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Methods for Improving the Efficiency of Estimating Total Osteon Density in the Human Anterior Mid-Diaphyseal Femur

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ABSTRACT In order to preserve whole bone integrity and minimize destruction, paleohistologists often rely on histomorphometric data obtained from small areas (1.5–50 mm^2) sampled within the anterior mid-diaphyseal femur. Because bone exhibits significant histological variation, the validity of results based on such sampling is questionable. The accuracy of various subareas (columns, rows, squares approximating dimensions and locations assessed by paleohistologists) in predicting total osteon density in the anterior mid-diaphyseal femur is assessed in the present study. Thirty-five specimens (12.7 mm wide, 100 μm thick, average area 56.7 mm^2) were chosen at random from a skeletal population of 94 Inuits and Pueblo agriculturists. The specimens were photographed and enlarged; an acetate grid (12 columns, 10 rows, 120 squares, square = 1 mm^2 of bone surface) was superimposed over the photograph; and secondary osteons and fragments were identified. Alternate columns (50% total area, T.Ar) predicted over 98% of entire section total osteon density. Two column combinations (15% T.Ar), separated by at least one column, predicted 91 to 95% of total osteon density. Individual column (8% T.Ar) predictability ranged from 48 to 86%. Two row combination (32 to 40% T.Ar) predictability values ranged from 86 to 95%. Individual rows ($<$1 to 20% T.Ar) predicted from 45 to 92% of total variation. Combinations of squares approximating areas and locations assessed by other paleohistologists ranged in predictability values from 80 to 94%. The results demonstrate that subareas of as little as 15% predict 95% of variation in total osteon density in the entire anterior mid-diaphyseal femoral section. A minimization of histological area evaluated without the loss of accuracy allows for a minimization of time invested in data collection and the utilization of partially damaged specimens. Am J Phys Anthropol 107:13–24, 1998.

Femoral cortical bone histomorphometry (quantitative histology) has been used to assess individual age at death (see Jackes, 1992, and Stout, 1992, for reviews) and infer various aspects of adaptation in past human populations. Examples of the latter include

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inferences regarding robusticity and mechanical strain among Late Archaic, Early Modern, and Recent humans (Abbott et al., 1996), nutritional stress among child-bearing females (Ericksen, 1980; Martin and Armelagos, 1979), lifestyle differences between ancient and modern populations (Burr et al., 1990), and inferences pertaining to possible dietary adaptations (Richman et al., 1979; Stout, 1983; Thompson and Gunness-Hey, 1981; Thompson et al., 1981). Yet, a standardized histological sampling method for the femur has not been established, limiting comparability among studies.

In the majority of cases, sampling is restricted to portions of the femur's mid-diaphysis and more specifically to a core (drilled biopsy) or wedge (chiseled biopsy) removed from the anterior part of the bone (Fig. 1). Sizes of the biopsies range from 4 to 12.7 mm in width. Dimensions subsequently sampled for histological evaluation vary from 1.5 to approximately 50 mm^2 and represent about 3 to 20% of total cross-sectional area.[1]

As bone exhibits a large degree of random and systematic histological variation both longitudinally and cross-sectionally (Amprino and Marotti, 1964; Frost, 1969; Iwaniec and Crenshaw, 1998; Martin et al., 1980; Pfeiffer et al., 1995; Raab et al., 1991; Tomerrup et al., 1993), the validity of results based on sampling limited areas within the anterior mid-diaphyseal femur has been questioned (Lazenby, 1984; Stout, 1989). Random histological variation from one part of a bone to an adjacent comparable part can introduce sampling error that ranges from 5 to over 300% if the areas examined within the site are inadequate in size (Frost, 1969). Systematic variation from one part of the bone to another can also be substantial. Periosteal fields within the anterior mid-diaphyseal femoral cortex, for example, exhibit significantly lower levels of remodeled bone than fields located closer to the endosteal surface (Pfeiffer et al., 1995). As such, an evaluation of subperiosteal fields in two or more skeletal populations subjected to differential periosteal postmortem weathering could consequently result in inaccurate conclusion of population level histological differences.

One way to circumvent some of the problems associated with long bone core histological sampling is through analysis of smaller bones, such as ribs, where complete cross-sections can be easily assessed (Stout, 1989, 1992). This may be appropriate and desired for the estimation of age at death based on bone histomorphometry (Stout and Paine, 1992), but because the rib is less affected by physical activity than are load-bearing long bones, like the femur (Raab et al., 1991; Tomerrup et al., 1993), it may not be appropriate for all investigations (Pfeiffer et al., 1995). If the anterior mid-diaphyseal femur is to serve as a standard, comprehensive, yet convenient sampling site for paleohistological studies, an understanding of variation in bone remodeling in the area and an analysis of the validity of sampling procedures is required.

