Quantitative Analysis of Cortical and Trabecular Bone in Three Human Populations

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ABSTRACT
Bone mass is known to vary as a result of age, sex, behavior, and diet; however, little is known about the differences between populations. This study's objective is to analyze cortical and trabecular bone structure in the proximal femur and to examine differences related to ethnicity and behavior. MicroCT scans were used to collect data from two Native American populations, the forager Black Earth and the agricultural Norris Farms, and an African agricultural population, the Kerma. Femoral neck cortical bone and 3D femoral head trabecular bone were examined quantitatively. Results show that the foragers have significantly more robust cortical and trabecular bone than the agriculturalists. These data support previous claims that a more sedentary lifestyle increases cortical and trabecular bone fragility, suggesting that susceptibility to osteoporosis is more prevalent now than it was then. Future research could be done to better understand the factors contributing to the variation across populations.

INTRODUCTION

Osteoporosis causes brittle and fragile bone resulting from loss of tissue due to hormonal changes, or deficiency of calcium or vitamin D. According to the American Academy of Orthopaedic Surgeons, "Osteoporosis is a global public health problem currently affecting more than 200 million people worldwide. In the United States alone, 10 million people have osteoporosis, and 18 million more are at risk of developing the disease. Another 34 million Americans are at risk of osteopenia, or low bone mass, which can lead to fractures and other complications." According to the National Osteoporosis Foundation, approximately one in two women and up to one in four men, age 50 and older, will break a bone due to osteoporosis. The most common sites of osteoporotic fracture are the distal forearm (wrist), vertebrae (spine), and proximal femur (hip). Hip fractures are strongly related to low bone mineral density (BMD), and therefore, have been used internationally to gauge osteoporosis (Cummings and Melton, 2002). The amount of bone mass a person has, along with bone structure, varies between individuals and populations due to ethnicity, sex, age, diet, or even behavior (Pollitzer and Anderson, 1989). It is important to understand how different factors affect bone composition so that we can more effectively treat osteoporosis and other bone related diseases.

Bone composition is determined by analyzing two types of bone tissue found in whole bones - cortical and trabecular bone. Cortical bone is dense and compact. It forms the outer layer of the bone. Trabecular bone, also known as spongy bone, is a complex network of rods and plates, and is present in joint regions, short bones, and flat bones. Variation in bone structure and bone mass determines bone strength. The amount of bone mass directly correlates with the strength of bone - more bone generally means stronger bones. Cortical thickness and the width of the femoral neck are two important factors of bone strength in the proximal femur and hip joint. Although there does not appear to be significant variation in cortical thickness between sexes, men tend to have wider femoral necks than women (Duan et al., 2003). Even though a wider bone may inhibit bending, the femoral neck is still at risk for fragility if trabecular and cortical bone is thin, especially at older ages. It has been shown that cortical bone in the femoral neck is thicker inferiorly (Ohman et al., 1997). Bone volume fraction (BV/TV) and degree of anisotropy (DA) are two variables of trabecular bone that are important to bone strength (Ryan and Walker, 2010; Hodgskinson and Currey, 1990a, b; Turner et al., 1990). Essentially, the amount of trabecular bone present and its distribution in space are great determinants of how strong the bone will be.

Past work has demonstrated that bone structure varies in populations with different behaviors. The shift from a hunter-gatherer lifestyle to an agricultural lifestyle has negatively impacted bone health because of a decrease in a nutritional diet and an increase in sedentism (Larsen, 1995). Becoming more sedentary causes a decrease in biomechanical loading, which causes more gracile bones. Studies have shown that people who are more active tend to have more robust cortical bone (Larsen, 1995; Ruff, 2005) and trabecular bone (Ryan and Shaw, 2014). With the shift to agriculture came a significant change in mobility patterns that had profound effects on bone mass and bone health (Bridges, 1989).

Clinical studies suggest bone also varies due to ethnicity or ancestry. It has been shown that people of African descent have greater bone mass than Caucasians (Pollitzer and Anderson, 1989; Wang et al., 1997; Ortiz et al., 1992). People of Hispanic origin are more similar to Caucasians with Asians having lower bone mass than Caucasians (Pollitzer and Anderson, 1989; Barrett-Connor et al., 2005; Cundy et al., 1995). While these groups are unnecessarily broad and

tend to follow traditional conceptions of racial categories, it is not clear how much variation in bone structure exists between groups or what is driving these differences. Few studies have examined the variation in cortical and trabecular bone in diverse human groups to determine the relative importance of behavioral versus genetic/ancestral differences.

