Intrapopulation variability in mineralization density at the human femoral mid-shaft

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Abstract

One of several microstructural variables known to affect the mechanical properties of bone is the degree of mineralization of bone matrix. The aim of this study was to examine mineralization density, and its variability with age and sex, from a biomechanical perspective. Histological sections, prepared from mid-shaft femora obtained at autopsy from 40 individuals, were imaged using quantitative backscattered electron microscopy. Each cross-section montage was divided into 48 segments according to anatomical position. Mean grey-level values were quantified for each segment. One-way ANOVA with Tukey HSD *post hoc* tests were used to test for differences in mineralization between segments, age groups and sexes. Results showed a decrease in overall degree of mineralization density with adult age, but an increase in its coefficient of variation. Degree of mineralization was significantly lower in the periosteal third of the cortex, particularly in the antero-lateral aspect. This pattern was most prevalent amongst the youngest individuals in the sample. Whereas males between ages 45–64 years had a higher average degree of mineralization than females, the opposite was true of the older age group. Mineralization significantly decreased between middle and older age groups in males, but not in females. Despite limited consistencies in the location of high and low average mineralization bone through the cortex, the degree of interindividual variation, even within a single age and sex group, overwhelmed population level trends. The patterns of variability identified in this study are consistent with results of an analysis of collagen fibre orientation using the same sample material.

Key words mineralization density; human femur; human variation; bone microstructure.

Introduction

The concentration of bone mineral in bone matrix is one of several properties to affect the mechanical efficacy of bone structure. Through the processes of modelling and remodelling, packets of bone (e.g. osteons) are constantly being laid down and turned over. As newly formed bone accumulates mineral with time, the most recently deposited bone will generally be less mineralized than bone deposited months or years earlier. Regions with high turnover, containing many newly formed osteons, will generally display lower average mineralization than areas of low turnover. This variability in the degree of mineralization may be important to our understanding of the biomechanical adaptation of bone and its age related changes.

Small increases in mineralization lead to relatively large increases in strength of the bone organ (Vose & Kubala, 1959), although at some point (roughly at 66% mineralization) increases in mineral will lead to brittleness and a reduction in strength (Currey, 1969; Bonfield & Clark, 1973). Despite this established relationship, studies that correlate the average degree of mineralization of a bone cortex with the predominant mechanical loading of that cortex have shown mixed results. Portigliatti-Barbos et al. (1983) found, in human femora, that regions of the bone predominantly loaded in tension demonstrated lower mineralization density due to a higher number of forming osteons. Riggs et al. (1993), by contrast, found that in the horse radius, the

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cortex experiencing predominantly higher tensile strains had a higher mineralization density and a lower remodelling rate than the cortex with predominantly compressive strains. The increased remodelling rate in the compressive cortex was associated with the development of a less longitudinal orientation of collagen fibres in the secondary osteons. These results were corroborated by Mason et al. (1995) for the horse radius and by Skedros et al. (1996) for the horse metacarpal. However, Skedros et al. (1997) found that in the calcaneus of horse, elk and sheep, cortices predominantly loaded in tension had a significantly lower mineral content and higher remodelling rate than those in compression, similar to the results of Portigliatti-Barbos et al. (1983) for human femora. Taken together, these studies may indicate that the relationship between mineralization density and loading may be complicated by particular growth and development differences between species and skeletal elements, as well as by the complex interaction of different aspects of the histological structure, such as tissue type.

Few researchers have studied age and sex variability in mineralization density. Most studies support an increased average mineralization density of the bone matrix with age, at least until the fourth decade in humans (Amprino & Engstrom, 1952; Dickenson et al. 1981; Reid & Boyde, 1987; Boyde et al. 1993; Grynpas, 1993) reflecting decreasing remodelling rates following the completion of growth. As remodelling continues through the lifespan, studies demonstrate increasing proportions of bone with low mineralization in older adults (Reid & Boyde, 1987; Simmons et al. 1991). Although age reduction in mineralization appears to occur earlier in females relative to males (Baud & Gossi, 1980; as cited in Meunier & Boivin, 1997), only limited research has been completed on mineralization differences within older age groups (Boyde et al. 1995b).

The primary objectives of the current study were (1) to determine whether mineralization density may show patterned variation around the cortex of the human femur, reflecting localized differences in remodelling rate as suggested by Portigliatti-Barbos et al. (1983) and (2) to characterize the variability in mineralization density patterning within a population sample of the adult age range. This study differs from most previous analyses of mineralization in that it focuses on regional variability in mineralization both within and between individuals by dividing the cortex into many segments and comparing mineralization between them. As this

regional patterning may reflect differences in localized remodelling rates, as well as processes of modelling and cortical drift, its detailed study might allow for improved understanding of age and sex trends in mineralization density.

Materials and methods

Specimen collection

Mid-shaft femur blocks of 40 individuals collected from the Victorian Institute of Forensic Medicine, Melbourne, Australia, were used in this analysis. This sample represents individuals of known age, sex, height, weight and cause of death. The individuals used in this study represent a subset of the same histological sections examined in Goldman et al. (2003) for variability in collagen fibre orientation. The sample was divided into three age groups, each containing equal numbers of males and females: younger (25–44 years, $n = 12$), middle (45–64 years, $n = 12$) and older (65+ years, $n = 16$), as described in Goldman et al. (2003). Further information on this sample can be found in Bertelsen et al. (1995), Feik et al. (1996, 2000) and Goldman (2001).