The current study determined the extent to which total osteon (sum of secondary osteons and secondary osteon fragments) density in subsamples of the anterior mid-diaphyseal femur, including those previously assessed by paleohistologists, predict total osteon density variation in the entire anterior mid-diaphyseal femur (average biopsy area $\pm$ SD, $56.7 \pm 12.4\ mm^2$). The study used 12.7 mm wide biopsy sections as these represent the largest specimens typically sampled in the anterior mid-diaphyseal femur (Fig. 1).

MATERIALS AND METHODS

Sample population

Histological anterior mid-diaphyseal femoral specimens (12.7 mm wide and 100 µm thick) and their respective microradiographs representing 117 adult Inuit and Pueblo agriculturists (Ericksen, 1980; Richman et al., 1979) were obtained from the Smithsonian Institution. Archaeological skeletal as opposed to modern cadaver remains were used as the results are intended for application in the analysis of skeletal remains possibly subjected to differential postmortem preservation (Hanson and Buikstra, 1987; Garland, 1989). The inclusion of two

[1]Based on mid-diaphyseal femoral cortical area measurements for Pecos Pueblo individuals (Ruff and Hayes, 1983), a 12.7 mm diameter core with an average area of $56.7 \pm 12.4\ mm^2$ (mean $\pm$ SD, n = 35) represents roughly 20% of a mid-diaphyseal femoral cross-sectional area.

Reference	Purpose	Type	Field location (not to scale)	Intra-cortical region examined	N fields	Field area mm^2	Total area mm^2
Richman et al., 1979	inter-population comparison	12.7 mm core		entire surface with exception of endosteal portion	-	-	<56.7±12.4
Erickson, 1980	inter-population comparison	12.7 mm core		outer third of cortex	3	0.49	1.47
Thompson and Gunness-Hey, 1981	inter-population comparison	4 mm core		adjacent to periosteum	4	0.99	3.96
Burr et al., 1990	inter-population comparison	4 mm core		entire surface	-	-	approx. 20[a]
Kerley, 1965 Kerley and Ubelaker, 1978	age regression	cross section		outer third of cortex	4	2.06	8.24
Ahlqvist and Damsten, 1969	age regression	cross section		adjacent to periosteum	4	1.00	4.00
Singh and Gunberg, 1970	age regression	10 mm wedge		outer third of cortex	2	3.14[a]	6.28[a]
Thompson, 1979	age regression	4 mm core		adjacent to periosteum	4	0.99	3.96
Samson and Branigan, 1987	age regression	2 10 mm wedges		not specified	2	?	?
Ericksen, 1991	age regression	10 mm wedge		adjacent to periosteum	5	0.89	4.45
This study	assessment of predictability	12.7 mm core		entire surface	66±12.3	0.86±0.25	56.7±12.4

[a]Estimated by authors of this article

Fig. 1. Comparisons of mid-diaphyseal femoral area locations and dimensions used in interpopulation histological comparisons or in generating regression equations estimating individuals' age at death.

groups as diverse as the Inuits and Pueblo agriculturists should increase population variability and burial environment heterogeneity and make results more applicable cross-culturally and/or cross-regionally.

Although it was desired that specimens showing postmortem weathering (e.g., periosteal erosion) be included, some sections had to be rejected from analysis. The rejected specimens (11 Inuit, 12 agriculturists) showed extensive microdamage and what appeared to be microbial damage (Hanson and Buikstra, 1987; Garland, 1989) that obliterated all or large portions of the surface histology and made data collection in the entire bone specimen impossible. A subsample of 35 sections was chosen from the remaining "histologically readable" popula-

TABLE 1. Age and sex distribution of Inuit and Pueblo populations[a]

Population	Inuit[b]			Pueblo[c]			
	M	F	Unknown[d]	M	F	Unknown[d]	Total
Young (18–25 years old)	7 (3)	8 (2)		6 (2)	11 (5)		32 (12)
Middle aged (30–50 years old)	5 (1)	9 (2)		10 (6)	9 (5)		33 (14)
Old (>50 years old)	4 (2)	6 (1)		7 (3)	10 (3)		27 (9)
Unknown			1 (0)			1 (0)	2 (0)
Total	16 (6)	23 (5)	1 (0)	23 (11)	30 (13)	1 (0)	94 (35)

[a] First value represents the number of individuals per group comprising the sample population from which the subsample used in this analysis was randomly derived (sex and age groups are represented equally in both populations, $P = 0.91$, chi square test). Second value (in parentheses) represents individuals analyzed in this study (age groups—sexes combined due to small sample size—are represented equally in both populations, $P = 0.54$, chi square test).
[b] Individuals from 12 coastal settlements in Northern Alaska (late 1700s to early 1900s).
[c] Individuals from the Colorado Plateau sites of Hawikuh (1300s to 1480), Puye (1300s to 1600s), and Pueblo Bonito (900s to 1100s).
[d] Unknown, sex and age could not be determined.

tion (n = 94) using a random number table. The subsample used in the current analysis consisted of 11 Inuits and 24 Pueblo agriculturists (Table 1).

