Cortical Bone Histomorphology of Known-Age Skeletons From the Kirsten Collection, Stellenbosch University, South Africa

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ABSTRACT

Objectives: Normal human bone tissue changes predictably as adults get older, but substantial variability in pattern and pace remains unexplained. Information is needed regarding the characteristics of histological variables across diverse human populations.

Methods: Undecalcified thin sections from mid-thoracic ribs of 213 skeletons (138 M, 75 F, 17–82 years, mean age 48 years), are used to explore the efficacy of an established age-at-death estimation method and methodological approach (Cho et al.: J Forensic Sci 47 (2002) 12-18) and expand on it. The ribs are an age-balanced sample taken from skeletonized cadavers collected from 1967 to 1999 in South Africa, each with recorded sex, age, cause of death and government-defined population group (129 "Colored," 49 "Black," 35 "White").

Results: The Ethnicity Unknown equation performs better than those developed for European-Americans and African-Americans, in terms of accuracy and bias. A new equation based solely on the study sample does not improve accuracy. Osteon population densities (OPD) show predicted values, yet secondary osteon areas (On.Ar) are smaller than expected for non-Black subgroups. Relative cortical area (Ct.Ar/Tt.Ar) is low among non-Whites.

Conclusions: Results from this highly diverse sample show that population-specific equations do not increase estimate precision. While within the published range of error for the method (±24.44 years), results demonstrate a systematic under-aging of young adults and over-aging of older adults. The regression approach is inappropriate. The field needs fresh approaches to statistical treatment and to factors behind cortical bone remodeling. Am J Phys Anthropol 160:137–147, 2016.

Skeletal growth and maturation is sufficiently predictable that normative standards can be established and applied clinically (Tanner and Whitehouse, 1975). This contrasts with patterns of considerable population variability associated with bone remodeling, once maturation is achieved. The variation that gets the most attention is associated with clinically relevant bone loss: osteopenia and osteoporosis (Nelson et al., 2011; Warden et al., 2013; Sheu et al., 2014; Shin et al., 2014; Zhou et al., 2014). The etiology of low bone mass in late adulthood is to some degree linked to the acquisition of peak bone mass in youth, which appears to be highly heritable (Burnham and Leonard, 2008), but is also influenced by environmental factors (Tveit et al., 2015; Weaver, 2015). The dynamics of bone maintenance are complex and are important to adult health. Within this framework, a relatively small research community has sought to characterize and predict the more-or-less linear remodeling patterns in cortical bone. These can be used to estimate age-at-death from undecalcified bone thin sections. While expanded reportage of human secondary bone histomorphometric variables can be useful (Keough et al., 2009), progress toward method refinement must include tests of existing approaches. We report here on the patterns and variability in standardized bone sections prepared from mid-thoracic ribs (normally R6) of a large and particularly diverse collection of skeletonized cadavers. The work describes the context of the collection, provides a validation study of an established age estimation method and expands on it. It explores unique sample characteristics of the variables that are combined in histological age estimation, including osteon population density (OPD), secondary osteon area (On.Ar) and relative cortical area (Ct.Ar/Tt.Ar).

Despite their existence for many decades in the tool kit for estimating adult age at death, assessment of cortical bone remodeling is an approach that is usually

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associated with contexts where only bone fragments are available, where methods based on gross morphology cannot be used (Garvin and Passalacqua, 2012; Trammell and Kroman, 2013). Among diverse proposed approaches, those that focus mid-sternal ribs are preferred. The rib's biomechanical environment is relatively invariable, rib bone sampling is cosmetically acceptable, and the small cross-section of bone cortex precludes the need to define a region of interest since it is feasible to assess the entire prepared surface (Crowder and Rosella, 2007). Despite the existence of histologically-based age estimates with inaccuracy and bias values that are comparable to several commonly used gross morphology methods (Crowder, 2009), there are concerns about the shortage of validation studies (Cho et al., 2002, 2006; Crowder, 2009) and the potential impact of varying investigator expertise (Crowder et al., 2012).

Current approaches to histological age-estimation are largely based on the methods developed by Cho et al. (Crowder et al., 2009; Cho et al., 2002; Kim et al., 2007; Pavón et al., 2010). It is often used as an exemplar in biological anthropology reference texts (Robling and Stout, 2008; Crowder, 2009; Crowder and Stout, 2012; Streeter, 2012). From 154 rib cross-sections of known age (ranges 17 to 95 years; African-American mean age 50.4 years, European-American mean age 37.8 years), Cho et al. (2002) developed three age-estimation formulae for European-American, African-American, and individuals of unknown ethnicity by incorporating three variables: osteon population density (OPD), osteon area (On.Ar), and relative cortical area (Ct.Ar/Tt/Ar). The validation sample within that study demonstrates that the error associated with the development sample is consistent with prior studies (Stout and Paine 1992), with a predictive interval of $\pm$24.44 years (Cho et al., 2002). This method strongly supports a population approach to histological age-estimation largely based on ancestry, and the variables used form the basis for more recent population specific histological age-estimation methods (Kim et al., 2007; Pavón et al., 2010; Cannet et al., 2011). An assessment by Crowder (2005) using 215 samples derived from the known age-at-death Spitalfields skeletal collection found that the histological age-estimation method developed by Cho et al. (2002) accurately estimated ages within the predicted interval. A portion of their African-American sample was derived from a cemetery population, leading to potential inaccuracy from use of civic age at death records. In this work we explore the proposition that the large predictive interval is linked to the statistical approach used. A larger, more firmly documented sample provides elucidation for the challenges and the potential of histological age estimation methods.