The goal of this study is to bring an anthropological perspective to these questions of bone structural variation by analyzing cortical and trabecular bone structure in the proximal femur, and examining differences related to ethnicity and behavior across multiple recent human populations. Specifically, this study attempts to assess how increases in sedentary lifestyles affect bone health and the effects of ancestry on bone structure. This study uses three different populations: Native American hunter-gatherer, Native American agriculturalist and African agriculturalist. I predict that the hunter-gatherers will have more robust femoral neck cortical bone and femoral head trabecular bone than the agriculturalists and that the African agriculturalists will have more robust bone than the Native American agriculturalist group.

MATERIALS AND METHODS

Skeletal Sample

The skeletal sample used in this study consisted of three populations with different behavioral patterns and ancestry. The Kerma individuals came from the Kingdom of Kerma located in Egypt and Sudan. The individuals date to about 3600 B.P and come from the Middle Kingdom period (Buzon, 2006). These individuals were primarily agriculturalists. The Norris Farms #36 site is a cemetery site from the central Illinois River valley from about 700 yrs. B.P. This site is linked to more sedentary agriculturalists of the Oneota cultural tradition (Ryan and Shaw, 2014). The Black Earth site dates to about 5000 yrs. B.P. and the individuals were highly mobile foragers (Ryan and Shaw, 2014). Details of the sample are listed in Table 1. Only adult individuals with no signs of pathologies were used in this study

Table 1. Skeletal sample used in this study

| Population	Cortical Bone			Trabecular Bone			
	N=	Males	Females	N=	Males	Females	Unidentified
Kerma	4	4	0	20	11	7	2
Black Earth	10	5	5	20	10	10	0
Norris Farms	10	5	5	19	10	9	0
Total	24	14	10	60	32	26	2

MicroCT Scan Data Collections

One femur from each individual was microCT scanned in order to quantify internal bone structure non-destructively. The Kerma individuals were scanned at the Cambridge Biotomography Centre at the University of Cambridge using a Nikon Metrology XT H 225 ST High Resolution CT Scanner. The Norris Farms and the Black Earth individuals were scanned at the Center for Quantitative Imaging (CQI) at Pennsylvania State University using the OMNI-X HD-600 industrial μCT system. High resolution scans of the proximal femur and low-resolution

scans of the entire femur were used in this study. The voxel sizes ranged from 0.0378 to 0.058 for trabecular bone and 0.110 to 0.125 for cortical bone.