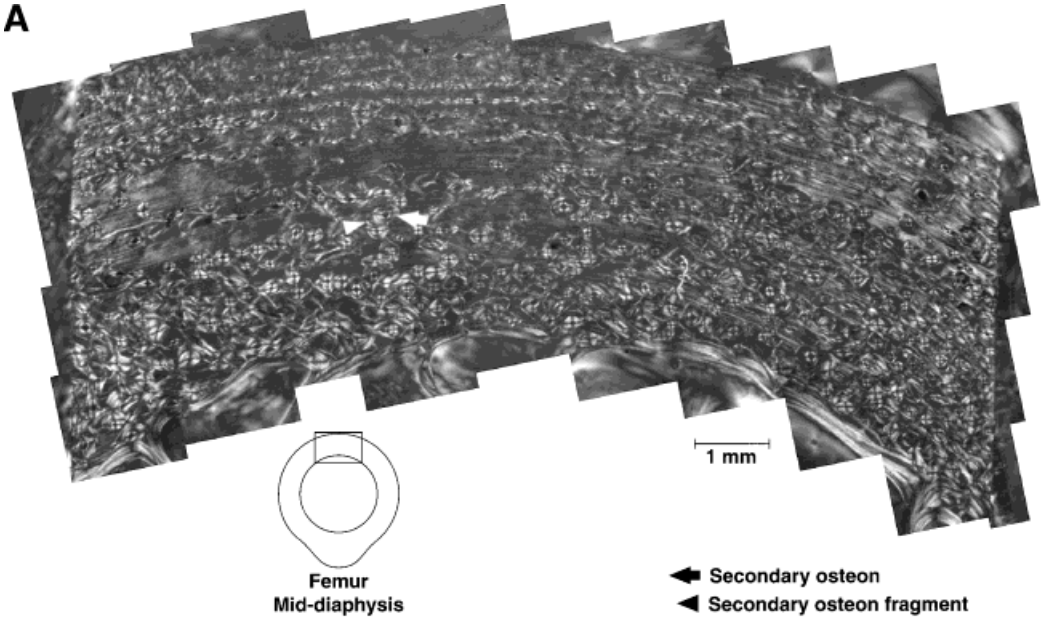
Sampling method

The entire surface of each bone section was evaluated for secondary osteons and secondary osteonal fragments. Although in histological cross-sections, secondary osteons (bone remodeling units) are characterized as circular structures made up of a central (haversian) canal surrounded by a series of concentric lamellae and a cement line, definitions of "complete" secondary osteons vary among researchers. The latter have been defined as 1) structures showing 80% or more of osteonal area intact around a complete haversian canal (Kerley, 1965), 2) structures showing concentric lamellae surrounding a haversian canal (Thompson, 1978), 3) structures with 100% of the haversian canal perimeter intact (Burr et al., 1990; Ericksen, 1980; Singh and Gunberg, 1970), and 4) structures with 90% of the haversian canal perimeter intact (Stout, 1983). Osteon fragments, or osteons that have been partially replaced by new osteons, are in turn defined with reference to the definition of a complete osteon. Following Stout (1983), for the purposes of this paper, secondary osteons (On) were defined as osteons with >90% of haversian canal intact and secondary osteon fragments (On.Fg) as remnants of secondary osteons with <90% of haversian canal intact.

Each histological section was photographed in polarized light at 20× using a Nikon UFX-II camera mounted on a Nikon Biophot microscope. On average, five photographs were required to image the entire specimen. The photographs were enlarged (57×) and combined as a photo-collage (Fig. 2A). To facilitate data collection, a clear acetate sheet with a grid (12 columns × 10 rows, 120 squares, Fig. 2B) was superimposed over the photo-collage. The top line of the grid was aligned with the outermost part of the periosteal envelope. The left line of the grid was aligned parallel to the left side of the bone section. Each grid square covered 1 mm^2 of surface. As such, bone area in squares totally filled with bone measured 1 mm^2. If the squares were only partially filled with bone (those intersecting periosteal, intracortical, or endosteal envelopes), the bone area was measured using a Nikon Optiphot microscope with a Carl Zeiss digitizing tablet and Zidas software (IBM PC).