In 11 individuals (five individuals aged 25–40 years, six individuals aged 60+ years) the femur block was removed from the right side, 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 and thus specimens were oriented in the medio-lateral plane based on the disposition of microstructural anatomical features as described in Goldman (2001).

Sample preparation

Bone blocks approximately 0.5 cm thick were removed from the samples and stored in 70% ethanol, prior to cleaning and dehydration in preparation for embedding in a poly methyl methacrylate (PMMA) and styrene mixture according to procedures described in Goldman et al. (1999). PMMA-embedded blocks were sectioned using a method developed to allow 100-μm thin sections to be imaged by both light and scanning electron microscopy (Goldman et al. 1999). Specimens were polished using a 0.01-μm alumina slurry on a Texmet pad (Buehler Ltd, Lake Bluff, IL, USA) to minimize surface topography. After all light microscopy imaging procedures were completed (for collagen

fibre orientation studies; see Goldman et al. 2003), specimens were carbon coated using a flash-evaporation process (Emitech, Inc.), in order to make them electrically conductive for backscattered electron microscopy (BSE-SEM) analysis. Each specimen was coated using standard carbon string lengths, out-gassing currents and evaporation times.

Image acquisition

Information on mineralization densities was obtained by BSE-SEM imaging using a LEO S440 Scanning Electron Microscope (LEO, Cambridge, UK). Digital grey-level images (each 1024×768 pixels, field width 2.2 mm; images later reduced to 33% of original size for ease of processing) were collected at a 15-mm working distance, 20 kV accelerating voltage and approximately 750 pA beam current using an LaB_6 filament that was allowed to warm and stabilize for 1 h before imaging. Leo Stage Scan software was used to provide automated tiling of BSE-SEM images across the entire bone cross-section. Following the method of Boyde et al. (1995a), halogenated dimethacrylate standards were used to monitor the stability of the electron beam during the imaging run and to correct for any errors due to drift in the system that would increase grey-level variability between images (Boyde & Jones, 1996; Boyde et al. 1998). An image of the standards was collected approximately after every 15 images. To standardize resulting images, the mean of the histogram peak for each standard was determined. Each of the bone images was then normalized based on the values for the nearest standards image, such that a value of 0 was assigned to the mean of the monobromo standard and a value of 255 was assigned to that of the mono-iodo standard (Boyde & Jones, 1996; Boyde et al. 1998). This procedure was automated using an in-house program developed using Visual Basic 6.0. (Microsoft) and Leadtools Imaging 16/32 Active X vs. 10, LEAD technologies, Inc., Charlotte, NC, USA. After normalization, tiled images were automatically montaged using another in-house program (Fig. 1).

In order to provide a visual map of mineralization density across the entire bone cortex, a colour look-up table (LUT) was applied to the montaged image using methodology similar to that of Boyde et al. (1998, 1999). Grey levels from 1 to 255 in the image were divided into eight intervals (bins), each represented by a corresponding colour (see Fig. 4 for a colour map and examples).

Areas of grey level 0 (non-bone) were assigned to black.

Each grey-level montage was automatically divided into segments using an Optimas Image Analysis (Version 6.5, Media Cybernetics, Inc.) software macro described in Feik et al. (2000) and Goldman (2001) (Fig. 2). This macro first traced an outline of the endosteal and periosteal bone surfaces produced using a Fourier shape descriptor technique, and then divided the cortex into three proportional rings (periosteal, mid-cortex and endosteal) and 16 sectors. Within each segment, the number of pixels within each grey level (of a total of 255 grey levels) was calculated, as well as the mean (BSE) grey-level value of the segment relative to the total area of the segment (not taking into account grey levels of 0, which would indicate unmineralized tissue, marrow, blood vessel canals, osteocyte lacuna spaces and cracks). Higher mean grey-level values correspond to regions of more highly mineralized bone, whereas lower mean grey level values correspond to regions of less mineralized bone. Whilst these values can theoretically be calibrated to actual mineralization density (Howell et al. 1998), data are presented on a relative scale.

Data analysis

To examine patterns of mineralization within individuals, mean grey levels were plotted by sector for each of the three rings (periosteal, mid-cortex, endosteal). A four-way ANOVA (with age, sex, ring and sector as factors) was run on the pooled sample, and a series of one-way ANOVA's were carried out for specific groups to examine further the variability in mineralization within the sample according to location within the cortex, and age and sex. Tukey HSD *post hoc* tests were used to determine significance of pair-wise comparisons.

