Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis

Baohua Li, Richard M Aspden

Abstract

Objective—To determine the material properties of the subchondral bone plate in patients with osteoarthritis or osteoporosis.

Methods—Femoral heads were obtained after surgical removal from age and sex matched groups of patients with either osteoporosis (OP), after a fractured neck of femur, or osteoarthritis (OA) and compared with a normal group. The mechanical stiffness, density, and composition of the subchondral bone plate from sites selected to represent areas of heavy, intermittent, and light loading were measured.

Results—Overall, OP bone was the least stiff and dense, followed by OA bone; normal bone was stiffer and more dense (p < 0.05). Though OP bone contained less mineral, the organic and water contents were increased in proportion suggesting no change in the relative amount of organic matrix. OA bone was also hypomineralised (p < 0.05) but had different organic and water fractions suggesting a defect in the matrix. Site variation of most properties was small, though across all the groups the superior region was significantly stiffer than the inferior.

Conclusion—This study shows that subchondral bone plate is less stiff than normal in both OP and OA and so cannot, by itself, explain the preserving of the overlying cartilage in OP while aiding its destruction in OA. However, the subchondral bone plate is only one part of the bony structure of the femoral head and changes in the cancellous bone need to be considered. The generalised changes in bone composition found in patients with OA support the hypothesis that the disease could involve the bone in the primary pathogenesis.

This study investigates the mechanical and material properties of the subchondral bone plate from the femoral head of patients with either osteoarthritis (OA) or osteoporosis (OP). The aims were to determine what changes occur in the properties of the bone in these patients. OA and OP together afflict a large proportion of the elderly population in the western world. They are among the greatest causes of morbidity in the elderly and place an enormous demand on orthopaedic services; about one quarter of all orthopaedic beds in the United Kingdom are occupied by patients who have sustained a fracture of the femoral neck resulting from OP and a further quarter by patients electing for a joint replacement for OA. Clinical studies have shown that these diseases are rarely present together in the same patient and that presence of one may be protective against the other. Byers et al noted that the progressive changes of OA were not seen in over 100 femoral heads removed after fracture of the femoral neck. In a study of a random population sample in Jerusalem, Pogrund et al found that the prevalence of OP and OA in 641 pelvic X rays separately was 16.1% and 4.1%, but in only 0.5% did OA and OP coexist in the same person. Epidemiological surveys have suggested a negative association between the two conditions and supported the hypothesis that these are two distinct diseases and not phenomena related solely to aging. However, it has not been established whether this apparent lack of association occurs by chance or if there are underlying causal factors.

The pathogeneses of both OA and OP are still not known. OA affects all the major joint tissues, including the synovium, articular cartilage, subchondral bone, and cancellous bone. It is generally regarded as a disease of cartilage, the bony changes being thought to be secondary to this, and most research and treatment is focused on trying prevent or reduce the degeneration of the articular cartilage. OP, however, is universally considered to be a disease of bone, though the reasons for the loss of bone and the inability of the cells to regulate properly the mechanical properties of the tissue are not fully understood. Part of the reason for our lack of understanding of both of these diseases is because of the difficulties in recognising early disease and obtaining human tissue for study at this stage.

Though it is widely held that OA is a primary disorder of articular cartilage with secondary change in the bone, it has been suggested that the health and integrity of the overlying articular cartilage depend on the mechanical properties of its bony bed. A defect in the subchondral bone, resulting in a reduction of its compliance, may result in greater stresses being sustained by the articular cartilage leading to overloading and consequent breakdown. Conversely, an increase in compliance, such as may be expected due to the loss of bone in OP, may have a protective effect. Previous studies of subchondral bone in...
relation to its possible role in the pathogenesis of OA have concentrated on morphological findings and we have not found any investigation into the mechanical properties and composition of the subchondral bone plate other than a study of the hardness of subchondral bone in the patella. This study investigates this question by measuring the stiffness, the density, and the composition of the subchondral bone plate itself and compares the results obtained from patients with OA or OP with a normal group to discover the importance of changes in the subchondral bone to the clinical presentation of OA and OP.

