A comparison of the stiffness, density and composition of bone from the calcar femorale and the femoral cortex

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The stiffness, density and composition were compared in bone from the calcar femorale and cortical bone from the mid-shaft of the femur from an elderly group of clinically normal patients. Variation of properties through the thickness of the bone and the stiffness, measured using ultrasound, in directions parallel and perpendicular to the bone axis were also investigated. The calcar was less stiff (30.5 GPa) than the cortical bone (33.0 GPa) ($P = 0.04$). It was also slightly less dense (2.01 compared with 2.05 g cm^{-3}, $P < 0.001$) and had a lower mineral content (56.7% of wet mass) than the femoral cortex (58.0%, $P < 0.05$). At both sites the bone stiffness, density and mineral content decreased from the outer layer towards the inside. The stiffness was greatest in an axial direction and least in a radial direction with the tangential stiffness slightly greater than the radial, indicating an orthotropic symmetry. These results show that bone from the calcar has similar material properties to cortical bone, and provide a baseline for further studies investigating possible changes in the calcar in osteoporosis which may increase our understanding of the mechanisms underlying fractures of the neck of the femur. © *1998 Kluwer Academic Publishers*

1. Introduction

This study compares the stiffness, density and composition of compact bone from the calcar femorale with the same properties of cortical bone taken from the mid-shaft of the femur. The calcar femorale is a prominent spur of compact bone running from the lesser trochanter towards the femoral head. It shows up clearly on radiographs as a dense area along the inferior aspect of the neck. An intertrochanteric fracture of the femoral neck, common in elderly people with osteoporosis, results in the fracture plane running across the calcar. Because of its evident density and position it would appear to be similar to cortical bone and to form a significant structural part of the neck of the femur. The aim of this investigation was to compare the properties of the calcar with those of cortical bone.

There has been considerable interest in determining the mechanical and structural properties of cancellous and cortical bone (for a review see Cowin [1]) and numerous *in vivo* studies of bone mineral content or density using photon absorptiometry. Age-related bone loss is believed to be a significant factor in the increasing incidence of fractures of the wrist, spine and hip that occur with age. There are also structural changes that accompany bone loss and it has been suggested that these reduce the strength of the bone, both trabecular and cortical, to a greater extent than the reduction in the amount of bone itself would suggest [2]. Cortical bone becomes thinner with increasing loss of bone [3–5] and the inner third appears to convert to a trabecular-like structure [6, 7]. The effects of these changes on the mechanical properties of the tissue are largely unknown.

2. Materials and methods

The proximal portion of the femur, from the femoral head to the middle of the shaft, was removed from cadavers during post-mortem examination. Ten femora were collected from seven cadavers. The median age was 79, and ranged from 58–93 y old. There were five females (seven femora) and two males (3 femora). The medical records were examined to exclude any patient with a history of metabolic disorders or drug therapies that could affect the bone, degenerative or inflammatory joint disease, or prolonged immobility.

Samples were prepared from two sites in each femoral segment. One site was in the calcar, defined as one fingerbreadth proximal to the lesser trochanter [8]. The other was at the medial aspect of the middle of the femoral shaft, the same side as the calcar. This is approximately where the cortical bone is thickest and was chosen as a representative site with which to make comparison. Samples from each site were divided first into three layers from the outside cortical surface to the inside medullary surface (Fig. 1). Each sample was then further subdivided for ultrasonic mechanical testing by cutting slices with faces oriented in axial, radial

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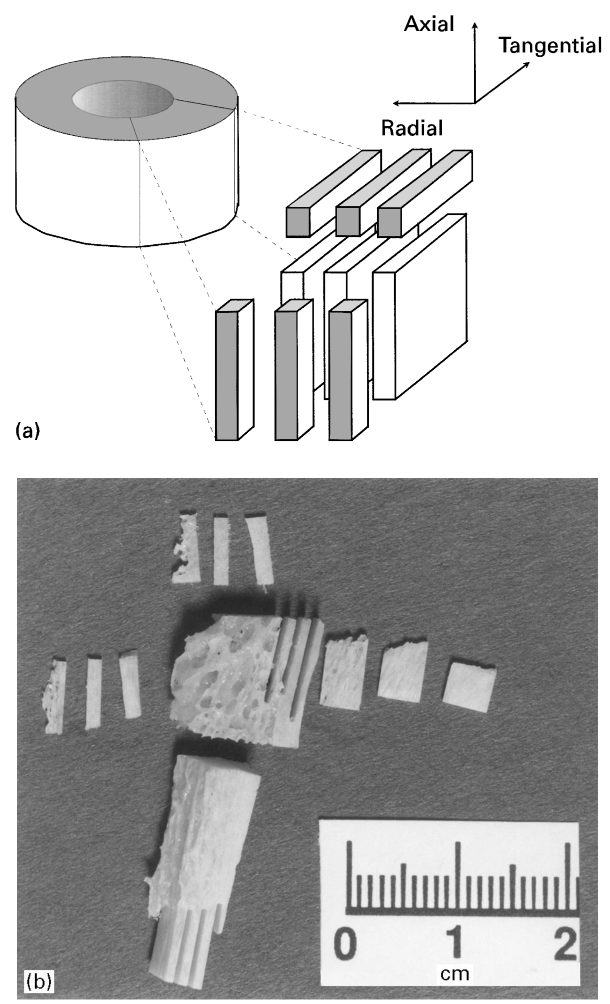

