individual dimensions does show some differences (*Figure 4 and 5*). For example, we see differences in radial and dorsal views. While the slope of the regression line is similar in both views for the recent women, the women in the medieval sample consistently have more MW in the dorsal view than in the radial view (which is consistently true on both the right and left side). That is, in medieval women, MW increased more in the radio-ulnar direction than in the dorso-palmar direction. In medieval women we also see more pronounced lateral differences in MW and HW in radial view, but (inconsistently) there is a greater increase in MW on the right side whereas HW on the left side. Thus, differences in the factors (e.g., physical load) underlying bone changes in adulthood cannot be ruled out in the two samples.

CONCLUSIONS

In our study, we observed a systematic change (increase) in the width of the 2nd metacarpal during adulthood in females. The rate of this change is similar in females from the medieval and recent samples. Although there were differences in the statistical significance of this relationship between these samples, they could be due to differences in the nature of the sampling and, in particular, the nature of the age-at-death values, which were accurately documented in the recent sample and cover the full range of life expectancy, whereas in the medieval sample they were estimated by different methods with varying accuracy and their range is (probably partially due to limitations of the age estimation methods) much smaller. The cross-sectional nature of the sample must also be taken into account. Nevertheless, in both samples, women consistently showed significantly greater changes with age than men in the same samples. This suggests some specificities of women's life history in adulthood that would be worth further investigation in terms of the influence of external factors such as social status, workload, nutritional status, etc. It will also be essential to examine the change in bone proportionality and shape with age, the possible association of these changes with absolute body size

(i.e., allometry), and the overall pattern of dimensional change of all metacarpals within the whole hand.

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APPENDIX 1: Descriptive statistics of Age at Death of the studied samples.

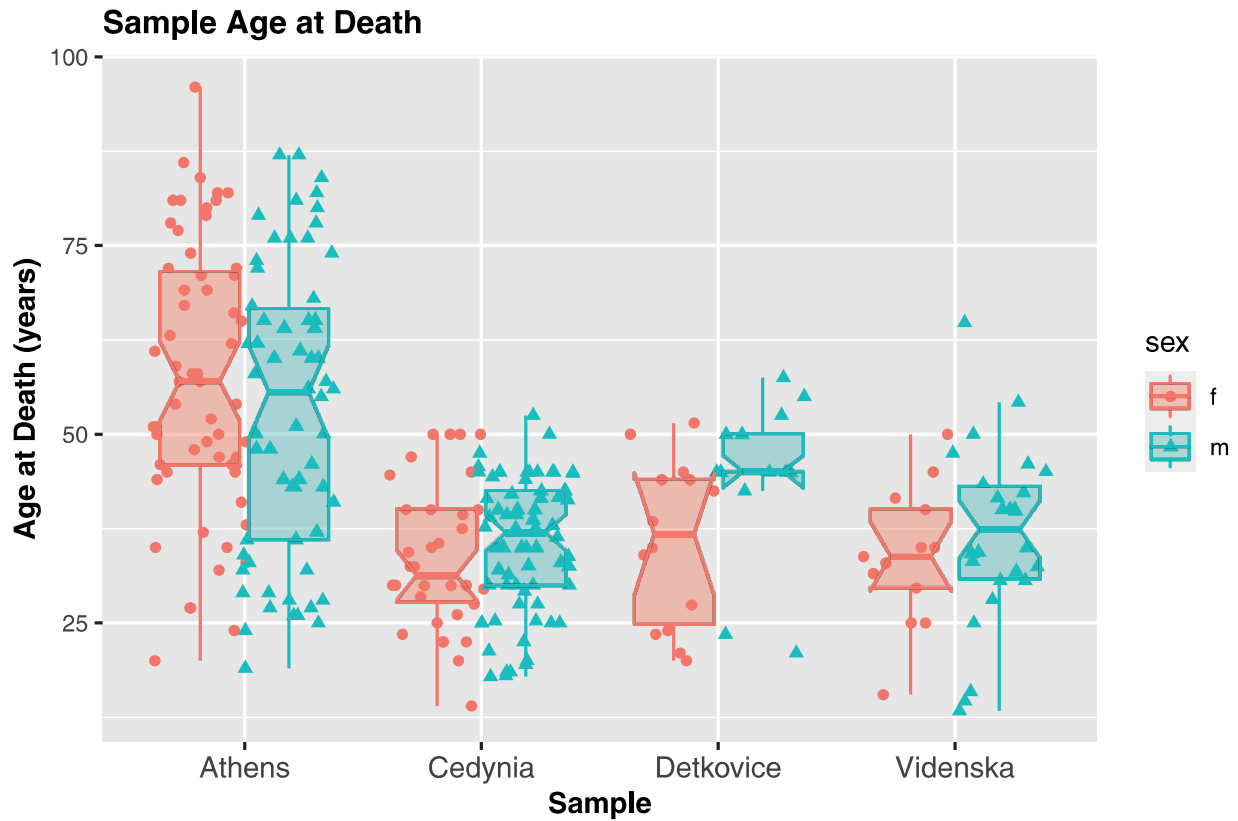


FIGURE A1-1: Boxplots augmented with scatter plots of Age at Death (AAD) in all included samples, separately for males (m, blue triangles) and females (f, red dots), thick horizontal – median, notch – 95%CI for median, box – interquartile range, whiskers – non-outlier range.

Table A1-1: Descriptive table of age at death of individuals from each site in the medieval sample; n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Sample	Sex	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se
Athens	f	59	57.31	17.88	57	57.57	17.79	20	96	76	-0.01	-0.86	2.33
Cedynia	f	34	33.68	9.56	31.25	33.52	10.38	14	50	36	0.21	-0.86	1.64
Dětkovice	f	14	35.75	10.92	36.75	35.75	12.97	20	51.5	31.5	-0.12	-1.61	2.92
Brno - Vídeňská Street	f	13	33.85	9.14	33.8	34.05	9.19	15.5	50	34.5	-0.14	-0.63	2.53
Athens	m	62	53.05	19.18	55.5	52.82	25.2	19	87	68	0.02	-1.23	2.44
Cedynia	m	70	35.42	8.81	36.95	35.98	10.3	17.9	52.5	34.6	-0.38	-0.87	1.05
Dětkovice	m	14	44.43	10.39	45	45.29	5.56	21	57.5	36.5	-1.13	0.32	2.78
Brno - Vídeňská Street	m	26	36.68	11.85	37.4	36.67	9.53	13.35	64.8	51.45	-0.04	-0.01	2.32

APPENDIX 2:
Detailed descriptive statistics of the measurements.

TABLE A2-1: Descriptive statistics of the length of the second metacarpals of samples from medieval sites from the dorsal view; dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Length – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	21	61.90	3.04	61.81	62.04	56.01	66.34	-0.25	-1.17	0.66
Detkovice	dex	f	11	65.37	3.74	65.46	65.40	59.03	71.46	-0.10	-1.11	1.13
Videnska	dex	f	11	65.42	1.96	65.55	65.35	62.51	68.96	0.07	-1.13	0.59
Cedynia	sin	f	26	62.65	3.22	61.52	62.67	55.73	69.61	0.11	-0.50	0.63
Detkovice	sin	f	6	64.08	3.47	64.34	64.08	58.81	69.32	-0.02	-1.23	1.42
Videnska	sin	f	8	64.57	2.56	64.51	64.57	61.09	69.06	0.30	-1.28	0.90
Cedynia	dex	m	60	68.18	3.23	68.07	68.10	61.39	76.13	0.19	-0.46	0.42
Detkovice	dex	m	11	68.60	2.83	68.55	68.87	61.65	73.08	-0.94	0.91	0.85
Videnska	dex	m	18	67.97	3.09	68.23	68.03	61.05	73.88	-0.41	0.01	0.73
Cedynia	sin	m	47	68.47	3.24	68.00	68.30	62.37	76.76	0.49	-0.24	0.47
Detkovice	sin	m	11	67.58	3.07	67.83	67.74	61.77	71.92	-0.32	-1.07	0.93
Videnska	sin	m	20	68.25	2.74	68.99	68.54	60.58	72.65	-0.97	0.86	0.61

TABLE A2-2: Descriptive statistics of the length of the second metacarpals of samples from medieval sites from the radial view; dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Length – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	20	64.49	3.20	65.56	64.62	58.89	69.13	-0.29	-1.33	0.72
Detkovice	dex	f	11	68.09	3.71	67.99	68.21	61.40	73.71	-0.19	-1.00	1.12
Videnska	dex	f	11	68.18	1.86	68.05	68.09	65.77	71.48	0.29	-1.26	0.56
Cedynia	sin	f	24	65.28	3.36	64.35	65.34	57.84	72.53	-0.02	-0.41	0.69
Detkovice	sin	f	6	66.60	3.72	67.12	66.60	61.07	71.97	-0.08	-1.42	1.52
Videnska	sin	f	6	66.96	2.86	67.02	66.96	62.91	71.38	0.12	-1.36	1.17
Cedynia	dex	m	58	71.14	3.23	71.37	71.14	64.53	77.74	-0.02	-0.71	0.42
Detkovice	dex	m	10	71.82	2.87	72.32	72.02	65.58	76.5	-0.60	0.01	0.91
Videnska	dex	m	17	71.21	3.52	71.27	71.20	64.18	78.38	-0.07	-0.35	0.85
Cedynia	sin	m	43	71.32	3.25	71.34	71.21	65.06	79.75	0.32	-0.29	0.50
Detkovice	sin	m	11	70.68	3.42	70.61	70.84	64.11	75.76	-0.29	-1.03	1.03
Videnska	sin	m	20	71.55	3.26	72.21	71.67	63.68	77.4	-0.41	-0.13	0.73

