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TECHNICAL NOTE

ANTHROPOLOGY

Margarita Valencia Pavón,[1] *M.A.; Andrea Cucina,*[2] *Ph.D.; and Vera Tiesler,*[2] *Ph.D.*

New Formulas to Estimate Age at Death in Maya Populations Using Histomorphological Changes in the Fourth Human Rib*

ABSTRACT: This study develops new histomorphological algorithms for Maya populations' human ribs and tests the applicability of published algorithms. Thin sections from the fourth rib of 36 individuals of known age were analyzed under polarized light microscopy. Osteon population density (OPD, the concentration of intact and fragmented osteons per mm^2), cortical area (CA), and osteon size (OS) were recorded. Seven algorithms were calculated, using all combinations of variables, and compared to the performance of published formulas. The OPD-based formulas deviate from the known age 8.7 years on average, while those from OS and CA deviate between 10.7 and 12.8 years. In comparison, our OPD-based algorithms perform better than the one by Stout and Paine and much better than Cho et al. In conclusion, algorithms should be developed using OPD for different ethnic groups; although Stout and Paine's can be used for Maya and maybe Mesoamerican individuals.

KEYWORDS: forensic science, age estimates, histomorphology, rib sections, osteon population density, Maya populations

The application of histomorphological age estimation techniques based on degenerative or remodeling processes of human skeletal segments are notoriously affected by limitations and biases imposed by the reference sample (1). The methods usually derive from few, relatively homogeneous populations and are applied worldwide to modern or archaeological samples with different genetic backgrounds, physical and dietary regimens, and remodeling dynamics. Therefore, the use of such methods may rather reflect the age-at-death distribution of the reference sample rather than that of the target series.

To limit the effect that external factors like physical activities may have in general remodeling or specific degenerative processes that come with aging, some methods were developed on specific portions of the skeletal structure that are theoretically less susceptible to external forces, like histomorphology on the fourth or sixth rib's midshaft (2–5). Differently from other bony segments, like appendicular long bones, this part of the skeleton is not likely to undergo specific mechanical loads that may influence the remodeling rates experimented by the appendicular skeleton.

A series of equations based on skeletal segments of the trunk has been developed during the last two decades. The formulas are built mainly on North American samples of African-American and European-American descent (2,5–7); despite their reduced susceptibility to mechanical factors, Robling and Stout (4) stress that "population-level differences in bone remodeling dynamics potentially can lead to complications in age estimates when equations based on one population are applied to others." In fact, bone turnover and remodeling rates may significantly vary among populations. Cho et al. (6) observed greater bone mass in African-American individuals than in European-Americans and at the same time present lower remodeling rates (5).

The present investigation addresses this problem for Yucatecan populations and, in a broader frame of reference, to modern Mexicans and Central Americans. The population of northern Yucatán (Mexico), like other parts of the world, faces ecological and environmental conditions typical of tropical and sub-tropical regions, with high temperatures and humidity. Here, living conditions in large segments of the population do not match the standards that characterize Western societies with consequences on the aging process. So far, the lack of reliable information on bone remodeling rates and bone mass that characterize the populations from this area does not permit to quantify the potential biases that originate by the application of generic age estimation formulas developed for other ethnic groups.

This work wants to contribute with a new set of regression formulas derived from the histomorphometry of midshaft rib sections in a modern autopsy sample obtained from Yucatecan adults. With it, we wish to improve our understanding of the physiological variables involved in the aging process of modern Yucatecan populations, assess the applicability of the published equations developed for the same bony segments respectively by Stout and Paine (2) and by Cho et al. (6), and provide a much-needed regionally adapted tool to assess age at death histologically in ancient Maya skeletons and modern Yucatecan populations.

[1]Servicio Médico Forense, Secretaría de Salud, Gobierno del Estado de Yucatán, Mérida, Mexico.

[2]Facultad de Ciencias Antropológicas, Universidad Autónoma de Yucatán, Mérida, Mexico.

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Materials and Methods

Osteon population density (OPD), cortical area (CA), and osteon size (OS) were recorded from the fourth left rib of 36 individuals of known sex and age (34 males and 2 females), spanning from age 20 to 87 (averaging 43.8 years at time of death), who mainly died in road accidents. Thirteen additional individuals (12 males and 1 female) formed the control sample; their ages span between 28 and 78 years of age with an average of 47.3 years (Table 1). Both the study cohort and the control sample are formed mainly by male individuals, since males, especially in Yucatán, are at a higher risk of being involved in fatal car accidents.