Current research provides an incomplete picture of the extent to which aging explains variability in the structures of interest. On.Ar variability has been documented to vary among diverse samples (Takahashi et al., 1965; Takahashi and Frost, 1966; Pfeiffer et al., 2006), without clear causative factors being identified. Variation has been reported at various organizational levels, from the bone to the population. A tendency for secondary osteons to be smaller in older people has been reported (Takahashi et al., 1965; Han et al., 2009), but the absence of this relationship has also been noted (Black et al., 1974; Burr et al., 1990; Pfeiffer et al., 2006; Lee et al., 2014). Recent study of cortical bone of the femoral neck region has reported a significant negative association between On.Ar and age (Tong et al., 2015), while another study reports a negative association between On.Ar and body weight, but not age (Britz et al., 2009). In a study of femora from a single baboon lineage, genetic effects account for 48 to 75% of phenotypic variance in On.Ar (Havill et al., 2013). Insofar as osteon population density (OPD) reflects tissue remodeling, On.Ar should be directly correlated with OPD, with smaller secondary osteons filling less of the rib cross-section space if bone turnover and cortical area are held constant.

The cross-sectional area of cortical bone, relative to total cross-sectional area, is relevant to our understanding of osteoporosis and osteopenia, in principle. However, cancellous bone sites are more important clinically, and the rib has not often been a focus of study. Relative cortical area varies across different skeletal elements intraindividually, but appears to be highly correlated in the long bones, especially those of the lower limb. Relative cortical areas are comparatively smaller in the ribs, suggesting that the same allometric relationship does not apply to this non-weight bearing bone (Stewart et al., 2015). Rib cross-sectional cortical area reaches its peak during the third decade and declines in later life, with average bone mass of males exceeding females (Sedlin et al., 1963; Takahashi and Frost, 1966; Dupras and Pfeiffer, 1996; Streeter and Stout, 2003; Agnew et al., 2013).

Histological patterns and structural variability within human cortical bone are also being explored in contexts that do not focus on aging. Variables such as collagen fiber orientation, osteon shape, and intra-cortical porosity (i.e. resorption spaces and Haversian canals) have all been linked to the mechanical competency of bone tissue (Sevostianov and Kachanov, 2000; Goldman et al., 2003, 2014; Bigley et al., 2006; Skedros et al., 2007, 2013). Counts and measurements along with regionally-specific concentrations of microstructural features may reflect different adaptations to bone strain (Rose et al., 2012). Anthropologists have used these measures to extrapolate habitual activity patterns of past and current populations (Kalmey and Lovejoy, 2002; Bromage et al., 2009).

MATERIALS AND METHODS

The Kirsten Collection is made up of skeletonized cadavers from the dissection teaching program of the medical school of Stellenbosch University, located in Tygerberg, a northern suburb of Cape Town, South Africa (Labuschagne and Mathey, 2000). Most individuals in the Kirsten Collection were born between 1920 and 1949, with the earliest born in 1855. Most of the individuals in the Collection died between 1970 and 1989. The skeletonization of cadavers began in 1957 and continues today. Common cadaver sources are the large public teaching hospitals, namely Tygerberg Hospital and Karl Bremer Hospital in the Bellville area north of Cape Town, and Groote Schuur Hospital in Cape Town. Less common sources are regional undertakers and bequeathments. There were some undocumented sources in the early years. The Kirsten Collection includes approximately 60% males, 40% females. The mean known age at death is 51 years (range 10–103 years), with men and women having very similar mean ages of death. The skeletons selected for this research were received as cadavers, used for dissection, then skeletonized by Stellenbosch University staff between the years of 1967 and 1999.

The socioeconomic status of most of the people represented in the Kirsten Collection was low, implying marginal to poor employment, housing and health care

experiences. While South Africa's group areas and group amenities acts of apartheid were repealed in the late 1980s, many conditions that had become established during the apartheid era continued for some time. Under apartheid, people were officially identified as "Black" (predominately of Bantu descent), "White" (European descent), or "Colored" (a heterogeneous group including people of Khoesan, Bantu, European, as well as South and East Indian descent). All South Africans older than 16 years were required to carry an identification book that provided information, including race. Blacks, who were not seen as South African citizens, had to carry a "passbook" with them at all times. While these identifiers do not ensure the accuracy of birth dates for donated bodies, the official focus on personal identity suggests accurate documentation. There were some institutional incentives to report births, and no institutional motivators to misrepresent age at death; there were no advanced ages that conferred particular benefits (like survivors' pensions). Blacks faced substantial barriers to migration to Cape Town, which was a designated White area (Bickford-Smith et al., 1999). Reflecting these social and political factors, approximately 12% of the skeletons are classified as White, 16.5% Black, and 60% Colored within the Kirsten Collection.

The Kirsten Collection encapsulates considerable genetic and socioeconomic diversity. The original classifications of each skeleton have been retained, to reflect that diversity. The use of these terms is not intended to legitimize race as a biological fact, but rather to reflect the impact of these categories on people's lived experiences and social identities. There continued to be a strong association between race classification and socioeconomic status immediately subsequent to the apartheid era. Evidence suggests that income poverty and racial inequality intensified during the transition to democracy (Seekings, 2011). In the post-apartheid era, residential segregation persists in the urban areas (Christopher, 2001), and differential access to both public and private health services underscores the difficulty of changing social policy (Harris et al., 2011).