Quantitative Analysis of Bone Structure

Cortical Bone

A multi-step process was used to define the femoral neck cross-section for this analysis. First, a maximum intensity projection of the proximal end of the femur, viewed anteriorly, was created. The femoral shaft axis was defined as a vertical line, viewed on the anterior portion of the bone, which extended from the midpoint of the shaft diameter to the superior portion of the bone (Fig. 1). Cortical bone thickness of the femoral neck was quantified on a single two dimensional slice positioned perpendicular to the long axis of the femoral neck. This slice was located at the midpoint of the femoral neck, which was defined as the distance between the center of the femoral head and the femoral shaft axis line. The center of the femoral head was defined as the intersection of the two lines at the midpoints of the mediolateral and superoinferior extents of the head. The midshaft slice produced contained trabecular bone and cortical bone (Fig. 1). A cortical bone separation algorithm used a three-step method to isolate the cortical bone from the trabecular bone. The Avizo 8.01 software was used to identify the slices needed for analysis of the cortical bone in the femoral neck.
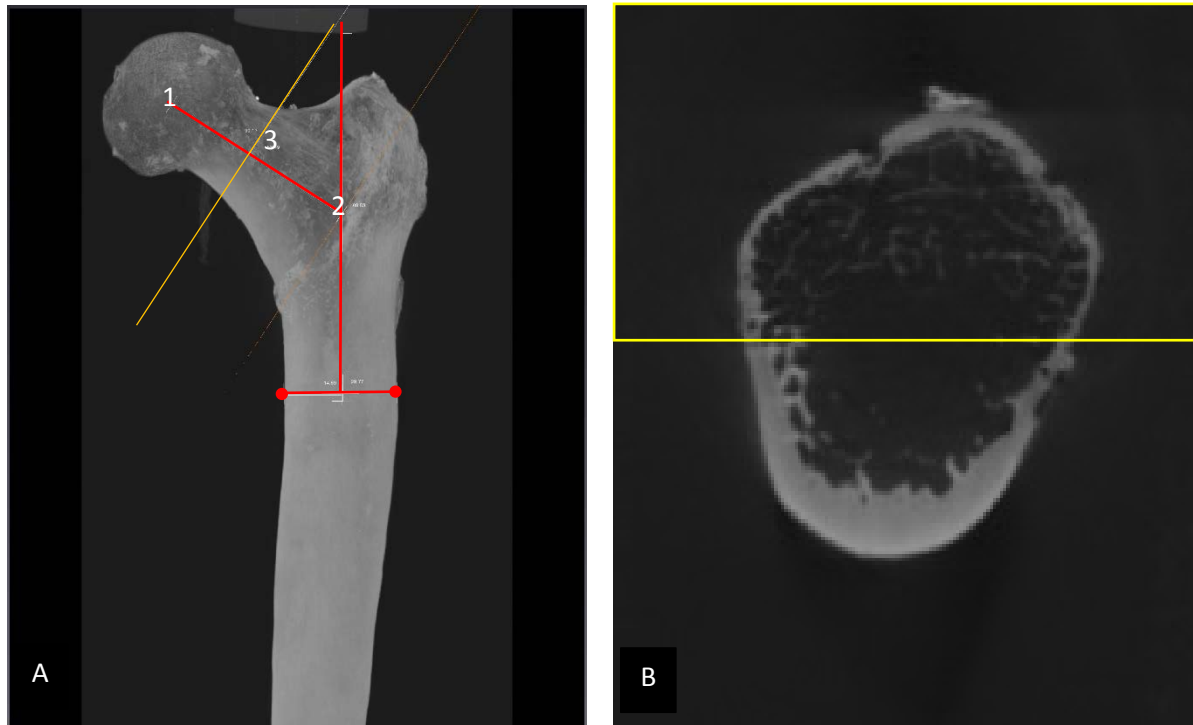

Fig. 1. A: Midshaft slice selection method. FNL was defined as the distance between the center of the femoral head (1) and a point on the femoral shaft axis line (2). The midpoint between (1) and (2) was calculated and labeled the midshaft slice (3). B: The midhsaft slice was split into a superior half and an inferior half based on superior-most and inferior-most "y" dimensions.

Each femoral neck cross-section was split in half along the midline in the superoinferior axis, producing two new datasets representing the superior and inferior halves of the femoral neck (Fig. 1). Each image was segmented using the iterative algorithm in ImageJ that determined

what was bone and what wasn't bone. The mean and maximum cortical bone thickness was calculated for the superior and inferior half of each cross-section using a model-independent method implemented in BoneJ within ImageJ.