TABLE A2-3: Descriptive statistics of the midshaft width of the second metacarpals from medieval sites from the dorsal view; dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Midshaft Width – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	21	7.54	0.63	7.45	7.51	6.60	8.76	0.22	-1.28	0.14
Detkovice	dex	f	11	7.47	0.68	7.37	7.50	6.31	8.42	-0.27	-1.38	0.21
Videnska	dex	f	11	8.15	0.47	8.21	8.13	7.41	9.06	0.17	-0.76	0.14
Cedynia	sin	f	26	7.42	0.57	7.39	7.43	6.39	8.34	-0.04	-1.22	0.11
Detkovice	sin	f	6	7.31	0.74	7.51	7.31	6.01	8.13	-0.64	-1.18	0.30
Videnska	sin	f	8	7.68	0.51	7.58	7.68	7.03	8.51	0.26	-1.53	0.18
Cedynia	dex	m	60	8.73	0.84	8.76	8.70	6.69	10.58	0.12	-0.25	0.11
Detkovice	dex	m	11	8.88	0.70	8.76	8.87	7.79	10.08	0.22	-1.08	0.21
Videnska	dex	m	18	8.81	0.78	8.66	8.81	7.37	10.24	0.24	-0.93	0.18
Cedynia	sin	m	47	8.48	0.81	8.38	8.45	6.69	10.54	0.38	-0.17	0.12
Detkovice	sin	m	11	8.41	0.66	8.34	8.43	7.28	9.36	-0.13	-1.46	0.20
Videnska	sin	m	20	8.46	0.77	8.38	8.41	7.37	9.99	0.46	-0.76	0.17

TABLE A2-4: Descriptive statistics of the midshaft width of the second metacarpals from medieval sites from the radial view dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean

Midshaft Width – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	20	7.69	0.56	7.79	7.70	6.39	9.02	-0.06	0.41	0.13
Detkovice	dex	f	11	7.99	0.55	8.13	8.00	7.11	8.76	-0.16	-1.59	0.16
Videnska	dex	f	11	8.42	0.93	8.42	8.41	6.98	10.03	0.16	-1.28	0.28
Cedynia	sin	f	24	7.85	0.61	7.83	7.83	6.48	9.19	0.17	0.00	0.12
Detkovice	sin	f	6	7.94	0.61	8.00	7.94	7.28	8.55	-0.05	-2.23	0.25
Videnska	sin	f	6	7.88	0.96	7.58	7.88	7.20	9.78	1.19	-0.36	0.39
Cedynia	dex	m	58	9.23	0.71	9.17	9.19	7.96	11.35	0.60	0.42	0.09
Detkovice	dex	m	10	9.13	1.11	9.14	9.11	7.62	10.80	0.07	-1.70	0.35
Videnska	dex	m	17	9.36	0.61	9.48	9.40	7.71	10.37	-0.98	1.00	0.15
Cedynia	sin	m	43	8.95	0.72	8.93	8.94	7.58	11.13	0.31	0.16	0.11
Detkovice	sin	m	11	8.67	0.67	8.51	8.62	7.79	9.99	0.44	-1.04	0.20
Videnska	sin	m	20	9.18	0.49	9.19	9.18	8.04	10.37	0.11	0.52	0.11

TABLE A2-5: Descriptive statistics of the head width of the second metacarpals from medieval sites from the dorsal view
dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Head Width – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	21	14.17	1.18	13.97	14.06	12.22	16.87	0.78	-0.20	0.26
Detkovice	dex	f	11	12.84	0.58	12.74	12.83	12.13	13.68	0.22	-1.63	0.18
Videnska	dex	f	11	13.86	1.02	14.07	13.93	11.97	15.17	-0.32	-1.25	0.31
Cedynia	sin	f	26	14.04	1.00	13.86	14.01	12.25	16.17	0.36	-0.46	0.20
Detkovice	sin	f	6	12.95	0.72	13.14	12.95	11.83	13.64	-0.42	-1.71	0.29
Videnska	sin	f	8	13.16	1.34	13.31	13.16	11.05	15.05	-0.22	-1.39	0.47
Cedynia	dex	m	60	15.94	1.19	15.79	15.84	13.89	20.07	0.95	1.34	0.15
Detkovice	dex	m	11	15.34	1.05	15.46	15.35	13.56	17.06	0.05	-1.16	0.32
Videnska	dex	m	18	15.37	0.79	15.15	15.36	14.17	16.73	0.14	-1.42	0.19
Cedynia	sin	m	47	15.53	1.04	15.63	15.56	13.00	17.95	-0.23	-0.32	0.15
Detkovice	sin	m	11	14.75	0.93	14.72	14.80	13.17	15.85	-0.15	-1.56	0.28
Videnska	sin	m	20	14.68	0.73	14.46	14.64	13.72	16.17	0.43	-1.21	0.16

TABLE A2-6: Descriptive statistics of the head width of the second metacarpals from medieval sites from the radial view;
dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Head Width – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	20	13.02	1.08	12.85	12.93	11.11	15.47	0.61	-0.29	0.24
Detkovice	dex	f	11	13.44	0.43	13.42	13.43	12.69	14.20	0.00	-0.97	0.13
Videnska	dex	f	11	13.52	1.04	13.13	13.41	12.38	15.66	0.74	-0.88	0.31
Cedynia	sin	f	24	13.03	0.74	12.96	13.07	11.39	14.34	-0.39	-0.18	0.15
Detkovice	sin	f	6	13.43	1.27	13.01	13.43	12.04	15.33	0.40	-1.76	0.52
Videnska	sin	f	6	12.88	0.73	12.74	12.88	12.15	14.09	0.50	-1.50	0.30
Cedynia	dex	m	58	15.03	1.10	14.79	14.98	12.38	18.35	0.52	0.29	0.14
Detkovice	dex	m	10	14.82	1.42	14.84	14.82	12.86	16.75	0.03	-1.72	0.45
Videnska	dex	m	17	14.79	1.13	14.77	14.80	12.92	16.57	-0.23	-1.31	0.28
Cedynia	sin	m	43	14.82	1.02	14.72	14.80	12.60	17.61	0.30	0.08	0.16
Detkovice	sin	m	11	14.04	1.08	14.49	14.22	11.30	15.23	-1.36	0.98	0.33
Videnska	sin	m	20	14.44	0.83	14.52	14.42	12.94	16.20	0.10	-0.71	0.19

TABLE A2-7: Descriptive statistics of the base width of the second metacarpals from medieval sites from the dorsal view; dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Base Width – Dorsal View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	21	14.50	1.16	14.29	14.45	12.56	16.99	0.58	-0.51	0.25
Detkovice	dex	f	11	14.08	0.65	13.96	14.02	13.21	15.49	0.73	-0.49	0.20
Videnska	dex	f	11	14.45	1.32	14.70	14.56	11.93	16.01	-0.55	-1.07	0.40
Cedynia	sin	f	26	14.34	1.00	14.27	14.36	12.56	15.85	-0.14	-1.11	0.20
Detkovice	sin	f	6	14.36	0.86	14.19	14.36	13.26	15.81	0.44	-1.21	0.35
Videnska	sin	f	8	14.20	1.28	14.30	14.20	11.51	15.45	-0.91	-0.32	0.45
Cedynia	dex	m	60	16.65	1.30	16.81	16.68	13.65	19.18	-0.21	-0.82	0.17
Detkovice	dex	m	11	16.59	1.22	17.06	16.62	14.69	18.21	-0.38	-1.55	0.37
Videnska	dex	m	18	16.65	1.35	16.87	16.70	13.70	18.83	-0.43	-0.69	0.32
Cedynia	sin	m	47	16.62	1.13	16.47	16.59	14.76	18.95	0.19	-1.26	0.17
Detkovice	sin	m	11	16.14	1.45	16.44	16.23	13.62	17.80	-0.54	-1.32	0.44
Videnska	sin	m	20	16.32	1.47	16.74	16.40	13.33	18.80	-0.46	-0.78	0.33

TABLE A2-8: Descriptive statistics of the base width of the second metacarpal from medieval sites from the radial view; dex – right, sin – left, f – females, m – males, n – number of individuals, sd – standard deviation, min – minimal value, max – maximal value, se – standard error of mean.

Base width – Radial View												
Sample	Side	Sex	n	mean	sd	median	trimmed	min	max	skew	kurtosis	se
Cedynia	dex	f	20	14.70	1.01	14.61	14.56	13.58	17.04	0.88	-0.17	0.22
Detkovice	dex	f	11	14.72	1.13	14.90	14.67	12.94	16.86	0.20	-1.02	0.34
Videnska	dex	f	11	15.08	1.31	15.15	15.09	12.95	17.10	0.08	-1.13	0.39
Cedynia	sin	f	24	14.45	0.69	14.44	14.45	13.06	15.83	-0.07	-0.75	0.14
Detkovice	sin	f	6	14.23	0.71	14.25	14.23	13.14	15.14	-0.20	-1.55	0.29
Videnska	sin	f	6	14.60	1.16	14.43	14.60	13.19	16.68	0.63	-0.90	0.47
Cedynia	dex	m	58	16.45	0.94	16.42	16.47	14.21	18.55	-0.17	-0.35	0.12
Detkovice	dex	m	10	16.45	1.03	16.60	16.46	14.61	18.22	-0.18	-0.90	0.33
Videnska	dex	m	17	16.27	0.84	16.21	16.29	14.4	17.71	-0.35	-0.43	0.20
Cedynia	sin	m	43	16.08	1.03	16.09	16.10	13.54	18.23	-0.24	-0.16	0.16
Detkovice	sin	m	11	16.08	1.21	16.38	16.12	14.12	17.62	-0.56	-1.37	0.36
Videnska	sin	m	20	16.11	0.85	16.21	16.15	14.62	17.30	-0.45	-1.11	0.19

APPENDIX 3: Descriptive boxplots and ANOVA.

