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Bone Quality In Prehistoric, Cis-Baikal Forager Femora: A Micro-CT Analysis of Cortical Canal Microstructure

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ABSTRACT Bone quality, a contributor to bone strength, is determined by structural and mechanical properties, which may be analyzed by gross and/or microscopic methods. Variables that contribute to bone quality, such as porosity, can provide insight into the health and lifestyles of people in prehistory. This study tests the ability of microcomputed tomography (μCT) to capture and characterize cortical canal systems in archaeological bone. Seven variables and 71 femora are analyzed to explore bone dynamics in prehistoric foragers from Lake Baikal, Siberia. The results indicate that canal number and canal separation differ significantly ($P < 0.05$) between age-at-death categories, but only for the pooled and male samples. When merged into a new variable by means of principal components analysis, canal diameter and canal surface to canal volume are also able to discriminate amongst age-at-death categories, as well as between the sexes. However, the overall lack of significant differences between the sexes and amongst age-at-death categories indicates that Baikal forager bone quality (i.e., canal architecture) did not change drastically throughout the lifespan. Interestingly, principal component one identified an untested variable that contributes to canal microstructure variability, and a sexual division of labor may promote divergent trends in canal degree of anisotropy between the sexes. Overall, μCT provides an alternate method for exploring bone quality in archaeological remains, complementing existing methods such as thin-sectioning and gross morphological analyses. Am J Phys Anthropol 154:486–497, 2014.

Skeletal remains provide insight into the sociocultural dimensions of past groups and the life histories of individuals. If skeletal remains are damaged or incomplete, microscopic analyses may be used to ascertain variables associated with bone quality in past populations, which may be used to address broader biocultural questions. The femur has often been employed for such analyses due to its size and, hence, its greater probability of surviving archaeological contexts. Traditionally, researchers have used thin-sections to perform histological analyses of the femur, particularly in reference to age-at-death estimation (e.g. Kerley, 1965; Ahlqvist and Damsten, 1969; Thompson, 1978, 1979; Stout and Gehlert, 1980; Ericksen, 1991). Recently, Cooper (2003, 2005) and colleagues (2007) introduced micro-CT (μCT) as a new method for analyzing bone histology, focusing on the three dimensional (3D) structure of cortical pore systems and their changes with advancing age. Additionally, Cooper (2005) suggested that μCT may be practical for analyzing archaeological bone due to the better preservation of cortical canals in archaeological contexts.

This study employs the approach of Cooper (2005) and colleagues (2007) to: 1) determine whether μCT is capable of reconstructing the microstructure of archaeological bone; 2) discern if any significant differences in porosity are present in Baikal forager femora between the sexes and amongst age-at-death categories; and 3) explore what bone quality may indicate in terms of the biocultural dynamics of past populations, with a focus on middle Holocene Lake Baikal foragers.

BACKGROUND

Bone is a dynamic material. In the majority of postcranial elements, bone is initially deposited on a cartilaginous matrix, with appositional and longitudinal growth of the element occurring via endochondral and subperiosteal ossification (Scheuer and Black, 2000; Hall, 2005). During this period, primary bone structures are created, including the formation of vascular canals and circumferential lamellar bone. With the cessation of longitudinal growth, the primary method of bone repair and structural modification is via remodeling (Hall, 2005; Robling and Stout, 2008). In this process, basic multicellular units (BMUs) resorb and deposit bone, creating osteons, also known as Haversian systems. With age and the accumulation of remodeling events, the number of osteons increases, until reaching an asymptote at a maximum of the seventh decade of life (Wu et al., 1970;

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Walker et al., 1994). At this point, newly forming osteons replace their older counterparts, as all primary bone has been replaced with Haversian systems.

Histological structures have been used to analyse bone quality and health in past populations. For example, abnormal bone formation and resorption patterns indicate the presence of various primary and secondary metabolic diseases (e.g., Aaron et al. 1992; Brickley et al., 2007; Mays et al., 2007; De Boer et al., 2013), including those induced by dietary stress (e.g., Martin and Armelagos 1979, 1985; Martin et al. 1987; Stout and Jackson, 1990; Paine and Brenton, 2006a,b; Brickley et al., 2007; Petrone et al., 2011). Histology has also been used to examine age-related bone loss (e.g., Martin and Armelagos 1979, 1985; Burr et al., 1990; De Boers et al. 2013), and differences in cortical microstructure between the sexes also underscore the impact of pregnancy and menopause on the female skeleton (e.g., Martin and Armelagos 1979, 1985; Cho and Stout, 2011). Additionally, histology can be used to explore culturally mediated dietary and/or activity-based differences between the sexes (e.g., Burr et al., 1990; Mulhern and Van Gerven, 1997; Robling and Stout, 2003; Cho and Stout, 2011; but see Pfeiffer et al., 2006).

Bone quality itself is a somewhat ambiguous term, encompassing several possible contributors to increased bone strength (Sievänen et al., 2007), defined as the amount of loading that an element can tolerate prior to failure (Carter and Beaupré, 2001; Felsendberg and Boonen, 2005; Knudson, 2007). Bone strength is determined by structural (e.g., architecture, size, shape) and mechanical (e.g., microdamage, collagen and mineral orientation, ratio of collagen to mineral, etc.) properties (Felsendberg and Boonen, 2005; Hernandez and Keaveny, 2006; Seeman and Delmas 2006; McNeil and Boyd, 2007; Boyd et al., 2011). An imbalance in these various factors can lead to bone fragility, in which an element is unable to withstand normal physiological loading (Carter and Beaupré, 2001; Felsendberg and Boonen, 2005). Although there are many ways in which to analyse bone quality, in this article, the term refers to porosity and the microstructural organization of bone, variables that contribute to fracture resistance (e.g., Turner, 2002; Augat and Schorlemmer, 2006; Seeman and Delmas, 2006; Chen et al. 2007, Kostenuick et al., 2011).