Figure 1 (a) Schematic diagram of how the bone samples were prepared from both the femoral shaft and the calcar femorale. All the surfaces were accurately cut and polished to be parallel and smooth prior to ultrasonic testing. (b) Photograph of the partly prepared pieces from the calcar (upper) and femoral shaft (lower) and typical sections that were obtained for testing.

and tangential directions. The axial direction was defined as parallel to the axis of the femur for the cortical samples and along the length of the spur of bone for the calcar. The radial direction was then perpendicular to this and directed towards the central axis of the femur or the neck, respectively. Finally, the tangential direction was perpendicular to both of these. All cutting was done using a rotary electric saw (Struers Accutom-2) fitted with an aluminium oxide cut-off wheel rotating at 300 r.p.m., cooled with distilled water. To enable ultrasound measurement of the stiffness, the parallel surfaces of the samples were polished by sequential grinding on a series of graded silicon carbide grinding papers, finishing with 4000 grit paper (grain size < 5 μm). The specimens were subsequently cleaned in an ultrasonic bath filled with calcium phosphate buffered saline solution to remove any traces of silicon carbide. The thickness of each specimen, d, was measured three times using a micrometer and the mean used in subsequent calculations. In total, 180 samples were prepared, 90 from each of the cortical bone and the calcar. The average final thickness of the prepared samples was 0.61 ± 0.11 mm (mean $\pm$ S.D.).

An ultrasonic method was used to determine the elastic stiffness modulus of the bone. Ultrasound, of 10 MHz frequency, was obtained using a pulsar/receiver (Model 5052 PR, Panametrics Inc.) and a straight beam contact transducer (Panametrics Inc.). Each specimen was firmly pressed on to the wear plate of the transducer with a drop of distilled water to ensure good acoustic coupling. The pulse transit time, T, was measured by a "pitch-and-catch" method using a separate transmitter and receiver and a dual-beam oscilloscope (Hitachi V-665A, Japan). The longitudinal sonic plesio-velocity was calculated using $v = d/T$. The effective path length for sound in an anisotropic, inhomogeneous medium, such as bone, is not known, and will generally be longer than the specimen. The estimation of longitudinal velocity will, therefore, probably be an underestimate and has been termed the plesio-velocity [9].

The density of each specimen, ρ, was measured using Archimedes' principle by weighing it in air, after removing excess water by gentle patting with a damp tissue, and then suspending it in calcium phosphate buffer solution of known density. Each measurement was repeated five times and the mean determined. The longitudinal elastic stiffness modulus was calculated from the formula $E = \rho v^2$ [9].

To determine the composition of the bone, the specimens were dehydrated at 105 °C for 24 h and weighed again. The water content was calculated as the difference between the wet weight and the dry weight. They were then ashed at 600 °C for 24 h and reweighed to determine the ash weight, which was taken to be the mineral content. The organic content was calculated by subtracting the ash weight from the dry weight. The mass of each component was then expressed as a fraction of the wet mass of the sample and also as mass per unit volume of tissue by multiplying the mass fraction by the density of the sample.

The results were analysed using a two-way analysis of variance (ANOVA) to determine first the overall differences between the shaft and the calcar, and secondly to investigate variation through the thickness of the bone. A two-way ANOVA was used because these may be inter-related. Normality of the distributions was assessed using the Kolmogorov–Smirnov test with the significance level, P, set at 0.05. Mean values $\pm$ standard deviations are shown for those data that are normally distributed, otherwise the median value (interquartile range) is shown. Pairwise comparisons were made using the Student–Newman–Keuls method for comparing all groups. Unconstrained linear regression was used to determine trends and possible relationships between variables.