Trabecular Bone

Trabecular bone structure was quantified in three dimensions in cubic volume of interest (VOI) extracted from the center of each femoral head (Fig. 2). The femoral head was isolated for each specimen by using a bounding box; this defined the region of interest (ROI). The size of each VOI was equal to 50% of the ROI to ensure that variability in femoral head size was accounted for. Further explanation on the method used to define VOI can be found in Ryan and Shaw (2012), Ryan and Walker (2010), and Ketcham and Ryan (2004). Seven morphometric variables were quantified using the BoneJ plugin in ImageJ: BV/TV, Tb.Th. Tb.Sp, Conn.D, DA, BS/BV, and SMI. BV/TV is defined as the amount of bone in the VOI. Tb.Th is the thickness of the trabeculae and Tb.Sp is the amount of separation between trabeculae. Conn.D determines how well the trabeculae are interconnected. DA defines how similarly oriented the trabeculae are and SMI measures the distribution of rod – to plate – like trabeculae. Lastly, BS/BV is defined as the amount of surface area the bone occupies in the VOI (Table 2). A correction factor was used to correct the variables for different voxel sizes. These variables were chosen because they were proven to be the best determinants of trabecular bone mechanical behavior, functional behavior, elasticity of structure, and strength (Ryan and Walker, 2010; Hodgskinson and Currey, 1990a, b; Turner et al., 1990, Kabel et al., 1990b; Odgaard et al., 1997; Cowin, 1997; Mittra et al., 2005).
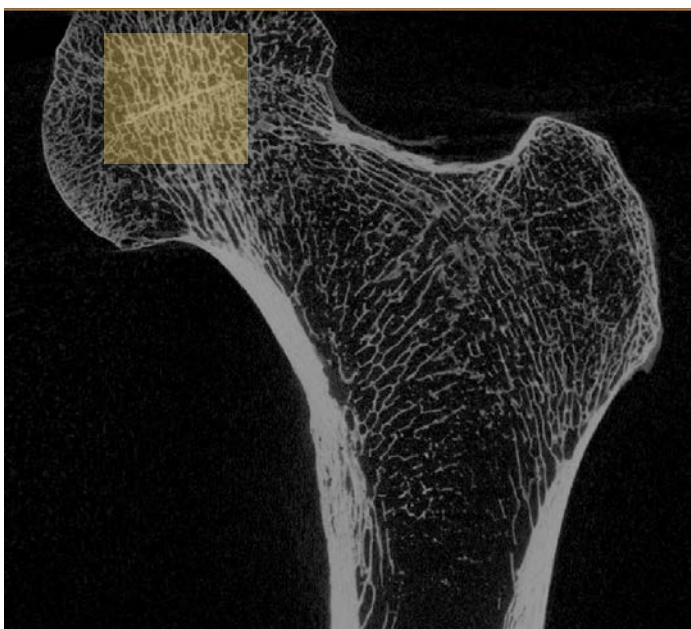
Fig. 2. The center of the femoral head was determined for each sample and a cubic volume of interest (VOI) was extracted.

Statistical Analyses

Mean and maximum thickness for the superior and inferior portions of the femoral neck were compared between populations using ANOVA. A paired-t test was used to compare

superior and inferior thickness means within each population. An ANOVA test, along with a post-hoc test, was used for the statistical analyses of trabecular bone. In order to determine what comparison test to use, Levene's test for equal variances was applied. Minitab 17.1 was used to run statistical analyses on all data.

Table 2. Measurements of trabecular architecture

Measurement	Abbreviation	Units	Description
bone volume fraction	BV/TV	(-)	ratio of bone volume to total volume of interest
degree of anisotropy	DA	(-)	extent to which trabeculae are similarly oriented
structure model index	SMI	(-)	measure of distribution of rod- to plate-like trabeculae
corrected trabecular thickness	Tb.Th	mm	measure of average strut thickness corrected for body size
trabecular separation	Tb.Sp	mm	measure of average distance between struts
connectivity density	Conn.D	mm^{-3}	relative quantity describing how well are the struts interconnected
bone surface to bone volume	BS/BV	%	the ratio of trabecular bone surface area to total trabecular bone volume in the VOI

(Fajardo et al., 2007; Ryan and Shaw, 2012)

RESULTS
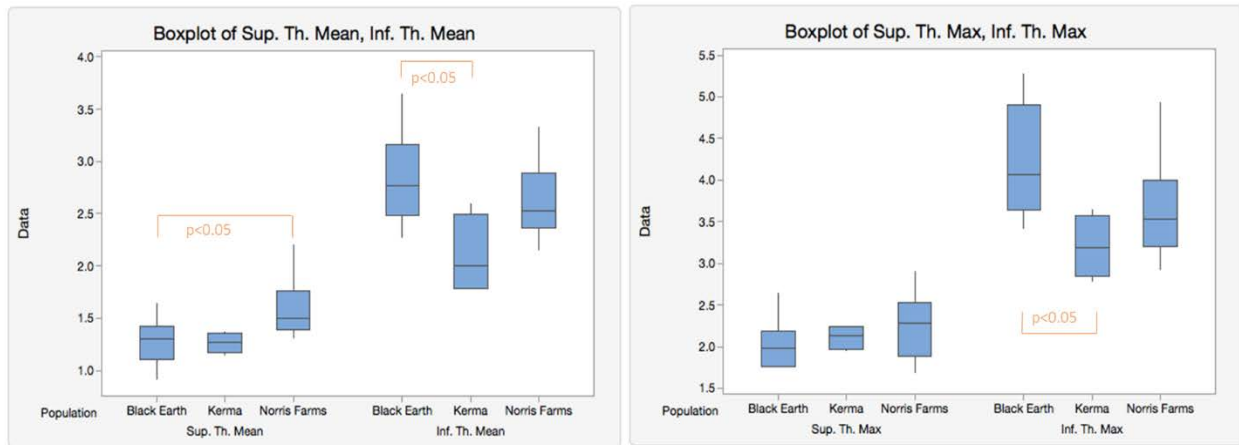
Femoral Neck Cortical Bone Thickness