Two microscopes placed side by side, one holding the bone section (Zeiss Standard microscope) and the other the respective microradiograph (Nikon Labophot-2 microscope), were used to identify the secondary osteons and secondary osteon fragments. Upon identification, the secondary osteons and secondary osteon fragments were recorded directly on the acetate sheet grid (Fig. 2b) overlaying the bone collage. The marking of osteons and osteonal fragments directly on the acetate grid assured that structures were counted only once. Secondary osteons and/or fragments intersecting left and upper grid lines of each square were recorded as part of the square. Those intersecting right or bottom grid lines were re-

A



B

Row \ Column	1	2	3	4	5	6	7	8	9	10	11	12
A	16	23	33	35	35	35	35	35	34	31	26	14
B	32	35	35	35	35	35	35	35	35	35	34	28
C	34	35	34	35	35	35	35	35	35	35	34	32
D	34	34	34	33	33	32	31	31	33	35	34	32
E	34	34	31	24	25	19	21	22	29	29	32	30
F	32	24	20	15	10	6	4	6	12	17	28	28
G	20	18	12	7	2	1	1	1	4	11	13	19
H	14	10	5	2					1	5	7	8
I	8	5	3							2	5	4
J	4	2	1								2	3

Fig. 2. **A:** A photo-collage of the anterior femoral mid-diaphyseal bone section. **B:** A representation of a 12 × 10 grid (120 squares) which when placed over a bone collage was used for recording secondary osteons and fragments. The grid was scaled such that each square represented 1 mm^2 of bone surface. The number in each square represents the total number of bones used for calculating osteon density in that specific square. The distribution is a result of variation in shape of individual sections.

corded as part of the right and bottom square, respectively. Osteons intersecting the lower right hand corner of a square were counted as part of the lower right diagonal square.

Statistics

For each individual, total osteon density (number of osteons + number of osteon fragments/mm^2, N.On + N.On.Fg/mm^2) was determined for the total sampling area (entire anterior femoral section) and subsamples of total sampling area [specific column(s), row(s), row-column combinations, and square combinations]. All data are expressed as mean ± SD.

The reliability of total osteon density in subsamples of total area as predictors of total osteon density in the anterior femoral section was tested using multivariate regression analysis (SAS, 1982). Total osteon density was the variable chosen for evaluating subsample predictability as it avoided discrepancies in the categorization of osteons into complete and fragment classes. The subareas evaluated included: columns (≈8 to 50% of total area, T.Ar, depending on number of columns included) (Fig. 2B), rows (≈<1 to 70% T.Ar, depending on number of rows included), combinations of columns and rows (≈18 to 26% T.Ar, depending on rows and columns included), and combinations of squares (5 to 14% T.Ar, depending on square combinations evaluated) approximating field locations and dimensions evaluated by other researchers (Fig. 1). Subsample means were regressed against total bone section means to generate a coefficient of determination (R^2). An example regression plot showing the relationship between total osteon density in the entire anterior femur section and a subsample of the area (columns 5 and 8 combined) is shown in Figure 3.

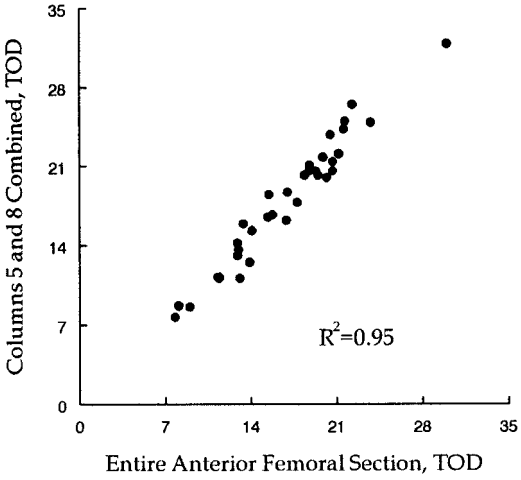


Fig. 3. A regression of the relationship between total osteon density (TOD, no. osteons per mm^2) in the total sampling area (entire anterior femoral section) and a subsample of the total sampling area (columns 5 and 8 combined). See Figure 2B for identity of columns.

RESULTS

Interindividual variation

The total anterior mid-diaphyseal femoral area averaged 56.7 ± 12.4 mm^2 (N = 35). Total osteon, secondary osteon, and secondary osteon fragment density means and interindividual variations, expressed as a CV, are presented in Table 2. All interindividual differences for total, secondary osteon, and secondary osteon fragment density were significant ($P < 0.001$).

Subsamples as predictors of anterior femoral section variability

Section columns, rows, combinations of columns and rows, and selected combinations of squares (Fig. 2B) approximating area dimensions and locations evaluated by other paleohistologists (Fig. 1) varied with respect to their predictability of total osteon density in the complete anterior femoral mid-diaphyseal section. Overall, row and column predictabilities showed similar ranges of variation in R^2.

