Preferred Collagen Fiber Orientation in the Human Mid-shaft Femur

HAVIVA M. GOLDMAN,[1]* TIMOTHY G. BROMAGE,[1] C. DAVID L. THOMAS,[2] AND JOHN G. CLEMENT[2]
[1]Hard Tissue Research Unit, Department of Anthropology, Hunter College of the City University of New York, New York, New York
[2]School of Dental Science, University of Melbourne, Melbourne, Australia

ABSTRACT

Collagen fiber orientation is one aspect of the microstructure of bone that influences its mechanical properties. While the spatial distribution of preferentially oriented collagen is hypothesized to reflect the effects of loading during the process of aging, its variability in a modern human sample is essentially unknown. In a large sample (n = 67) of autopsied adults, the variability of collagen fiber orientation in the mid-shaft femur was examined in relation to age and sex. Montaged images of entire 100 μm thick cross-sections were obtained using circularly polarized light microscopy (CPLM) under standardized illuminating conditions. An automated image-analyzing routine divided images into 48 segments according to anatomical position. Average gray values (varying with orientation) were quantified for each segment, and one-way ANOVA with Tukey HSD post hoc tests were applied to assess differences between segments. Collagen fiber orientation appeared to be nonrandomly distributed across the mid-shaft femur sample; however, no single "human" pattern was identified. Individual variation, unexplainable by age, sex, or body size, exceeded population-level trends. Differences between age and sex groups suggest there is a strong correspondence between collagen fiber orientation and tissue-type distributions. The minimal consistencies demonstrated here may reflect mechanical forces induced at the femoral mid-shaft. However, the myriad of other factors that may influence collagen fiber orientation patterning, including growth trajectories, metabolic and nutritional status, and disease states, must be explored further. Only then, in conjunction with studies of other structural and material properties of bone, will we be able to elucidate the linkages between microstructure and functional adaptation in the human mid-shaft femur. Anat Rec Part A 272A:434–445, 2003.

Key words: collagen fiber orientation; human femur; human variation; bone microstructure

Recent research indicates that the preferred orientation of collagen fibers within bone is a particularly good indicator of bone strength (Martin and Ishida, 1989; Boyde and Riggs, 1990; Martin and Boardman, 1993; Riggs et al., 1993a,b; Mason et al., 1995). Within a bone cross-section, regions with the greatest proportion of transversely oriented fibers best withstand high compressive strain perpendicular to the section axis, while regions with the greatest proportion of longitudinally oriented fibers best withstand high tensile strain perpendicular to the section axis. These hypotheses were first tested by Gebhardt (1905) and were later tested by Ascenzi and Bonucci (Ascenzi and Bonucci, 1964, 1967, 1968a,b) and Pidaparti and Burr (1992). The spatial distribution of collagen fiber orientation has been hypothesized to reflect the effects of loading during the process of aging in several nonhuman mammals, as supported by experimental strain data in

Grant sponsor: National Science Foundation; Grant numbers: SBR-9512373; SBR-9727689; Grant sponsor: Louis B. Leakey Foundation.

*Correspondence to: Dr. Haviva M. Goldman, Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129. Fax: (215) 843-9082.
E-mail: hgoldman@drexel.edu

Received 3 April 2002; Accepted 27 December 2002
DOI 10.1002/ar.a.10055

the horse radius (Boyde and Riggs, 1990; Riggs et al., 1993a,b; Mason et al., 1995), the macaque circumorbital region (Bromage, 1992), and the calcanei of horse, sheep, and elk (Skedros et al., 1997).

Few studies have quantified preferred collagen fiber orientation and its relationship to mechanical loading in human bone. Evans and Vincentelli (1969) and Vincentelli and Evans (1971) found a significant positive correlation between tensile stress and strain and the percentages of osteons with predominantly longitudinal collagen fibers, in cross-sections taken near the fracture sites of mechanically tested tibiae. More recently, studies of the tibia and fibula (Carando et al., 1989) and femur (Portigliatti-Barbos et al., 1983, 1984) provided the first quantitative assessments of preferred distribution of collagen fiber orientation in entire human bone cortices. As the distribution of preferentially oriented collagen may provide useful information concerning bone quality, its characteristics and variability with respect to age and sex in humans should be investigated further.

The Portigliatti-Barbos et al. (1983,1984) studies of the human femur included data from only two individuals (both males in their forties). The authors described a pattern of collagen fiber distribution in which lamellae containing transverse fibers (the so-called "transverse lamellae") underwent a rotation from the medial to the posterior cortex, as viewed from the proximal to the distal end of the femoral shaft. In the mid-shaft of the femur, transverse lamellae were primarily found posteromedially. They also predominated along the endosteal surface and in a small sector periosteally along the anterolateral border. The linea aspera and anterior and lateral cortices exhibited a pattern in which lamellae containing longitudinal fibers (the so-called "longitudinal lamellae") predominated. The authors offered a biomechanical explanation for these observations based on available data on bending forces operating in the femoral shaft. For example, the authors suggested that bending at the mid-shaft would produce anterolateral tension and posteromedial compression. This would explain the observed increased longitudinal lamellae anteriorly and laterally, and the increased transverse lamellae medially and posteriorly.

Variability in human collagen fiber patterning with age has also rarely been investigated. In a study of human femora and tibiae, Smith (1960) suggested that secondary osteons may change their fiber orientation as they age. However, this concept, which would require a complete change in the orientation of collagen and crystallites following completed mineralization, has not been supported by subsequent studies. Vincentelli and Evans (1971) and Vincentelli (1978) examined collagen fiber orientation in a sample of young and old male tibiae. They suggested (in support of earlier observations by Amprino and Bairati (1936)) that as individuals age, the proportion of osteons with transverse lamellae increases. Moreover, Vincentelli (1978) clearly showed that newly formed osteons (as determined by relatively low mineralization revealed in microradiographs) could be found with either transverse or longitudinal orientation. This supported Smith's (1960) observation of a change in predominant orientation with age, but not his proposed mechanism.

In the current study we sought to determine whether the human mid-shaft femur demonstrates a predictable pattern of collagen fiber orientation that is consistent with the forces encountered in bipedal locomotion. We also investigated whether there was variability within such a pattern, and how it related to age and sex, and body weight and height. Various factors, including mechanical ones that may influence patterns of collagen fiber orientation, are also discussed.

MATERIALS AND METHODS

Specimen Collection

Mid-shaft femur blocks from 67 individuals, obtained from the Victorian Institute of Forensic Medicine, Melbourne, Australia, were used in this analysis. The sample included individuals of known age, sex, height, weight, and cause of death. The sample was divided into three age groups: younger (25–44 years), middle (45–64 years), and older (65+ years). Each group had approximately equal numbers of males and females. The age ranges of the three groups were chosen to reflect biological changes. The "younger" group included adults of both sexes who had generally completed the growth phase, but no females who would have been menopausal. The "middle" group included females who may have been peri- or postmenopausal. The "older" group included females who were probably fully postmenopausal. Further information on this sample can be found in Bertelsen et al. (1995), Feik et al. (1996,2000), and Goldman (2001).

In 17 individuals, the femur block was removed from the right thigh, 1 cm superior to the measured mid-point of the femur. Orientation was not recorded at the time of collection in the remainder of the sample; therefore, the specimens were oriented in the mediolateral plane based on of the disposition of microstructural features as described by Goldman (2001).