Results

Variability in whole cross-section mineralization density

Data were pooled to provide information on average mean grey levels across whole cross-sections, an indicator of overall degree of mineralization for each individual. Variability in mean grey levels, considering all sectors and rings of the cortex, was relatively low (mean ± SD grey level = 139.2 ± 7.9), indicative of a relatively
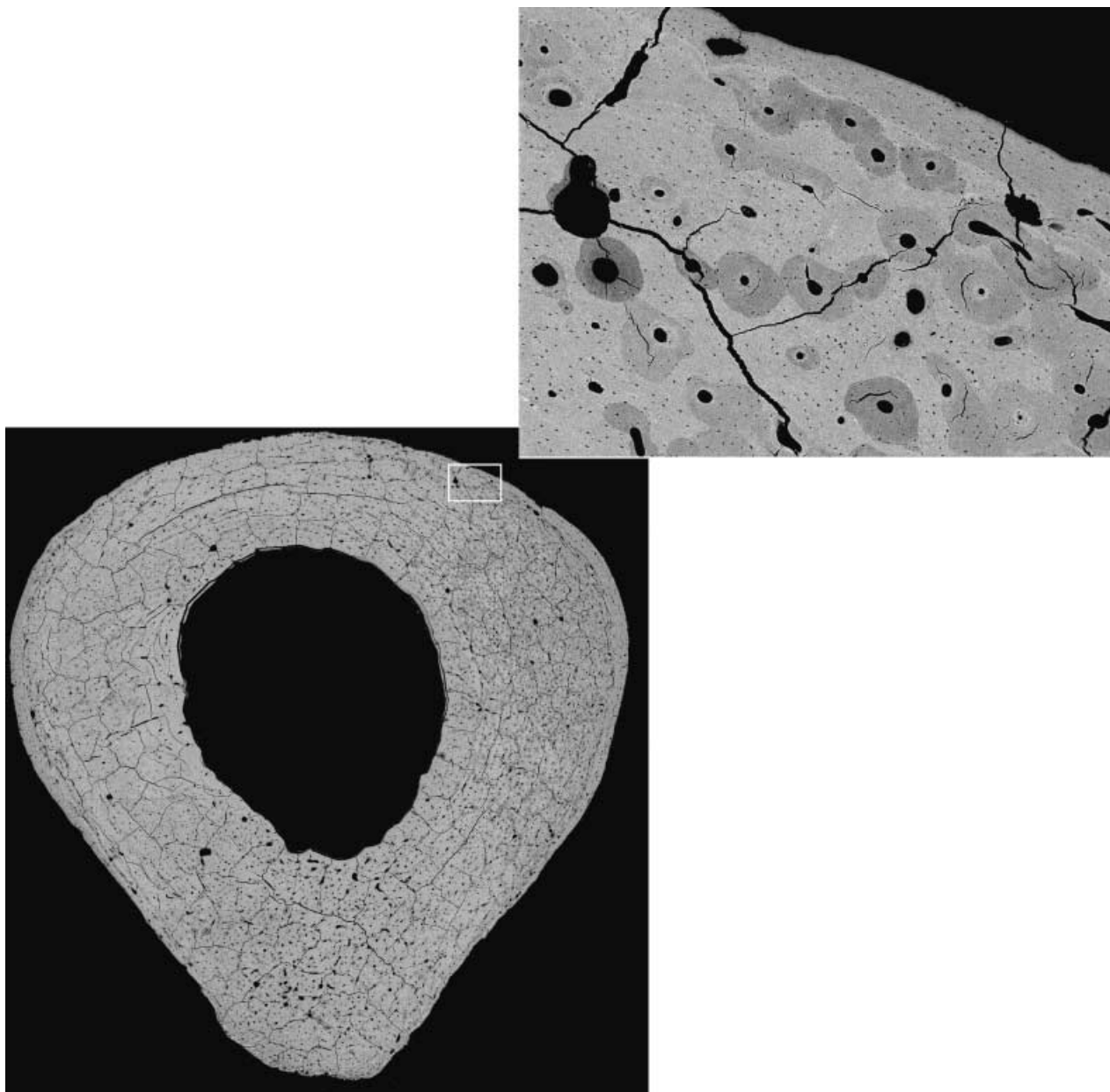
Fig. 1 Individual images obtained with BSE-SEM to determine mineralization density were each standardized and then montaged into a single image of the entire cross-section using an automated program. Cracks are artefacts of preparation and imaging techniques.

narrow range of mineralization characterizing the sample. Coefficient of variation (CV) data demonstrate significant increases in variability of mineralization density with age (Table 1). Significant decreases in mineralization density with age were found between each age group in males, and between young and middle age group females (Table 1). *t* tests demonstrated that females in the oldest age group had a significantly higher mean degree of mineralization than males (*P* < 0.001) of the same age group.