Although several features of cortical structure (i.e., distribution of bone, bone mineral density, etc.) and microstructure (i.e., percentage remodeled bone, collagen orientation, etc.) are not analyzed with the method of Cooper (2005) and colleagues (2007), it does hold the promise of proving useful in better understanding bone quality in past populations. More specifically, it offers a 3D view of the pore systems in cortical bone, which are affected by factors such as genetics (e.g., Weinstein and Bell, 1988; Cho et al., 2006; Schnitzler et al., 2009), sex (e.g., Cho et al., 2006; MacDonald et al., 2011; Nishiyama et al. 2012; Kazakia et al., 2013), age (e.g., Jowsey, 1966; Cho et al., 2006; Cooper et al., 2007; Burghardt et al., 2010; MacDonald et al., 2011), diet (e.g., Martin et al. 1987; Stout and Jackson, 1990; Paine and Brenton, 2006a,b), environment (e.g., Cho et al., 2006; Brickley et al., 2007; Petrone et al., 2011), and activity (e.g., Skedros, 1994, 1997, 2004; van Oers et al., 2008a,b). Additionally, μCT has the potential to circumvent problems associated with diagenetically altered bone because it relies on the preservation of Haversian canals, which are less susceptible to damage due to highly mineralized canal walls (Hanson and Buikstra, 1987; Samson and Branigan, 1987). It is automated, which removes a degree of interobserver error (Bousson, 2000; Thomas et al., 2006; Matrille et al., 2009), and the amount of bone required for analysis is small, thereby minimizing damage to archaeological collections. The bone analyzed is not affected by chemical processing and can subsequently be used for additional tests, ultimately reducing the total amount of sampling necessary.

MATERIALS

The Lake Baikal region of Russia is rich in archaeological sites, including many forager burial areas. Located in the south of eastern Siberia (52°–58° north latitude, 101°–110° east latitude), *Cis*-Baikal (Michael, 1958) is defined as the regions to the north and west of the lake, including portions of the Angara and Lena drainage basins, as well as the west coast of the lake and Ol'khon Island. The Baikal Archaeology Project (BAP) is an international collaboration of researchers that has excavated mortuary sites in this region, including five forager cemeteries on the western and southern coasts of Lake Baikal (Fig. 1). The cemeteries are dated to the middle Holocene (Weber and Bettinger, 2010; Weber et al., 2010) and include burials attributed to the Kitoi (8000-6800 calBP), a Forest Neolithic culture.

Kitoi individuals were included in the analysis if femora were fused, indicating cessation of linear growth in this element, and if the skeletons were preserved well enough to determine sex and age-at-death using standard gross morphological methods [see Buikstra and Ubelaker (1994) for a compilation of methods]. All sex and age-at-death[1] determinations were made by KF to reduce interobserver error. Sex was determined using pelvic and skull morphological characteristics, including the shape and presence or absence of features on the pubic symphysis (Phenice, 1969), the appearance of the preauricular and auricular surfaces (Milner, 1992; Buikstra and Ubelaker, 1994), the angle of the sciatic notch (Buikstra and Ubelaker, 1994; although see Walker, 2005), and mandibular and cranial characteristics, such as the relative size of the mastoid process and the appearance of the glabella (Acsádi and Nemeskéri, 1970). Overall, due to taphonomic factors and mortuary customs of skull removal, these determinations were more heavily weighted toward pelvic characteristics. A multifactorial approach was adopted to determine the assignment of an individual to an age-at-

[1]The accuracy of age-at-death prediction varies depending on reference sample composition [see Bocquet-Apple and Masset (1982), as well as overviews by Jackes (2000) and Milner et al. (2008)], as well as genetic and epigenetic factors, such as ethnicity, nutrition, activity, and health status. Therefore, although ages-at-death treated as "known" in the remainder of this study are determined by gross morphological methods, the actual accuracy of these predictions is unknown, although most methods tend to overestimate age-at-death in the young, and underestimate age-at-death in older individuals. The pelvic methods used in this analysis, specifically the Suchey-Brooks method (Brooks and Suchey 1990) for pubic symphyses and the Meindl and Lovejoy (1989) method for auricular surface analyses, have been tested in modern Asian populations, with mixed results (Sakaue 2006, Schmitt 2004). At the very least, these methods have provided a means for seriating the collection into the age-at-death categories used for this study.

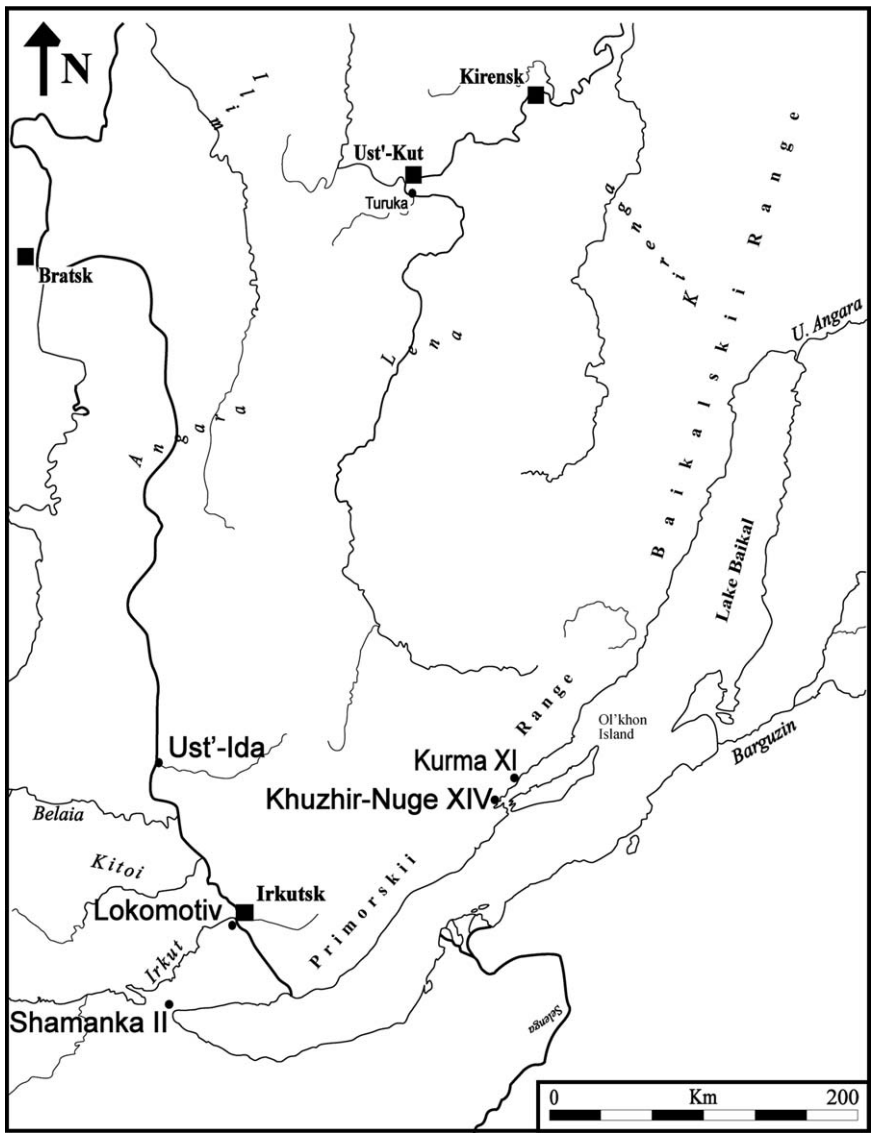

Fig. 1. The location of the five cemeteries used in this analysis. Lake Baikal, Siberia, Russian Federation.