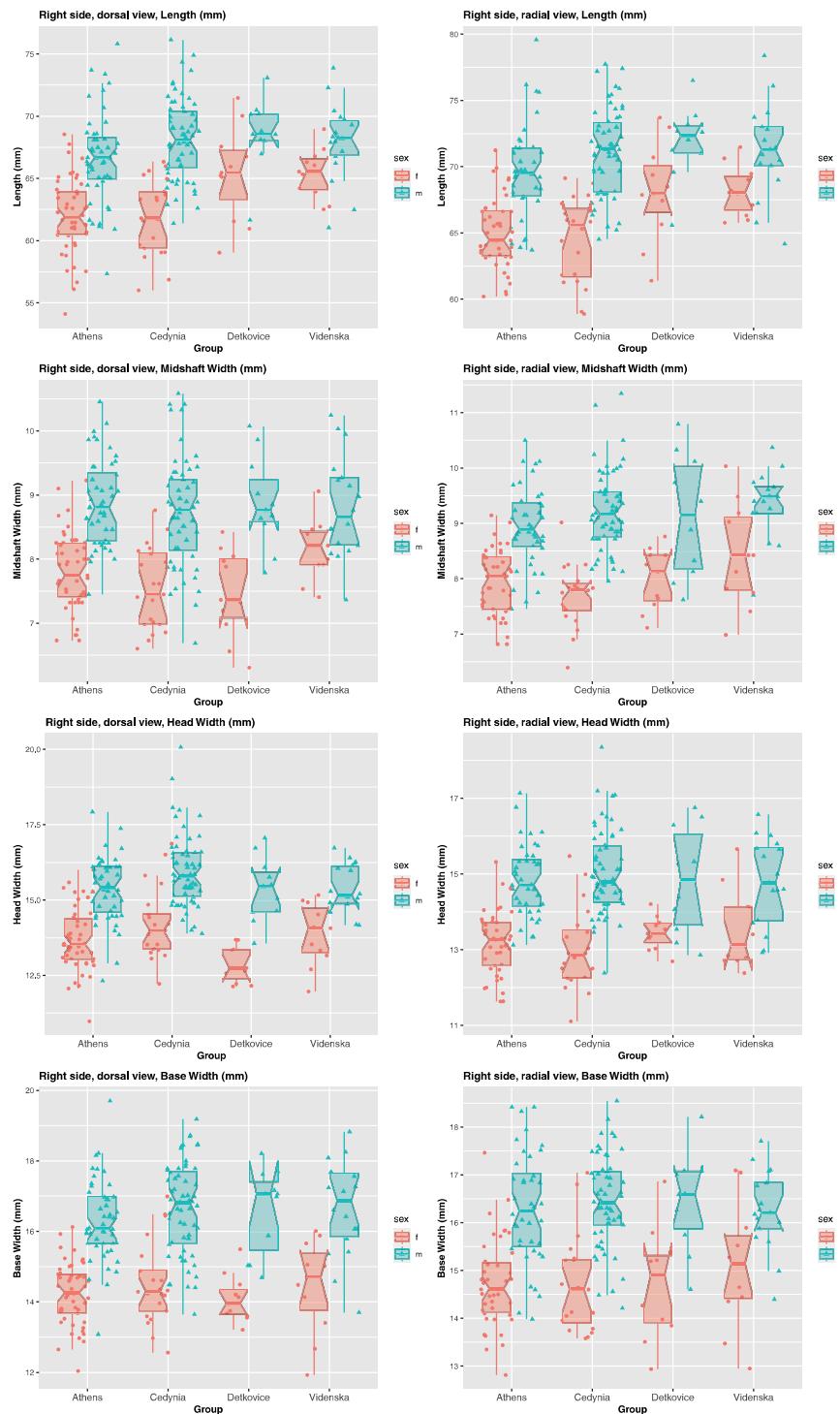


FIGURE A3-1: Boxplots augmented with scatter plots of the right second metacarpal measurements in all four studied samples, separately for males (m, blue triangles) and females (f, red dots), left column – dorsal view, right column – radial view, thick horizontal – median, notch – 95%CI for median, box – interquartile range, whiskers – non-outlier range.

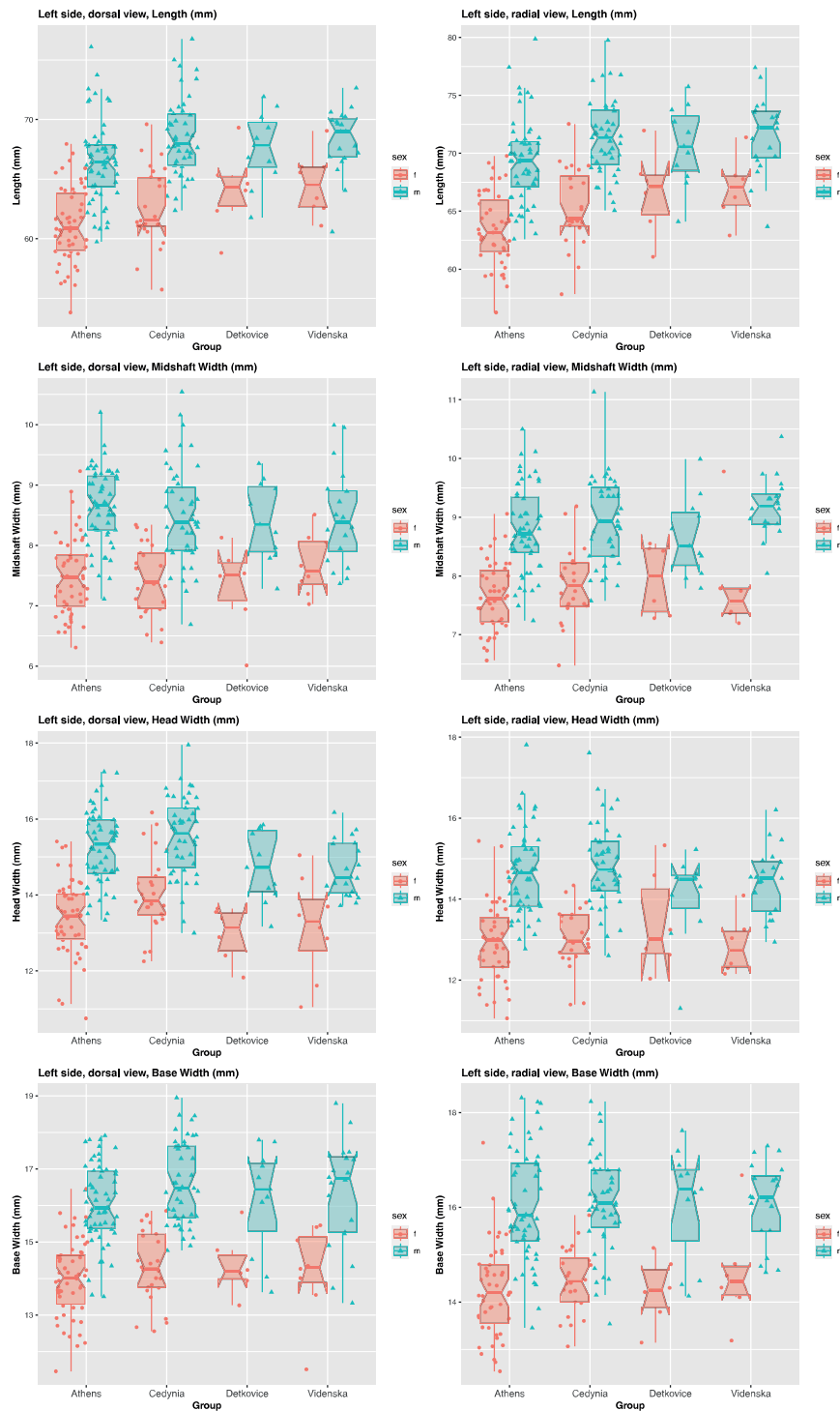


FIGURE A3-2: Boxplots augmented with scatter plots of the left second metacarpal measurements in all four studied samples, separately for males (m, blue triangles) and females (f, red dots), left column – dorsal view, right column – radial view, thick horizontal – median, notch – 95%CI for median, box – interquartile range, whiskers – non-outlier range.

TABLE A3-1: Results of Art ANOVA of the right second metacarpal measurements for each view separately; presented are F-values and p-values explained by factors (Sex, Sample) and their interaction terms; for p-values expressed as 0.00 the true value was lower than 1.0E-06.

Right hand							
		Sex		Sample		Sex:Sample	
		F-value	p-value	F-value	p-value	F-value	p-value
Dorsal view	Length	93.59	0.00	10.55	0.0000017	2.67	0.048
	Midshaft width	89.04	0.00	1.45	0.23	1.42	0.24
	Head width	132.41	0.00	15.36	0.00	0.67	0.57
	Base width	149.35	0.00	7.82	0.000056	0.23	0.87
Radial view	Length	101.47	0.00	10.57	0.0000017	2.32	0.08
	Midshaft width	113.26	0.00	6.86	0.00020	1.73	0.16
	Head width	114.87	0.00	2.55	0.057	0.99	0.40
	Base width	90.55	0.00	2.82	0.040	0.61	0.61

TABLE A3-2: Results of Art ANOVA of the left second metacarpal measurements for each view separately; presented are F-values and p-values explained by factors (Sex, Sample) and their interaction terms; for p-values expressed as 0.00 the true value was lower than 1.0E-06.

Left hand							
		Sex		Sample		Sex:Sample	
		F-value	p-value	F-value	p-value	F-value	p-value
Dorsal view	Length	99.55	0.00	12.57	0.00	0.86	0.46
	Midshaft width	81.04	0.00	0.29	0.83	0.64	0.59
	Head width	97.94	0.00	6.98	0.00017	0.45	0.72
	Base width	120.60	0.00	7.02	0.00016	0.09	0.96
Radial view	Length	99.45	0.00	12.03	0.00	0.38	0.77
	Midshaft width	90.59	0.00	4.56	0.0041	0.86	0.46
	Head width	87.62	0.00	1.85	0.14	0.69	0.56
	Base width	89.53	0.00	2.04	0.11	0.17	0.92

APPENDIX 4: Relationship of measurements with age at death – Correlograms.