Sample Preparation

Bone blocks (approximately 0.5 cm in height) were removed from the samples and stored in 70% ethanol. They were then cleaned and dehydrated before they were embedded in a poly methyl methacrylate (PMMA) and styrene mixture according to procedures described by Goldman et al. (1999). The PMMA-embedded blocks were sectioned in such a way as to allow thin sections to be imaged by both light and scanning electron microscopy (Goldman et al., 1999). The resulting ground, thin sections (100 μm thick, noncoverslipped) were mounted to a glass slide with components of dental adhesive systems (Bisco, Schaumburg, IL, and Dentsply, York, PA). Uniform (100 μm, ±2μm) section thickness was determined using an Edge R400 (Microscience Technologies, Marina del Ray, CA) real-time 3D microscope fitted with precision staging in the "Z" direction. The specimens were polished to minimize surface topography, as required for subsequent backscattered electron microscopy imaging procedures to examine mineralization density in these same sections (Goldman, 2001).

Image Acquisition

Images of entire mid-shaft femur cross-sections, suitable for determining collagen fiber orientation, were obtained at high resolution by automated montaging of tiled images across the entire specimen. The sections were temporarily coverslipped in ethylene glycol to improve image quality. Gray-level images (each 1,024 × 768 pixels, field width = 2.2 mm (later reduced to 33% of original size for ease of processing)) were obtained with a Leica DMRX/E

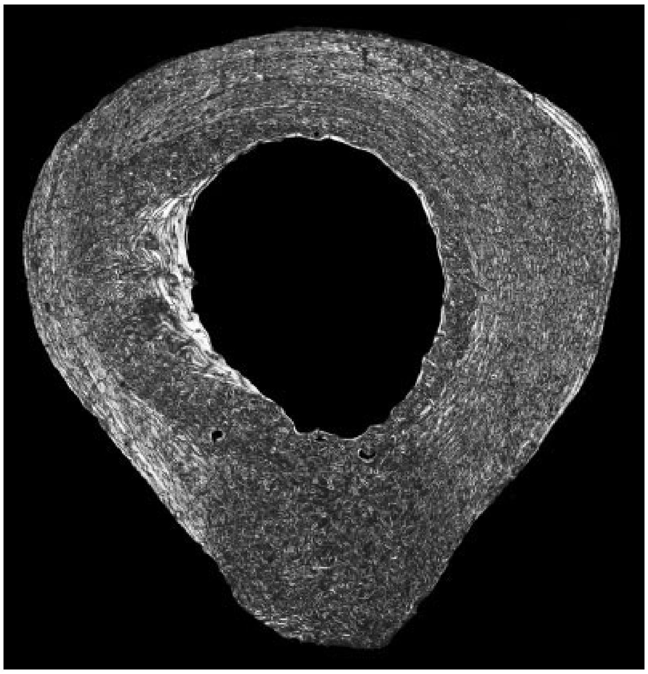

Fig. 1. This figure demonstrates a complete gray-scale image montage after masking, but prior to further processing. Figure 5a features this same specimen after image processing and application of a color look up table (LUT). In the LUT, gray levels above 0 are divided into 8 color intervals (bins), using a variation of a thermal color scheme, adapted from that used by Riggs et al. (1993a). Section is oriented so that medial is to the left and posterior is towards the bottom of the page.

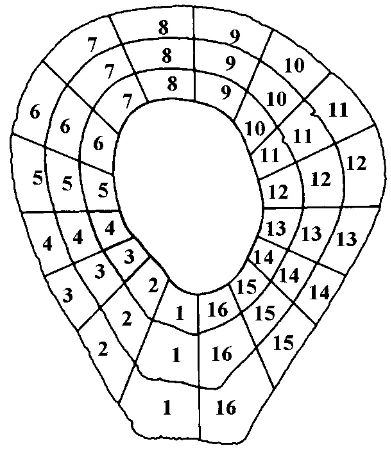

Fig. 2. To quantify visually observed pattern differences, a customized macro within the Optimas image analysis program (Media Cybernetics, Inc.) was used to segment the cortex into 16 radial sectors and three rings (periosteal, mid-cortex, and endosteal), for a total of 48 segments. Within each segment, quantitative data on gray-level values were obtained, which provided a means of quantifying differences between bone regions. Sections are oriented so that the medial is to the left and the posterior is toward the bottom of the page.

(Leica Microsystems, Baunnockburn, IL) universal microscope configured with circularly polarized light (CPL) filters and an automated high-resolution Martzhauser X-Y stage. The images were transferred to a Leica Quantimet high-resolution image analysis system (Q600) via a Kodak Megaplus CCD camera. Lighting was adjusted to a standard illumination with a neutral density filter, the gray level of which was checked and reset to a predetermined setting before imaging each specimen. Immediately after the specimen was imaged by CPL microscopy (CPLM), the CPL polarizer was removed and the specimen was reimaged. (These transmitted light microscopy (LM) images would later be used for masking procedures, as described below.) Tiled images from both the CPLM and LM runs were automatically montaged using a dedicated software program developed in our laboratory with Visual Basic 6.0 (Microsoft) and Leadtools Imaging (16/32 ActiveX v. 10, LEAD Technologies, Inc., Charlotte, NC).

Image Analysis

The transmitted light montage of each specimen was used to produce a background mask for its respective CPLM montage (see Goldman, 2001, for details on this method), resulting in the assignment of most non-bone areas (the medullary cavity, resorption bays, and larger Haversian canals) to a gray-level value of zero, so that they could easily be excluded from analysis. Without such masking procedures, it would have been impossible to differentiate the gray level of pores within the bone from that of dark longitudinal lamellae, which would have biased the quantitative analysis. Once the CPLM image was masked, a color look-up table (LUT) was applied to the processed CPLM image using an adaptation of the methodology of Riggs et al. (1993a) to provide a visual map of collagen fiber orientation across the entire bone cortex (Goldman, 2001) (Fig. 1).

The processed gray-level CPLM image was automatically divided into 48 segments within the periosteal, mid-cortex, and endosteal rings using an Optimas Image Analysis (Media Cybernetics, Inc., Silver Spring, MD) software macro, as described in Feik et al. (2000) and Goldman (2001) (Fig. 2). Within each segment the number of pixels within each of 256 gray levels, and the mean gray value of the segment were calculated. Results were transferred via a dynamic data exchange (DDE) link to a Microsoft Excel spreadsheet.

Data Analysis

A "brightness index" was calculated for each segment by subtracting the percentage area of dark pixels (pixels between gray values 1–48) from the percentage area of bright pixels (pixels between values 209–255), and adding the value of 100 to the result. Segments with a greater proportion of bright pixels (more transverse lamellae) received an index value of >100. Segments with a greater proportion of dark pixels (more longitudinal lamellae) received an index value of <100. This data set had a 0.99 correlation coefficient with the mean gray-level values. However, as the index scale provided information concerning the relative proportion of bright and dark pixels, these values provided a more easily interpretable indication of the distribution of transverse and longitudinal lamellae.