Factors relating to variation in mineralization density

A four-factor ANOVA with mean grey level as the independent variable indicated that age, ring and sector were all significant factors in the variability of degree of mineralization (*P* < 0.0001). Although sex itself was not a significant factor, an interaction between age and sex was shown (*P* < 0.0001). Height and weight were not significant factors in mineralization variability. To investigate these relationships further, the sample
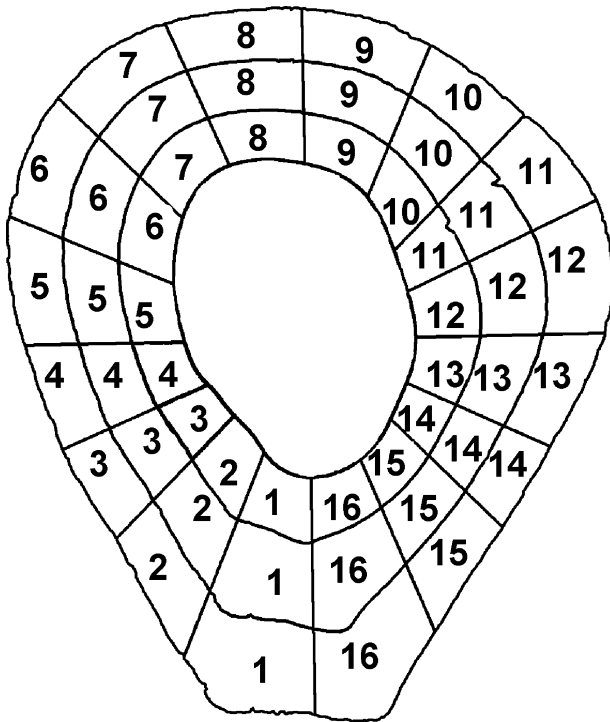
Fig. 2 Using a customized macro cortex was divided into 16 radial sectors and three rings (periosteal, mid cortex and endosteal), for a total of 48 segments. All sections were orientated so that medial is to the left and posterior is towards the bottom when viewed on screen or print.

Table 1 One-way ANOVA of mineralization and coefficient of variation by age

	Mean grey	CV
Females		
Younger	145.04*†	20.86*†
Middle	138.47*	22.38*‡
Older	138.11†	24.71†‡
Males		
Younger	145.11*†	20.27*†
Middle	139.88*‡	22.77*
Older	135.28†‡	22.94†

*Significant difference between younger and middle age groups at $P < 0.01$.
†Significant difference between younger and older age groups at $P < 0.01$.
‡Significant difference between middle and older age groups at $P < 0.01$.

was examined in age- and sex-matched groups for each ring and sector.

Regional variation in mineralization density

Younger age group (age 25–44 years)

Statistical analysis of data from this age group revealed no sex differences in the degree of mineralization, as evidenced by mean grey levels. Sexes were therefore pooled for further analysis. Within this age group, bone in the periosteal ring was significantly less mineralized than that of the mid-cortical or endosteal rings, primarily due to less well-mineralized bone along the antero-lateral aspect (see Table 2 and Fig. 3). This antero-lateral aspect was significantly less mineralized than the postero-medial aspect in both the periosteal and the mid-cortical rings (see Table 3).

Although not quantified, secondary osteons appeared to be more abundant in the antero-lateral aspect of the cortex of individuals in this age group, corresponding to the least mineralized areas quantified here. In other

aspects of the cortex, many individuals in the sample displayed high proportions of primary circumferential bone characterized by a high degree of mineralization (Fig. 4).

Middle age group (45–64 years)

With data from all sectors and rings combined, males in this age group were shown to have significantly ($P = 0.03$) higher mean grey levels (mean = 139.9) than females (mean = 138.5) and were significantly more variable. No significant sex differences in mean grey levels were identified for individual sectors or rings.

Within females, the periosteal ring was significantly less mineralized than the mid-cortical ring, and the mid-cortical ring was significantly less mineralized than the endosteal ring (Table 2). Medial and antero-lateral aspects of the cortex were predominantly responsible for these differences. In males, no significant differences were detected in the mineralization of each ring of bone. In addition, no significant differences were detected between individual sectors of each ring with sexes analysed separately or combined.

Interestingly, four individuals in this age group (three female, one male) showed a pattern of mineralization characterized by localized areas of retained primary (unremodelled) bone and increased mineralization (particularly anteriorly). This observation may be reflected in the data by the trend towards higher mean grey-level values in that cortex amongst females (Fig. 4).

Table 2 Differences in mean grey-level values between circumferential rings

	Sexes pooled				
	Mean grey level	Individual sectors§ showing significance at $P < 0.05$			
Young age (25–44 years) group					
Periosteal	142.79‡*	10,11			
Mid-cortex	146.56‡	10,11			
Endosteal	145.89*	10,11			

	Females		Males		
	Mean grey level	Individual sectors showing significance at $P < 0.05$	Mean grey level	Individual sectors showing significance at $P < 0.05$	Sex differences
Middle age (45–64 years) group					
Periosteal	137‡	5,6,11	138.27	n.s.	n.s.
Mid-cortex	140.19†‡	5,6,11 (‡only)	141.55	n.s.	n.s.
Endosteal	138.21†	n.s.	139.81	n.s.	n.s.
Old age (65+ years) group					
Periosteal	136.78‡	n.s.	133.82‡	n.s.	$P < 0.01$ (sectors pooled)
Mid-cortex	140.06†‡	n.s.	136.79‡	n.s.	$P < 0.01$ (sectors pooled)
Endosteal	137.48†	n.s.	135.3	n.s.	$P < 0.05$ (sectors pooled)