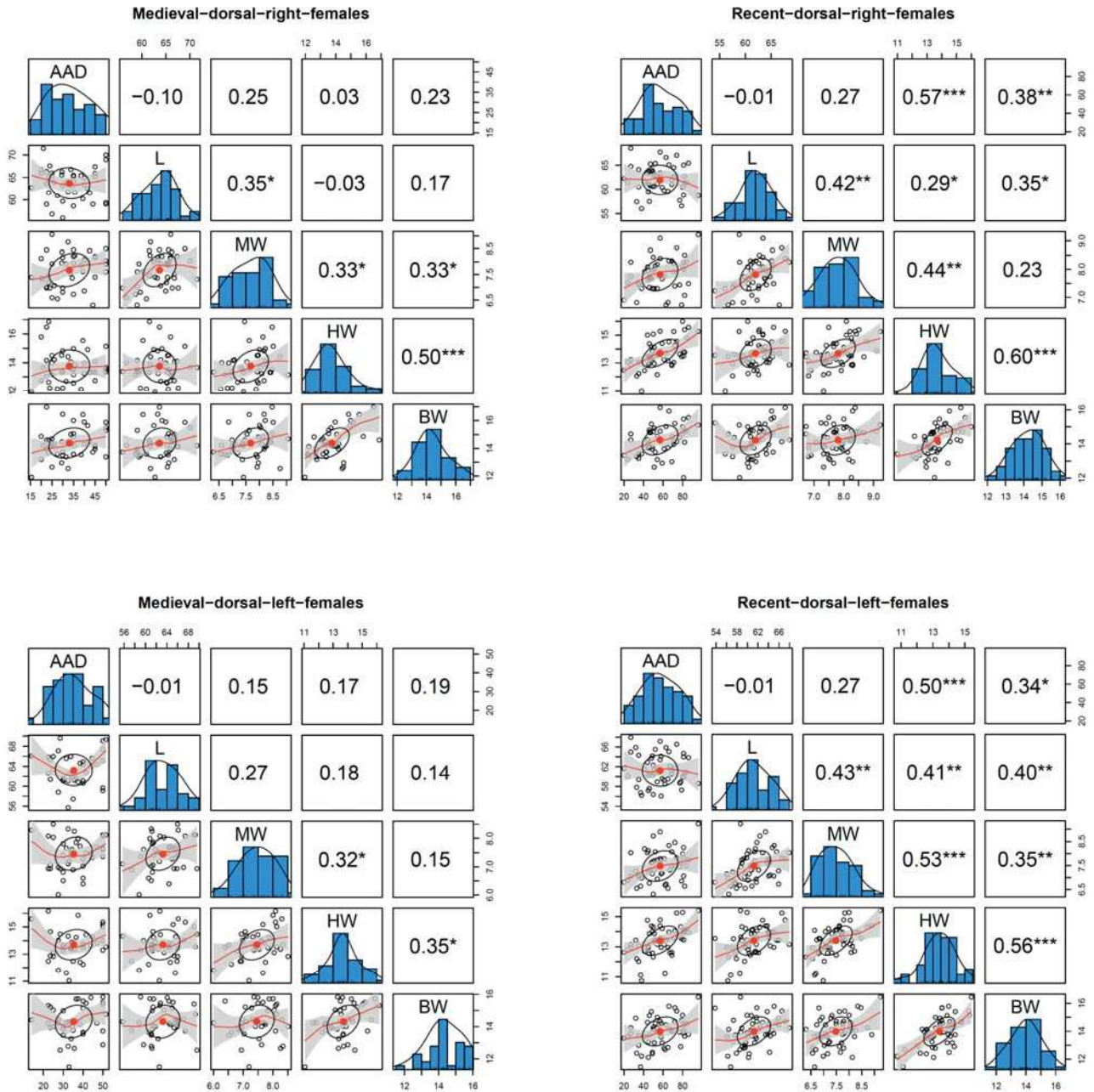


FIGURE A4-1: Correlograms for the second metacarpal measurements and age at death (AAD) for dorsal view on the bone in females; diagonal – histograms, above diagonal – Spearman rank order correlation coefficients with marked significance (asterisks), below diagonal – scatterplots augmented with loess smooths models and correlation ellipses.

Metric differences in adult second metacarpal bones related to age-at-death and their comparison between recent and historical populations

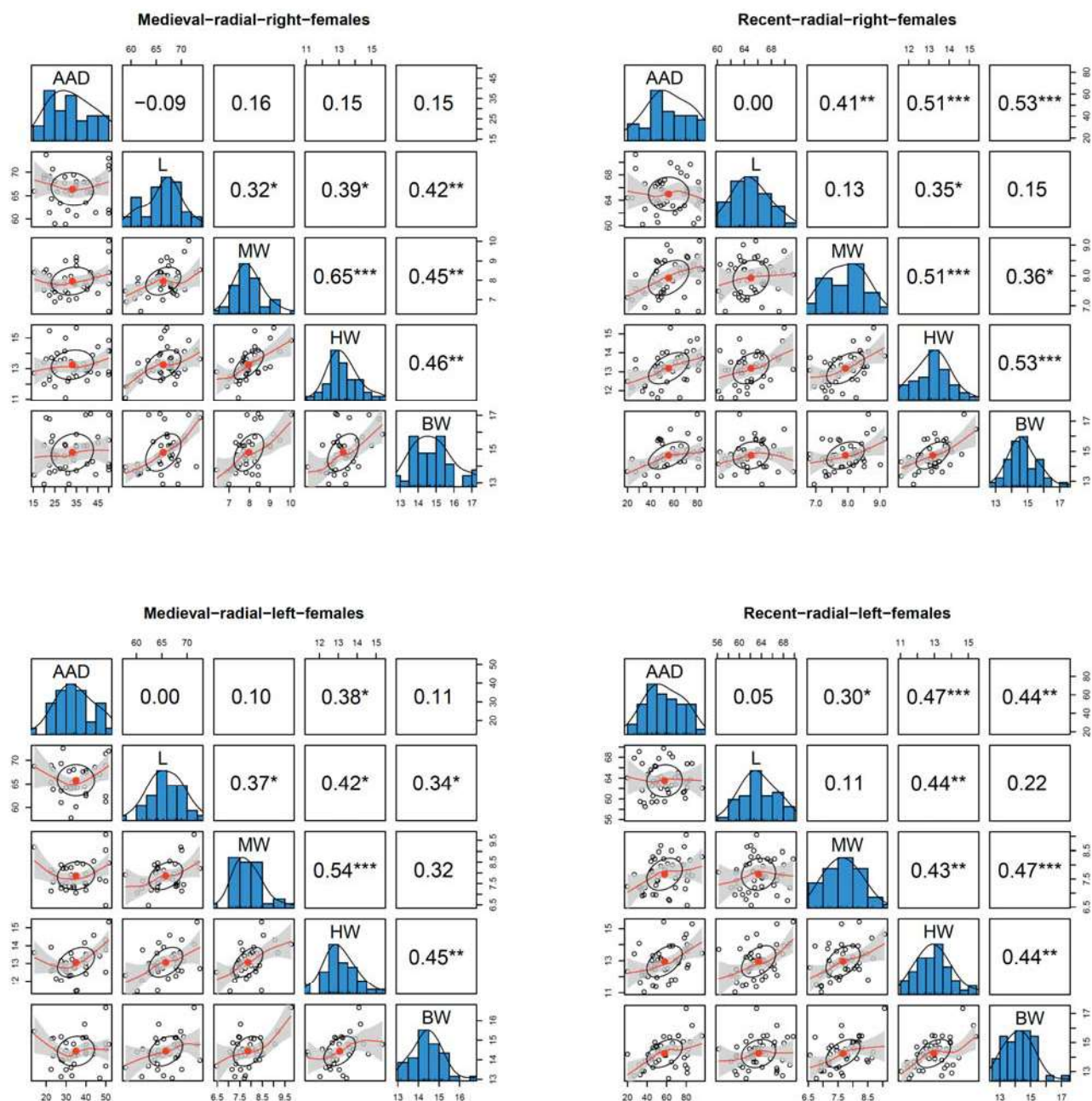


FIGURE A4-2: Correlograms for the second metacarpal measurements and age at death (AAD) for radial view on the bone in females; diagonal – histograms, above diagonal – Spearman rank order correlation coefficients with marked significance (asterisks), below diagonal – scatterplots augmented with loess smooths models and correlation ellipses.