In the early 1990s, Cape Town had a population of about three million people. Only 31% of the population was deemed to have adequate housing. Impoverished regions suffered from the ravages of gangs, alcohol, and drug addiction, and domestic violence throughout the period represented by this sample. One reflection of alcohol addiction is the sustained high frequency of fetal-alcohol syndrome (Croxford and Viljoen, 1999). In addition to alcohol, mandrax (methaqualone) and dagga (cannabis) would have been the most commonly available intoxicants, with cocaine, LSD and other drugs becoming more available in the late 1990s (Bickford-Smith et al., 1999).

In the Cape Town suburbs, the Colored genetic distribution has been characterized as foremost Khoesan, followed by Black African, White and Asian (de Wit et al., 2011; Daya et al., 2013). The Khoesan are the most ancient ancestral peoples of southern Africa, as reflected in their distinctive genome (Schlebusch et al., 2013). The Black Africans of South Africa, often referred to as Bantu, are genetically distinctive from the West Africans that dominate the gene pool of African-Americans (Tishkoff et al., 2009; Silva et al., 2015). Thus, both the Colored and Black components of the Kirsten Collection introduce aspects of genetic diversity that are rarely explored in studies of human bone remodeling (Pratte and Pfeiffer, 1996; Paine and Brenton 2006a,b). Reported causes of death for the sample are consistent with those of the larger Kirsten Collection (Labuschagne and Mathey, 2000). According to death certificates, causes of death are mainly cardiovascular diseases and cancer of various origins, although respiratory diseases such as pulmonary tuberculosis, bronchiectasis, pneumonia and asthma account for a large number of intakes during the cold, wet winters. Other co-morbid conditions include cardiopulmonary failure, renal failure, and diseases of the liver and gastrointestinal tract.

The extent to which living conditions affected developmental milestones is difficult to gauge. Available information suggests general similarities among the three identified groups. In 1977, average menarcheal age was 13.9 years for South African Blacks and 13.1 years for South African Whites (Jones et al., 2009). Peak bone mass attainment may have been delayed among some youths under apartheid if they had dietary deficiencies in vitamin D like those observed among contemporary youths from the region (Naude et al., 2012). Dietary information for the apartheid era is limited. It has been suggested that a high percentage of the population was malnourished (Wisner, 1989) caused at least in part by grain shortages. Fruit, vegetables, and offal from red meat hawker stands constituted major sources of the food in informal townships during the apartheid era (Karaan and Myburgh, 1992). A more recent study (Drimie et al., 2013) indicates that the diet in urban informal households consists mostly of cereals and meat/poultry/fish; dietary diversity is low. With increasing urbanization, food insecurity, and malnutrition continue to be of concern in South Africa (Battersby and McLachlan, 2013).

While slight ethnic differences in the timing of menopause in South Africa have been reported, the average age of menopause in urban Black women was 48.9 years during the era under study, not significantly different from values reported for White women (Walker et al., 1984). Use of hormone-based treatments for birth control and menopause symptoms is likely to have been rare, despite government interventions directed toward limiting births among non-Whites (Kaufman, 1998, 2000). Variability in bone turnover may be expected within the Kirsten sample, reflecting generally higher bone mass, as in African Americans (Cauley and Nelson, 2013). In the post-apartheid context, osteoporosis is seen more commonly in White women (Hough, 2006). Differences between Black and White South African women have been noted in risk factors including body mass, physical activity, smoking, and contraceptive use (Conradie, 2008). However, in a recent study, values for bone mineral density (BMD) between healthy Black and White South African women were similar, showing higher BMD in Black women only at the proximal femur, a weight-bearing site (Conradie et al., 2014).

Mid-thoracic rib samples were selected from the Kirsten collection using a stratified sampling protocol based on population group, sex, and age. The target was to include 15 samples of each sex per 10-year age increment for each population group (i.e. 20–29 years, 30–39 years... 70–79 years). This target was sometimes unmet because of limited representation within the collection or insufficient rib tissue associated with a skeleton. Rib samples were only selected if they were R5, R6 or R7, had a complete cross-section, and represented the midshaft portion of the rib shaft. Rib samples exhibiting evidence of ante-mortem trauma were not included in this study. In all instances, the curatorial procedures of

TABLE 1. Kirsten Collection sample composition and ages at death

	N	Mean (yr)	S.D.	Range (yr)
Colored men	73	47.86	16.41	17–78
Colored women	56	43.59	15.04	18–72
Black men	42	47.24	15.11	18–79
Black women	7	36.71	11.37	22–53
White men	23	58.04	11.71	35–78
White women	12	58.67	12.00	42–82
All men	138	49.37	15.72	17–79
All women	75	45.36	15.45	18–82
Total	213	47.96	15.7	17–82

Stellenbosch University were followed. A total of 213 rib samples were selected for this study, ages 17 to 82 years, mean age 48 years (Table 1).

Rib tissue samples were exported with the permission of the Western Cape Government Inspector of Anatomy. At the University of Toronto, they were prepared for histological analysis following methods outlined by Crowder et al. (2012). Cross-sectional tissue samples were removed from selected rib samples using a rotary saw and embedded in an epoxy resin under vacuum. Thick sections (approximately 700 μm) were cut from these embedded samples using a Buehler Isomet precision saw, and ground to approximately 100 μm using a Buehler Ecomet grinding wheel. These thin sections were then polished using a diamond suspended paste (1 μm grit) and mounted onto slides using mounting medium, and cover slips were then applied.