*Significant difference between endosteal and periosteal rings at $P < 0.05$.
†Significant difference between endosteal and mid-cortex rings at $P < 0.05$.
‡Significant difference between periosteal and mid-cortex rings at $P < 0.05$.
§Sectors defined as: 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 Fig. 2.
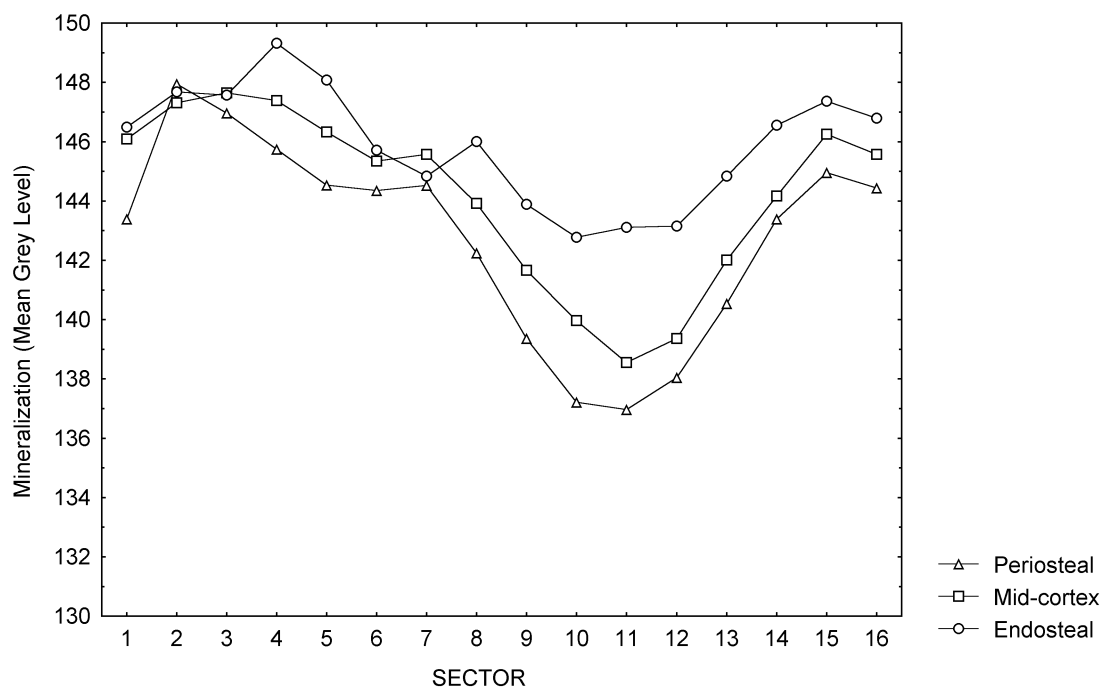

Fig. 3 Comparison between circumferential rings by sector, young age group, sexes pooled. Abbreviations for sectors: Posterior = sectors 1,16; Postero-medial = 2,3; Medial = 4,5; Antero-medial = 6,7; Anterior = 8,9; Antero-lateral = 10,11; Lateral = 12,13; Postero-lateral = 14,15.

Table 3 Differences in mean grey levels between sectors around the cortex

Periosteal			Mid-cortex			Endosteal		
Sector	Mean grey level	Sectors significantly different†	Sector	Mean grey level	Sectors significantly different†	Sector	Mean grey level	Sectors significantly different†
Age 25–44 years (sexes pooled)								
1	143.9		1	148.4		1	146.5	No
2	148.0	9,10,11,12	2	149.5	11	2	147.7	significant
3	147.0	10,11,12	3	149.9	11	3	147.6	differences
4	145.8	11	4	149.8	11	4	149.3	
5	144.5		5	148.5		5	148.1	
6	144.4		6	147.3		6	145.7	
7	144.5		7	147.6		7	144.8	
8	142.3		8	146.0		8	146.0	
9	139.4	2	9	144.1		9	143.9	
10	137.2	2,3	10	142.5		10	142.8	
11	137.0	2,3,4	11	141.3	2,3,4	11	143.1	
12	138.1	2,3	12	142.0		12	143.2	
13	140.6		13	144.7		13	144.8	
14	143.4		14	146.8		14	146.6	
15	145.0		15	148.8		15	147.4	
16	144.4		16	148.0		16	146.8	

*Sectors defined as: 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 Fig. 2.
†Results considered significant at $P < 0.05$.

Table 4 Differences in mean grey levels between age groups by ring

Ring	Younger	Middle	Older
Females			
Periosteal	142.50*†	137.00*	136.78†
Mid-cortex	146.59*†	140.19*	140.06†
Endosteal	146.05*†	138.21*	140.48†
Males			
Periosteal	143.04*†	138.27*‡	133.48†‡
Mid-cortex	146.54*†	141.55*‡	136.79†‡
Endosteal	145.76*†	139.81*‡	135.25†‡

*Significant difference between younger and middle age groups at $P < 0.01$.
†Significant difference between younger and older age groups at $P < 0.01$.
‡Significant difference between middle and older age groups at $P < 0.05$.