death category. For each skeleton, an age-at-death range was produced by the overlap in estimated age ranges derived from several independent methods; these included the metamorphosis of the pubic symphysis (Brooks and Suchey, 1990), auricular surface (Lovejoy et al., 1985; Meindl and Lovejoy, 1989), and sternal rib end (Işcan et al., 1984), as well as the stage of fusion of late fusing epiphyses (e.g., sternal clavicular epiphyses, spheno-occiptial synchondrosis, etc.). For the present analysis, and in line with research already conducted on this population (e.g., Lieverse, 2005, 2010; Lieverse 2007; Lieverse et al., 2007a,b, 2009; Waters-Rist et al., 2010, 2011; Waters-Rist, 2011), individuals were divided into the following age-at-death categories: adolescent (<20 years), young adult (20–34 years), middle adult (35–49 years), and old adult (50+ years) (Table 1).

Samples were always procured from the anterior aspect of the femur and, when possible, from the midshaft. However, in fragmented remains, the sample was sometimes derived from other locations along the length of the shaft[2], but as close to midshaft as possible. In order to minimize the amount of destruction inflicted on the archaeological collection, if an adequate sample of the anterior aspect of the femur was previously collected for other analyses, this was used in lieu of incurring more damage by unnecessary oversampling.

METHODS

Micro-CT analysis

Sections of femora, measuring 4 mm in width, 5 mm in length, and the full thickness of the cortical envelope, were placed in an 11.4 mm diameter specimen holder. Images were generated using a μCT 35 scanner (Scanco

[2]According to Chan et al. (2007), who used Thompson's (1978) method to compare age-at-death estimates along the length and around the circumference of cadaveric femora, samples taken from the anterior aspect of the femur produced comparable results, regardless of location along the diaphysis.

TABLE 1. Kitoi sample by age-at-death and sex categories

	Age category				
Sex	Adolescent	Young adult	Middle adult	Old adult	Total
Female	4 (2)	16 (7)	1 (0)	5 (3)	26 (12)
Male	2 (2)	23 (14)	14 (8)	6 (4)	45 (28)
TOTAL	6 (4)	39 (21)	15 (8)	11 (7)	71 (52)

Numbers outside parentheses represent the sample that includes modified images (i.e., the 5VDS), whereas those inside parentheses represent the sample with unmodified images only (i.e., the 7VDS).

TABLE 2. Scanning protocol for micro-CT analysis, based on Cooper (2005), modified for the μCT 35

Femoral scanning protocol, Scanco μCT 35	
E (kvp)	70
1 (uA)	114
Resolution	High
Samples	2048
Proj/180deg	1000
Model	Conebeam
Tube Diameter	14.9 mm
Slice Number	462
Increment	6 um
Integration Time	800 ms
Average Data	1
Measure Time	276.4 min
Calibration	70 kVp, BH: 1200 mg HA/ccm, scaling

Medical, Switzerland; Micro-CT Laboratory at the University of Calgary), using the scanning protocol developed by Cooper (2005), but modified here for the μCT 35 (Table 2). A total of 462 transverse *X*-rays of each sample were acquired and then constructed into a 3D image, producing images with a 6 μm nominal isotropic resolution.

For each sample, a circular region of interest (ROI) was chosen, abutting the periosteal border on the most anterior aspect of the shaft. This ROI was defined on each transverse X-ray image (i.e., the 462 slices, Fig. 2), producing a cylindrical volume of interest (VOI) that followed a linear path through the length of the femoral sample. Each ROI was 3 mm (325 × 325 pixels) in diameter, resulting in VOIs with a volume of 78.38 mm^3. If possible, the ROI and resulting VOI were derived from a region of good preservation (i.e., free of cracks, low bone mineral density, canal inclusions).

Once the VOI was defined, each sample was individually thresholded using Scanco Medical analysis software (Image Processing Language, v5). The threshold value was chosen according to its ability to best produce a binary image where bone (white) and pore space (black) most accurately reflected these structures in the slices (Fig. 3). Samples were smoothed by a Guassian filter in order to reduce noise, as well as to most accurately reflect the correct shape and size of the bone and pore spaces in the binary image. Cracks, canal inclusions, and other problematic regions were removed from the sample using postprocessing techniques that essentially "erase" areas with such defects. Samples were discarded from the analysis if it was impossible to produce a binary image that accurately represented the sample's porous architecture.

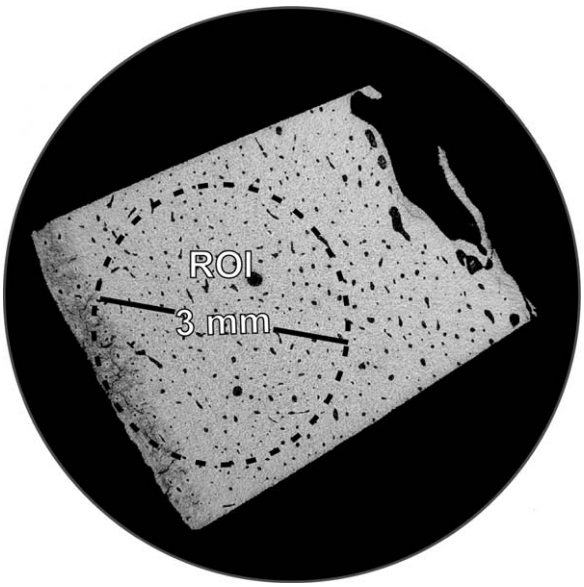

Fig. 2. An example of a region of interest (ROI) on one transverse X-ray of bone. Low BMD along the periosteal surface may complicate analyses and, if so, is removed if the region is not too large.