The choice to study the fourth rib lies after the correspondence with the age estimates from the bone's sternal end (3) that will be object of future investigations. Nonetheless, as (8–10) reported, no differences were highlighted among different rib numbers.

The rib specimens were collected by the senior author from autopsies performed by the Servicio Médico Forense (SEMEFO) of the State of Yucatán. The individuals had to be of Maya origin (in order to avoid genetic admixture) and be free from clear pathological conditions that may alter the regular bone metabolic activity. The biographic information was obtained by interviewing the relatives of the deceased. One of the criteria of inclusion was that the individuals' family had to be Yucatecan for at least the three previous generations.

Each rib section underwent a cleaning process that started by removing the soft tissue from the cortical surface. To this end, the samples were embedded for 12 h in an alkaline 10% solution of Extran™, a liquid detergent for hand washing commonly used in clinical laboratories. After this initial stage, the bone fragments were brushed with a soft toothbrush to remove macroscopic portions of soft tissue, were inserted in a small cotton bag together with an indelible tag and then boiled in the same alkaline solution for 12 h. Further toothbrushing was carried out to remove the remnants of tissue. The macerated samples were left to dry for 24 h at room temperature, labeled, and left in acetone or dichloromethane for 2 more hours before being embedded with Biodur™ resin, prepared mixing 100 g of resin with 30 g Hardener™. Air bubbles were removed promptly and penetration secured by placing the casts for 20 min in a high-pressure vacuum. The samples were left to dry in the polymeric medium for at least 3 weeks. Thin sections were obtained from the cross-sections by cutting the embedded specimens with an Isomet™ slow-speed saw and polishing then until the desired thickness of *c.* 80–100 microns was reached. For the microscopic analysis, a Leica® DM EP polarizing microscope was employed.

Osteon population density was calculated by counting twice the total number of intact and fragmented osteons in alternating fields throughout the whole cortical surface of the rib's thin section with polarized light microscopy and 10× magnifications. The CA was determined microscopically by counting twice the number of hash marks of a 6×6 Merz Grid on the cortical surface. By knowing the size of the grid, which varies according to magnification, the size of the CA and of the total area of the rib section were calculated. From there, we could compute the CA as it appears in Table 1, which corresponds to the percent of CA out of the rib section's total area. Finally, OS corresponds to the average size of an intact osteon. It is calculated using the above procedure and by averaging the number of hash marks of at least twenty randomly distributed osteon structures throughout the whole rib's section. For more detailed procedures, see Stout and Paine ([2], see also [11]). Intraobserver and interobserver error were tested systematically before the final scoring was made and statistical elaborations performed.

The data were processed to develop regression formulas for Maya populations using individual variables (OPD, CA, and osteon area), as well as a combination of two and three of the parameters. These algorithms were then tested on the control sample. Regression formulas were calculated using Statistica 7.0 and SPSS 14.5. The results were compared to the existing formulas by Stout and Paine (2) and Cho et al. (6).

TABLE 1—*Individual data for OPD, OS, and CA for the main group and the control group.*

Main Group				Control Group			
Age	OPD	OS	CA	Age	OPD	OS	CA
20	24.94	0.036	36.34	28	24.82	0.034	43.58
22	14.47	0.037	39.60	30	29.75	0.039	58.80
22	16.58	0.039	44.87	32	28.16	0.034	27.91
27	19.60	0.040	31.73	33	26.95	0.032	42.90
28	25.84	0.041	35.26	40	36.58	0.026	23.21
28	17.64	0.057	42.93	42	32.79	0.034	33.98
28	23.04	0.043	56.15	44	28.45	0.041	32.56
29	23.03	0.036	45.34	50	31.72	0.029	32.01
29	22.87	0.042	53.67	50	33.28	0.032	40.94
29	19.39	0.041	48.15	60	28.75	0.024	17.27
30	25.53	0.045	40.59	64	31.57	0.014	14.14
30	23.69	0.035	36.63	64	36.65	0.026	31.65
34	27.20	0.040	34.34	78	44.83	0.026	28.55
35	29.26	0.034	35.95				
35	21.21	0.049	34.16				
36	32.59	0.036	35.76				
37	25.76	0.040	34.34				
40	23.08	0.041	48.15				
40	24.02	0.040	36.91				
41	28.40	0.042	33.74				
45	32.80	0.040	28.52				
45	30.51	0.038	31.20				
46	33.37	0.039	23.27				
48	36.60	0.039	28.69				
54	31.41	0.041	32.40				
55	35.80	0.038	41.46				
56	36.72	0.033	23.44				
58	35.30	0.041	34.08				
58	36.58	0.029	31.03				
60	32.54	0.025	30.07				
62	39.36	0.029	31.03				
65	34.47	0.035	34.41				
67	32.94	0.036	42.48				
70	34.16	0.035	30.07				
84	43.05	0.033	25.21				
87	37.13	0.027	28.12				