Column predictability. The distribution of total osteon density among columns is presented in Figure 4. Because of interindividual variation in bone shape, core sample size, and method of data collection, total osteon density variation in all of the columns is not directly comparable. The biopsied tissue in the four medial columns (5 through 8) expanded from the periosteal to the endosteal surface and as such represents a periosteal to endosteal continuity in bone remodeling (postmortem weathering not controlled). A periosteal to endosteal

TABLE 2. Interindividual variation in total osteon density, secondary osteon density and secondary osteon fragment density

Item	Code	N[a]	Mean ± SD[b]	Interindividual CV[c]
Total osteon density	(N.On + N.On.Fg)/mm^2	35	16.6 ± 4.7	28.3[d]
Secondary osteon density	N.On/mm^2	35	12.0 ± 3.1	25.8[d]
Osteon fragment density	N.On.Fg/mm^2	35	4.6 ± 2.4	52.2[d]

[a] Total number of individuals.
[b] Mean pooled across population, sex, and age ± standard deviation (SD).
[c] Coefficient of variation calculated as the (SD/mean) * 100.
[d] $P < 0.001$ (ANOVA test; SAS, 1982).

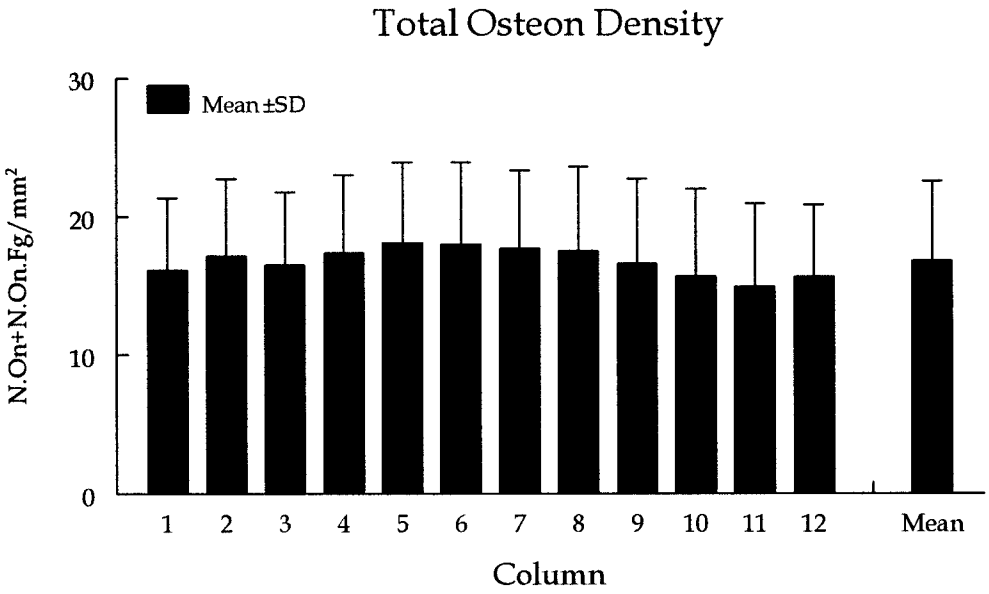


Fig. 4. Distribution of total osteon density in the anterior mid-diaphyseal femur by column (N = 35 for columns 1–10, N = 34 for column 11, and N = 32 for column 12; see Figure 2B for identification of columns). The data do not represent a lateral to medial distribution for total osteon density in the anterior mid-diaphyseal femur (see text).

continuity was not necessarily the case in the left and right columns. Many left and right columns tended to terminate within the intracortical envelope. Although in some large specimens both the right and left columns terminated in the cortex, in the majority of sections the intracortical termination was restricted to one side only. As such, columns 1 through 12 as a set do not represent lateral to medial variation in anterior mid-diaphyseal femoral total osteon density. A lateral to medial distribution of total osteon density can, however, be assumed for columns 5 through 8 as periosteal and endosteal surfaces were roughly parallel in these columns.

Individual column (8% T.Ar) predictability (R^2) of anterior femoral section total osteon density ranged from 0.48 to 0.86, depending on column evaluated (Table 3). An association was noted between R^2 values and column location with the medial four columns (5, 6, 7, 8) exhibiting higher predictability values (R^2, 0.80–0.86) than either the four left (1, 2, 3, 4; R^2, 0.55–0.76) or the four right (9, 10, 11, 12; R^2, 0.48–0.75) columns. Kruskal-Wallis testing with planned post-hoc comparisons (student-Neuman-Keuhls test) showed that the three groups were significantly different ($P < 0.05$) with the medial columns being better predictors of anterior section total osteon density than either the four left ($P < 0.007$) or the four right columns ($P < 0.003$).