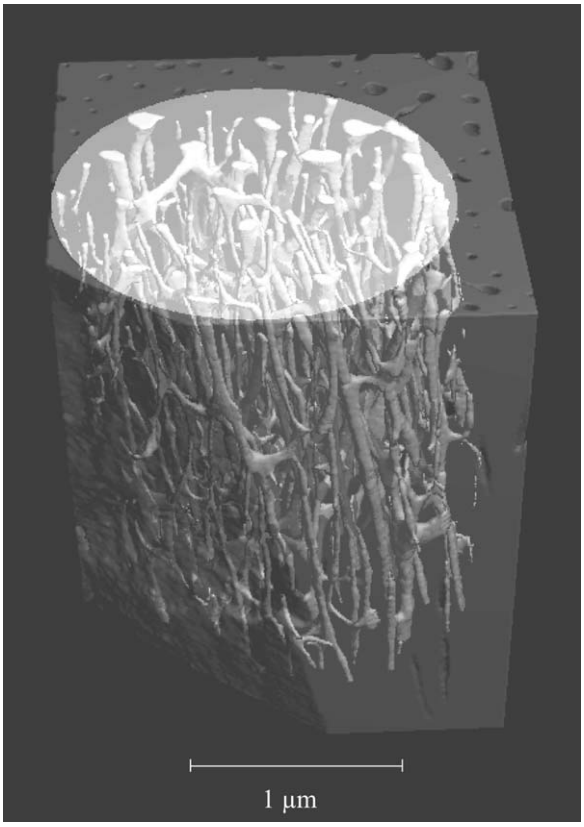

Fig. 3. Individual X-rays are compiled into a 3D image, and then the colors assigned to bone and pore space are reversed, making the pore networks visible.

Subsequently, the remaining manipulation and quantitative analysis of each VOI was conducted via customized scripts. As with Cooper (2005) and colleagues'

TABLE 3. Trabecular variables and analogous canal variables with units [after Cooper et al. (2003), with abbreviations based on Parfitt et al. (1987)]

Trabecular variable	Canal variable	Unit
Bone Volume Fraction (BV/TV)	Cortical Porosity (Ca.V/TV)	%
Trabecular Number (Tb.N)	Canal Number (Ca.N)	1/mm
Trabecular Separation (Tb.Sp)	Canal Separation (Ca.Sp)	μm
Trabecular Thickness (Tb.Th)	Canal Diameter (Ca.Dm)	μm
Bone Surface to Total Volume (BS/TV)	Canal Surface to Canal Volume (Ca.S/Ca.V)	%
Connectivity Density (ConnD)	Canal Connectivity Density (Ca.ConnD)	$1/mm^3$
Degree of Anisotropy (DA)	Canal Degree of Anisotropy (Ca.DA)	1=isotropic, >1 anisotropic

(2007) method, the VOI was inverted so that bone appeared as black space, and pores appeared as white bone, allowing the software to analyze the canal architecture using parameters originally intended for trabecular analyses (Table 3, left column.). The parameters were subsequently relabelled to reflect the pore structures analyzed (Table 3, right column).

STATISTICAL ANALYSIS

Changes in bone porosity were analyzed between the sexes and amongst age-at-death categories. All statistical analyses were performed using JMP 9.0.3 and 10.0.0 (JMP, 1987–2010; JMP 1987–2012), with a probability of <0.05 considered significant. The distribution of variables was analyzed using the Shapiro–Wilk W goodness of fit test (Shapiro and Wilk, 1965). Outliers, defined as values greater than 1.5 times the interquartile range (SAS Institute, Inc., 2010), were removed from the analysis. Non-normally distributed variables and/or those of unequal variance were transformed using the Johnson Su (JSU), which normalizes data characterized by a skewed (or kurtotic) distribution (SAS Institute, Inc., 2010), which was particularly relevant for Ca.V/TV and Ca.ConnD. If no transformation resulted in normality, variables were analyzed by nonparametric statistics. Equality of variance was analyzed using Levene's (1960) test.

Significant differences between the sexes were tested using the Student's *t*-test (all were normally distributed). In the pooled and male samples, differences between age-at-death categories were analyzed using analysis of variance (ANOVA) with Tukey HSD post hoc for parametric variables, and Steel-Dwass All Pairs for nonparametric variables. For females, because of the small sample size and instances of single individuals representing an age-at-death category, only the Steel-Dwass statistic was used. Further multivariate methods were not employed because of female sample size constraints, as well as the inability of several variables to meet the assumptions inherent in covariance statistics. However, principal component (PC) analysis with Varimax rotation was performed to explore the relationships amongst μCT variables, as well as their patterns according to age-at-death and sex. Multivariate outliers were identified using a Jackknife Distances analysis, and only PCs explaining greater than 5% of the variation were considered in subsequent analyses (Zelditch et al., 2004). Principal components were then analyzed for differences amongst age-at-death categories and between the sexes using ANOVA with Tukey HSD post hoc and Student's *t*-test, respectively.

RESULTS

Micro-CT analysis

Seventy-nine Kitoi samples were scanned, eight of which were deemed too degraded for μCT analysis, resulting in a total of 71 samples available for statistical analyses (Table 1). In reviewing the data, abnormal Ca.V/TV and Ca.ConnD values were detected for 23 individuals, all of which had regions removed due to the presence of cracks and/or low bone mineral density. Thus, for Ca.V/TV and Ca.ConnD (which are significantly correlated, $r = 0.737$, $P < 0.0001$) modified samples ($n = 31$) were excluded from analysis in order to avoid erroneous results, resulting in a sample size of 40 individuals (Table 1, from here referred to as 7VDS, i.e., the seven variable dataset). However, there is no reason to assume that the other five variables were affected by the removal of cortical areas; for example, Ca.Dm should not be influenced by the removal of nonporous regions, as the variable relies on canal characteristics alone. A review of data confirmed this, as data for these five canal variables in modified samples fell within the normal range of values for unmodified samples. Therefore, the full sample of 71 individuals was used to analyze the five remaining canal variables (Table 1, from here referred to as 5VDS, i.e., the five variable dataset). For the PC analysis, both datasets were tested in order to ascertain the influence of Ca.V/TV and Ca.ConnD, as well as to increase the power of the analysis for all other variables.