Brightness indices were plotted against the sector (location around the cortex) for each of the three rings examined (bone located toward the periosteal surface, mid-cortex, or endosteal surface). Because brightness indices

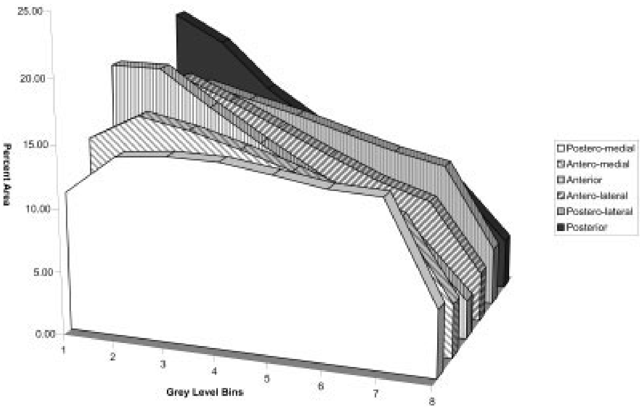

Fig. 3. Gray-level distributions, divided into eight gray-level bins, between selected sectors. Note that the greatest differences in the height of these distributions is at the darkest (bins 1 and 2) and brightest (bins 6 and 7) grays, while very little difference is found in the middle bins of the distribution. For this reason, the brightness index, a calculation of the proportion of brightest and darkest pixels, is a good representative index of sector differences. Moreover, note how the majority of sectors show a skewed distribution with increased proportions of longitudinal lamellae. Posteromedial = sectors 2 and 3; anteromedial = sectors 6 and 7; anterior = sectors 8 and 9; anterolateral = sectors 11 and 12; posterolateral = sectors 14 and 15; and posterior = sectors 1 and 16.

were normally distributed for the whole sample, parametric ANOVAs with Tukey HSD post hoc tests were used to determine the effect of location in the cortex (i.e., sector and ring) and body-size variables (height and weight) on collagen fiber orientation. The ranges of the brightness index values were compared between age and sex groups to determine whether variability in collagen fiber orientation was significantly higher or lower in any group. ANOVA was also used to determine the effects of age and sex on collagen fiber orientation variability.

RESULTS

Variability in Brightness Indices

The data were pooled to provide information on the average brightness indices across whole cross-sections, which is an indicator of the overall prevalence of longitudinal vs. transverse collagen fibers. The mean brightness index for the entire sample, including all sectors, was 89.94 (±14.1 S.D.), indicating a trend toward a predominance of longitudinal collagen fibers in whole cross-sections. The data were skewed toward lower brightness indices in most sectors, indicating that most regions of the cortex within individuals contained a predominance of longitudinal collagen fibers (see Fig. 3). This result characterized all age groups and sexes, and was not significantly correlated with any body-size variable (height, weight, or body mass index).

Although the data were skewed toward lower brightness indices on average, there were large differences between individuals in the range of brightness indices within the cross-section. An examination of these ranges provided additional information on the degree of heterogeneity in collagen fiber orientation that could not be appreciated by examination of the mean alone. When data from whole cross-sections were pooled, individuals from the younger (mean range of values = 56.0) and middle age groups (mean range = 57.7) showed a significantly greater range of brightness index values than older individuals (mean range = 38.7) ($P < 0.001$), while the younger and middle groups were not significantly different from one another.

Factors Related to Variation in Collagen Fiber Orientation

A four-factor ANOVA (with age group, sex, ring, and sector as factors, and the brightness index as the independent variable) indicated that age group, sex, ring, and sector are each significant contributors ($P < 0.01$) to the variability of the brightness index. Moreover, this analysis demonstrated an interaction between age and sex. To investigate these relationships further, the sample was first examined in age- and sex-matched groups for each ring and sector. Sexes were pooled if no sex differences were identified.

Regional Variability in Collagen Fiber Orientation: Younger Group (25–44 Years)

Females in the younger group showed significantly higher brightness indices (i.e., more transverse lamellae) than males only within bone of the endosteal ring (female mean = 98.6; male mean = 94.2, $P < 0.05$). As a whole, the endosteal ring contained significantly more transverse collagen fibers than either the periosteal or mid-cortical rings (see Table 1); however, only a few sectors medially and posteriorly could account for this difference (see Fig. 4). Within each ring, sector differences reflected an increase in transverse collagen fibers medially and laterally relative to the posterior, anterior, and anterolateral sectors. These differences were found predominantly in the periosteal ring, with fewer significant differences in the mid-cortical and endosteal rings (see Table 2).

Of note, high proportions of primary circumferential bone were typical of many individuals in this age group, particularly those under the age of 35. These lamellae were predominantly transverse in orientation and generally found in the periosteal third of the bone cortex (Fig. 5a). These areas were most often found medially and anteromedially, but in some cases large amounts of circumferential lamellae could be found irrespective of the radial position in the cortex (with the exception of the posterior aspect). Secondary osteons in these regions did not appear to follow the same orientation as the circumferential bone, particularly in the anterolateral aspect (Fig. 6). There appeared to be much variability in the proportion of the cortex encompassed by primary circumferential bone; however, this was not quantified in the present study.

Regional Variability in Collagen Fiber Orientation: Middle Group (45–64 Years)

The females in this age group showed statistically higher brightness indices than the males in each circumferential ring, when all sectors were combined ($P < 0.001$). Table 1 demonstrates that these differences were primarily due to increased brightness indices in anteriorly and medially located sectors. Differences between rings were significant in both males and females, with increasing transverse lamellae toward the endosteal surface, particularly in the posterior and medial sectors in females, and in the posterior, anterior, and lateral sectors in males (Fig. 4).

TABLE 1. Differences in brightness index between circumferential rings

	Females		Males		
	Mean B.I.[a]	Individual sectors showing significance at $P < 0.05$[b]	Mean B.I.	Individual sectors showing significance at $P < 0.05$	Sex differences
Young age (25–44) group					
Periosteal	88.9[c]	1,12,16	89.6[c]	16	–
Mid-cortex	89.4		87.7[d]		–
Endosteal	98.6[c]	1,12,16	94.2[c,d]	16	$P < 0.05$ (sectors pooled)
Middle age (45–64) group					
Periosteal	84.4[c]	1,4,5	76.5[c,e]	1,8,9,10,11,12,13,14,15,16	$P < 0.05$ (sectors pooled and sectors 8–9)
Mid-cortex	88.5[d]		82.2[d,e]		$P < 0.05$ (sectors pooled and sectors 4,9)
Endosteal	94.8[c,d]	1,4,5	88.6[c,d]	1,8,9,10,11,12,13,14,15,16	$P < 0.05$ (sectors pooled and sectors 3,4,5)
Old age (65+) group					
Periosteal	87.2[c]		89[c,e]		–
Mid-cortex	90.7		93.5[e]		–
Endosteal	91.9[c]		96.4[c]		$P < 0.05$ (sectors pooled)

[a]B.I., Brightness Index; see Methods for definition of measurement.
[b]Posterior, 1,16; postero-medial, 2,3; medial, 4,5; antero-medial, 6,7; anterior, 8,9; antero-lateral, 10,11; lateral,12,13; postero-lateral, 14,15; see Figure 2.
[c]Significant difference between endosteal and periosteal rings at $P < 0.05$.
[d]Significant difference between endosteal and mid-cortex rings at $P < 0.05$.
[e]Significant difference between periosteal and mid-cortex rings at $P < 0.05$.
–, no significant differences.

The females showed significantly more transverse collagen fibers in the medial cortex relative to the posterior aspect of the periosteal ring, and the anterior and antero-lateral aspects of the endosteal ring. Similarly, the males showed increased transverse collagen fibers in medial sectors, in both the periosteal and mid-cortical rings. In both sexes, most of the significant differences were limited to the periosteal ring (see Table 2 for a summary of the results).

Although many significant differences in brightness indices between regions of the cortex were identified in this study, the middle group demonstrated remarkable variation in overall patterning of collagen fiber orientation. A minority of individuals within this group (n = 5) possessed areas of extensive primary circumferential bone, predominantly transverse in orientation, most frequently located in the anterior aspect of the bone cross-section (see Fig. 5b), and endosteally along the medial aspect. This bone likely represents remnant primary circumferential and coarse cancellous bone from the growth process, as evidenced by the high degree of mineralization in this bone (as determined by backscattered electron microscopy (Goldman, 2001)).