Older age group (65+ years)

In this age group, females showed significantly higher average mineralization than males in each of the three rings (Table 2). Although differences in mean grey level were identified between rings within both sexes of this age group, it was impossible to demonstrate statistically which aspects of the cortex contributed to these differences. Figure 4 shows examples of individuals from this age group.

Regional variation in degree of mineralization between age groups

In females, significant decreases in mineralization density were identified between young and middle-aged individuals. These were predominantly due to the postero-medial aspect of the periosteal ring (Sectors 2 and 3; see Table 4). In males, the older age group was significantly less mineralized than the younger age group in several sectors of the periosteal, mid and endosteal cortices, as shown in Table 4.

Discussion

The objectives of this study were to determine if mineralization density demonstrated patterned variation around the cortex of the human femur, and to characterize this variability in mineralization density patterning within an adult population sample. The BSE-SEM methodology afforded high resolution, so that even
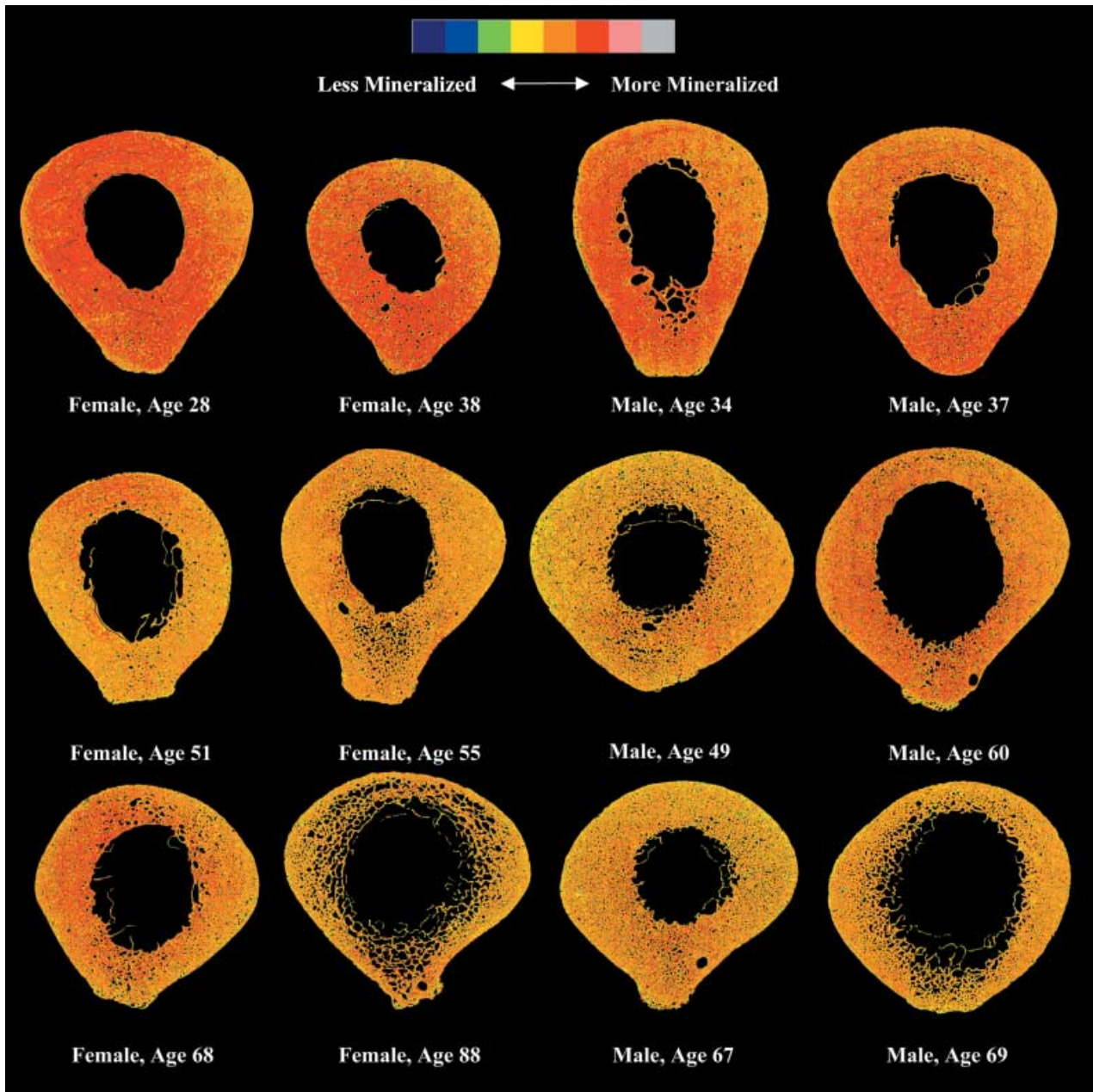
Fig. 4 Examples of montages from each age group, with colour look-up table applied. Top row = younger age, middle row = middle age, bottom row = older age.

small changes in mineralization density could be detected (Boyde & Jones, 1996). Significant age and sex differences were demonstrated, along with limited consistencies in the location of high and low average mineralization bone through the cortex. These were broadly similar to the observations of Portigliatti-Barbos et al. (1983) of mineralization patterning at the mid-shaft femur. However, the most interesting finding of this study may be the characterization of the degree of individual variation in mineralization density

that cannot be explained by age or sex. After discussing each of the contributing factors analysed in explaining the variation (age, sex and location in the cortex), the significance of the additional variation will be addressed.