Data for the seven μCT variables were plotted according to age-at-death category for the sex-stratified and pooled samples (Fig. 4a–c) in order to observe whether trends were evident with advancing age (see Table 4 for summary statistics). In the sex-stratified samples, males and females were similarly characterized by increasing values of Ca.N and Ca.ConnD, decreasing values of Ca.Sp, and relatively constant levels of Ca.Dm[3] and Ca.S/Ca.V. Conflicting trends were present in Ca.V/TV and Ca.DA, which increased in females, but decreased in males. When trends conflicted, the pooled results tended to mirror those of the male sample, reflecting the larger sample size.

In the univariate analyses, significant differences were noted for Ca.N and Ca.Sp. When the sexes were pooled, significant or near significant differences were identified between adolescents and middle adults, adolescents and old adults, young and old adults, and young and middle adults (Table 5). When the sexes were stratified, significant differences were noted between adolescent and old adult males (Ca.N $P = 0.047$; Ca.Sp $P = 0.028$), with near significant differences between

[3]Because the calculation of Ca.Dm is automated, it is possible that forming osteons are incorporated into the analysis, with unfilled canals positively skewing values. Because Ca.Dm is the average of all canals in a sample, the effect is assumed to be slight, particularly in older adults. See Cooper et al. (2006) for a discussion of μCT and BMU-related resorption spaces.

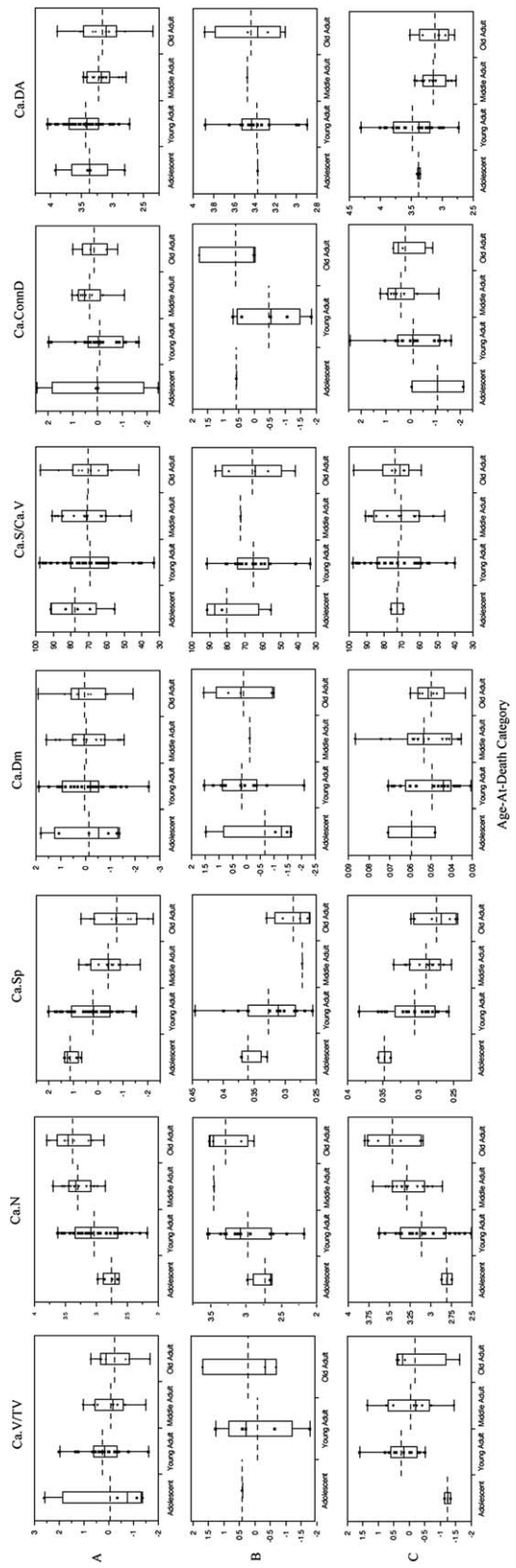

Fig. 4. (**a–c**) Boxplots of μCT variables by age-at-death category for the (a) pooled, (b) female, and (c) male sample. Hashed line represents the mean, solid line the median.

young adult and old adult males (Ca.N $P = 0.064$) and adolescent and middle adult males (Ca.Sp $P = 0.071$). No significant or near significant differences were found between female age-at-death categories, nor for any other variables in the male and pooled samples. When age-at-death categories were pooled, there were no significant differences between the sexes for any μCT variable, although Ca.N neared significance ($P = 0.064$).

Principal components results for the 5VDS and 7VDS were similar in structure (Table 6), with 98.36% and 98.45% of the variance explained by three and four PCs, respectively. For both analyses, scatterplots did not produce overtly obvious groupings; however, for PC2, younger individuals tended to fall toward the more positive range of the axis, and older individuals grouped toward its more negative aspect. These observations were echoed using ANOVA, where significant differences were noted for PC2 only (see Fig. 5a–b for boxplots of PC2 by age-at-death category). In the 5VDS, adolescents were significantly different to middle adults ($P = 0.017$), and young adults to old adults ($P = 0.016$), with no significant differences between the sexes. In the 7VDS, significant differences were found between adolescents and middle ($P = 0.025$) and old ($P = 0.020$) adults, between young and old adults ($P = 0.030$), and between the sexes ($P = 0.002$; female $\bar{x} = 1.300$, male $\bar{x} = -0.312$). In both PCAs, the correlation between PC2 and Ca.Dm was positive ($r = 0.67$ 5VDS; $r = 0.722$ 7VDS), and between PC2 and Ca.S/Ca.V negative ($r = -0.596$ 5VDS; $r = -0.693$ 7VDS). This indicates that pooled Ca.Dm values decreased with advancing age, while pooled Ca.S/Ca.V increased, and that females were generally characterized by greater Ca.Dm values, and lesser Ca.S/Ca.V values, than males.