Histomorphometric measurements for each rib sample follow definitions provided by Cho et al. (2002), henceforth termed the reference study. These variables include intact osteon population density (N.On), fragmentary osteon population density (N.On.Fg), On.Ar, and Ct.Ar/Tt.Ar. Values for N.On and N.On.Fg (summed and divided by Ct.Ar to generate OPD) were collected using an Olympus BX-41 light microscope fitted with a Mertz eye-piece reticle. A single well-trained research assistant did all the counts. On.Ar and Ct.Ar/Tt.Ar were measured using image Olympus cellSens software package (v. 1.9), a Wacom digitizing tablet and virtual slides. A virtual slide of the complete cortical cross-section was generated for each sample using the Olympus BX-41 light microscope, an Olympus SC30 camera, a PriorOptiScan II automated stage, and Olympus cellSens software package. On.Ar was calculated from a minimum of 25 secondary osteons of roughly circular shape, from diverse locales within the cross-section. Images were captured and stored at 100× magnification under both bright field (BF) and linearly polarized light (LPL). Area measurements were collected using the LPL image montages. A single researcher traced all the osteon areas.

The European-American formula (age = 38.029 + 1.603 (OPD) – 882.21(On.Ar) – 51.228(Ct.Ar/Tt.Ar) + 57.441 (Ct.Ar/Tt.Ar)), the African-American formula (age = 38.029 + 1.603 (OPD) – 51.228(Ct.Ar/Tt.Ar)), and Ethnicity Unknown formula were applied to each sample. The Ethnicity Unknown formula was modified following recommendations by Cho et al. (2002) to match the demography of the sample population. In 1980, the Cape Peninsula region's population was composed of approximately 33% of individuals with European ancestry, and approximately 67% of individuals with Colored and African (non-European) ancestry (Labuschagne and Mathey, 2000). This ratio results in an Ethnicity Unknown equation of Age = 34.191 + 1.658(OPD) – 203.9(On.Ar) – 33.414(Ct.Ar/Tt.Ar). Each formula was qualitatively and quantitatively assessed for accuracy and bias for each racial grouping by sex.

In addition to the European-American formula, African-American formula, and Ethnicity Unknown formula, a fourth formula was generated for the Kirsten Collection sample following the forward step-wise linear approach outlined by Cho et al. (2002). Predictor variables (OPD, On.Ar, Ct.Ar/Tt.Ar), a categorical variable for group, and predictor variables with a group interaction term (OPD-group, On.Ar-group, Ct.Ar/Tt.Ar-group) were added in a step-wise manner. Acknowledging the inappropriate use of a ratio as a parametric variable, we transformed Ct.Ar/Tt.Ar values to *z*-scores for this regression. This new step-wise regression formula was assessed in the same manner for accuracy and bias for each racial group by sex.

RESULTS

A sample of 213 ribs representing ages at death from 17 to 82 years, mean age 48 years, was used to test equations based on a sample with similar age ranges (Cho et al., 2002). Three previously published age estimation equations were applied to the complete Kirsten Collection sample, as well as a newly generated equation. Mean estimated ages for the European-American, African-American, Ethnicity Unknown and new step-wise regression formulae are 42.3, 50.5, 47.6, and 48.0 years, respectively (Table 2). Relative to the predicted 95% error of ±24.44 years, 11%, 7%, 6%, and 6% of the age estimates fall outside that range, respectively. The break point between under- and over-estimation is closest to the middle of the distribution when the Ethnicity Unknown equation or new step-wise regression equation is used. The four formulae underestimate known ages in 64.3%, 41.7%, 51.6%, and 47.9% of cases, respectively. These values suggest that the European-American formula tends to underestimate age, the African-American formula tends to overestimate age, and the Ethnicity Unknown and new step-wise regression formulae are relatively unbiased (Fig. 1).

Overall, the equations perform better for females than males. While the European-American equation is generally the least accurate, there is considerable variability among subgroups. In terms of accuracy and bias, the best performance came from the equations developed for samples of Unknown Ethnicity and the new step-wise regression, and the least satisfactory estimates came from the equation developed for European-Americans. All the equations follow the pattern of underaging young adults and over-aging old adults (Fig. 1).

With regard to the subdivisions within the Kirsten Collection sample, the observation that ages of females are predicted more accurately than those of the males applies to all three groups. In all group subdivisions, the coefficient of determination (R^2) values from each of the three equations are higher for the females than for the males (Table 2). This contrast is especially notable between Colored men and Colored women. For most subdivisions, the R^2 values of the four age-estimation equations are higher than 0.30. However, for Colored men, the European-American, African American, Ethnicity Unknown, and new step-wise regression equations have R^2 values of 0.15, 0.01, 0.03, and 0.27 respectively. These values are much lower than the corresponding values for

TABLE 2. Results of age estimations following the European-American (E), African-American (A), and Ethnicity Unknown (U) equations provided by the reference study (Cho et al., 2002), as well as the newly generated equation (N)