Combinations of two adjacent medial section columns (15% T.Ar) predicted between

TABLE 3. Coefficients of determination (R^2) for individual columns and combinations of columns

No. of columns[a]	Column relationship[b]	Column identification[c]	N[d]	Area (mm^2, mean ± SD)	≈% total area	R^2
1	Individual	1	34	5.5 ± 1.7	8	0.55
		2	35	5.4 ± 1.6		0.72
		3	35	5.0 ± 1.5		0.66
		4	35	4.6 ± 1.2		0.76
		5	35	4.4 ± 1.0		0.86
		6	35	4.2 ± 1.0		0.80
		7	35	4.2 ± 0.9		0.83
		8	35	4.2 ± 1.0		0.85
		9	35	4.4 ± 1.1		0.75
		10	35	4.7 ± 1.3		0.67
		11	34	5.1 ± 1.7		0.60
		12	32	4.9 ± 2.0		0.48
2	Adjacent (mid-section[e])	5, 6	35	8.5 ± 1.9	15	0.88
		6, 7	35	8.3 ± 1.9		0.88
		7, 8	35	8.4 ± 1.9		0.89
2	Alternate (mid-section[e])	5, 7	35	8.6 ± 1.9	15	0.92
		6, 8	35	8.4 ± 1.9		0.91
		5, 8	35	8.6 ± 1.9		0.95
2	Spaced	4, 9	35	9.0 ± 2.1	15	0.95
4	Adjacent (total section)	1, 2, 3, 4	35	20.6 ± 5.8	30–35	0.73
		5, 6, 7, 8[f]	35	16.9 ± 3.7		0.94
		9, 10, 11, 12	32	19.1 ± 5.7		0.68
6	Alternate (total section)	1, 3, 5, 7, 9, 11	34	28.6 ± 6.3	50	0.99
		2, 4, 6, 8, 10, 12	32	28.0 ± 6.2		0.98

[a] Number of columns (of possible 12, see Figure 2b) regressed against entire bone section total osteon density means to generate R^2.
[b] Column relationship defines the position of the columns in relation to each other.
[c] See Figure 2b for column identification.
[d] Total number of individuals.
[e] The four medial section columns were selected for further analysis based on the significantly higher column predictability values associated with this area in relation to either the four left or the four right columns.
[f] Fields approximating location and area dimensions evaluated by Burr et al., 1990.

88 and 89% of anterior section total osteon density. Combinations of two medial section columns, separated by one column, predicted between 91 and 95% of total osteon density. Ninety-five per cent of total osteon density was predicted by two columns (4 and 9) separated by four columns (5–8). The predictability of total osteon density in the anterior section by combined columns 1 through 4, 5 through 8 [approximates Burr et al. (1990) sampling procedure], and 9 through 12 was 0.73, 0.94, and 0.68, respectively. Alternate columns (50% of section area) predicted 98 to 99% of anterior section total osteon density.

Row predictability. The row distribution of total osteon density in the anterior mid-diaphyseal femur is presented in Figure 5. This distribution was used for assessing row predictability of total visible osteon density variation in the anterior mid-diaphyseal femur. The data do not represent a true periosteal to endosteal total osteon density distribution for the anterior femur. The distribution is a result of the data collection method outlined previously for the 12.7 mm wide anterior femoral biopsy section.

As for individual column predictability, row predictability of anterior femoral section total osteon density differed depending on row(s) assessed (Table 4). Osteon densities associated with row A or the periosteal row (12% T.Ar) accounted for 79% of the entire anterior mid-diaphyseal femoral sections' total osteon density variation. Total osteon density in rows B and C (20% T.Ar each) accounted for 92 and 88% of total variation, respectively. Predictability values for rows D through J (19 to <1% T.Ar) ranged from 45 to 71%, depending on row examined. Combinations of two adjacent or alternate rows (32–40% T.Ar) ranged in predictability values from 86 to 95%. Combinations of four adjacent rows A through D [71% T.Ar, approximates Richman et al. (1979) sampling procedure] and E through H (28% T.Ar) predicted 93 and 90% of the variation in total osteon density in the anterior mid-diaphyseal femur, respectively.

Combination column and row predictability. Column and row combinations were also evaluated. Various combinations of the four medial columns (5, 6, 7, 8) with rows A through E ranged in predictability