DISCUSSION

Using micro-CT in archaeological bone

Overall, μCT was successful in recreating the cortical pore structure of femora belonging to middle Holocene foragers, although a small number ($n = 8$) of samples were too affected by taphonomic changes for analysis. Additionally, the removal of problematic regions during post-processing affected Ca.V/TV and Ca.ConnD values. Generally, the trajectories of μCT variables in the archaeological sample were similar to those found in the modern cadaveric sample analyzed by Cooper (2005) and colleagues (2007), indicating that the trends observed were real. However, in the modern cadaveric femora, males and females followed similar trajectories for all variables analyzed with respect to advancing age. This differed in the archaeological femora, in which divergent trends for the sexes were noted for Ca.DA, Ca.V/TV, and Ca.Dm with advancing age.

Cortical remodeling in Baikal femora: Age and sex

Canal number and Ca.Sp differed significantly between age-at-death categories, which, according to their heavy weighting on PC1, are correlated with one another. Generally, adolescents and young adults differed from middle and old adults, with significant differences noted in males, but not females. This discrepancy may be an artifact of sample size, as a greater number of males was analyzed. Alternatively, it could also result from a more linear trend in male values, compared with the nonlinear trend in middle adult female values.

TABLE 4. Summary statistics for all variables for the pooled, female, and male samples

Category		Ca.V/TV (%)	Ca.N (1/mm)	Ca.Sp (μm)	Ca.Dm (μm)	Ca.S/Ca.V (%)	Ca.ConnD (1/mm^3)	Ca.Da (≥1)
Pooled	Mean	7.23	3.12	309.43	54.15	70.31	17.36	3.37
	SD	8.6	0.37	44.44	16.67	15.01	18.86	0.35
	Max	49.89	3.80	445.40	98.60	97.81	102.72	4.31
	Min	2.61	2.17	244.70	30.60	33.24	3.28	2.73
Females	Mean	10.5	3.01	321.90	57.61	67.82	16.16	3.39
	SD	14.03	0.39	49.11	19.47	16.63	14.08	0.26
	Max	49.89	3.54	445.40	98.60	91.52	58.26	3.89
	Min	2.66	2.17	256.10	34.50	33.24	4.31	2.89
Males	Mean	5.14	3.20	297.30	51.14	71.79	15.07	3.34
	SD	2.00	0.32	33.67	1.31	14.61	11.84	0.37
	Max	11.35	3.80	383.20	86.50	97.81	65.17	4.31
	Min	2.61	2.51	244.70	30.60	40.04	3.28	2.73

Ca.V and Ca.ConnD are derived from the 7VDS, whereas all other variable values are derived from the 5VDS. SD = standard deviation, Max = maximum value, Min = minimum value.

TABLE 5. Steel-Dwass All Pairs results for canal number and canal separation for the pooled sample

	Steel-Dwass All Pairs					
	Young adult		Middle adult		Old adult	
Age category	Ca.N	Ca.Sp	Ca.N	Ca.Sp	Ca.N	Ca.Sp
Adolescent	0.280	0.104	0.005	0.004*	0.008*	0.008*
Young adult			0.090	0.140	0.071	0.056
Middle adult					0.787	0.0697

*Significant difference between age-at-death categories.

Although Ca.N and Ca.Sp were effective at discriminating between a few of the age-at-death categories, the variables lost this ability when combined and transformed into PC1. This indicates that another untested variable yields a greater influence on cortical microstructure variability than age-at-death or sex.

In contrast, PC2 was heavily weighted toward Ca.Dm and Ca.S/Ca.V, which were unable to discern between age-at-death or sex categories when serving as independent variables, but were successful when combined and transformed. Not only were adolescents discernable from middle and old adults, but young adults were distinguished from these older age categories as well, as were females from males. The ability of PC2 to discriminate amongst additional age-at-death categories, as well as between the sexes, most likely reflects the mirrored trends in Ca.Dm and Ca.S/Ca.V between the sexes, as well as the additional power achieved when multiple variables are used in tandem. For example, PC4 was unable to discriminate between the sexes, despite the fact that the trajectories for Ca.DA differed between males and females.

Although some significant differences were found, the majority of variables proved ineffective at discriminating amongst age-at-death categories and between the sexes. This indicates that these factors did not exhibit a powerful effect on pore microstructure and, by proxy, bone quality. Although it is acknowledged that the strength of this statement would be enhanced with larger sample sizes, particularly for females and Ca.V/TV and Ca.ConnD variables, there are several scenarios that may have contributed to this pattern, assuming it is real. For example, health may not have declined with age to an extent that it significantly affected bone pore microstructure. Foragers may not have lived much past 50 years of age, thus minimizing the amount of age-related bone loss experienced [although see Gurvan & Kaplan (2007) and Tuljakpurkar et al. (2007) who argue for greater longevity in foragers than is commonly assumed]. Or, because activity impacts bone remodeling in humans, Kitoi foragers may have been active enough to maintain similar bone porosity values throughout the lifespan [see Maïmoun and Sultan (2011) for a review of activity-related remodeling studies in modern populations, and Pfeiffer and colleagues (2006) for an example of problems identifying activity-related remodeling patterns in archaeological collections]. Additional research is needed to clarify whether the lack of significant differences is real, whether it is an artifact of sample size, particularly for the female subset, or whether it is related to the method used, particularly the size of the ROI/VOI.

TABLE 6. Results of the principal components analysis for the 7VDS and 5VDS

	7VDS				5VDS			
	Variance (%)	Variable	Correlation (r)	*p* value	Variance (%)	Variable	Correlation (r)	*p* value
PC1	47.35	Ca.N	0.73	<0.0001	52.10	Ca.N	−0.76	<0.0001
		Ca.Sp	−0.73	<0.0001		Ca.Sp	0.71	<0.0001
PC2	30.33	Ca.Dm	0.73	<0.0001	30.92	Ca.Dm	0.68	<0.0001
		Ca.S.S/Ca.V	−0.69	<0.0001		Ca.S.S/Ca.V	−0.60	<0.0001
PC3	10.75	Ca.V/TV	−0.75	<0.0001	15.34	Ca.DA	0.78	<0.0001
		Ca.ConnD	−0.44	0.01				
PC4	10.02	Ca.DA	0.74	<0.0001				
Total variance	98.45				98.36			