Results

The histomorphological data obtained from the cohort of 36 scrutinized individuals were used to develop seven different regression formulas using all possible combinations of the three indicators (Table 2). Each formula was then tested on the control sample.

TABLE 2—*Algorithms developed from the main group of Yucatecan population.*

Algorithm 1	Age = 119.05–1,179.47*OS −0.84*CA
Algorithm 2	Age = −3.07 + 1.98*OPD − 294.76*OS + 0.04*CA
Algorithm 3	Age = −1.12 + 1.96*OPD −291.88*OS
Algorithm 4	Age = −17.46 + 2.11*OPD + 0.02*CA
Algorithm 5	Age = 87.28–1.20*CA
Algorithm 6	Age = 105.78–1,629.05*OS
Algorithm 7	Age = 10.258784*exp(0.048198*OPD)

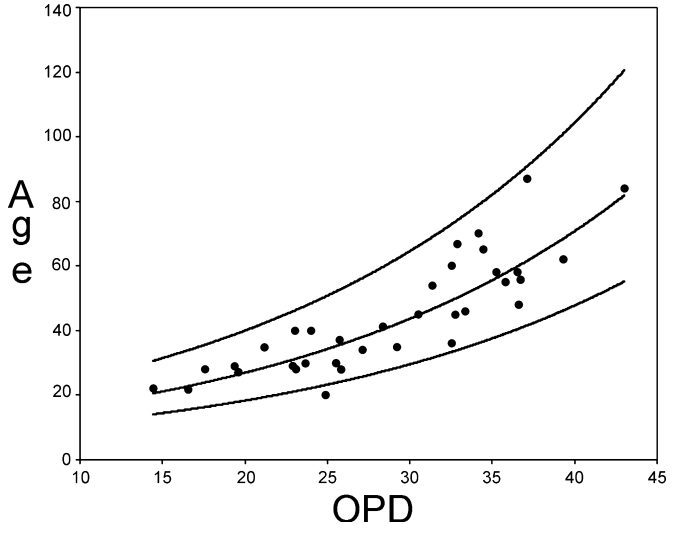

FIG. 1—*Exponential regression line with 95% limits of confidence. In this case the independent variable is the OPD while age is the dependent variable.*

Figure 1 shows the graphic OPD distribution and the optimized regression line with the upper and lower limits of variability. Figures 2 and 3 represent the relationship between age at death in the main group and respectively OS and CA. Similarly to Stout and Paine's equation (1992) the OPD-only algorithm is the result of an exponential regression, because OPDs follow a curvilinear pattern that tends to an asymptote in older ages, while the other variables (or multiple variables) produced linear single or multiple regression equations. The seven algorithms were then tested on the 13 individuals forming the control group. Age estimates were also obtained for both series using the algorithm by Stout and Paine (2) and by Cho et al. (6).

Table 3 reports the mean of the absolute residuals as well as the standard deviation of the means. All formulas produced better results when applied to the main group of 36 individuals rather than the control group. The control group indicates that the closest estimates are those that consider the OPD in the equations from this study. In all the algorithms that incorporate OPD (algorithms no. 2, 3, 4, and 7—Table 2), the mean of the differences range between 8.5 and 9.0 years, and the standard error is around