				Estimated ages (yrs)						
	N	Mean (yr)	Equation	Mean	SD	Abs. diff.	S.D. resid.	% w/in	*r*	R^2
Colored men	73	47.86	E	43.48	11.03	12.93	15.47	87.67	0.3886	0.1510
			A	52.15	10.05	12.05	14.31	91.78	0.0896	0.0080
			U	49.04	9.99	11.58	14.32	91.78	0.1829	0.0334
			N	47.09	7.52	11.58	14.09	91.78	0.52	0.27
Colored women	56	43.59	E	43.06	11.52	9.96	12.29	96.43	0.6336	0.4014
			A	47.69	11.39	9.64	12.65	91.07	0.7486	0.5605
			U	45.87	10.95	9.28	12.25	92.86	0.7251	0.5258
			N	44.60	8.09	9.91	12.38	92.86	0.57	0.32
All Coloreds	129	46.01	E	43.30	11.20	11.64	14.26	91.47	0.4817	0.2320
			A	50.21	10.84	11.00	13.57	91.47	0.3791	0.1438
			U	47.66	10.50	10.58	13.42	92.25	0.4145	0.1718
			N	46.01	7.84	10.85	13.35	92.25	0.55	0.30
Black men	42	47.24	E	37.07	14.72	14.22	14.85	80.95	0.5044	0.2544
			A	48.08	13.94	9.60	12.56	92.86	0.6286	0.3952
			U	44.37	13.81	10.45	12.80	95.24	0.6116	0.3741
			N	46.78	10.29	9.42	11.79	95.24	0.63	0.39
Black women	7	36.71	E	33.10	7.84	6.66	6.57	100.00	0.8274	0.6847
			A	36.82	10.42	4.39	5.54	100.00	0.8745	0.7647
			U	35.24	9.10	3.75	4.47	100.00	0.9283	0.8617
			N	39.44	7.23	4.69	5.57	100.00	0.92	0.84
All Blacks	49	45.73	E	36.50	13.95	13.14	14.11	83.67	0.5268	0.2776
			A	46.47	13.98	8.86	11.78	93.88	0.6718	0.4513
			U	43.07	13.55	9.49	11.94	95.92	0.6544	0.4282
			N	45.73	10.19	8.74	11.13	95.92	0.67	0.45
White men	23	58.04	E	46.08	10.66	13.83	12.40	82.61	0.4186	0.1752
			A	57.84	8.31	10.24	13.74	95.65	0.5012	0.2512
			U	53.95	8.34	10.44	13.07	95.65	0.5001	0.2501
			N	58.70	6.18	9.74	12.71	95.65	0.09	0.01
White women	12	58.67	E	47.52	12.76	12.56	10.67	91.67	0.5996	0.3595
			A	55.29	13.95	8.27	9.40	100.00	0.5717	0.3269
			U	52.52	13.13	8.63	9.41	100.00	0.5955	0.3546
			N	57.42	9.71	6.33	8.33	100.00	0.73	0.53
All Whites	35	58.26	E	46.58	11.25	13.40	11.68	85.71	0.4920	0.2421
			A	56.97	10.45	9.56	12.37	97.14	0.5410	0.2927
			U	53.46	10.06	9.82	11.84	97.14	0.5486	0.3009
			N	58.26	7.45	8.57	11.31	97.14	0.37	0.14
All men	138	49.37	E	41.96	12.59	13.47	15.07	84.78	0.4506	0.2031
			A	51.86	11.52	11.00	13.75	92.75	0.5268	0.2775
			U	48.44	11.45	11.05	13.76	93.48	0.5243	0.2749
			N	48.93	9.32	10.61	13.12	93.48	0.55	0.31
All women	75	45.36	E	42.84	11.85	10.07	12.16	96.00	0.6313	0.3986
			A	47.89	12.43	8.93	11.94	93.33	0.6524	0.4256
			U	45.94	11.80	8.66	11.67	94.67	0.6630	0.4396
			N	46.17	9.67	8.85	11.30	94.67	0.68	0.47
Total	213	47.96	E	42.27	12.31	12.27	14.28	88.73	0.5024	0.2524
			A	50.46	11.97	10.27	13.11	92.96	0.5799	0.3363
			U	47.56	11.61	10.20	13.06	93.90	0.5785	0.3346
			N	47.96	9.51	9.99	12.50	93.90	0.61	0.37

Summary values include: mean estimated ages and standard deviations, the standard deviation residuals, the percent of the sample where estimated ages fall within the 95% confidence interval of the equation (±24.44 years), and values for *r* and R^2.

Colored women ($R^2_{\text{European-American}} = 0.40$, $R^2_{\text{African-American}} = 0.56$, and $R^2_{\text{Ethnicity Unknown}} = 0.53$). Similarly, the inaccuracy of the Ethnicity Unknown equation is lower for females than males. While this difference is observed in all groups, it is only statistically significant between Black males and females (two-tailed $t = 4.41$, $P < 0.001$).

Linear regression equations fit to the Kirsten Collection sample (Table 3). With the information provided by the variables describing histological structures and bone mass, we generated forward step-wise linear regressions for the Kirsten Collection sample and its groups. The final model took the form: Age ~ OPD + Ct.Ar/Tt.Ar + group. In all step-wise instances, the largest contributor to approximating age is OPD. There is no interactive effect of group on any of the "independent" variables, so the regression slope is the same for all groups. Similar to the African-American equation, On.Ar was not incorporated in the final model. The best fit equation, applied to the full sample, is plotted in Figure 2. The confidence interval is not appreciably narrowed, when compared with that of the equations provided by Cho, et al., (2002).

Characteristics of the pertinent variables are given in Tables 4–6 and each is summarized below.