3. Results

The median (interquartile range) density of the cortical bone, 2.05 (2.13, 1.99) g cm^{-3}, calculated from all the samples from the shaft, was slightly, but significantly, greater than that of the calcar bone, 2.01 (20.6, 1.90) g cm^{-3} ($P < 0.001$). There was also a significant difference ($P < 0.05$) between the layers, irrespective of site, with the outer layer being the most dense (2.08 cm^{-3}) and the inner layer the least (1.99 g cm^{-3}). A two-way ANOVA showed there to be no significant

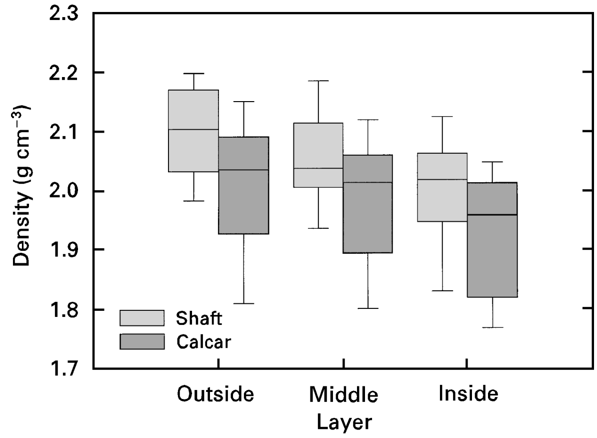

Figure 2 The median density of bone from the femoral shaft and the calcar on passing from the outermost layer to the inner. Shaded regions show the 25% and 75% confidence limits, the error bars the 5% and 95% limits.

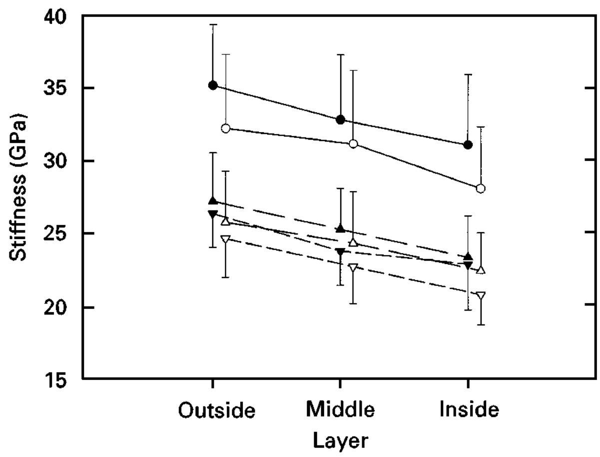

Figure 3 Variation in stiffness (mean and standard deviation) from the outside to the inside layers of cortical bone from the femoral shaft and bone from the calcar, shown slightly displaced for clarity. In each case the stiffnesses are in the order axial > tangential > radial, (○) Calcar axial, (●) shaft axial, (△) calcar tangential, (▲) shaft tangential, (▽) calcar radial, (▼) shaft radial.