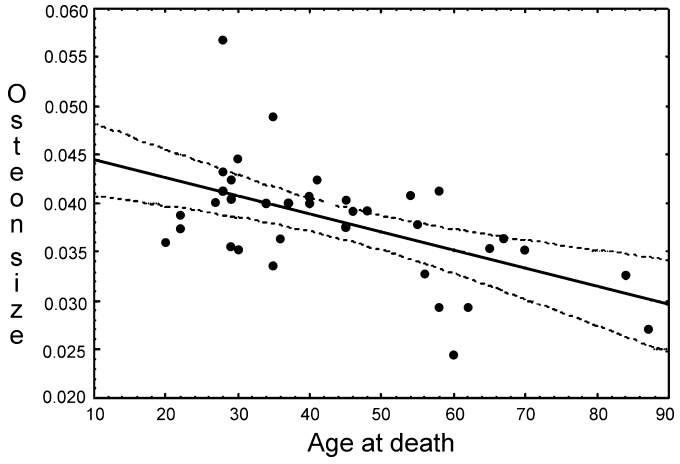

FIG. 2—*Regression line between age at death and OS.*

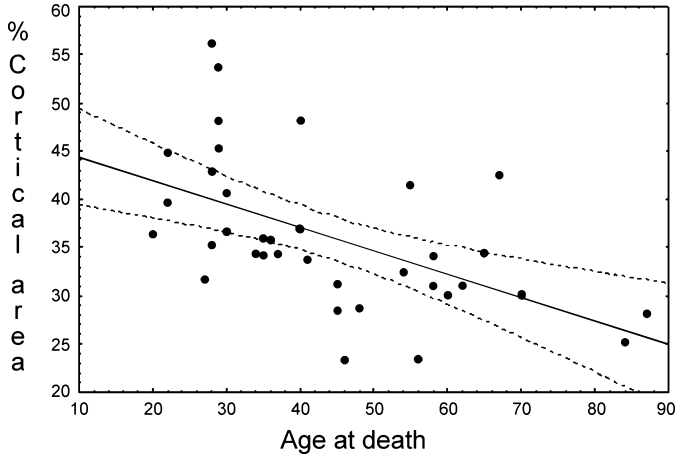

FIG. 3—*Regression line between age at death and CA.*

6.5 years. Stout and Paine (2) produces reliable estimates on the main group but skewed the control group by almost 11.5 years. Cho et al.'s formulas (6) do not produce reliable estimates in neither sample, as they overestimate the age of almost 11.7 and 13.6 years (on average) for the main group and 15.2 and 17.6 years (on average) for the control group. Poor estimates were also produced when using Cho's formulas for OS and CA (alone or in combination).

Figure 4 compares the real age with the OPD estimates obtained in this study as well as the results from Stout and Paine's (2) method and two algorithms presented by Cho et al. (6). The comparisons stress the poor estimates obtained using Cho et al.'s formulas. The OPD estimates from this study run parallel to Stout and Paine's estimates. Stout and Paine's formula tends to overestimate more than our equation, but at the same time they tend to underestimate less. The differences among the algorithms are also evident in Table 4, which shows the results of a Wilcoxon nonparametric test for dependent samples between real age and estimated age at death in the control group for the four algorithms used in Fig. 4.

Table 5 presents the average histomorphometric variables for the Maya main group in comparison with the values calculated by Cho et al. (6) for Euro-Americans and African-Americans.

Discussion

The present work was developed upon the need to verify the reliability of previously published algorithms in the process of estimating age at death in genetically, socially, and environmentally different populations.

Before discussing the results and assessing their applicability to this or other populations, it is worth spending a few lines on the sex issue of the main group used in this analysis. As mentioned above, our sample is mainly formed by adult males, with only two females in the main group, one aged 27 years and the other 56 years. In Robling and Stout's (4) brief discussion on the effect that sex has on the OPD and the other variables, they underscore that it is not yet very clear whether females present different parameters than males, as the previous studies that they cite reached contradictory results (12–15). In young adult age cohorts, for example, females who complete their cortical bone formation at an earlier age than males should theoretically present a higher OPD value. Nonetheless, with only one exception, when the two individuals from our main sample are extrapolated from their own age

TABLE 3—*Mean of differences and standard deviation of the mean of differences between the actual age and the estimates using the seven different equations plus Stout and Paine (2) and the two algorithms developed by Cho et al. (6).*

		OS+CA	OPD+OS +CA	OPD+OS	OPD+CA	CA	OS	OPD	Stout and Paine	Cho et al. (formula 1)	Cho et al. (formula 2)
Mean of differences	Group 1	10.7	7.4	7.4	7.2	22.9	11.5	6.2	7.7	11.7	13.6
	Control group	12.8	8.7	8.5	8.6	10.7	12.8	9.0	11.5	15.2	17.6
St. error	Group 1	8.1	5.2	5.1	5.3	12.4	8.4	5.7	5.2	7.1	7.9
	Control group	9.3	6.5	6.5	6.8	7.7	7.8	6.4	8.0	8.9	9.8