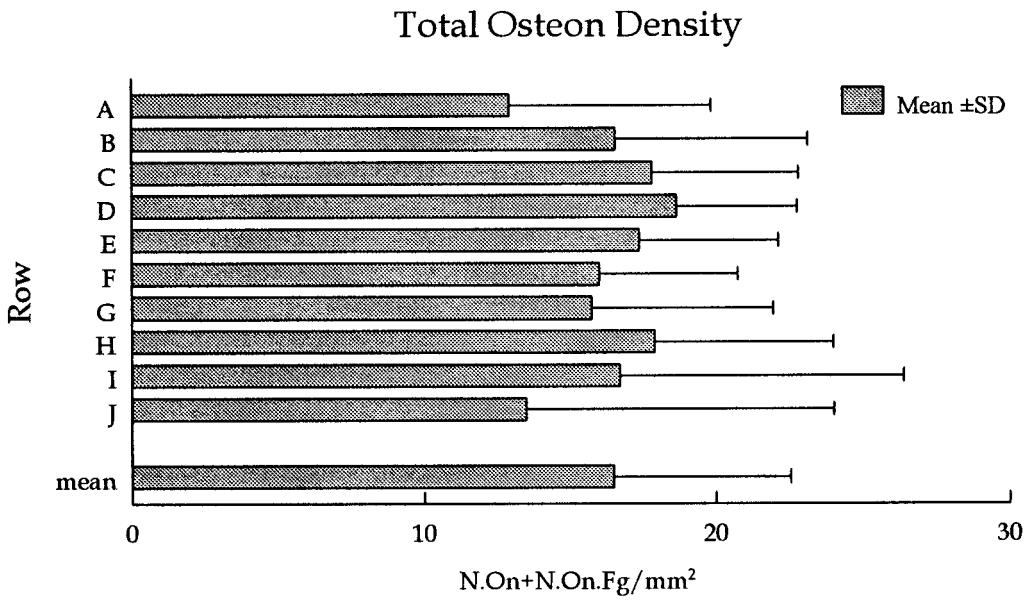


Fig. 5. Distribution of total osteon density in the anterior mid-diaphyseal femur by row (N = 35 for rows A through E, 34 for row F, 27 for row G, 17 for row H, 10 for row I, and 6 for row J; see Figure 2b for identification of rows). The data do not represent a periosteal to endosteal distribution for total osteon density in the anterior mid-diaphyseal femur (see text).

TABLE 4. Coefficients of determination (R^2) for individual rows and combinations of rows

No. of rows[a]	Row relationship[b]	Row identification[c]	N[d]	Area (mm^2, mean ± SD)	≈% total area	R^2
1	Individual	A	35	7.0 ± 1.2	12	0.79
		B	35	11.1 ± 0.8	20	0.92
		C	35	11.6 ± 1.1	20	0.88
		D	35	10.6 ± 2.3	19	0.69
		E	35	7.7 ± 3.3	14	0.71
		F	34	4.6 ± 3.0	8	0.45
		G	27	2.3 ± 2.1	4	0.60
		H	17	1.1 ± 1.4	2	0.45
		I	10	0.5 ± 0.9	<1	0.49
		J	6	0.2 ± 0.6	<1	0.53
2	Adjacent	A, B	35	18.1 ± 1.9	32	0.86
		B, C	35	22.7 ± 1.5	40	0.93
		C, D	35	22.2 ± 3.2	39	0.95
2	Alternate	A, C	35	18.6 ± 1.6	33	0.87
		B, D	35	21.7 ± 2.6	38	0.95
4	Adjacent	A, B, C, D[e]	35	40.3 ± 4.1	71	0.93
		E, F, G, H	17	15.6 ± 9.0	28	0.90

[a] Number of rows (of possible 10, see Figure 2b) regressed against entire bone section total osteon density means to generate R^2.
[b] Row relationship defines the position of the rows in relation to each other.
[c] See Figure 2b for row identification.
[d] Total number of individuals.
[e] Fields approximating location and area dimensions evaluated by Richman et al., 1979.

from 89 to 96%. Results for a combination of one of the columns (column 5) and the five rows are presented in Table 5. Similar patterns and R^2 values were observed when the remaining columns from the medial section (columns 6, 7, or 8) were combined with rows A, B, C, D, or E (data not shown).

Combination square predictability. Predictability results using combinations of squares approximating areas and locations assessed by paleohistologists (Fig. 1), other than those already mentioned, are presented in Table 6. A combination of three approximately equidistant squares (2.8 ± 0.3 to 2.9 ± 0.02 mm^2) from the outer third of

TABLE 5. Coefficients of determination (R^2) for individual column and row combinations

Column identification[a]	Row identification[a]	N[b]	Area (mm^2, mean ± SD)	≈% total area	R^2
5	A	35	10.4 ± 1.9	18	0.92
5	B	35	14.1 ± 2.2	25	0.96
5	C	35	14.9 ± 1.7	26	0.92
5	D	35	14.0 ± 3.6	25	0.90
5	E	35	11.6 ± 3.8	20	0.89

[a] See Figure 2B for column and row identification.
[b] Total number of individuals.

the cortex [approximates Ericksen's (1980) sampling location; size of the area evaluated in the current study is, however, twice that of Ericksen] predicted from 83 to 89% of anterior section total osteon density, depending on squares examined. More than one combination of squares approximating the Ericksen (1980) sampling location was evaluated to show the range in R^2 based on a slight change in area location assessed. A combination of five approximately equidistant squares (4.3 ± 0.5 mm^2) located near the periosteal border [approximates Ericksen (1991) sampling procedure] predicted 90% of total anterior section osteon density. Predictability of total osteon density by four adjacent periosteal squares (3.6 ± 0.4) in the middle third of the bone section [approximates Thompson (1979) sampling procedure] was 80%. Two randomly chosen sites composed of four adjacent squares each [7.8 mm^2, approximates Singh and Gunberg (1970) sampling procedure] accounted for 86% of section variation in total osteon density.