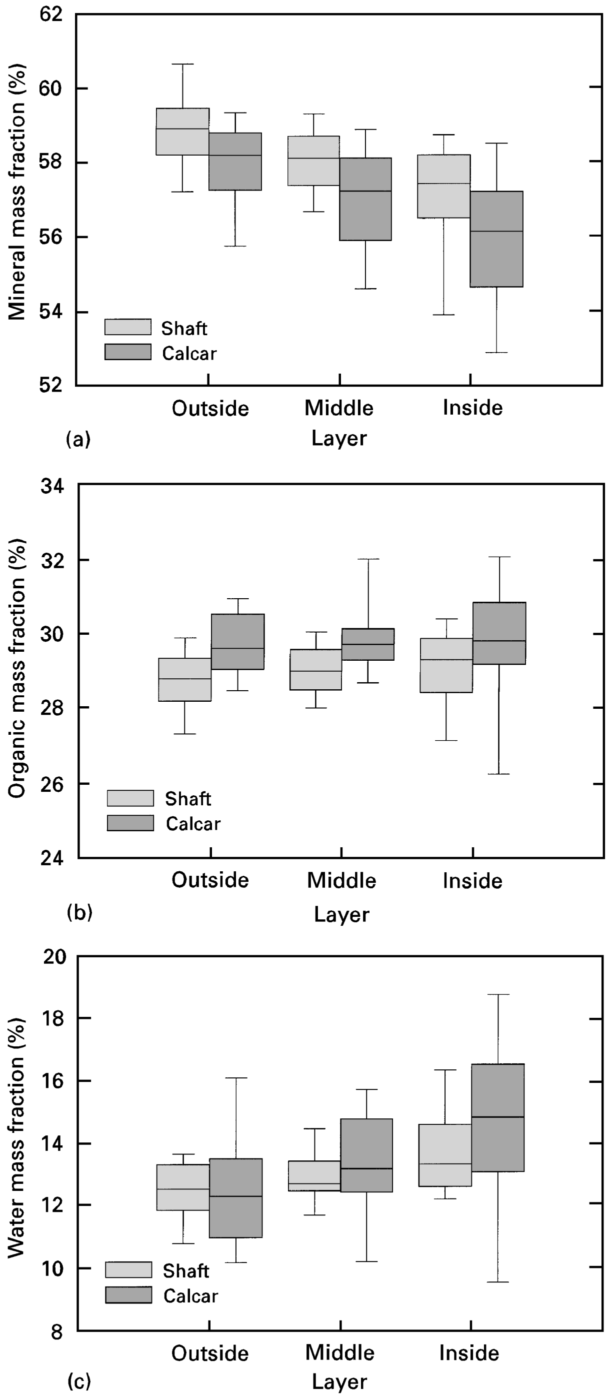

Figure 4 Mass fractions of (a) mineral, (b) organic and (c) water in the different layers from the calcar and femoral shaft cortical bone. Median values are shown; the shaded regions show the 25% and 75% confidence limits and the error bars the 5% and 95% limits.

interaction between layer and site. Fig. 2 shows the variation with layer at each site, with the cortical bone being always more dense than the calcar bone in each layer.

The stiffness of each sample was calculated from the measured density and ultrasound velocity and the results are shown in Fig. 3. Two-way ANOVA showed that, after allowing for differences in layer, the stiffnesses in each of the axial and radial directions were greater in the shaft than the corresponding stiffnesses measured in the calcar ($P < 0.05$). The difference between the tangential stiffnesses failed to reach significance, but showed the same trend with the shaft slightly stiffer than the calcar. The greatest stiffness was found in the axial direction for both of the calcar (30.5 ± 1.5 GPa, mean $\pm$ S.E.M.) and the shaft (33.0 ± 1.4 GPa). In each layer, after allowing for differences in site, the outside layer was the stiffest and the inner layer the least stiff ($P < 0.05$) with the middle layer having intermediate, though not always significantly different, values. At each site and in each layer the tangential and radial stiffnesses were similar in magnitude and lower than the axial stiffness. However, the difference between the radial and tangential stiffnesses was significant ($P < 0.05$) with the bone appearing stiffer in a tangential direction than radially.

The lower stiffness and density of the calcar bone are reflected in the composition. Bone from the calcar contained a smaller mineral mass fraction (56.7%) than the cortical bone (58.0%, $P < 0.05$). This difference was enhanced when expressed as mass per unit volume, when the median values were 1.20 (1.15, 1.25) $g\,cm^{-3}$ for the femoral shaft and 1.14 (1.07, 1.20) $g\,cm^{-3}$ for the calcar (Figs 4 and 5). Mineralization was greatest