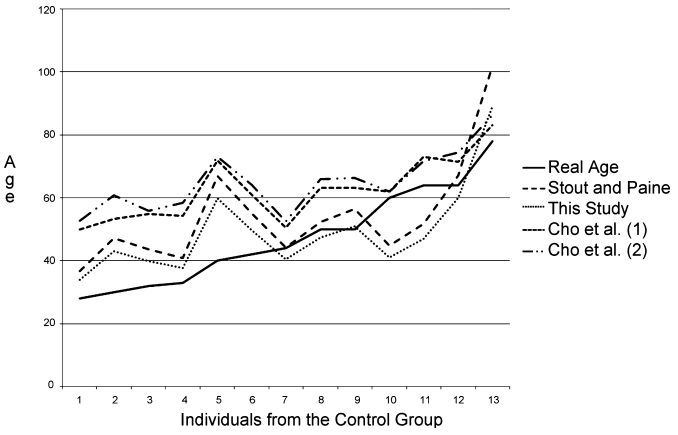

FIG. 4—*Comparison between real age and the estimated age at death in the control group using the OPD-only formula for Yucatecan populations, the algorithms from Stout and Paine (2) and the two by Cho et al. (6).*

TABLE 4—*Nonparametric Wilcoxon test for dependent samples between real age and estimated age at death.*

	No. of Non-ties	% v>V*	z	*p*-level
Real age—This study	13	61.53846	0.554700	0.579100
Real age—Stout and Paine	13	84.61538	2.218801	0.026500
Real age—Cho et al. (formula 1)	13	100.0000	3.328201	0.000874
Real age—Cho et al. (formula 2)	13	100.0000	3.328201	0.000874

*Frequency of estimated ages that overestimate real ages.

TABLE 5—*Comparison of the histomorphological parameters obtained from European-American and African-American samples (6) and the Yucatecan sample.*

Variable*	European-Americans	African-Americans	Yucatecans
Tt.Ar (mm^2)	69.51 ± 2.39	62.83 ± 1.78	52.10 ± 12.14
Ct.Ar (mm^2)	21.43 ± 0.73	20.92 ± 0.75	18.07 ± 4.831
En.Ar (mm^2)	48.09 ± 2.20	41.91 ± 1.60	34.09 ± 11.76
CA	0.33 ± 0.01	0.35 ± 0.001	0.35 ± 0.08
OPD (#mm^2)	21.02 ± 0.61	22.54 ± 0.83	28.76 ± 6.48
OS (#mm^2)	0.04 ± 0.001	0.03 ± 0.001	0.03 ± 0.007
Ac.f (#mm^2/year)	1.17 ± 0.09	0.79 ± 0.003	2.64 ± 2.81
BFR (mm^2/year)	0.05 ± 0.01	0.003 ± 0.001	0.099 ± 0.115
Net BFR (mm^2/mm^2)	1.01 ± 0.08	0.82 ± 0–03	2.86 ± 3.91

*Tt.Ar (total area); Ct.Ar (cortical area); En.Ar (endosteal area); OPD (osteon population density); On.Ar (osteon size); Ac.f (activation frequency per year); BFR (bone formation rate); Net BFR (net bone formation rate).

cohorts and their values *z*-scored to the mean and SD of the males, they tend to fall well within the male range of variability. Specifically, the 27-year-old individual, compared to the 20- to 29-year-old males, falls into the 37.54th percentile for OPD, 42.9th and 42.5th percentiles respectively for OS and CA. The 56-year-old female was compared to the 50+ years of age cohort (this age was expanded in comparison to the previous one because there were very few 50–59-year-old males). Her percentile values are respectively 61.79 for OPD, 44.43 for OS, and 3.75 for CA (*z* score −1.79). With the only exception of the OS in the elderly female, the overall values registered for these two females do not differ from those encountered in males, stressing the fact that their inclusion in the sample did not introduce particular biases.