Regional Variability in Collagen Fiber Orientation: Older Group (65+ Years)

In the older group, the only statistically significant differences in brightness indices between sexes were in the endosteal ring (female mean = 91.9; male mean = 96.4, $P = 0.02$), although no particular sector accounted for these differences (see Table 1). Significant differences in brightness index were identified between rings in both males and females, indicating a general increase in transverse lamellae toward the endosteal surface. However, no significant differences were found between rings of individual sectors (Fig. 4). When each ring was considered separately, the only significant sector differences identified within this age group were in the periosteal ring: the medial sectors were significantly brighter than the anterior and posterior sectors (Table 2).

It was visually apparent that collagen fiber orientation patterning was much less regular in the older group, and the lack of statistical significance in the results supports this observation. Of note, this age group displayed a reduced range of brightness values (see first section of Results), which indicates there is a greater homogeneity of the cortex in this group (see Fig. 5d).

Regional Variation in Collagen Fiber Orientation Between Age Groups

Table 3 summarizes the results for analyses of brightness index between age groups for males and females. Within the periosteal ring of females, transverse collagen fibers proportionately decreased between the younger and middle groups, but no significant differences were identified between the older group and any other group. Within males, the younger and older groups had significantly more transverse lamellae in the periosteal ring than the middle group. When data were analyzed for individual sectors of the periosteal ring, no significant differences between age groups were identified for either sex, with the exception of two sectors in males of the older and middle groups (see Table 3). When only the mid-cortical ring was considered, no significant age differences were found

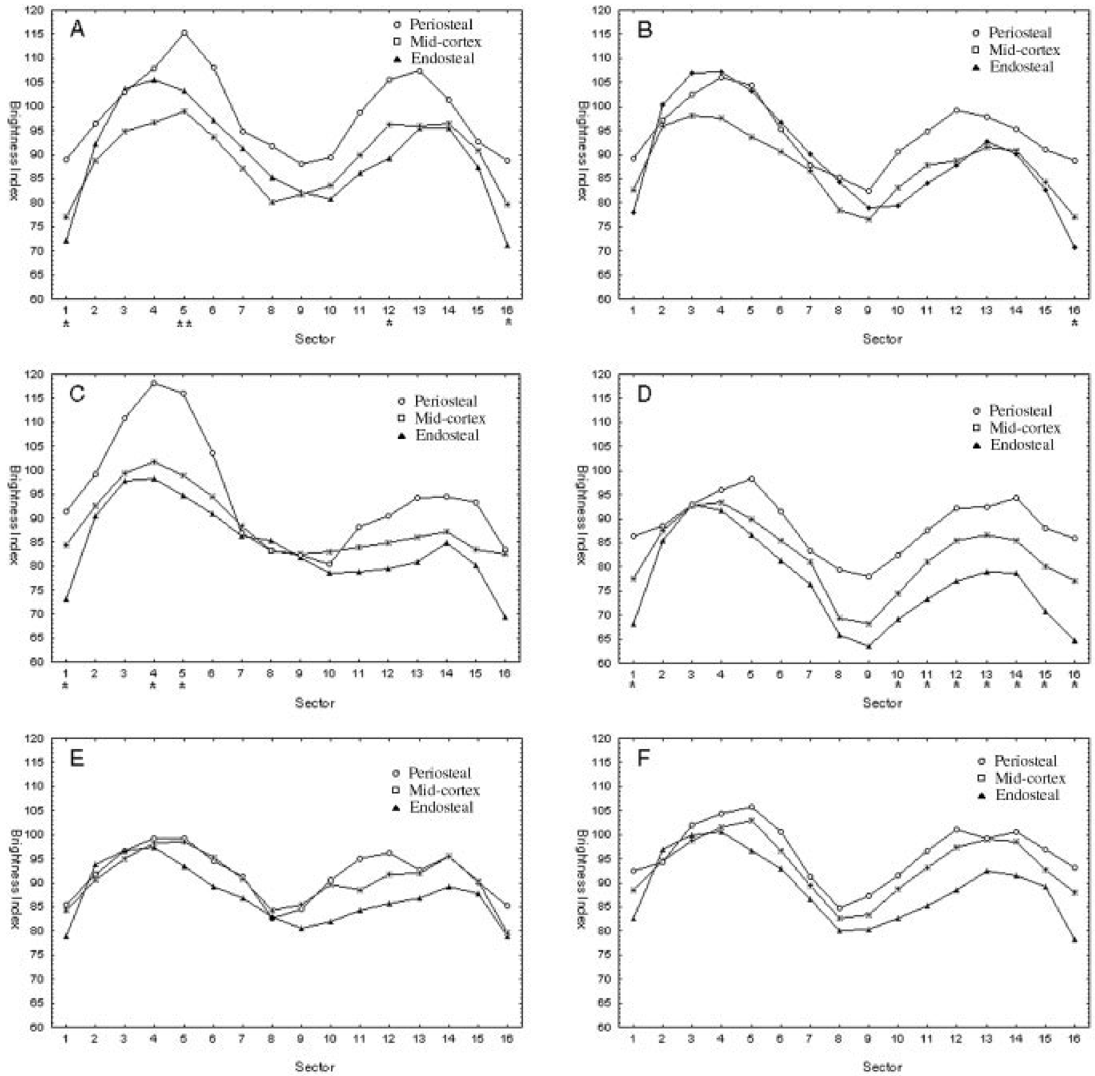

Fig. 4. Comparison of the brightness index between circumferential rings by sector: (**a**) younger females only, (**b**) younger males only, (**c**) middle females only, (**d**) middle males only, (**e**) older females only, and (**f**) older males only. Posterior = sectors 1 and 16; posteromedial = 2 and 3; medial = 4 and 5; anteromedial = 6 and 7; anterior = 8 and 9; anterolateral = 10 and 11; lateral = 12 and 13; and posterolateral = 14 and 15. The analyses of sector and ring differences are summarized in Tables 2 and 3. * Indicates a sector with significant differences between the periosteal and endosteal rings. ** Indicates a sector with significant differences between the mid-cortex and endosteal rings.

among females. Among males, the proportion of transverse collagen fibers decreased between the younger and middle groups, and then increased between the middle and older groups. However, none of these age differences were significant when the sectors were considered individually. When only the endosteal ring was considered, younger females were found to have significantly more transverse collagen fibers than older females with all sectors considered together. In males, both the younger and older groups were found to have significantly more transverse collagen fibers than the middle group. Neither sex showed any significant differences when each sector was considered separately.

DISCUSSION

This analysis of a relatively large study sample, representing a broad age range in both sexes, allows variability in collagen fiber patterning across entire femoral shaft cross-sections to be examined more extensively than ever before. Previous studies of whole cross-sections were limited to very small sample sizes of less than four individuals each (Vincentelli and Evans, 1971; Portigliatti-Barbos et al., 1984; Carando et al., 1989). The only other quantitative study in which human collagen fiber orientation was investigated in a large sample (>50 human tibiae) was conducted by Vincentelli (1978). That study was limited to newly formed osteonal bone from sample regions of the cortex, in contrast to the whole-bone cross-sectional patterning of collagen fiber orientation examined here.