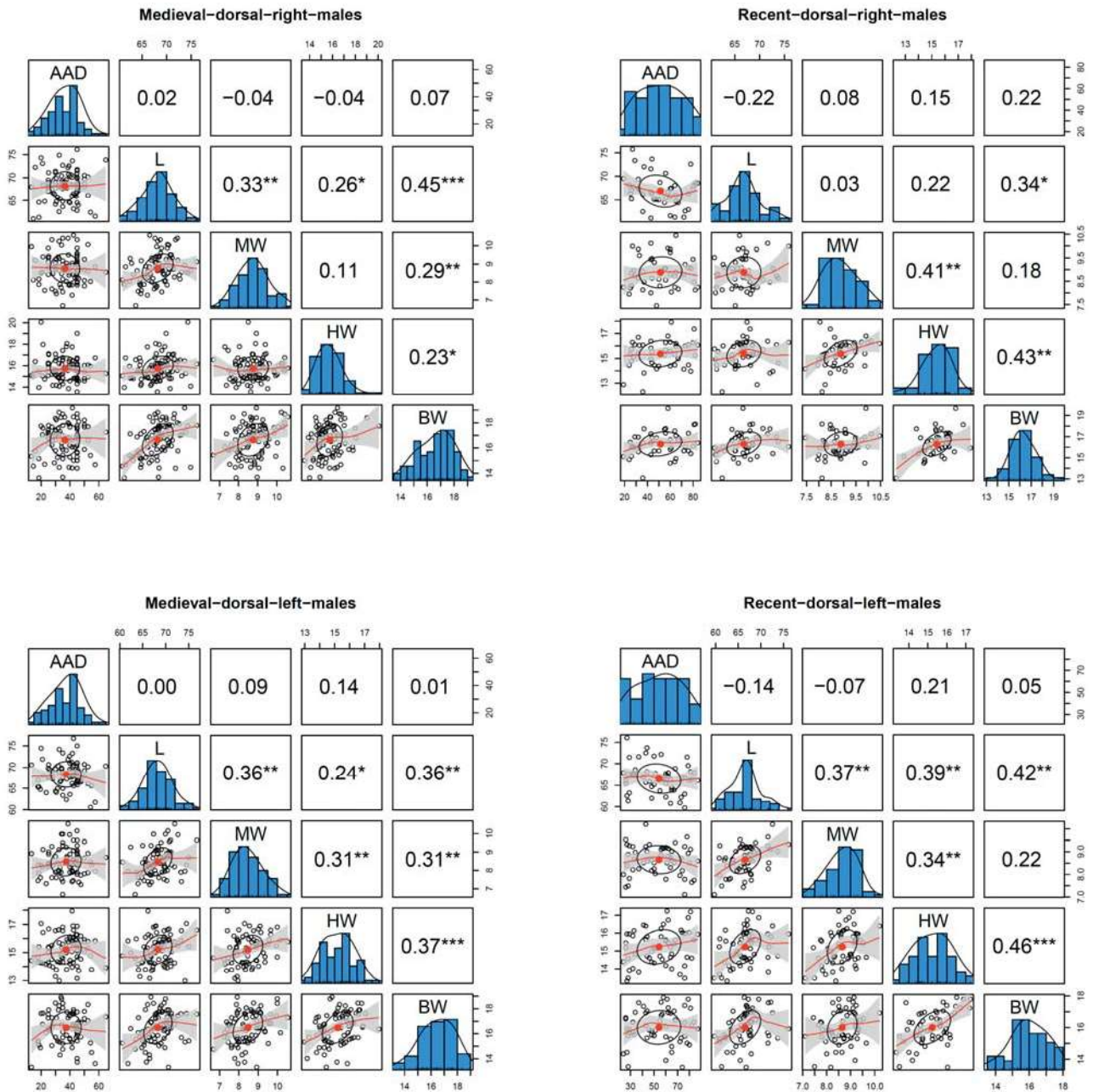


FIGURE A4-3: Correlograms for the second metacarpal measurements and age at death (AAD) for dorsal view on the bone in males; diagonal – histograms, above diagonal – Spearman rank order correlation coefficients with marked significance (asterisks), below diagonal – scatterplots augmented with loess smooths models and correlation ellipses.

Metric differences in adult second metacarpal bones related to age-at-death and their comparison between recent and historical populations

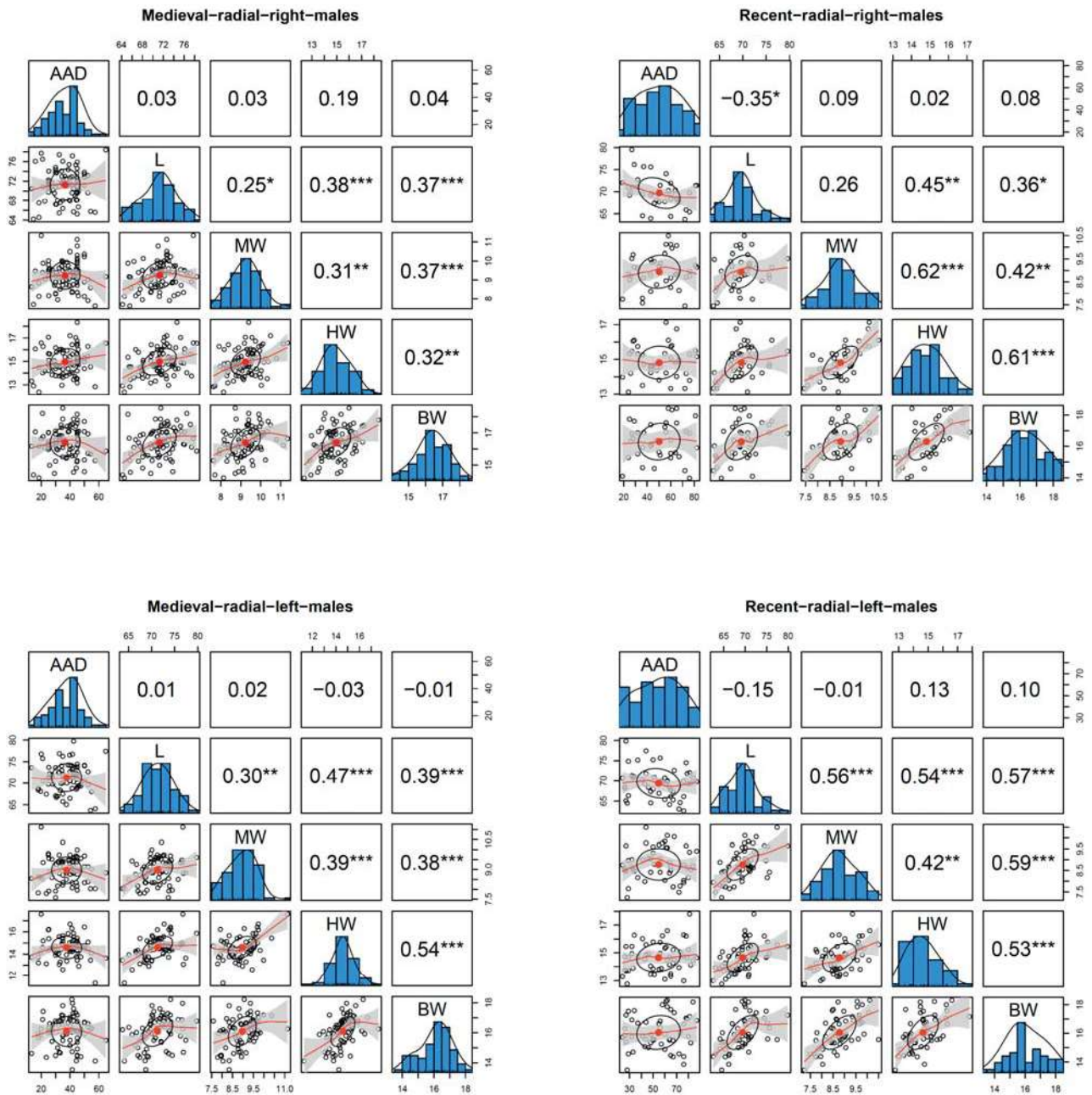


FIGURE A4-4: Correlograms for the second metacarpal measurements and age at death (AAD) for radial view on the bone in males; diagonal – histograms, above diagonal – Spearman rank order correlation coefficients with marked significance (asterisks), below diagonal – scatterplots augmented with loess smooths models and correlation ellipses.

APPENDIX 5: Relationship of measurements with age at death – Regression analysis.

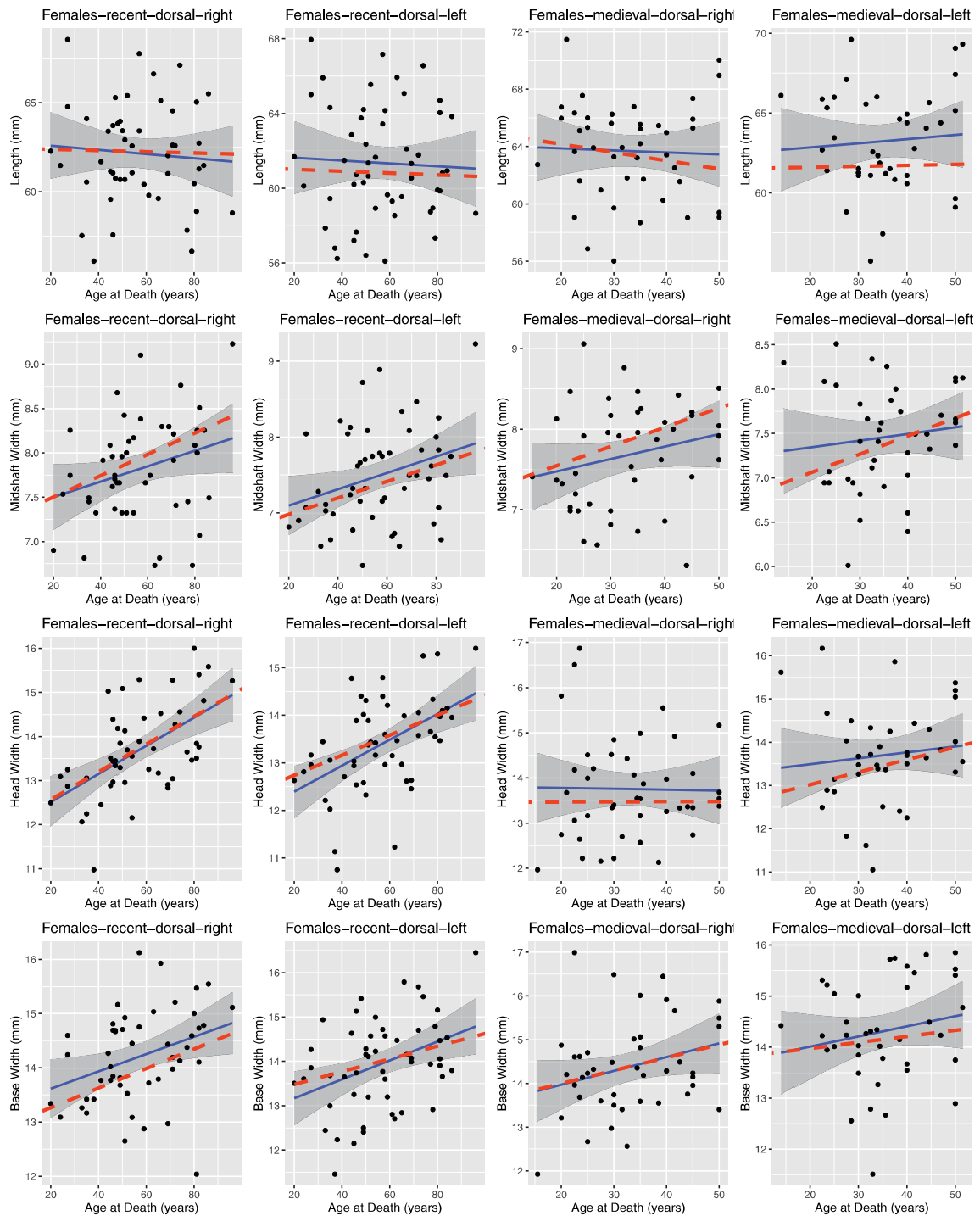


FIGURE A5-1: Scatterplots of relationships between second metacarpal measurements and age at death dorsal view on the bone in females augmented with linear regression models; blue solid line – parametric linear model with 95% confidence interval, red dashed line – nonparametric Kendall–Theil regression.