DISCUSSION

The results demonstrate that total osteon density in subareas of as little as 15% predict 95% of variation in total osteon density in the entire anterior mid-diaphyseal femoral section (20% of total cross-section). The femoral histomorphometric analyses completed to date, although limited in number, have generated insightful hypotheses regarding adaptation in various human populations. A minimization of the histological area evaluated without the loss of accuracy increases the feasibility of population-level histomorphometric analyses due to reduction in data collection time. Alternately, a reduction in the data collection time per specimen allows for the analysis of a greater number of specimens.

The reduction in the quantity of tissue required for analysis also has positive implications for assessment of damaged specimens, assuming preservation is adequate for a specified subarea evaluation. Histological bone preservation varies between and within archaeological sites (see Jackes, 1992, for review). Although archaeological bone histology may be exceptionally well preserved (Pfeiffer, 1980), in the majority of cases some specimens will show various degrees of post-burial histological alteration (Garland, 1987; Hanson and Buikstra, 1987). In the current study, 20% of specimens had to be rejected from analysis due to postmortem damage making the histological assessment of the entire anterior femoral mid-diaphyseal section impossible. Upon reexamination it was determined that a majority of the specimens were adequately preserved for a subarea evaluation (e.g., 2 column count), allowing for the inclusion of these specimens in future studies.

Choice of subarea for evaluation should depend on level of predictability desired, time available for data collection, and extent of histological preservation. For 12.7 mm wide anterior femoral mid-diaphyseal sections with good preservation, the counting of remodeling units in two, periosteal to endosteal 1 mm wide columns, separated by 2 mm of bone may be the most efficacious choice. In specimens where preservation is inadequate for such a sampling, three to five equidistant squares, for example, may still be assessed (assuming adequate preservation) to account for 80 to 90% of variation in total osteon density in the anterior femoral section.

Predictability results (R^2) based on evaluations of area dimensions and locations approximating those examined in earlier studies varied depending on size and location of area evaluated. For the sampling techniques assessed, however, total osteon density in the area specified predicted over 80% of variation in anterior section total osteon density. Because osteons are not distributed uniformly, it is not known to what extent

TABLE 6. Coefficients of determination (R^2) for various fields approximating location and area dimensions evaluated by researchers using the anterior mid-diaphyseal core sampling method (Fig. 1)

No. of squares[a]	Squares relationship[b]	Square identification[c]	N[d]	Area (mm^2, mean ± SD)	≈% total area	R^2	Ref.
3	Equidistant	C2, B6, B11	35	2.8 ± 0.3	5	0.89	Eriksen, 1980[e]
		B3, B6, B10	35	2.9 ± 0.2		0.88	
		C2, A6, C11	35	2.9 ± 0.2		0.83	
		C3, A6, C11	35	2.9 ± 0.2		0.87	
5	Equidistant	B2, A4, A6, A8, B11	35	4.3 ± 0.5	8	0.90	Ericksen, 1991[f]
4	Adjacent	A5, A6, A7, A8	35	3.6 ± 0.4	7	0.80	Thompson, 1979[g]
8	Random	B4, C4, B5, C5, B10, C10, B11, C11	35	7.8 ± 0.5	14	0.86	Singh and Gunberg, 1970[h]
		A5, B5, A6, B6, B9, C9, B10, C10	35	7.8 ± 0.3		0.86	

[a] Number of squares (of possible 103, see Figure 2b) regressed against entire bone section total osteon density means to generate R^2.
[b] Squares relationship defines the position of the squares in relation to each other.
[c] Square = row * column, see Figure 2b for square identification.
[d] Total number of individuals.
[e] Three fields in outer third of cortex; although locations approximate those of Ericksen (1980), the area dimensions evaluated in the current study are twice as large.
[f] Five equidistant fields as close to the periosteal edge as possible.
[g] Four fields along periosteal border.
[h] Two fields from outer third of cortex.

predictability results based on total visible osteon density distributions apply to type II osteon (Richman et al., 1979), forming osteon (Martin and Armelagos, 1979; Richman et al., 1979), complete secondary osteons as defined by various researchers (Burr et al., 1990; Ericksen, 1991; Singh and Gunberg, 1970; Stout, 1983; Thompson, 1979), or double zone osteon (Martin and Armelagos, 1985) distributions. The subsample predictability of total anterior femoral section type II osteon, complete secondary osteons as defined by various researchers, and double zone osteon distributions requires future testing.

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