Population means and standard deviations for each measured cortical bone variable are listed in Table 4 and boxplots for each variable in each population are provided in Figure 3. A statistically significant difference was found between thickness means for the superior and inferior portions in each population. There are no statistically significant differences between populations for maximum thickness in the superior neck. The Norris Farms group has a significantly higher mean superior thickness than the Black Earth. However, the Kerma are not significantly different from Norris Farms or Black Earth in this variable. The Black Earth foragers have a significantly higher mean and maximum thickness in the inferior neck than the Kerma. The Norris Farms agriculturalists, however, are not significantly different than either the Black Earth or the Kerma in the inferior femoral neck thickness (Fig. 4).

Table 4. Basic Statistics for Cortical Neck Thickness Analysis, Mean (St Dev)

| Population | Superior | | Inferior | |
	Thickness Mean	*Thickness Max*	*Thickness Mean*	*Thickness Max*
Black Earth	1.286 (0.234)	2.008 (0.278)	2.847 (0.468)	4.188 (0.665)
Kerma	1.266 (0.097)	2.110 (0.147)	2.092 (0.387)	3.202 (0.374)
Norris Farms	1.586 (0.268)	2.245 (0.377)	2.603 (0.352)	3.645 (0.598)

**Table 5. Basic Statistics for Trabecular Thickness Analysis, Mean (St Dev)**

Population	BV/TV	Tb.Th	Tb.Sp	Conn.D	DA	SMI	BS/BV
Black	0.465	0.438	0.696	1.959	0.627	1.130	4.670
Earth	(0.060)	(0.074)	(0.101)	(0.505)	(0.059)	(0.761)	(0.630)
Kerma	0.302	0.258	0.731	5.684	0.597	2.575	7.942
	(0.045)	(0.033)	(0.091)	(2.289)	(0.067)	(0.488)	(0.670)
Norris	0.387	0.337	0.712	2.619	0.702	2.350	5.465
Farms	(0.034)	(0.057)	(0.087)	(0.696)	(0.056)	(0.415)	(0.392)

Fig. 3. Boxplots showing the results of morphometric analyses of cortical bone in the femoral neck for each population. Statistically significant population differences, based on the ANOVA, are marked with their respective p-values.