Age

Age was a significant contributor to the variability identified in this sample. Mineralization density

decreased with adult age, most evident between males of all age groups and between young and middle-aged females. This finding is consistent with previous studies that focused on age trends in mineralization among adults (e.g. Reid & Boyde, 1987; Simmons et al. 1991), and probably reflects accumulations of increasing numbers of remodelling events. Qualitative examination of the images in this study suggests that the high degree of mineralization in the young age individuals relates to the retention of large amounts of primary circumferential bone in this group, particularly on the medial aspect of the bone cortex. As this primary bone eventually becomes remodelled with increasing age, mineralization decreases.

Not all aspects of the cortex demonstrated such age-associated decreases. For instance, whereas posterior and medial aspects decreased in mineralization with age, the antero-lateral aspect did not. These regional differences may be a reflection of localized differences in modelling and remodelling rates through the lifespan (see discussion of location in cortex, below).

Sex

Sex differences were identified in the sample in both middle and older age groups. On average, middle age group males showed a higher degree of mineralization than females, but only when all circumferential rings and sectors were pooled. Some regions of the cortex (i.e. anteriorly) of females of this age group tended to have higher mineralization density than males. In the oldest age group, females showed significantly higher mineralization than males within each of the circumferential rings. Moreover, mineralization significantly decreases between middle and older age groups in males, but not in females.

Few studies have investigated sex differences in mineralization density with age. Boyde et al. (1995b) identified higher mineralization values in the iliac crests of females relative to males, but this study did not employ external standards and the tissue studied was taken at autopsy immediately deep to the iliac crest, and therefore not comparable with all other 'histomorphometric' studies of iliac crest biopsies, which are taken 1–2 cm below the crest. The crestal zone contains much residual calcified fibrocartilage and Sharpey fibre bone. Baud & Gossi (1980; as cited in Meunier & Boivin, 1997) suggested that females show decreased mineralization density in the iliac crest at an earlier age than

males. Hobson (1998) demonstrated a small shift towards higher mineralization bone in the mandibles of males relative to females, whereas Kingsmill & Boyde (1998) found no gender differences in the mandible. It is difficult to compare these studies to one another, as they represent different skeletal elements, but Kingsmill & Boyde (1999) used the same methodology as the present study. They found that mineralization density in different skeletal elements within the same individual did not correlate with one another, suggesting that mineralization differences do not primarily reflect systemic factors. Rather, Kingsmill & Boyde (1998) found that site-specific loading experiences showed a strong relationship to mineralization variability. Examination of the localized sex differences in mineralization density identified in the present study may be examined in the future relative to lower limb functional loading differences between the sexes (e.g. Kerrigan et al. 2000).

We can also examine the results of the current study in light of their possible relationship to bone turnover differences between adult males and females, particularly at female peri- and post-menopausal ages. Some studies of histomorphometric indices have found lower bone turnover, especially lower bone formation, in adult females relative to adult males (Meunier et al. 1976; Dahl et al. 1988; Schnitzler et al. 1990). Schnitzler et al.'s (1990) study of iliac crest biopsies also demonstrated that males lacked erosion surface increases with age, suggesting a closer balance between resorption and formation. Studies of biochemical markers of bone turnover, specifically those of bone formation, are inconsistent. Some have reported higher levels of turnover in males relative to females (Duda et al. 1988; Vanderschueren et al. 1990; Resch et al. 1994; Henry & Eastell, 2000), whereas others report the opposite (Epstein et al. 1984). One study demonstrated higher levels of bone resorption markers in males relative to females, but the difference was not significant when body size was taken into account (Henry & Eastell, 2000).

Together, these studies tend to support an increase in bone turnover in males relative to females, suggesting more frequent and more efficient renewal of bone in males. Athough females may lose bone at an equal or higher rate than males, particularly along the endosteal surface of the bone, they do not replace it to the same extent as males. Consequently, females may accumulate areas of hypermineralized bone that escape the

remodelling process (Schnitzler, 1993). Unfortunately, the automated methods employed in this study (in which rings and sectors used to divide the cortex were generated by the Optimas macro) limited our ability to interpret equivalent areas of bone tissue at the endosteal surface, or to account for tissue lost from endosteal resorption (for further discussion of this issue, see Goldman et al. 2003). Nevertheless, the present results demonstrate higher mineralization density bone in older females relative to males and highlight a potentially important difference in bone ageing that is rarely addressed in the bone biology literature.

In order to study this further, it is worthwhile to consider variation in mineralization density relative to that of porosity, as the distribution of both relate to variations in remodelling rate. Previous studies of this same research sample have demonstrated increases in total subperiosteal porosity with age, due primarily to increases in pore size, rather than pore number (Stein et al. 1999). Moreover, this increased porosity is seen at an earlier age in females than in males (Feik et al. 1997). The present study demonstrates increased proportions of bone with high mineralization density bone in older females relative to males of the same age group. Focusing on the relationship between the distributions of these two variables will provide added information on age and sex differences in remodelling rate.