This study illustrates that a *single* pattern of collagen fiber orientation patterning, as suggested by Portigliatti-Barbos et al. (1983,1984), is not typical in the human mid-shaft femur. Although examples of the pattern de-

TABLE 2. Differences in brightness index between sectors around the cortex

	Age 25–44 (Sexes pooled)		Age 45–64 (Females)		Age 45–64 (Males)		Age 65+ (Males)[c]	
Sector	Mean B.I.[a]	Sectors significantly different[b,d]	Mean B.I.	Sectors significantly different[b,d]	Mean B.I.	Sectors significantly different[b,d]	Mean B.I.	Sectors significantly different[b,d]
Periosteal								
1	75.0	2,3,4,5,6 and 13,14	73.1	–	68.2	3,4,5	81.1	3,4
2	96.1	16	90.4	–	85.4	8,9	95.5	–
3	105.2	1,8,9,10,11,15,16	97.6	15,16	92.9	1,8,9,10,11,16	98.5	8,9,16
4	106.4	1,8,9,10,11,12,15,16	98.1	15,16	91.7	1,8,9,10,11,16	99.2	8,9,16
5	103.2	1,8,9,10,11,15,16	94.8	–	86.7	1,8,9,10,16	95.1	–
6	96.9	16	91.0	–	81.4	9	91.3	–
7	90.7	–	86.3	–	76.4	–	86.7	–
8	84.7	3,4,5	85.4	–	65.9	2,3,4,5	81.3	3,4
9	80.5	3,4,5	81.7	–	63.5	2,3,4,5,6	80.4	3,4
10	80.2	3,4,5	78.4	–	69.2	3,4,5	82.3	–
11	85.2	3,4,5	78.8	–	73.3	4,5	84.9	–
12	88.5	4	79.4	–	77.1	–	87.2	–
13	94.1	1,16	80.9	–	78.9	–	90.0	–
14	92.9	1,16	84.8	–	78.6	–	90.5	–
15	85.1	3,4,5	80.3	3,4	70.8	–	88.7	–
16	71.0	2,3,4,5,6,13,14	69.4	3,4	64.7	2,3,4,5	78.6	3,4
Mid-cortex								
1	79.8	3,4,5	84.4	–	77.5	–	86.7	–
2	92.2	–	92.6	–	87.6	8,9	92.9	–
3	96.3	1,8,9,16	99.3	–	92.8	8,9,10	97.2	–
4	97.1	1,8,9,16	101.8	–	93.5	8,9,10	100.2	–
5	96.4	1,8,9,16	98.8	–	89.8	8,9	101.0	–
6	92.2	–	94.5	–	85.4	9	96.0	–
7	86.8	–	88.3	–	81.1	–	90.1	–
8	79.3	3,4,5	83.1	–	69.3	2,3,4,5	83.4	–
9	79.3	3,4,5	82.5	–	68.1	2,3,4,5,6	84.3	–
10	83.3	3,4,5	82.9	–	74.5	3,4	89.1	–
11	88.8	–	83.9	–	81.1	–	91.1	–
12	92.5	–	84.9	–	85.4	–	94.9	–
13	93.9	–	86.0	–	86.7	–	95.9	–
14	93.8	–	87.2	–	85.5	–	97.2	–
15	87.8	–	83.4	–	80.0	–	91.6	–
16	78.4	3,4,5	82.5	–	77.1	–	84.2	–
Endosteal								
1	89.1	4,5	91.5	–	86.4	–	89.2	–
2	96.8	–	99.2	–	88.4	–	93.1	–
3	102.7	9	110.8	8,9,10,16	92.8	–	99.6	–
4	107.0	1,8,9,10,16	118.1	7,8,9,10,11,12,16	96.0	–	102.0	–
5	110.1	1,7,8,9,10,15,16	116.0	7,8,9,10,11,16	98.3	8,9	102.7	8
6	102.1	9	103.7	–	91.4	–	97.8	–
7	91.5	–	86.9	4,5	83.5	–	91.3	–
8	88.6	–	83.1	3,4,5	79.4	5	83.8	5
9	85.3	3,4,5,6,12,13	82.2	3,4,5	77.9	5	86.0	–
10	89.9	4,5	80.4	3,4,5	82.4	–	91.1	–
11	96.9	–	88.2	4,5,	87.7	–	95.8	–
12	102.6	9	90.6	4	92.3	–	98.8	–
13	102.8	9	94.2	–	92.4	–	96.2	–
14	98.5	–	94.5	–	94.4	–	98.2	–
15	92.0	5	93.4	–	88.0	–	93.8	–
16	88.7	4,5	83.5	3,4,5	85.9	–	89.5	–

[a]B.I., Brightness Index; see Methods for definition of measurement.
[b]Posterior, 1,16; postero-medial, 2,3; medial, 4,5; antero-medial, 6,7; anterior, 8,9; antero-lateral, 10,11; lateral, 12,13; postero-lateral, 14,15; see Figure 2.
[c]Old age (65+) females not shown because no results were significant.
[d]Results considered significant at $P < 0.05$.
–, no significant differences.

scribed by Portigliatti-Barbos et al. (1983,1984) in 43- and 46-year-old males can be found in the present study sample, particularly among male individuals between the ages of 35 and 64, this pattern is not typical of all individuals in this age range, or of individuals in the other age ranges investigated. Rather, it appears that although some consistencies in pattern can be found within and between age and sex groups, individual variation (unexplainable by