Compared to the formulas published in the literature, our results show that the new equations using OPD (alone or in conjunction with the other parameters) to estimate age at death performs slightly better than the ones developed by Stout and Paine (2) from U.S. white and U.S. black autopsy series. Stout and Paine's algorithm tends to produce slightly older estimates. If we rule out inter-observer differences in scoring intact and, more so, fragmentary osteons, we consider that the reason for such older estimates could be a slightly lower osteon formation rate in their North American sample. Even though such value was not reported in the 1992 publication, we did estimate the OPD for the 12 individuals forming the control group, whose data are shown in Stout and Paine's Table 3 (2). This group ranges between 17 and 44 years of age and shows an average of 16.42 OPD. The first individual's age in the series was 21 years and was estimated to be 20.3 years old (OPD = 13.12). Our equation would have estimated an age of 19.3 years for this individual. If we consider that the Stout and Paine's original sample is formed by 32 whites, 4 blacks, and 4 more individuals of unknown ethnic origin, it is clear that the algorithm reflects mainly the European-American segment of the series. Also the data published by Cho et al. (6) indicate that the Euro-American sample presents lower values of OPD as well as bone and net formation rates in comparison with the Yucatecan group (Table 4). Therefore, the lower average OPDs in Stout and Paine's (2) sample might explain, at least to some extent, why their algorithm produces older estimates when applied to the Yucatecan populations, with differences that are statistically significant (Table 4).

On the other hand, the methods developed by Cho et al. (6) tend to overestimate significantly the individual's age. The CA and OS appear to be comparable with those of our sample; in fact, the slopes for OS and CA tend to mirror those obtained by Cho et al. (6) for their African-American and European-American samples. The similarities encountered in the values of CA and OS indicate that those parameters are fairly similar among ethnicities. Unfortunately, their distribution within every population is highly variable so that their use (alone or in reciprocal combination) is not recommended for reliable age estimations. Therefore, once again, OPD and net formation rate, that are lower in the African-American and in the European-American groups used to develop the algorithms, should be responsible for the systematic differences in age estimates.

As previously mentioned, it must be underscored that Stout and Paine (2) and Cho et al. (6) used the sixth rib instead of the fourth

one. Yet, (8–10) indicated that the two rib segments can be used interchangeably, due to a reduced difference in their remodeling rates. Therefore, we tend to rule out that the selection of the fourth or sixth rib can be responsible for the minor differences with Stout and Paine (2) and major differences with Cho et al. (6).

On the contrary, the comparison in Table 5 clearly shows that ethnicity is an issue in the development and application of such equations to different populations, a factor that has been repeatedly highlighted (see [5] for a review of the literature). If CA and OS are similar among the three groups, OPD in the Yucatecan sample appears to exceed that of the other groups as CA is smaller, while activation frequency and net bone formation rate are higher.

Also dietary factors and subsistence patterns can influence remodeling rates. Stout (16,17) noted that agricultural populations with a high maize consumption present higher remodeling rates; Stout and Lueck (18) found the same tendency when comparing traditional agricultural societies with hunter-gatherer, and modern series, even though the parameters in archaeological populations were always lower than those of the modern cohort. Ericksen (19) and Simmons (20), among others, claim that nutrition is responsible for remodeling rates and bone growth. We cannot rule out the possibility that a high dependence on maize, which is common in the everyday diet of average Yucatecan population, may have an effect on the results on remodeling. However, we feel confident that it only constitutes one of many factors that add to genetic differences. Unfortunately, little information is available of bone mass in Yucatecan people and we do not know the extent of genetic factors in this population's high remodeling rates. Similarly, the age of onset of new compact bone formation may differ between the Yucatecans and North Americans (of either European or African ancestry), leading to different estimates with each equation. On the other hand, our results are consistent with those encountered by Thompson and Gunness-Hey (21) who noted a markedly higher rate of turnover in the femur of a 19th-century Eskimos sample in comparison with European-Americans. In this study, the Eskimo populations even surpassed the bone turnover rates of Native-American populations like Arikara and Pueblo Indians (4). Obviously, these studies were performed on the individuals' long bones and not the ribs that undergo a different level of mechanical stress, so we cannot rule out that the Eskimo ribs would provide different results from the femur and tibia.

In conclusion, overall results indicate that the fourth rib is a relatively safe bony segment to estimate age at death because it is not subject to particular work-load even though its remodeling rate may not be the same in different populations. The analysis on the Yucatecan sample points to little differences with the results obtained from the Stout and Paine (2) formula, while major and significant discrepancies occur when using the Cho et al. (6) equations. OPD remains the most reliable indicator while the other variables are not as reliable. We think that for Maya populations, and Mesoamerican populations in general (modern and prehistoric alike), this algorithm and the one by Stout and Paine (2) should produce fairly reliable results, while the others should not be applied.

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Additional information and reprint requests:
Andrea Cucina, Ph.D.
Facultad de Ciencias Antropológicas
Universidad Autónoma de Yucatán
Carretera Mérida-Tizimin
97305, Cholul
Merida, Yucatán
México
E-mail: acucina@yahoo.com