On a related note, given that the bone cortex generally becomes more porous with age, a methodological concern of any study relying on edge-detection technology to differentiate bone from non-bone (as was done here) might be that the proportion of edges to bone would increase with age, thereby increasing error in older individuals. However, because increases in porosity appear to be primarily due to increases in pore size, rather than pore number, this is unlikely to be an issue. Moreover, the resolution of the BSE-SEM images, combined with the relatively low magnification of the images, renders any small increase in the proportion of edge to bone to be of little consequence.

Location in cortex

Location (ring and sector) was also significant in explaining the variability in degree of mineralization. Specifically, the periosteal ring was consistently the least mineralized relative to bone located in the mid-cortex and endosteal cortex. In all age groups, the antero-lateral aspect of the bone tended to be the least

mineralized and the postero-medial aspect most mineralized, though these results were only significant in the periosteal and mid-cortex of the youngest age group. The significant differences in mineralization density between aspects of the cortex may reflect regional differences in bone turnover within the femur, at least within younger adults in the sample. This pattern is broadly consistent with that identified by Portigliatti-Barbos et al. (1983) in two male specimens, aged 43 and 46 years. These authors suggested a relationship between low mineralization in the anterior and lateral aspects of the bone with high remodelling rates induced by tensile stresses. Studies of other skeletal elements – for instance the human proximal femur (Loveridge et al. 2000), human mandible (Kingsmill & Boyde, 1998) and horse radius (Riggs et al. 1993), have also demonstrated consistent locational variation in mineralization density that has been attributed to regional differences in functional loading. Further study of loading at the human femoral mid-shaft should improve our ability to interpret the regional variability identified in this study.

Range of mineralization density variation

The range of mineralization densities characterizing this sample was similar to results reported in previous studies of adult humans (Baud & Gossi, 1980; Roschger et al. 1998; as cited in Meunier & Boivin, 1997). In the present study, coefficients of variation were shown to increase with age, particularly among females. This finding is supported by Sharpe (1979), but contradicts studies that have observed a decrease in variability in mineralization with age (i.e. Amprino & Engstrom, 1952; Reid & Boyde, 1987, in human ribs). The latter studies, however, included subadults in their samples as well.

The increase in variability in the middle and older age groups in this study may reflect increased heterogeneity in the degree to which different individuals of the same age turnover their bone. Whereas some individuals in the middle age group retain large portions of highly mineralized primary circumferential bone, others have extensively remodelled their cortices. The increased variability in degree of mineralization, particularly among older females, may point to an increase in areas of both hypo- and hyper-mineralization (Sharpe, 1979). Alternatively, as this sample is likely to contain both osteoporotic and 'normal' individuals, mineralization

differences between normal and osteoporotic bone may account for some of this variability (Jowsey, 1964; Parfitt, 1993; Boyde et al. 1995b). Moreover, although most specimens in this sample were obtained at a date prior to extensive use of osteoporosis preventive treatments (e.g. bisphosphonates), the effect of osteoporosis treatment on mineralization amongst the elderly of this sample is unknown. To examine the relative changes in mineralization variability with age further, studies focusing on smaller regions of the cortex, at higher resolution, would clarify the relative abundance of hypo- and hyper-mineralized areas. In addition, separation of the oldest age group in this study into older and younger subgroups may help to identify any trends in mineralization within the elderly, such as those described in Simmons et al. (1991).

Implications of this research

This study clearly demonstrates that although degree of mineralization of the mid-shaft femur has a limited range within adult individuals, interindividual variation is important within a single age and sex group. Moreover, regional variability in mineralization even within a single bone element of a single individual is extensive. This result is consistent with other human studies (Frost, 1969), and even with studies utilizing animal models in which more control over the genetics, activity patterns and environment was possible (Iwaniec & Crenshaw, 1998). This study of mineralization density is also consistent with dynamic and static histomorphometric analyses illustrating variations in remodelling rates between regions of cross-sections of single elements (Jowsey, 1966; Martin et al. 1980; Raab et al. 1991; Bertelsen et al. 1995; Pfeiffer et al. 1995; Feik et al. 1997), and variability within a region of a given element cross-section (Pfeiffer et al. 1995).

The results of this study of mineralization density in many ways mirror the results of a similar study examining collagen fibre orientation variability utilizing this same material (Goldman et al. 2003). Analysis of that histocompositional variable demonstrated broad age and sex trends, along with some consistencies in patterning across the mid-shaft femur cortex. It also demonstrated overwhelming variability within age and sex groups that could not be explained assuming similar function of the lower limbs. An exploration of mechanical explanations was undertaken in that paper, the discussion of which could equally apply to the current

study. Given the broad similarities observed in these two data sets (i.e. in overall variability and patterning), further consideration of the relationships between bone mineralization density, remodelling rates and preferred collagen fibre orientation may allow us to explain better the variability that has been characterized in these studies. Ongoing integrative research utilizing these same research samples will address these issues further.

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