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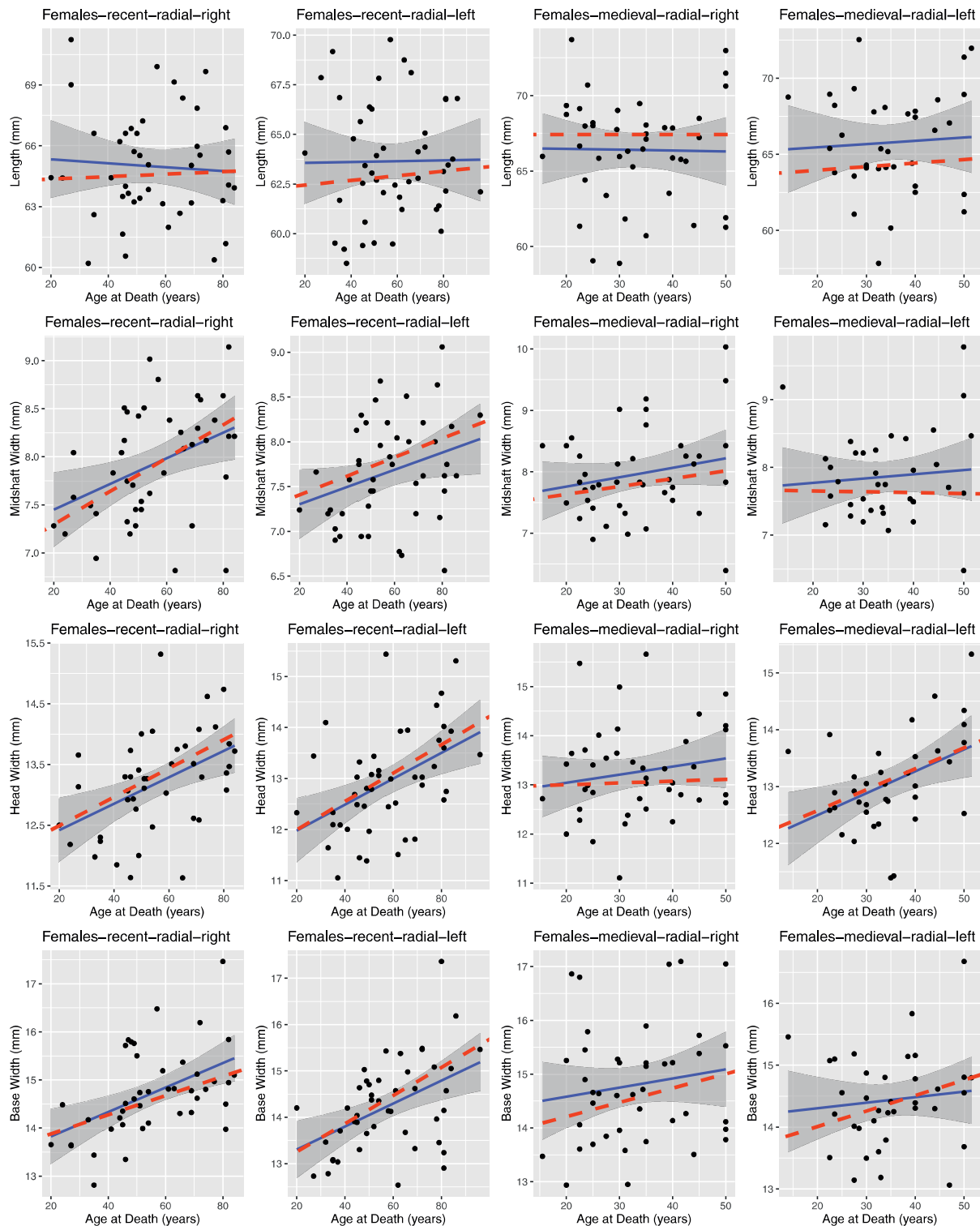


FIGURE A5-2: Scatterplots of relationships between second metacarpal measurements and age at death radial view on the bone in females augmented with linear regression models; blue solid line – parametric linear model with 95% confidence interval, red dashed line – nonparametric Kendall-Theil regression.

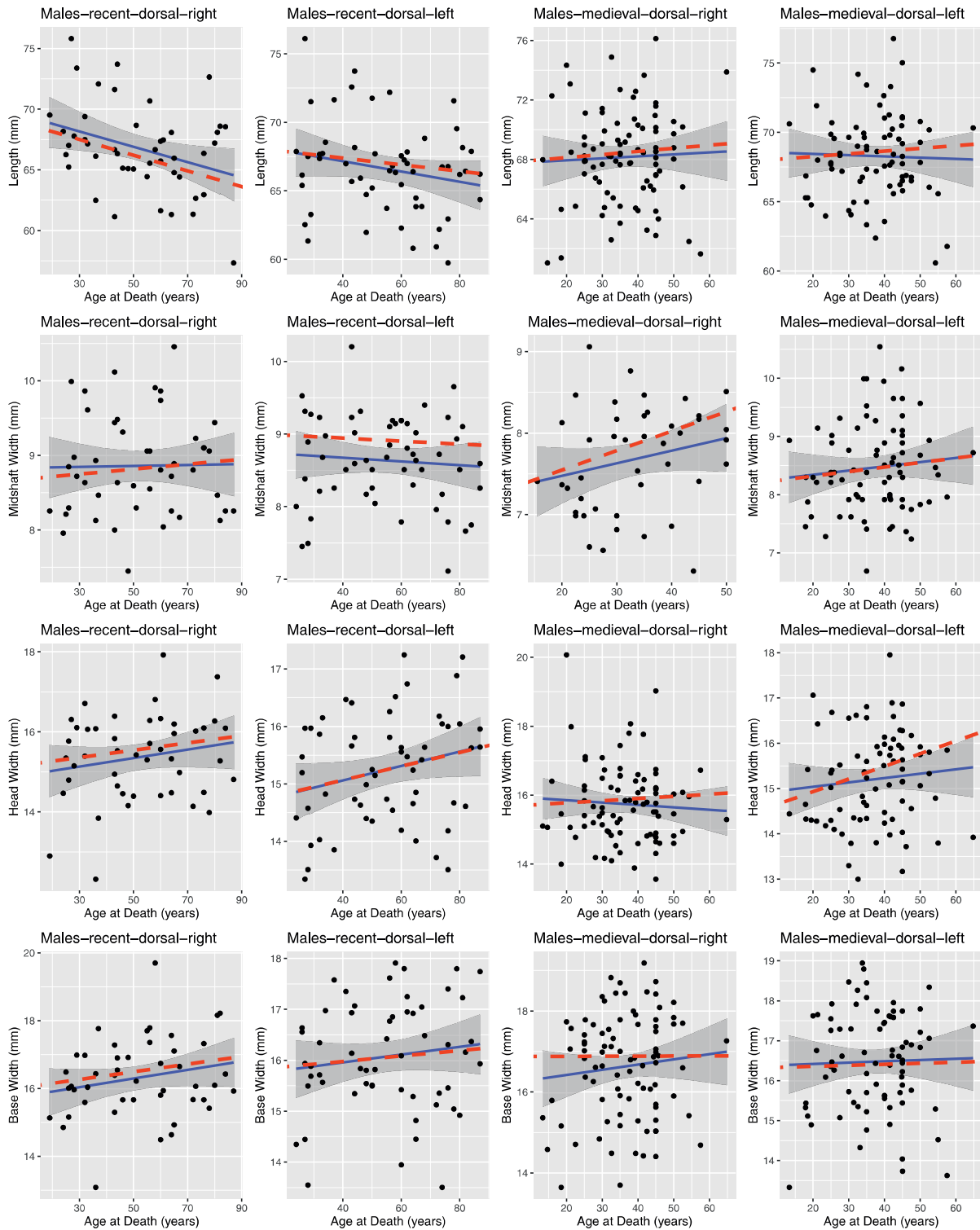


FIGURE A5-3: Scatterplots of relationships between second metacarpal measurements and age at death dorsal view on the bone in males augmented with linear regression models; blue solid line – parametric linear model with 95% confidence interval, red dashed line – nonparametric Kendall–Theil regression.