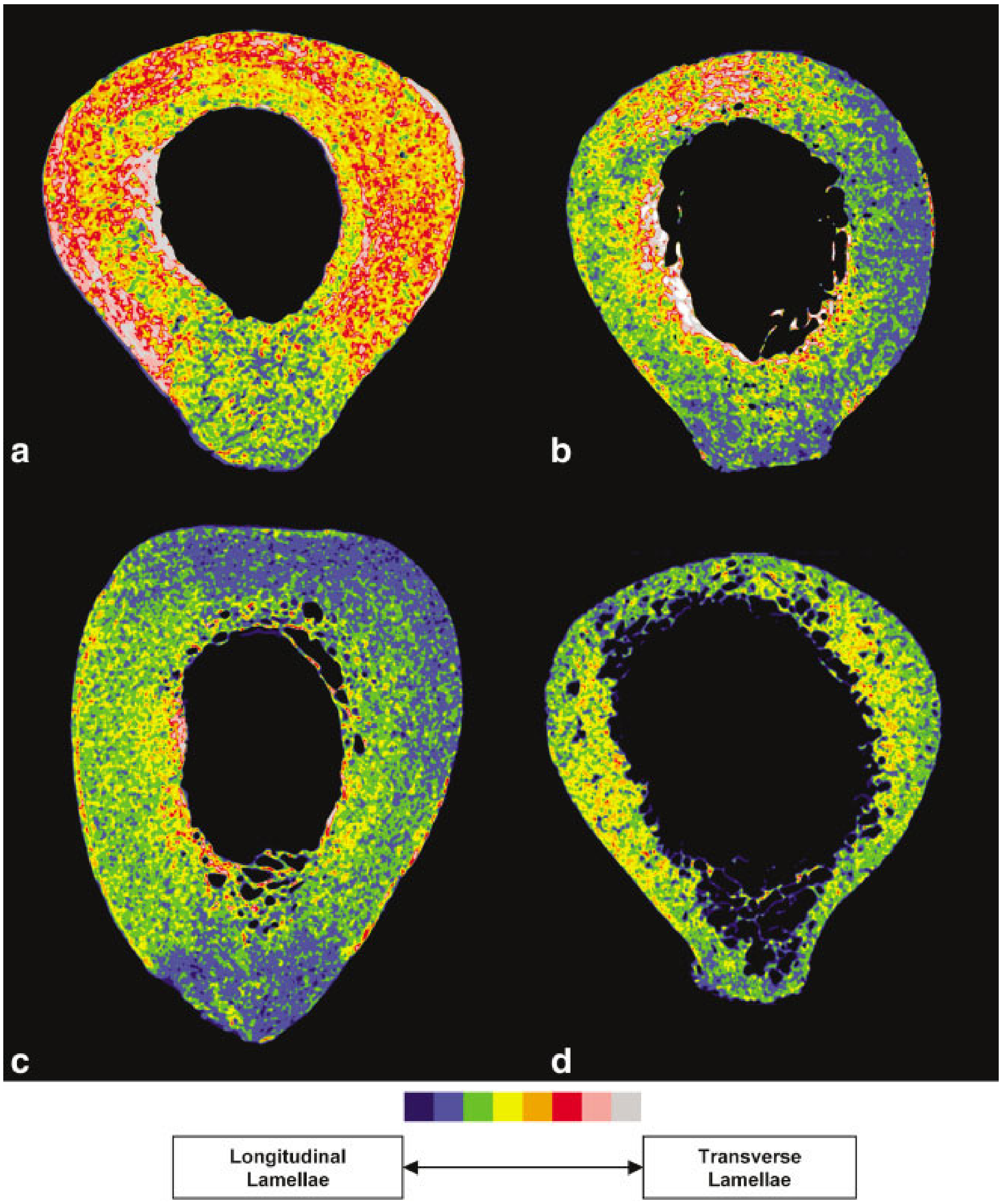

Fig. 5. Examples of color LUTs of whole cross-sections. **a:** Top left, a 28-year-old female individual demonstrating a high proportion of transverse collagen fibers, predominantly located within circumferential lamellar bone. **b:** Top right, a 51-year-old female individual demonstrating a high proportion of transverse collagen fibers in circumferential lamellar bone of the anterior cortex. **c:** Bottom right, a 27-year-old male demonstrating a pattern of collagen fiber orientation similar to that observed by Portigliatti-Barbos et al. (1983,1984). **d:** Bottom left, an 88-year-old female demonstrating little patterning of collagen fiber orientation around the cortex.

age, sex, or body size) tends to overwhelm any trend at a population sample level.

The results of this study demonstrate that age and sex are significant contributors to the variability identified in this sample. These results indicate a complex pattern of variability between groups. For instance, while previous research demonstrated an increase in transverse collagen fibers with age (Amprino and Bairati, 1936; Smith, 1960; Vincentelli and Evans, 1971; Vincentelli, 1978), the current study shows that the proportion of transverse collagen fibers actually decreased between the young and middle groups, following a later increase between the middle and older groups. In addition, there were sex differences in this trend. For instance, females in the middle group tended to have higher proportions of transverse collagen fibers than males, particularly in the anterior aspect of the cortex.

Another interesting age-related trend identified in this study was that the frequency of significant differences in

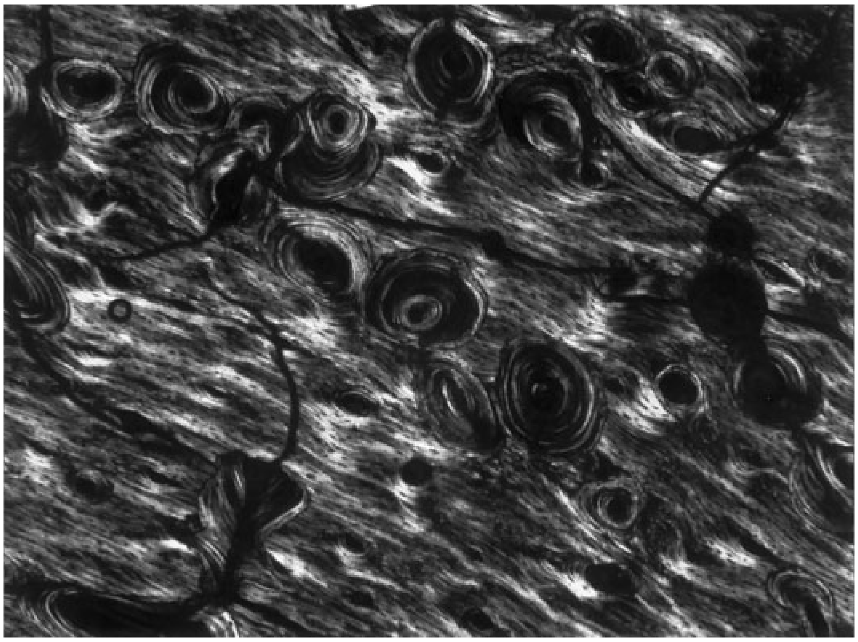

Fig. 6. High-magnification view (field width = 2.2 mm) of the anterolateral cortex of the specimen shown in Figure 5a. Note the difference in predominant orientation of the circumferential lamellar bone (more transverse lamellae) relative to the osteonal bone (more longitudinal lamellae).

TABLE 3. Differences in brightness index between age groups by ring

	Younger	Middle	Older
Ring	Mean B.I.[a]	Mean B.I.	Mean B.I.
Females			
Periosteal	89.9[b]	84.4[b]	87.2
Mid-cortex	89.4	88.5	90.7
Endosteal	98.6[c]	94.8	91.9[c]
Males			
Periosteal	89.5[b]	76.5[b,d]	89.0[d]
Mid-cortex	87.7[b,c]	82.2[b,d]	93.5[c,d]
Endosteal	94.2[b]	88.6[b,d]	96.4[d]

[a]B.I., Brightness Index; see Methods for definition of measurement.
[b]Significant difference between younger and middle age groups at $P < 0.05$.
[c]Significant difference between younger and older age groups at $P < 0.05$.
[d]Significant difference between middle and older age groups at $P < 0.05$.

collagen fiber orientation around the cortex (i.e., between sectors and rings) decreased with age; hence the older group in this study showed little patterning in preferred collagen fiber orientations, and very high intragroup variability. Moreover, older individuals tended to show lower ranges of brightness indices within each section, indicating an overall increase in cross-sectional homogeneity. This increasing intragroup variability and intraspecimen homogeneity must be examined further relative to mechanical factors, such as reduced muscle strength and changes in gait patterns with age (Craik, 1989; Sinclair and Dangerfield, 1998).

Despite the significant differences identified between age and sex groups in this sample, an extensive variation within groups, which was not attributable to body size (height and weight), was also identified in this study. With the little information we had concerning the lifestyles and health of the individuals in this sample, it was impossible to test the influence of such factors on this variability. However, these factors need to be considered in future studies.

Although the research design of this study did not permit the assignment of preferential collagen fiber orientations to different tissue types (for example, circumferential lamellar or secondary osteonal), visual examination of these specimens demonstrated extensive variability in the amount and location of different tissue types between individuals of the same age and sex groups. Interestingly, the relatively high proportion of transverse collagen fibers in the younger individuals appeared to reside predominantly within primary circumferential bone tissue. It was not possible to determine from this analysis whether the later increase in transverse collagen fibers identified between the middle and older groups was due to the introduction of secondary osteons with a preferential transverse orientation, or was a result of a preferential maintenance of interstitial bone of transverse orientation.