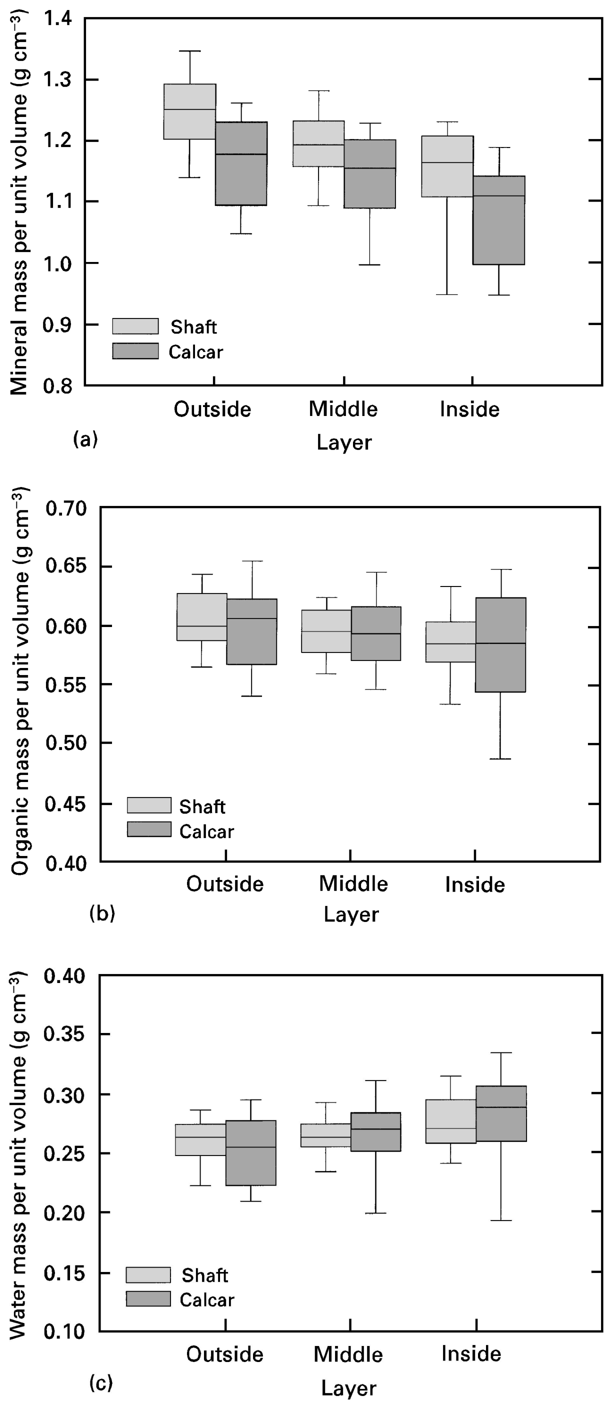

Figure 5 Mass per unit volume of (a) mineral, (b) organic and (c) water in the different layers from the calcar and femoral shaft cortical bone. Median values are shown; the shaded regions show the 25% and 75% confidence limits and the error bars the 5% and 95% limits.

in the outer layer of both sites and decreased through the thickness of the bone to the inner surface. The mean organic content, expressed as a fraction of the mass of the sample, was greater in the calcar bone, 29.8%, compared with the cortical bone 28.9% ($P < 0.05$), but if these values were expressed as mass per unit volume, this difference disappeared (Fig. 5). There was no variation in organic content with layer at either site. There was no significant difference in mean water content between sites, 13.1% in the shaft and 13.5% in the calcar ($P = 0.19$), though between the outside and the inside layers there was a significant increase in water content ($P < 0.05$) at both sites. In

TABLE I Linear regression equations for the relationships between stiffness and density for bone from different sites in the femoral shaft and the calcar. E denotes stiffness in GPa, ρ is the density in g cm^{-3}

	Shaft	Calcar
Axial	$E = -37 + 34\rho$	$E = -36 + 34\rho$
	$R^2 = 0.93$	$R^2 = 0.87$
Radial	$E = -29 + 26\rho$	$E = -30 + 27\rho$
	$R^2 = 0.87$	$R^2 = 0.92$
Tangential	$E = -45 + 34\rho$	$E = -31 + 28\rho$
	$R^2 = 0.91$	$R^2 = 0.95$

the shaft it increased from 12.3% in the outer layer to 13.3% in the inner layer and in the calcar from 12.5% to 14.8%.

A linear regression analysis of the relationship between stiffness and density showed that there was no difference between bone from the calcar and that from the shaft apart from in a tangential direction. Here, for the shaft, the stiffness increased with density at the same rate as that for the axial direction, whereas for the calcar the regression equation was indistinguishable from that for the radial stiffness (Table I). Analysis of the residuals showed them to be normally distributed and to have no trend with density. Non-linear regression did not produce a better fit to the data over the range of density values found.

4. Discussion

It is commonly assumed that the dense bone of the calcar femorale is similar to cortical bone. This would appear to be a reasonable assumption but has never, to our knowledge, been directly tested. Its alignment with the stress trajectories in the femoral neck and its higher radiographical density compared with the predominantly cancellous bone of the rest of the neck, indicate a significant mechanical role in the transmission of loads in the hip. An intertrochanteric fracture of the hip, commonly sustained after a fall by an osteoporotic patient, often results in the calcar being broken close to its junction with the femoral shaft just proximal to the lesser trochanter.