Femoral Head Trabecular Bone Structure

Population mean and standard deviations for each measured trabecular bone variable are listed in Table 5 and boxplots for each variable can be found in Figure 5. There are no statistically significant differences between populations for average distance between struts (Tb.Sp) and body mass. Norris Farms has significantly higher DA than Black Earth and Kerma, while Black Earth and Kerma were not significantly different. Black Earth has significantly higher Tb.Th than both Norris Farms and Kerma. Norris Farms has significantly higher Tb.Th than the Kerma population. Kerma and Norris Farms has significantly higher SMI than Black Earth. Kerma has significantly higher Conn.D than Norris Farms and Black Earth. Norris Farms has significantly higher Conn.D than the Black Earth population. Kerma has a significantly higher BS/BV than Norris Farms and Black Earth. Norris Farms has a significantly higher BS/BV than Black Earth. Black Earth has a significantly higher BV/TV than Norris Farms and Kerma. Norris Farms has a significantly higher BV/TV than the Kerma population.
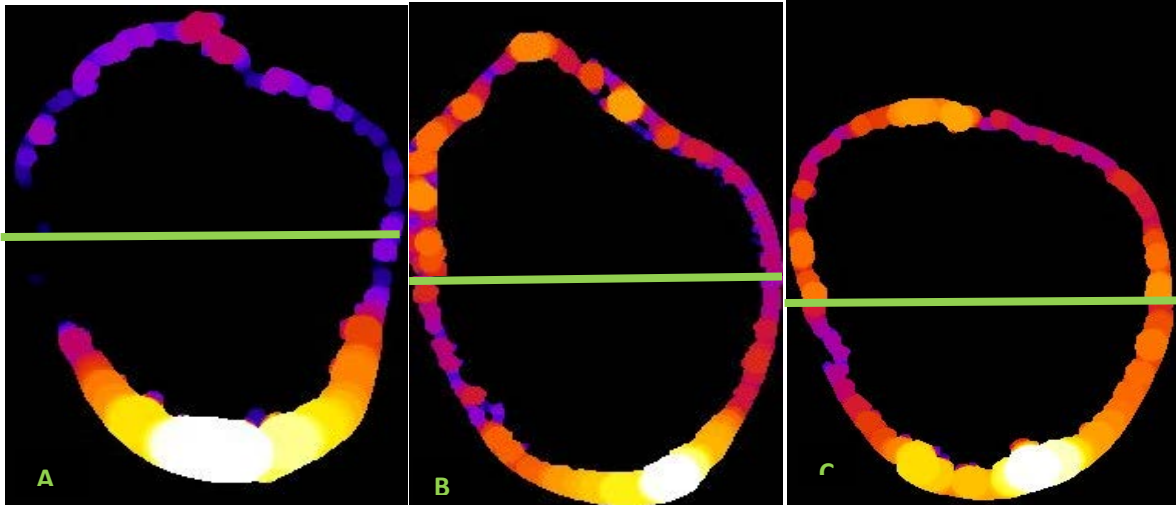
Fig. 4. Thickness Maps for cortical bone from the three populations: (A) Black Earth, (B) Kerma, and (C) Norris Farms. The line separates the superior half from the inferior half and represents where the cortical bone was split. The images have been adjusted to show the thickest point (shown in white) of the inferior region.

DISCUSSION

The goal of this study was to better understand human skeletal variation and its relationship to age-related bone loss in contemporary human populations. Osteoporosis is a systemic bone disease with significant global health impacts that is characterized by low bone mass and microarchitectural deterioration, leading to increased bone fragility and fracture risk (Ryan and Shaw, 2014). Although trabecular architecture is usually more susceptible to bone loss, this study analyzed cortical and trabecular bone because they both contribute to bone strength. The thicker the trabeculae found in the femoral head of bones, along with thicker cortical bone in the femoral neck, corresponds with greater bone mass and ultimately stronger bones.