Osteon population density (OPD). This variable is the sum of intact osteon density (N.On) and fragmentary osteon density (N.On.Fg). The Pearson correlation of

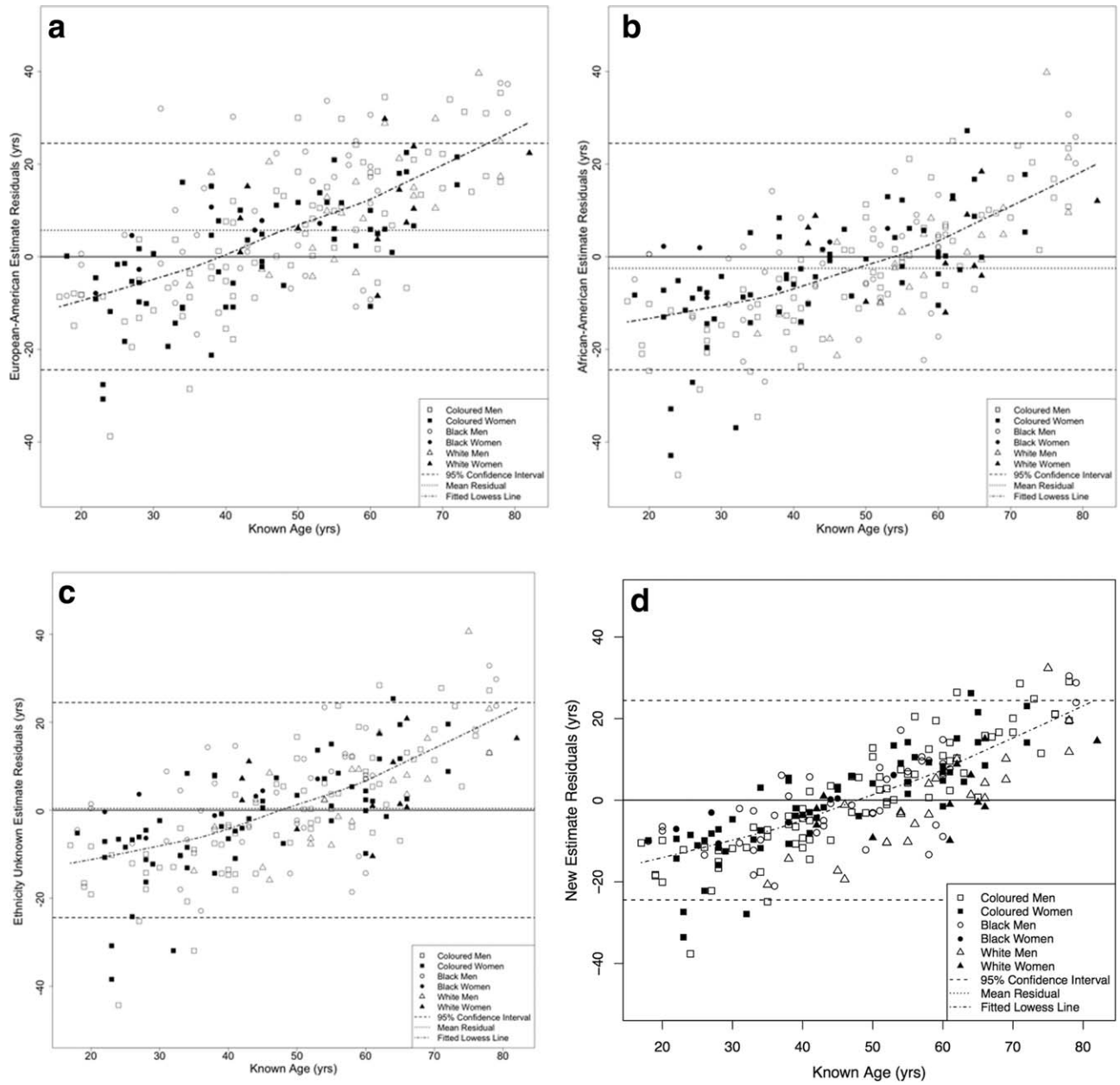

Fig. 1. Residuals of age at death estimates from three predictive equations (Cho et al., 2002) and a newly generated predictive equation, each compared with known ages at death ($N = 213$; 138 males, 75 females). **a**: European-American equation. **b**: African-American equation. **c**: Ethnicity Unknown equation. **d**: Newly generated equation.

OPD with age at death is significant ($r = 0.539$, $P < 0.0001$). Both components of OPD are significantly correlated as well (N.On $r = 0.417$; N.On.Fg $r = 0.526$). Clearly, the accumulation of remodeling events within the rib cortex is strongly correlated with adult age. The mean value for OPD, 19.9, is of the same general magnitude as values reported in the reference study in which average age is about one decade younger. There, the mean OPD for the African-American sample is 18.7, and that for the European-American sample is 20.1.

Secondary osteon area (On.Ar). The rib cross-sections assessed here generally have secondary osteons that are about ten percent smaller in area than those of the reference sample. While most Kirsten Collection subgroups have mean On.Ar values of 0.033 to 0.034 mm^2, the reference study reports 0.036 mm^2 for African-Americans and 0.039 mm^2 for European Americans (Cho et al., 2002). Of the various components within the Kirsten Collection sample, only Black men and women have mean values for rib On.Ar of magnitude comparable to those of the reference sample. Both sexes in both of the other groups (Colored and White) have smaller, and rather similar values for On.Ar (Table 3).

For the Kirsten Collection study sample, On.Ar and age at death have a significant negative association ($r = -0.314$, $P < 0.0001$). The age ranges are similar for this study and the reference study. It is unlikely that slight differences in mean age at death can explain the

substantial difference in On.Ar. The proposition of a genetic component appears weak, insofar as the White subset of this study is genetically similar to the European-American component of the reference study, yet the On.Ar values are significantly different (two-tailed $t = 4.24$, $df = 67$, $P < 0.0001$).