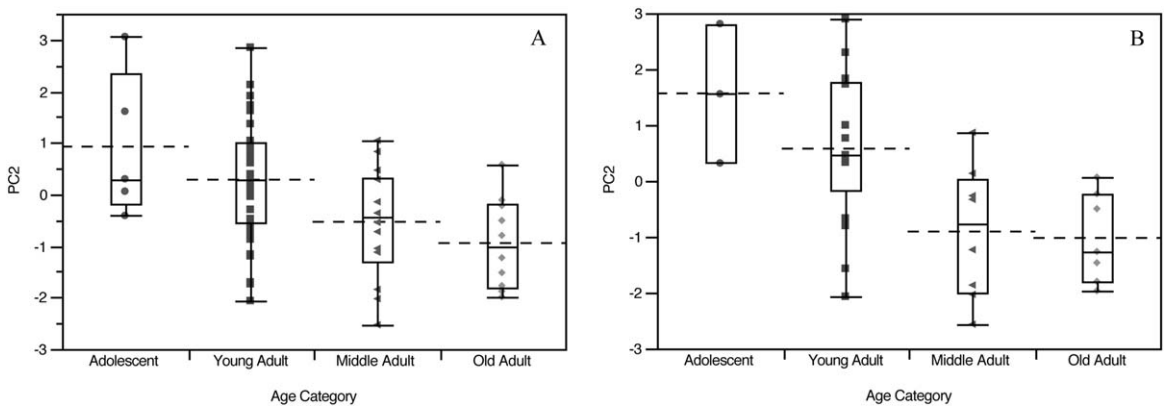

Fig. 5. (**a** and **b**) PC2 boxplots for the (a) 5VDS and (b) 7VSD. Hashed line represents the mean, solid line the median.

Porosity

In this study, it also appears that porosity decreased in males, which is contrary to the female trend, as well as patterns observed in the studies by Cooper (2005) and colleagues (2007). However, neither male Ca.N nor Ca.Dm decreased over time, two factors that should influence porosity. It is argued that the appearance of diminishing Ca.V/TV is an artifact of the data, with values derived from the 7VDS, while Ca.N and Ca.Dm were derived from the 5VDS. When Ca.Dm is analyzed in the 7VDS, it decreases with advancing age, which coincides with the Ca.V/TV results. Thus, at this stage, particularly when taking into account trends in Ca.N and Ca.Dm, there is no reason to believe that male forager Ca.V/TV values decreased over time, and it is likely that, with a greater sample size, increasing values with age would be observed.

Degree of anisotropy

Canal degree of anisotropy is a measurement of directionality. In the cadaveric studies by Cooper (2005) and colleagues (2007), the authors argued that the transition to a more isotropic structure was due to increasing communication between Haversian systems via Volkmann's canals. In the Baikal sample, this same trend is present in males, whereas female Ca.DA increased with age. What could explain the directional differences in trends between the sexes?

When characteristics of female and male cortical porosity were explored, differences between the sexes were insignificant. However, when stratified by age-at-death category, female cortical thickness was less than, and canal diameter was slightly greater than, male values (Table 7). This indicates that the VOI in the female sample is incorporating larger canals, including those located toward the endosteal surface. According to Thomas and colleagues (2005), remodeling is generally more active toward the endosteal surface, and according to Cooper and colleagues (2007), large canals tend to coalesce in the process of trabecularization, resulting in a shift toward anisotropy. Therefore, the "deviant" female pattern could be the result of more endosteal aspects incorporated into the analysis. However, all other factors being equal, one would expect a similar pattern in modern females, which was not the case in the sample studied by Cooper (2005) and colleagues (2007). Thus, other possibilities for increasing Ca.DA in females should be explored.

TABLE 7. Mean canal diameter and cortical thickness (CTHICK) values by age-at-death and sex

	Ca.Dm (μm)		CTHICK (mm)	
Age category	Female	Male	Female	Male
Adolescent	517.75	593.00	5.48	6.55
Young adult	598.67	493.14	5.49	6.34
Middle adult	492.00*	532.57	5.32	6.67
Old adult	598.40	496.83	5.02	5.14

Values should be viewed as illustrative only, as cortical thickness values are only represented by 2–4 individuals each. *$n = 1$.

Signatures of activity?

Could activity be influencing cortical canal microstructure? Is it the unknown variable that corresponds to PC1? Could it be responsible for the divergent trends in Ca.DA observed between forager males and females, and between prehistoric and modern females? Although a sexual division of labor is not a likely candidate for PC1 (as there were no significant differences between the sexes), it is possible that the habitual activity patterns undertaken by individuals or task groups could be influencing cortical canal microstructure and the component heavily weighted toward Ca.N and Ca.Sp. Additional ways of testing this, such as comparing cortical pore microstructure to femoral cross-sectional geometry, could help to answer this question. However, a sexual division of labor might have influenced the sex-based differences in Ca.DA trends, and a more active lifestyle in the Kitoi may be responsible for the differences observed between forager and modern females.

According to Lieverse (2009) and colleagues (2007, 2011) and Stock and colleagues (2010), musculoskeletal markers, the distribution of osteoarthritis in the skeleton, and differences in bone shape provide evidence for gendered activity and mobility strategies in the Kitoi. Additionally, Lieverse (2009) and colleagues (2007, 2011) argue that these patterns support a logistical foraging strategy, where males form task groups, travel away from base camps in order to procure various resources, and likely traversed complex terrain, particularly in the

winter months. If this explanation is entertained, then the difference in Ca.DA trends could be due to the sexes engaging in different habitual activity patterns. If strain patterns differed on a consistent basis, then this could have influenced the directionality of canals, which align in the principal direction of strain (Skedros, 1994, 1997, 2004; van Oers et al. 2008a,b). This suggests that strain was more multidirectional in males, versus unidirectional in females (and, by extension, more multidirectional in modern versus prehistoric females). Additionally, greater strain results in smaller canals (Skedros, 1994, 1997, 2004; van Oers et al. 2008a,b). This could imply that strain in male femora was higher than that in female femora, resulting in a remodeling strategy that incorporates smaller Ca.Dm, although other factors, such as hormonal changes associated with pregnancy and menopause, cannot be ruled out. More data are needed, particularly in the female category, to make more concrete arguments, and further exploration of these patterns is warranted to better understand causation and variation in remodeling characteristics in human populations.