The goal of this analysis was to investigate cortical and trabecular bone structure in the proximal femur and to examine differences related to ethnicity and behavior. As expected, the results show that the Black Earth hunter-gatherers have the thickest inferior femoral neck cortical bone. They have the highest inferior thickness max and mean with the Norris Farms following close behind. This suggests that mobility and associated loading have effects on the cortical bone of the femoral neck. Surprisingly, there are no significant differences between the Norris Farms agriculturalists and the African agriculturalists. This suggests that behavior may be more important than ancestry when it comes to cortical bone. Overall, the results indicate that patterns of trabecular structural variation differ between the three populations (Fig. 6). As predicted, the Black Earth hunter-gatherers have more robust trabecular bone. It was shown that the Black Earth had the highest BV/TV, meaning they have more bone overall than the other populations. However, the Kerma were shown to have the highest Conn.D and BS/BV values but the lowest Tb.Th. This means that even though the Kerma have the greatest number of trabeculae, their trabeculae are significantly thinner than the other two populations. This suggests that loading and activity may be a significant factor in building robust bone.
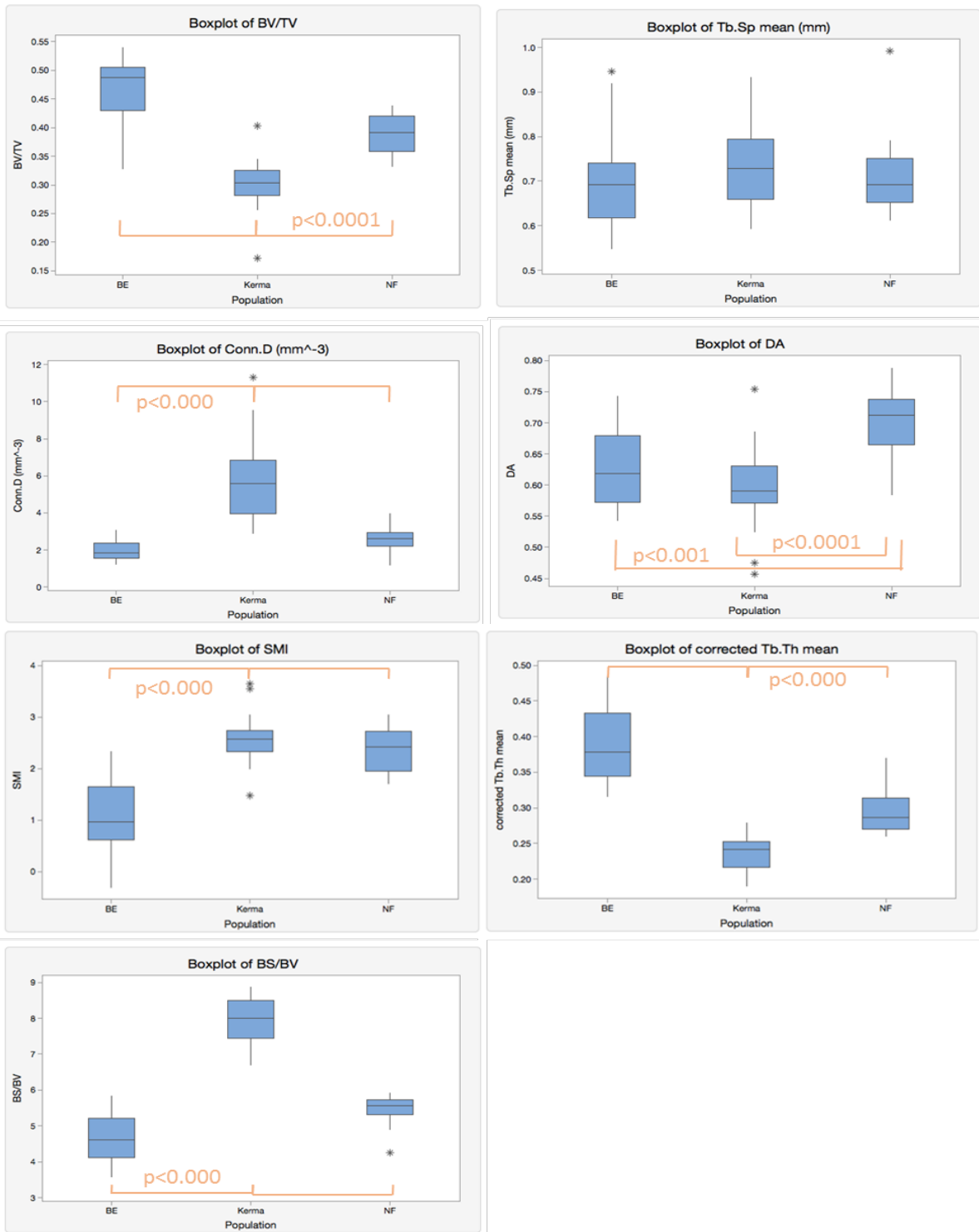
Fig. 5. Boxplots showing the results of morphometric analyses of trabecular bone in the femoral head for each population. Statistically significant population differences, based on the ANOVA, are marked with their respective p-values. Outliers are marked by asterisks (*).

Many factors could explain the variation in cortical and trabecular bone: ancestry, complexity of societies, behavior, age, sex, etc. Studies have suggested that people who are more sedentary, agriculturalists, have more gracile bone when compared to more active individuals, hunter-gatherers (Ryan and Shaw, 2014). The shift from a foraging lifestyle to an agricultural lifestyle led to more gracile human bones for a number of reasons. The main reason was the decrease in physical activity. Hunter-gatherers were naturally more mobile because of the work they had to perform to find food. As humans adopted agriculture, the amount of mobility decreased, therefore, less biomechanical loading on our bones, especially the femur. With the shift to agriculture came the change in the division of labor, as well as, eventually, more stratified and societies. Another potential force in gracilization is the significant change in diet. By moving to an agricultural lifestyle, much of the nutritional diversity and possibly quality was lost, for instance, the high levels of proteins and carbohydrates (Agarwal and Grynpas, 1996; Ryan and Shaw, 2014).