TABLE 3. Results of a new step-wise linear regression based on entire data set

Step	Variable Entered	R^2
1	OPD	0.2875
2	Ct.Ar/Tt.Ar	0.3308
3	Group	0.3548

Coefficients	Estimate	Std. Error	t value	$P > \|t\|$
(Intercept)	16.602	3.631	4.573	8.27E-06
OPD	1.315	0.183	7.176	1.24E-11
Ct.Ar/Tt.Ar	−10.4	3.123	−3.33	0.00103
Group2	2.854	2.166	1.318	0.189
Group3	7.533	2.464	3.057	0.00253

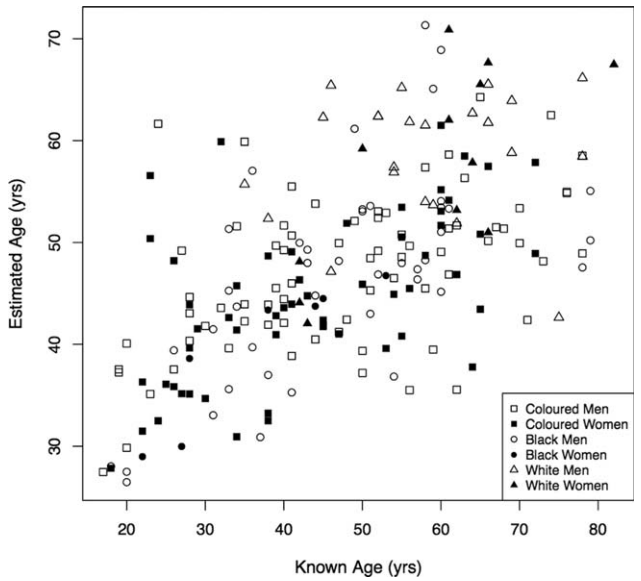

Fig. 2. Age at death estimates from a step-wise linear regression predictive equation generated from variables of Kirsten Collection rib cross-sections, compared with known ages at death ($N = 213$; 138 males, 75 females).

Relative cortical area (Ct.Ar/Tt.Ar). The mean relative cortical area for the Kirsten Collection sample is 38.1%. As would be predicted, there is a negative correlation between relative cortical area and age ($r = -0.284$, $P < 0.0001$). While total cross-sectional area does not correlate with age ($r = 0.131$, $P = 0.057$), medullary area shows a positive correlation with age ($r = 0.259$, $P < 0.0001$). As medullary area increases, relative cortical area decreases. Values for relative cortical area are higher for non-whites, perhaps reflecting the slightly older age of the White subsample. Comparing values to the reference study (Cho et al., 2002), the mean value for the White subsample is very similar to that reported for European-Americans (33% compared with 34%) but the values for non-whites in the Kirsten collection are higher than that reported for African-Americans (39% compared with 35%).

DISCUSSION AND CONCLUSIONS

This research provides a unique perspective on an established method for ascertaining adult age at death. Samples of rib tissue represent a large, culturally diverse, age-balanced sample. Indeed, this sample is larger, and has more reliable age-at-death estimates, than the sample on which the method being tested is based. Each histological variable was assessed by a single person (thereby minimizing interobserver error), using image capture methods that maximize visual acuity. The European-American, African-American, and Ethnicity Unknown equations successfully estimated ages within the prediction interval (±24.44 years) for most rib samples. While this overall success rate is high, the predictive interval is large. Since the standard deviation of known age is 15.7 years, the predictive interval is only ±7 years smaller than the 95% confidence interval for age. A newly generated step-wise linear regression equation to fit this sample does not improve the results. While each age-estimation equation performed relatively well with respect to the criteria stated by Cho et al. (2002), the large breadth of the prediction interval reduces the predictive value of histological methods.

The value of ancestry-specific age estimation equations is not demonstrated by these results. Based on R^2 values, population specific equations do not improve performance over more general equations; the

TABLE 4. Osteon population density (OPD), secondary osteon areas (On.Ar), and relative cortical area (Ct.Ar/Tt.Ar) for ribs in the Kirsten Collection sample

	N	Mean OPD	S.D.	Mean On.Ar (mm^2)	S.D.	Range (mm^2)	Mean Ct.Ar/Tt.Ar (%)	S.D.
Colored men	73	20.2	5.0	0.03303	0.006	0.0207–0.0497	35.6	9.2
Colored women	56	20.0	4.8	0.03377	0.006	0.0205–0.0486	43.8	11.0
All Coloreds	129	20.1	4.9	0.03335	0.006	0.0205–0.0497	39.2	10.8
Black men	42	18.1	6.4	0.03654	0.008	0.0229–0.0609	36.9	10.9
Black women	7	15.3	4.4	0.03690	0.004	0.0299–0.0418	50.1	13.2
All Blacks	49	17.7	6.2	0.03659	0.007	0.0229–0.0609	38.8	12.1
White men	23	22.4	3.9	0.03380	0.008	0.0179–0.0541	31.4	7.2
White women	12	22.3	5.0	0.03222	0.008	0.0204–0.0465	35.9	15.1
All Whites	35	22.4	4.2	0.03326	0.008	0.0179—0.0541	33.0	10.6
All men	138	19.9	5.5	0.03422	0.007	0.0179–0.0609	35.3	9.6
All women	75	19.9	5.0	0.03381	0.006	0.0203–0.0485	43.1	12.3
Total	213	19.9	5.3	0.03408	0.007	0.0179–0.0609	38.1	11.3