Micro-CT: a unique perspective of bone dynamics and bone quality

According to Frost (1983), the skeletal system is comprised of various levels of skeletal intermediary organization (IO). This approach to skeletal organization posits that there is a hierarchical structure to bone composition, beginning at the cellular level, and ultimately culminating at the skeletal level. Beginning with cells, each subsequent stage of organization is a product of the more "basic" organizational units, but each stage is also indivisible, possessing "quantum" properties associated with that level of organization alone. Gross morphological methods focus on the higher and organ levels of organization, referring to epiphyses and whole elements, respectively, and rely on the gradual degeneration of joint surfaces, the obliteration of cranial sutures, and the erosion of dental surfaces. In contrast, microstructural analyses focus on the middle level of IO, or the processes associated with bone growth, maintenance and repair. At this level, bone remodeling units are responsible for resorbing and replacing bone, whether related to nutritional requirements, activity patterns, or the repair of microfractures within the element. Therefore, gross morphological and histological approaches are based on unique levels of skeletal organization, analyzing distinctive processes within bone. Thus, histological analysis provides not only an alternative method for discerning age-at-death in archaeological bone, but it allows for a more nuanced perspective of bone dynamics in past populations.

As bone is a dynamic material, its changing composition affects its overall quality. Traditional histological studies focus on the 2D analysis of bone microarchitecture, namely the ratio of primary to secondary bone, and the structural parameters of secondary osteons and fragments. Although these methods provide insight into the processes of bone resorption and deposition, the 2D nature of such studies limits the information one can derive regarding 3D architecture and porosity variables. The value of μCT is in the visualization and quantification of 3D spatial variation in the cortical canal network. It provides information regarding microarchitecture in more than one plane, and it offers a different perspective of porosity within bone, a parameter considered important in contributing to bone quality. In addition, μCT provides several new variables for understanding cortical composition that can be used to complement data derived from thin-sectioning and gross morphological methods. However, despite its benefits, μCT is limited by its focus on pore networks alone, and the fact that it is difficult to identify whether an osteon is in the remodeling process or complete (i.e., whether the Ca.Dm of a pore represents an incomplete remodeling phase or the finalized product). Thus, μCT and traditional histological methods are complementary techniques which, in combination, may provide an even greater perspective of bone dynamics and quality in the past.

Benefits and limitations of methods

Ultimately, whether to use traditional histology or μCT may rest on practical considerations, such as the cost, time, and knowledge necessary to collect the desired data. Depending on the number of samples needed, and the requirements for technical staff, costs associated with μCT could quickly exceed those needed for histological analyses. In this study, the scan time per femoral sample was in the range of three to four hours, with a maximum of about five femoral samples possible for batch scanning. Three-dimensional reconstructions of samples were generally available within 12 hours. The most time-consuming aspect of data collection was the post-processing period. In this phase, each slice per sample was reviewed for regions of low BMD, cracks, and inclusions in the Haversian canal networks, which were subsequently removed in order to reduce overall error. In well-preserved bone, samples were process quickly, generally in less than five minutes. In poorly preserved samples, several days could be required per sample in order to thoroughly remove any areas of concern. Additionally, a degree of subjectivity is introduced when determining whether particular regions are salvageable or not, and the extent that excluding various regions impacts the final results must be considered, as seen in the problems associated with Ca.V/TV and Ca.ConnD in this study. Therefore, although μCT scanning can offer novel information, costs and time constraints may preclude its use, and preservation issues can complicate the process.

With the cost and time involved under consideration, there are benefits conferred by using μCT analysis. First, samples can be reused for alternate analyses, such as stable isotope and radiocarbon testing, and only a small unit is required for scanning. Second, μCT analysis is semi-automated, which, theoretically, removes subjectivity in analysis. Criteria may be set regarding when to include canals in the analysis (such as when canals communicate with marrow) and structures under a certain size may be excluded, as the chance exists of detecting "false" canals due to variation in bone mineral density. Additionally, although not employed in this study, recalibration of bone values (i.e., grey values ascribed to bony structures) may be used to identify and remove inclusions in bone, as well as incorporate more poorly preserved (i.e., lower density) bone (Spoor et al., 2000; Zonneveld, 2002). These alternate methods of binarization may be more efficient and effective than global thresholding. In an analysis of archaeological ribs, undertaken subsequent to this femoral analysis, a hysteresis thresholding technique was

found effective at including bone of varying density values (Faccia, 2011). If employed, this strategy would likely have produced more accurate Ca.V/TV and Ca.ConnD values in those samples that were modified in the current study.

Ultimately, the question being addressed may dictate which approach is preferable, as both data collection techniques have distinct benefits and limitations. For example, thin-sectioning allows for a more detailed view of the microstructure of bone, albeit in 2D. Primary and secondary bone structures are visible, and boundaries for Haversian canal systems are generally readily detectable. Additionally, with regards to diagenetic changes, inclusions may be more easily diagnosed, fungus and bacteria can be visualized, and confusion over whether regions are representative of lower BMD or diagnetic change is avoided. Finally, if comparative data is the objective, then the literature is more replete with traditional histological studies, as μCT has only recently been introduced into bioanthropological studies. In μCT analysis, the inability to identify most microstructural components of bone may be outweighed by the benefits of 3D visualization. This allows for a different perspective into the dynamic nature of bone, including the directionality of canal networks and remodeling events, as well as the connectivity of Haversian canals, which could tailor to studies addressing activity-induced bone remodeling, the progression of bone-related disease processes, the dispersion of diagnetic change through bone, and so forth. Additionally, if the microstructure of bone is obscured in 2D, then using this 3D approach can allow for the generation of multiple variables on the microscopic level. Because Haversian canals are more likely to remain intact due to their hypermineralized walls, basing a study on these structures might allow one to address questions which otherwise may be precluded from analysis due to poor preservation.

CONCLUSIONS

This study tested the ability of μCT to identify the pore structure of archaeological bone and explores the concept of bone quality in archaeological remains. Age-related trends largely mirrored those in a modern sample, indicating that the data were real. Overall, when using 3D pore microstructure as a proxy for bone quality, there were very few significant differences with advancing age, indicating that Baikal forager bone quality changed slowly throughout the lifespan. Principal components analysis identified an as-yet unidentified variable that explains the greatest amount of variability in the data, and it is posited that this could be related to differences in activity patterns between individuals and/or task groups. Likewise, a sexual division of labor may be responsible for the divergent trends in Ca.DA observed between the sexes. Whether one chooses μCT depends on the question being asked, as well as the resources available. Ultimately, μCT analysis provides a novel way of investigating and comparing bone quality in archaeological populations.

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