Metric differences in adult second metacarpal bones related to age-at-death and their comparison between recent and historical populations

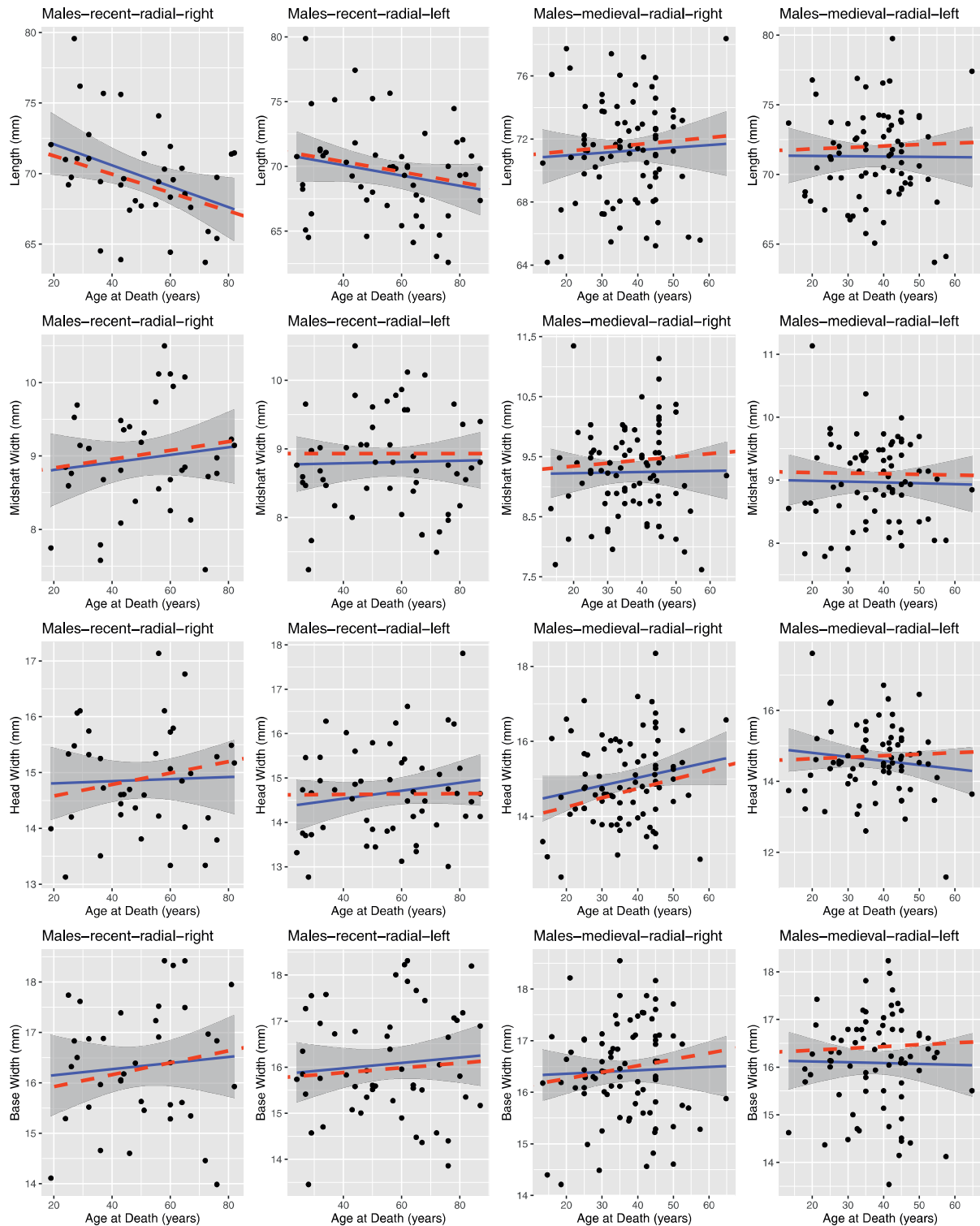


FIGURE A5-4: Scatterplots of relationships between second metacarpal measurements and age at death radial view on the bone in males augmented with linear regression models; blue solid line – parametric linear model with 95% confidence interval, red dashed line – nonparametric Kendall-Theil regression.

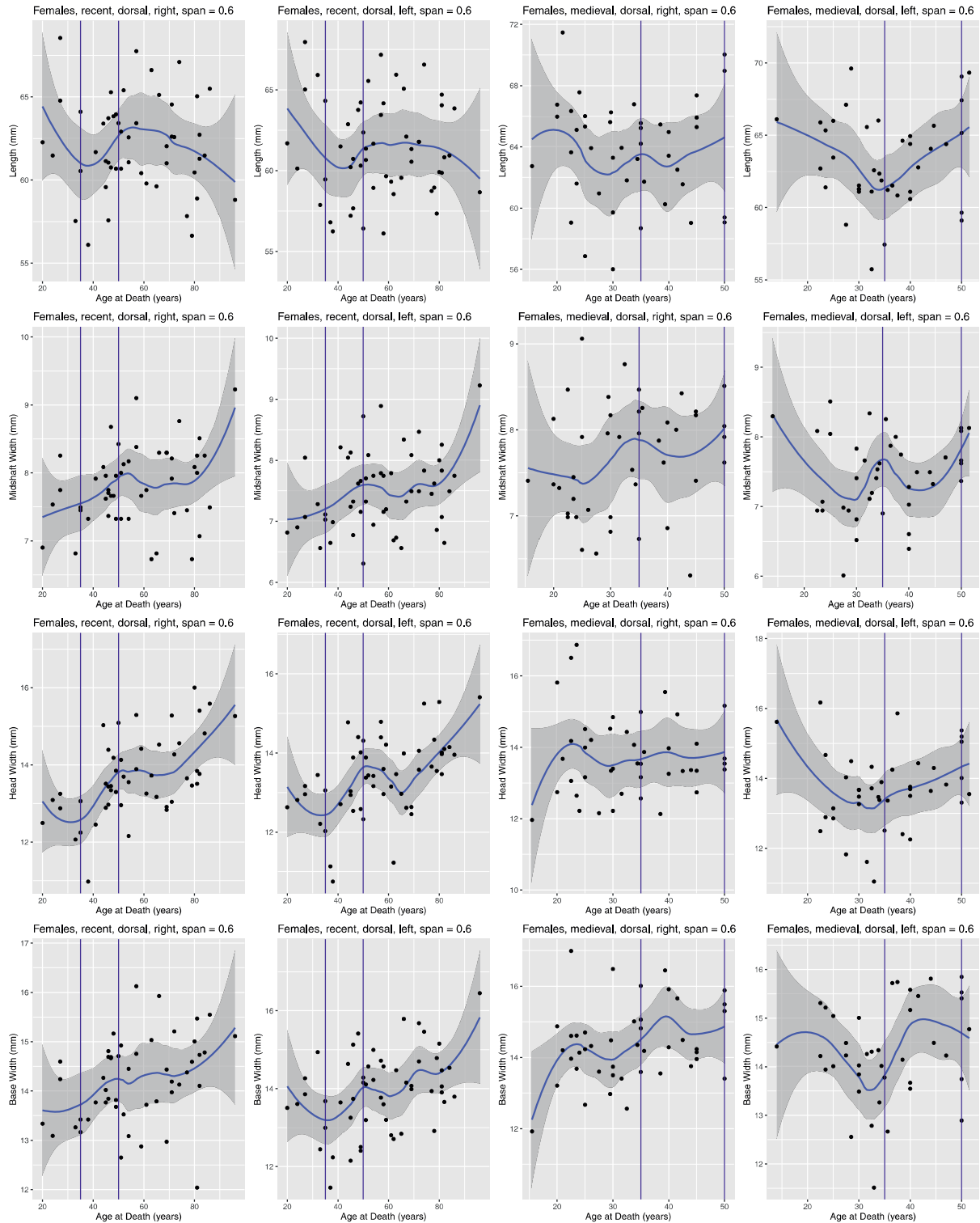


FIGURE A5-5: Scatterplots of relationships between second metacarpal measurements and age at death dorsal view on the bone in females augmented with smoothing spline; blue solid line – smoothing spline (span=0.6) with 95% confidence interval, blue verticals at 35 and 50 years.

Metric differences in adult second metacarpal bones related to age-at-death and their comparison between recent and historical populations