African-American equation performed better than the European-American equation for White men, the European-American equation performed better than African-American equation for Colored men and Colored women, and the Ethnicity Unknown equation performed better than the African-American equation for Black women. For all subdivisions, the Ethnicity Unknown equation performs relatively well, and, in most subdivisions, better than the population specific equation. Despite the noted differences in mean On.Ar between the reference study and this data set, there is no apparent relationship between the use of On.Ar in specific equations and their success. The European-American and Ethnicity Unknown equations both incorporate On.Ar, the African-American equation does not. The two equations that incorporate On.Ar are the best and worst performers. As with other established methods for estimation of age-at-death from the skeleton, we agree with colleagues who have noted, "more emphasis needs to be placed on collecting data on age changes in large samples, rather than focusing on the possibility of interpopulation variation in rates of aging" (Konigsberg et al., 2008; p 541).

While the variables of interest show correlations with age-at-death, the linear regression approach to age estimation does not accurately capture these correlations. Perhaps because age reflects time, and time is the ultimate linear variable, we are forcing age-related processes into a linear model. If we linearly regress AGE $\sim$ OPD using our data, we get: Age $= 16.093 + 1.599$ (OPD). Thus, if we didn't observe a single secondary osteon, we would already estimate age-at-death to be 16 years. For this equation to accurately predict the age of a 20-year-old, the OPD has to be around 2.5 osteon/mm^2. None of the 20-year-old individuals in our sample have an OPD lower than 7 osteons/mm^2 ($N_{age} = 20$: 4; mean $OPD_{age} = 20$: 9.55 osteons/mm^2) It is almost impossible to under-age a 20 years old. On the other end of the spectrum, these equations would require an observation of 40 osteons/mm^2 to accurately estimate the age of an 80 year old. This is likely to be well past the asymptote; our maximum OPD observation was 34.79 osteons/mm^2. The commonly followed regression approach that reverses causality—regressing age (as dependent) onto observed variables (treated as independent)—is inappropriate. A maximum likelihood approach should be explored, using a suite of variables that remain to be delimited.

Measures of bone mass and histological structures are sufficiently varied and predictable to form the basis of strong methods in the future, but methods must be improved. The results of this study demonstrate the need to better characterize the remodeling process, in which it is expected that primary cortical bone will be replaced by secondary bone, which in turn may undergo subsequent remodeling. The OPD variable is a count of remodeling events divided by the cortical area of the cross-section. It is roughly equivalent to percent remodeled bone. In some but not all predictive equations, a measure of average secondary osteon area (On.Ar) contributes additional information about individual variability. This information should be important to characterization of bone turnover. It is intriguing that Kirsten Collection average values for OPD are similar to those in the reference study, despite the smaller secondary osteons in White and Colored subsets. The similarity in OPD implies that secondary osteons may have been accumulating at a different pace in at least some subsets of the South African sample. Values for Ct.Ar/Tt.Ar are larger in most of the South Africans than in the Americans of the reference sample, suggesting a difference in the pace of bone turnover. However, rates cannot be compared between the two studies using the variables available. All three variables are proxy measures of bone dynamics. As such, they provide limited information about cortical bone tissue response during adulthood. Some systems of age estimation rely on the assessment of percent remodeled bone (Ahlqvist and Damsten, 1969; Kerley and Ubelaker, 1978; Thompson, 1979, 1981; Maat et al., 2006). If secondary osteon size is as globally variable as it appears to be, that approach may be just as efficacious, at least in principle. However, to date those methods are not widely used. None of them focus on rib cross-sections.

The equations developed from study of a sample of 154 American adult skeletons can be applied to a sample of 213 South African skeletons with results that are almost as accurate as the original results. This suggests that the development of population-specific age estimation equations is unnecessary. The equations explored here incorporate three variables, each of which differs in the South African sample from the patterns observed in the reference study. Nevertheless, an equation built on values from the South African sample does not perform appreciably better. Until it is clear how each variable contributes to a successful age estimate, and until we escape the arbitrary nature of linear regression, it will be difficult to hone our methods toward greater precision. The exploration of other morphological features within adult cortical bone is a positive development in this field. Adjustments to our perspective about age-

TABLE 5. Covariance matrix of the histological variables incorporated into the age estimation equations, including age at death, osteon population density (OPD), secondary osteon areas (On.Ar), and relative cortical area (Ct.Ar/Tt.Ar) for ribs in the Kirsten Collection sample

	Age	OPD	On.Ar	Ct.Ar/Tt.Ar
Age	246.6444	44.8698	−0.0337	−1.9449
OPD	44.8698	28.063	−0.0178	−0.6221
On.Ar	−0.0337	−0.0178	4.65E-05	7.87E-04
Ct.Ar/Tt.Ar	−1.9449	−0.6221	7.87E-04	0.0929

TABLE 6. Correlation matrix of the histological variables incorporated into the age estimation equations, including age at death, osteon population density (OPD), secondary osteon areas (On.Ar), and relative cortical area (Ct.Ar/Tt.Ar) for ribs in the Kirsten Collection sample

	Age	OPD	On.Ar	Ct.Ar/Tt.Ar
Age	1	0.53932686	−0.314461195	−0.406251979
OPD	0.53932686	1	−0.494042526	−0.385247975
On.Ar	−0.314461195	−0.494042526	1	0.378589468
Ct.Ar/Tt.Ar	−0.406251979	−0.385247975	0.378589468	1

contingent change during remodeling may contribute to more precise, yet generally applicable methods.

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