Differences in the distribution of preferred collagen fiber orientations between tissue types have been observed in previous studies. In a study of the macaque mandible, Bromage and Boyde (1998; unpublished data) found divergent collagen fiber orientations between tissues of endosteal vs. periosteal origin, as well as between bone deposited during the growth process vs. intracortically remodeled bone. They suggested that species- and bone-specific developmentally constrained construction rules, unrelated to mechanically induced strains during life, would govern collagen orientation during growth, while intracortical remodeling would result in preferred collagen fiber orientations in response to functional strains. Riggs et al. (1993a) reported similar results in the juvenile horse radius. If various bone tissue types influence global patterning of collagen fiber orientation differently, the ability to distinguish them in future studies would help elucidate the relationship between collagen fiber orientation and the mechanical properties of bone, and may explain some of the variability in collagen fiber patterning identified in this study. Data necessary for quantifying tissue-type variability can readily be extracted from images obtained in the present study, which will allow these issues to be addressed in further studies.

In explaining the variability identified in the current study, a methodological component of this study must also be critically considered. The rings and sectors used to divide the cortex were automatically generated by the Optimas macro, and therefore the size of each segment depended on the size and shape of individual cross-sections. Irrespective of whether an individual had a thick, robust cortex or one that had thinned with age, the cross-section was still represented by three computationally determined circumferential rings. The bone sampled within these rings may not provide homologous tissue portions of bone (i.e., of periosteal or endosteal origin). Thus, the influence of bone loss or gain at either surface cannot be accounted for. This may be an important consideration, because studies have indicated that older individuals experience bone loss at the endosteal surface (Ruff and Hayes, 1982; Stein et al., 1998), thereby reducing the equivalence of bone tissues at the endosteal margin between individuals of different ages and sexes. It is hoped that the present method can be refined in such a way as to address some of these issues in the future.

To better understand the variability identified in this study, it is important to examine it with respect to lifestyle and the mechanical "environment." The current sample represents a modern urban population, which likely included individuals with highly variable activity levels and lifestyles. The average mechanical loads on the femur in this population may be relatively low and have less consistency than those in rural individuals who have experienced relatively higher workloads. Studies of prehistoric hunter-gatherer populations relative to modern urban samples demonstrate that the latter experience less regular loading and lower overall activity levels, which may result in a more circular cross-sectional shape, and reduced cortical thickness and second moments of area (Ruff and Hayes, 1983a,b; Ruff et al., 1984; Brock and Ruff, 1988). The extent to which variability in collagen fiber orientation pattern may relate to lack of mechanical regularity should be examined by comparing this population sample to one in which activity patterns are more regular and better documented.

Finally, the significance of collagen fiber orientation in the human mid-shaft femur must be evaluated relative to the expected mechanical forces in the mid-shaft femur. Most previous studies have suggested that bending is an important force that acts on the mid-shaft femur (Blaimont, 1968; Amtmann, 1971; Pauwels, 1980). If, as Pauwels (1980) suggested, bending incurred by standing (in a one-legged stance) is unidirectional and of predominant importance to the morphology of the mid-shaft, then tensile forces would be expected to predominate anteriorly and laterally, and compressive forces would be expected posteriorly and posteromedially. Moreover, bone located farthest from the neutral axis would be most important for resisting the forces induced by stance and gait.

To some extent, the minimal consistencies in the collagen fiber orientation pattern identified in this study reflect this model of bending in the femur. In the current sample, the anterior periosteal cortex did tend to contain more longitudinal fibers than either the medial or lateral cortices. The anterolateral periosteal cortex was more variable in degree of brightness, but in the younger group and in males of the middle group, it contained significantly more longitudinal collagen fibers than the opposing posteromedial (and medial) aspect. In this respect, the posteromedial aspect would be optimized to resist compression, and the anterior and anterolateral cortices would be optimized to resist tension. The posterior mid-shaft femur morphology is dominated by the presence of the linea aspera, which as a muscle attachment site would induce tensile forces and preferential longitudinal collagen fiber orientation. Indeed, sectors in the linea aspera contained predominantly longitudinal collagen fibers. Moreover, the most significant differences between sectors (in all age and sex groups) were found within the periosteal third of the bone cortex, which suggests that remodeling events farthest from the neutral axis are more likely to incorporate collagen fibers of a preferred orientation based on the loading induced on that cortex. However, perhaps even more interesting than these trends, which are identifiable at the population level, and consistent with the results of Portigliatti-Barbos et al. (1983,1984), is the fact that few individuals actually reflect this pattern.

The difficulty of providing a mechanical explanation for the results of this study may relate to the current debate concerning the importance of bending forces in the mid-shaft femur (Taylor et al., 1996; Aamodt et al., 1997; Duda et al., 1998). Muscle activity (particularly by action of the ilio-tibial tract) can serve to counteract bending stresses at the mid-shaft (Rybicki et al., 1972; Pauwels, 1980), and thus reduce actual bending loads. Moreover, the direction and magnitude of bending can change during different phases of locomotion (Duda et al., 1997). The importance and regularity of bending at the mid-shaft may also change with age. Decreases in muscle strength with age (Burr, 1997; Frost, 1997) may reduce the ability of muscle to counteract bending forces. Age changes in posture and gait (Craik, 1989; Sinclair and Dangerfield, 1998) may result in decreased regularity of the bending direction. The degree to which the variability within and between age groups in this sample may be a reflection of the complex mechanical environment of the mid-shaft femur, and its changes with age, needs to be addressed in future studies. In particular, if bending itself is indeed less significant at the mid-shaft than previously thought, perhaps these data on collagen fiber orientation should be reconsidered in the light of the more complex mechanical environment. Further development of better biomechanical models of the femoral shaft, and a better understanding of the linkages between microstructure and macrostructure will help elucidate these problems. The sample used in this study can be used in future investigations to address many of these questions. The analysis presented here is part of a larger, ongoing study of microstructural, cross-sectional geometric and biomechanical analyses of this population.

By examining broad patterns of variability, we have demonstrated that a single pattern of collagen fiber orientation does not exist in the mid-shaft femur. While some consistencies within and between age groups may be relevant to the particular influence of bipedal locomotion and the forces it induces at the femoral mid-shaft, the overwhelming variation detected in this study suggests that this aspect of the microstructure is very sensitive to individual adaptation. Despite significant age and sex differences, sample variability clearly prohibits the use of this variable as a predictor of age or sex. The organization of collagen fibers within the cortex reflects a myriad of factors that affect the microstructure of bone at the individual level, e.g., lifestyle, diet, metabolic and disease states, and growth trajectories. These factors were not controlled for in this sample, and therefore their influence could not be tested. Nevertheless, the present findings will be of significance in future studies using collagen fiber orientation and other microstructural parameters to examine human bone microstructural adaptation. Ongoing studies that are using this same well-documented autopsy sample are providing much-needed data concerning variability in microstructural parameters (such as mineralization density and tissue type distribution), and aspects of cross-sectional geometry. These continuing research efforts will enable us to better understand and explain the variability in bone structure that characterizes modern humans throughout the aging process.

ACKNOWLEDGMENTS

The authors thank the mortuary staff at the Victorian Institute of Forensic Medicine for their efforts in collecting the material used in this study. We also acknowledge Dr. Rita Bruns and Ms. Sherie Blackwell, University of Melbourne, for their support and contributions to specimen preparation, and Mr. Aron Blayvas, Hunter College, for his participation in certain aspects of programming. This project built directly upon the ground-breaking work of Dr. Alan Boyde, and benefited from his advice and mentoring. The manuscript was greatly improved by the extensive advice and commentary provided by Dr. Mitch Schaffler during the completion of the dissertation project (by H.M.G.) from which this work stems.

LITERATURE CITED

Aamodt A, Lund-Larsen J, Eine J, Andersen E, Benum P, Husby OS. 1997. In vivo measurements show tensile axial strain in the proximal lateral aspect of the human femur. J Orthop Res 15:927–31.