There were some limitations to this study. As mentioned before, diet potentially has a dramatic effect on bone strength and bone growth. Because we weren't aware of each population's specific diet, we were not able to determine if that was a factor that significantly affected their bone structure. Also, our sample size was limiting in two other ways. The sample consisted of only two Native American groups (one hunter-gatherer and one agriculturalist) and one African group (agriculturalist). If we were able to obtain a hunter-gatherer African group, and possibly even samples from Europe or Asia, then we would be able to determine the relative importance of behavior and ancestry on bone structure and composition. Also, including samples from different regions of Africa could help to determine more specifically how ancestry affects bone composition.
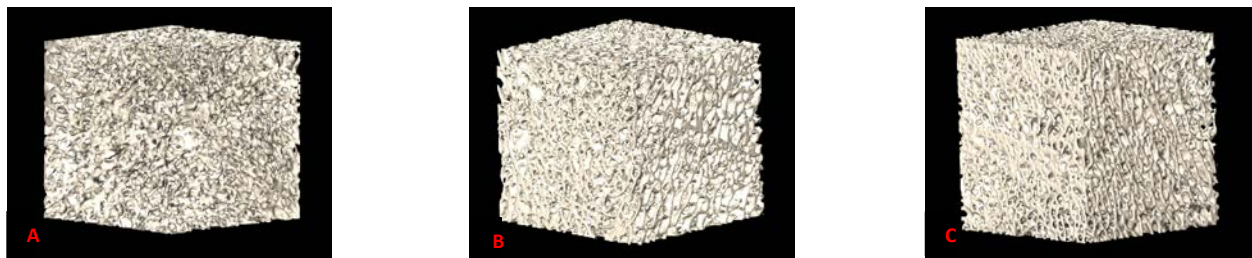
Fig. 6. Three-dimensional reconstructions of cubic trabecular bone specimens from the femoral head for each population: (A) Kerma, (B) Black Earth, and (C) Norris Farms. These cubes were used for analysis of trabecular bone.

Further analyses are needed in order to better assess whether or not behavior or ancestry is more important to bone composition. If the sample variation was increased to include more ethnic groups and more diverse behavioral styles, we could better address which factor, behavior or ancestry, affects bone composition more. Bone samples from older individuals within each population would be useful to analyze as well. This would tell which populations were affected by osteoporosis, or other related bone diseases, more frequently. From that information, along with the analysis of the populations' behavior, diet, economic status, etc., it could be determined why they experienced more osteoporosis. Lastly, the research could potentially help better treat osteoporosis and other related bone diseases. If it were determined that ancestry played a bigger role in bone composition, then medical treatment could become more personalized. Hopefully

with a more specific treatment, more people would not need artificial bone and/or joints or die from the fractures.

CONCLUSION

Overall, the main conclusion made from this study is that mobility and associated loading (behavior) seems to play a bigger role in building more robust bone than ancestry. However, the variability in bone composition still exists and it affects the health of the bone and the person, specifically in the occurrence of osteoporosis. It increases the chance of fracture risk and is a common concern among people in the medical field. Ancestry is often excluded when discussing bone health in past and present human populations. Understanding bone loss and bone health, as it pertains to ethnicity, presents a new and exciting direction and could help conclude why people experience common bone disorders differently. Understanding this will help determine how, or if, people should be treated for these disorders and if they can be prevented.

Although first recognized more than 250 years ago, the clinical and epidemiological knowledge about osteoporosis is largely limited to the last 70 years. The study of osteoporosis in past populations increases knowledge about bone modifications related to age, menopausal status or lifestyle (Curate, 2014). For instance, studies have shown that the way osteoporosis is experienced today is different than how past populations experienced it. Past *Homo sapiens* populations had cases of osteoporosis in both sexes and across all age ranges. Today, osteoporosis is found more in women, and usually in older women. In more modern *Homo sapiens* populations, osteoporosis tends to cause fragility fractures in the skeleton. The fractures that occur due to osteoporosis tend to be more fatal and are the reason the medical field wants to learn more about the disease; however, past *Homo sapiens* populations did not have a large occurrence of fragility fractures (Agarwal, 2008). Although the risk factors for osteoporosis between blacks and whites are similar, osteoporosis and related fractures occur half as much in African American women than Caucasian women (Bohannon, 1999; Barrett-Connor et al., 2005).

ACKNOWLEDGMENTS

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