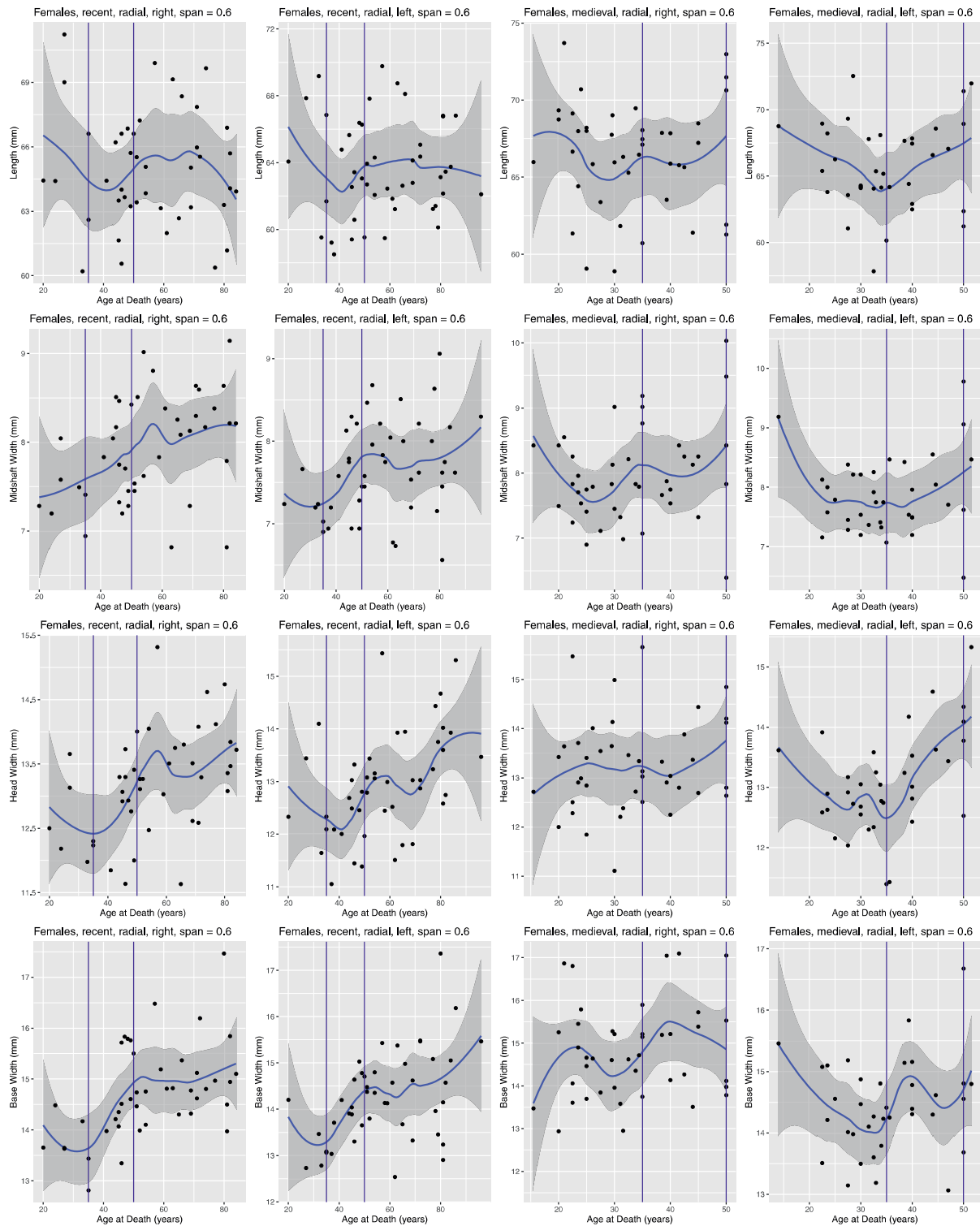


FIGURE A5-6: Scatterplots of relationships between second metacarpal measurements and age at death radial view on the bone in females augmented with smoothing spline; blue solid line – smoothing spline (span=0.6) with 95% confidence interval, blue verticals at 35 and 50 years.

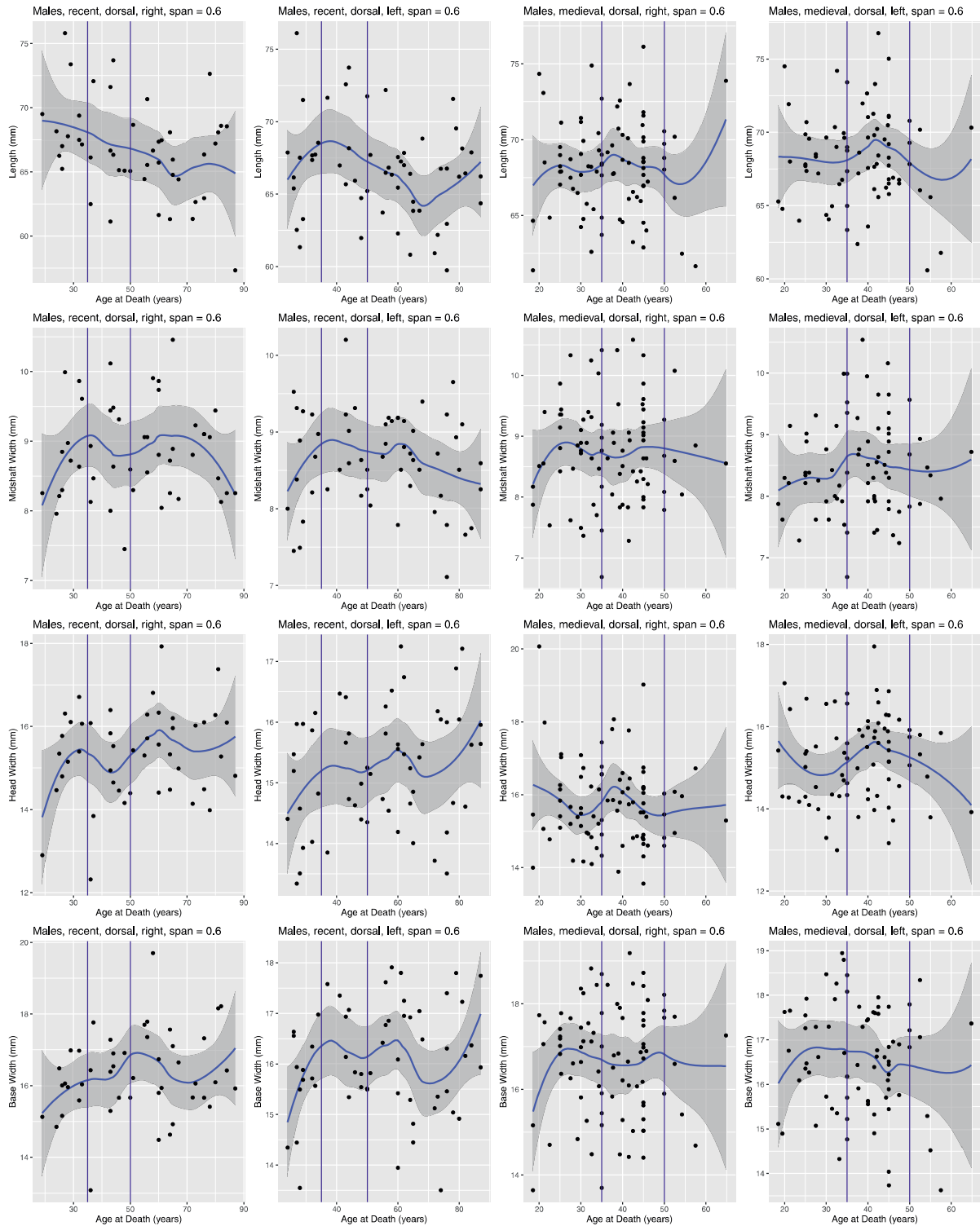


FIGURE A5-7: Scatterplots of relationships between second metacarpal measurements and age at death dorsal view on the bone in males augmented with smoothing spline; blue solid line – smoothing spline (span=0.6) with 95% confidence interval, blue verticals at 35 and 50 years.

Metric differences in adult second metacarpal bones related to age-at-death and their comparison between recent and historical populations

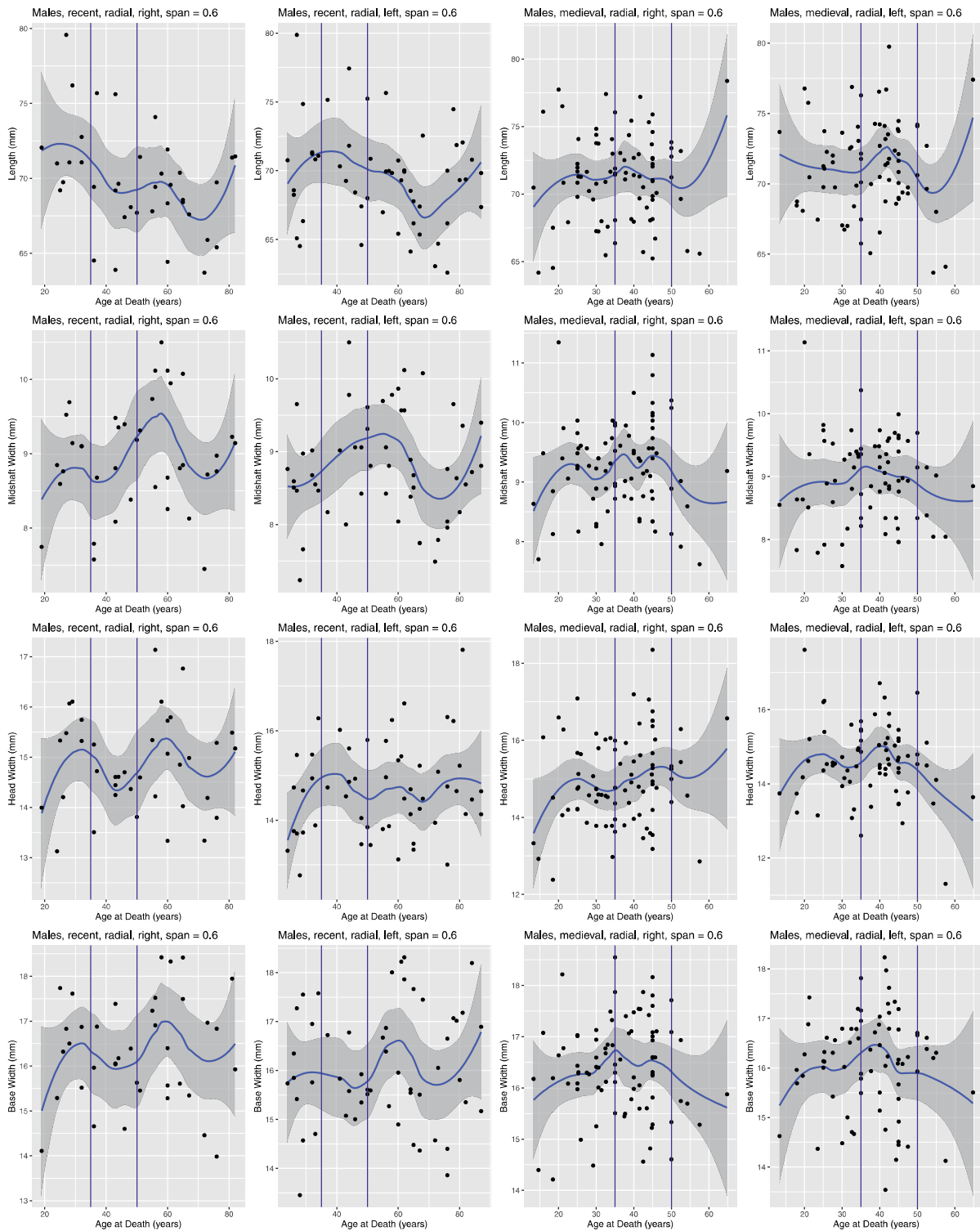


FIGURE A5-8: Scatterplots of relationships between second metacarpal measurements and age at death radial view on the bone in males augmented with smoothing spline; blue solid line – smoothing spline (span=0.6) with 95% confidence interval, blue verticals at 35 and 50 years.