Amprino R, Bairati A. 1936. Processi di ricostruzione e di riassorbimento nella sostanza compatta delle ossa dellúomo. Z Zellforsch mikrosk 24:439–511.

Amtmann VE. 1971. Mechanical stress, functional adaptation and the variation structure of the human femur diaphysis. Erg der Anat und Ent 44:1–87.

Ascenzi A, Bonucci E. 1964. The ultimate tensile strength of single osteons. Acta Anat 58:160–183.

Ascenzi A, Bonucci E. 1967. The tensile properties of single osteons. Anat Rec 158:375–386.

Ascenzi A, Bonucci E. 1968a. The compressive properties of single osteons. Anat Rec 161:377–392.

Ascenzi A, Bonucci E. 1968b. The shearing properties of single osteons. Anat Rec 172:499–510.

Bertelsen PK, Clement JG, Thomas CDL. 1995. A morphometric study of the cortex of the human femur from early childhood to advanced old age. Forensic Sci Int 74:63–77.

Blaimont P. 1968. Contribution á l'étude biomécanique du fémur humain. Acta Orthop Belg 34:665–844.

Boyde A, Riggs CM. 1990. The quantitative study of the orientation of collagen in compact bone slices. Bone 11:35–39.

Brock SL, Ruff CB. 1988. Diachronic patterns of change in structural properties of the femur in the prehistoric American Southwest. Am J Phys Anthropol 75:113–127.

Bromage TG. 1992. Microstructural organization and biomechancs of the macaque circumorbital region. In: Smith P, Tchernov P, editors. Structure, function and evolution of teeth. London: Freund. p 257–272.

Bromage TG, Boyde A. 1998. Intracortical remodeling and growth of the macaque mandible. Scanning 20:240.

Burr DB. 1997. Muscle strength, bone mass, and age-related bone loss. J Bone Miner Res 12:1547–1551.

Carando S, Portigliatti-Barbos M, Ascenzi A, Boyde A. 1989. Orientation of collagen in human tibial and fibular shaft and possible correlation with mechanical properties. Bone 10:139–142.

Craik R. 1997. Changes in locomotion in the aging adult. In: Woollacott M, Schumway-Cook A, editors. Development of posture and gait across the lifespan. Columbia, SC: Univeristy of South Carolina Press. p 176–201.

Duda GN, Schneider E, Chao YS. 1997. Internal forces and moments in the femur. J Biomech 30:933–941.

Duda GN, Heller M, Albinger J, Schulz O, Schneider E, Claes L. 1998. Influence of muscle forces on femoral strain distribution. J Biomech 31:841–846.

Evans FG, Vincentelli R. 1969. Relation of collagen fiber orientation to some mechanical properties of human cortical bone. J Biomech 2:63–71.

Feik SA, Thomas CDL, Clement JG. 1996. Age trends in remodeling of the femoral midshaft differ between the sexes. J Orthop Res 14:590–597.

Feik SA, Thomas CD, Bruns R, Clement JG. 2000. Regional variations in cortical modeling in the femoral mid-shaft: sex and age differences. Am J Phys Anthropol 112:191–205.

Frost HM. 1997. On our age-related bone loss: insights from a new paradigm. J Bone Min Res 12:1539–1546.

Gebhardt W. 1905. Über funktionell wichtige Anordnungsweisen der feineren und gröberen Bauelemente des Wirbeltierknochens. Arch Entwickl Org 20:187–322.

Goldman HM, Kindsvater J, Bromage TG. 1999. Correlative light and backscattered electron microscopy of bone. I. Specimen preparation methods. Scanning 21:40–43.

Goldman HM. 2001. Histocomposition and geometry at the human mid-shaft femur. Ph.D. dissertation, City University of New York, New York, NY.

Martin RB, Ishida J. 1989. The relative effects of collagen fiber orientation, porosity, density, and mineralization on bone strength. J Biomech 22:419–426.

Martin RB, Boardman DL. 1993. The effects of collagen fiber orientation, porosity, density, and mineralization on bovine cortical bone bending properties. J Biomech 26:1047–1054.

Mason MW, Skedros JG, Bloebaum RD. 1995. Evidence of strain-mode-related cortical adaptation in the diaphysis of the horse radius. Bone 17:229–237.

Pauwels F. 1980. Biomechanics of the locomotor apparatus: contributions on the functional anatomy of the locomotor apparatus. Berlin: Springer Verlag. 518 p.

Pidaparti RM, Burr DB. 1992. Collagen fiber orientation and geometry effects on the mechanical properties of secondary osteons. J Biomech 25:869–880.

Portigliatti-Barbos M, Bianco P, Ascenzi A. 1983. Distribution of osteonic and interstitial components in the human femoral shaft with reference to structure, calcification and mechanical properties. Acta Anat 115:178–186.

Portigliatti-Barbos M, Bianco P, Ascenzi A, Boyde A. 1984. Collagen orientation in compact bone. II. Distribution of lamellae in the whole of the human femoral shaft with reference to its mechanical properties. Metab Bone Dis Relat Res 5:309–315.

Riggs C, Vaughan L, Evans G, Lanyon L, Boyde A. 1993a. Mechanical implications of collagen fibre orientation in cortical bone of the equine radius. Anat Embryol 187:239–248.

Riggs CM, Lanyon LE, Boyde A. 1993b. Functional associations between collagen fibre orientation and locomotor strain direction in cortical bone of the equine radius. Anat Embryol 187:231–238.

Ruff CB, Hayes WC. 1982. Subperiosteal expansion and cortical remodeling of the human femur and tibia with aging. Science 217: 945–948.

Ruff CB, Hayes WC. 1983a. Cross sectional geometry of Pecos Pueblo femora and tibiae—a biomechanical investigaiton: II. Sex, age, and side differences. Am J Phys Anthropol 60:383–400.

Ruff CB, Hayes WC. 1983b. Cross sectional geometry of Pecos Pueblo femora and tibiae—a biomechanical investigation: I. Method and general patterns of variation. Am J Phys Anthropol 60:359–381.

Ruff CB, Larsen CS, Hayes WC. 1984. Structural changes in the femur with the transition to agriculture on the Georgia Coast. Am J Phys Anthropol 64:126–136.

Rybicki EF, Simonen FA, Weis EBJ. 1972. On the mathematical analysis of stress in the human femur. J Biomech 5:203–215.

Sinclair D, Dangerfield P. 1998. Human growth after birth. Oxford: Oxford University Press. 251 p.

Skedros JG, Su SC, Bloebaum RD. 1997. Biomechanical implications of mineral content and microsructural variations in cortical bone of horse, elk, and sheep calcanei. Anat Rec 249:297–316.

Smith JW. 1960. The arrangement of collagen fibres in human secondary osteones. J Bone Joint Surg 42:588–605.

Stein MS, Thomas CDL, Feik SA, Wark JD, Clement JG. 1998. Bone size and mechanics at the femoral diaphysis across age and sex. J Biomech 31:1101–1110.

Taylor ME, Tanner KE, Freeman MA, Yettram AL. 1996. Stress and strain distribution within the intact femur: compression or bending? Med Eng Phys 18:122–131.

Vincentelli R, Evans FG. 1971. Relations among mechanical properties, collagen fibers, and calcification in adult human cortical bone. J Biomech 4:193–201.

Vincentelli R. 1978. Relation between collagen fiber orientation and age of osteon formation in human tibial compact bone. Acta Anat 100:120–128.