The results of this study show that in this elderly group, the bone of the calcar has properties similar to those of cortical bone from the middle of the femoral shaft. It is slightly less stiff and dense, but not to an extent that would suggest a marked mechanical deficiency. Because our specimens were all from elderly patients, this study cannot determine whether the calcar is always less stiff than the cortical bone or whether there is greater loss of bone stiffness in the calcar with ageing than there is in the femoral shaft. A study of femoral bone, using quantitative bone scintigraphy, showed an increased turnover of bone in both the femoral neck and femoral shaft in an osteoporotic group compared with normal [10]. There also appeared to be a slightly greater turnover in the neck, which is predominantly cancellous bone, than in the shaft, in accordance with other studies which have reported a greater rate of turnover in cancellous than cortical bone [11]. However, whether the turnover of bone in the calcar is properly represented in the scintigraphy study cannot be determined from their results. Clearly, the best test of age-related changes would require a study group containing a wide range of ages. Although in this study the range was almost 40 y, these were all from relatively elderly patients and were too few to be able to detect such variation. More samples are required from some much younger patients but these are very difficult to obtain. Other studies are in progress to compare the calcar in an osteoporotic group with a normal group.

Ultrasound is well established as a technique to measure the elastic modulus of bone [9, 12–16]. One advantage of ultrasonic measurement is that small, thin samples can be tested. Although macroscopic samples of cortical bone can be machined for conventional testing in a materials testing machine, cutting thin samples, as described here, enables the variation of properties in different layers and in different directions to be determined. The ordering of the measured stiffnesses, axial > tangential > radial, is the same as that found in previous studies ([17, 18] and agrees with the generally accepted description of the symmetry of cortical bone being orthotropic [1]. Our results are similar to those previously reported for both human and bovine bone using this method, which generally produce stiffness values slightly higher than those obtained using materials testing machines because of the large strain-rates developed in the material by the high-frequency sound. The increase in stiffness found from inside to outside could be a consequence of the bone adapting its stiffness to the loading demands being placed upon it. Bending of a tube or a bar generates the largest strains on the outside, because of the larger distance from the bending axis, and so material on the outside has a greater effect on the overall properties of the structure. This, for instance, is the reason why rods lose little of their bending stiffness if the centre is removed to make a tube while resulting in a considerable saving in mass.

Another advantage of ultrasound is that it measures the elastic properties of the material; viscoelasticity and its associated time-dependency are effectively removed due to the high frequencies involved. It is also non-destructive, although this latter also means that it cannot directly provide information on yield properties or ultimate strength. Successive demineralization of bone pieces has shown that the longitudinal sonic plesio-velocity increases linearly with density [13]. However, it is also affected by the anisotropy and porosity of the bone [12]. We found a good correlation between velocity and density at both sites, R^2 values of between 0.69 and 0.87, which suggests that alterations in the structure of the bone are small and most of the variance is explained by changes in the mineral content. Other experimental results support the idea that, in bone, stiffness and strength are approximately linearly proportional to each other [1]. So, although actual values for strength cannot be measured by this technique, a greater stiffness may indicate a correspondingly greater strength.

The difference in density between the cortical and the calcar bone is small, about 2%, whereas the calcar is about 7.5% less stiff than the cortex. Some of this difference is clearly due to mineral content which is 2% lower by mass but 5% less in terms of mass per unit volume of bone in the calcar. Interpreting changes in mass fractions requires some care, as a change in the absolute amount of one component will result in apparent changes in the others even if their total quantity has not changed. Calculations show that a reduction in mineral mass fraction from 58.0% to 56.7% will result in an apparent increase in organic mass fraction from 28.9% to 29.8% and an apparent increase in water mass fraction from 13.1% to 13.5% even if there are no changes in the absolute amounts present. These are exactly the differences found between the cortical bone and the calcar, which suggests that the basic organic matrix is unchanged but may be mineralized to a lesser extent in the calcar. Calculating the mass per unit volume for each component reflects this by showing no differences in either organic or water content between the shaft and the calcar.

This study also showed that the outer layers of both the femoral shaft and the calcar had a greater density than the inner layers and that this corresponded to a greater mineral content and lower water content. These results are compatible with an increase in the porosity of the bone from outside to inside [6, 7], although a direct method of measurement is the only conclusive way of testing this.

In conclusion, this study shows that the stiffness and material properties of bone from the calcar femorale are similar to but slightly less than that of cortical bone from the midshaft of the femur. These results suggest that it is capable of providing a support to the femoral neck and acting as a transitional structure to transfer stress from the trabecular bone of the femoral head and neck to the cortical bone of the femoral shaft.

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