Methods
Samples of subchondral bone were taken from femoral heads collected from three clinical groups, OA, OP, and normal, which were matched for age and sex. The femoral heads from both disease groups were obtained in the operating theatre from patients undergoing a hip replacement for either a fractured neck of femur attributed to OP or for OA of the hip. Cases with roentgenographic, biochemical, or histological evidence of osteomalacia, multiple myeloma, rheumatoid arthritis, or secondary osteoporosis due to corticosteroids were excluded from the osteoporotic group. Patients with rheumatoid arthritis, osteomalacia, congenital or acquired dysplasia, gout, or avascular necrosis were excluded from the osteoarthritic group. A control group was collected from hips removed during postmortem examination and the medical records were examined to exclude known disorders affecting bone metabolism. A clinical definition of each disease was used to determine what overall changes occur in the bone of patients judged suitable for surgery as National Health Service patients. No attempt was made to grade the severity of disease. Table 1 shows the median ages and the oldest and youngest of each of the patient groups. The ratio of female to male was not significantly different in any group and there was no difference in the age distributions between the groups. All samples were stored at 4°C in a calcium phosphate buffered saline solution containing sodium azide as this has been shown to preserve the structure and composition of the bone.

From each femoral head, cylindrical cores of 9 mm diameter were removed from sites chosen to represent regions subjected to different amounts of loading in vivo (fig 1): superior being the most heavily loaded; posterior, anterior and medial from the partially loaded region; and inferior being the least loaded. Care was taken to maintain the bone at all times in a fully hydrated condition. Any articular cartilage on the surface of the specimen was removed with a scalpel. The subchondral bone was cut from each core using a rotary electric saw (Struers Accutom-2) fitted with a silicon carbide cut off wheel rotating at 300 rpm, cooled with distilled water. This resulted in a disc of bone less than 1 mm thick. In preparation for ultrasonic measurement of the stiffness, the remains of the trabeculae and the cartilage were removed and the surfaces made parallel and polished by sequential grinding on a series of graded silicon carbide grinding papers, finishing with 4000 grit paper. 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 five times using a micrometer and the mean used in subsequent calculations.

An ultrasonic method was used to determine the elastic stiffness modulus of the bone.

Table 1 Details of groups from which femoral heads were removed. Significance values for sex and age matching are from χ² and non-parametric ANOVA tests respectively enabling acceptance of the null hypothesis of no difference between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of femoral heads</th>
<th>Sex (female/male)</th>
<th>Age (median(younger, oldest))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoporotic</td>
<td>17</td>
<td>13/4</td>
<td>77 (66, 91)</td>
</tr>
<tr>
<td>Osteoarthritic</td>
<td>16</td>
<td>12/4</td>
<td>73 (49, 86)</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>5/2</td>
<td>79 (58, 93)</td>
</tr>
</tbody>
</table>

p = 0.97

p = 0.44

Figure 1 Schematic diagram of the sites on the human femoral head from which samples of subchondral bone were removed.

Figure 2 Median values of the stiffness of the subchondral bone plate determined from all sites over the femoral head of patients undergoing a hip replacement for osteoarthritis (OA), or osteoporosis (OP) compared with normal controls. The extent of the shaded bars shows the 25% and 75% confidence limits, the error bars show the 5% and 95% limits. (*) p<0.05.
Ultrasound, of 10 MHz frequency, was obtained using a pulsar/receiver (Model 5052 PR, Panametrics Inc) and a straight beam contact transducer (Panametrics Inc). The specimen was firmly pressed onto the wear plate of the transducer with a drop of distilled water to ensure good acoustic coupling and the transit time, T, was measured using a single transmitter/receiver and a dual beam oscilloscope (Hitachi V-665A, Japan) by the pulse-echo method. The cartilage surface of the bone was always placed adjacent to the transducer. The longitudinal sonic plesio-velocity was calculated using $v = \frac{2d}{T}$. 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 for this reason.17

To determine the composition of the bone, the specimens were dehydrated at 105°C for 24 hours and weighed again. The water content is the difference between the wet weight and the dry weight. They were then ashed at 600°C for 24 hours 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 specimen, as determined above. The results were analysed first to determine differences in the overall results in each patient group and then to investigate site variation within each group. Because these may be interrelated a two way analysis of variance (ANOVA) was performed. Normality of the distributions was assessed using the Kolmogorov-Smirnov test with the significance level, $p$, set at 0.05. Mean values and associated standard deviations are shown for those data that are normally distributed, otherwise median values and associated 5%, 25%, 75%, and 95% intervals are shown. Pairwise comparisons were made using Student-Newman-Keuls method for comparing all groups or Dunnett’s method for comparison with a control. Unconstrained linear regression was used to determine trends and possible relations between variables.

Results

THICKNESS AND APPEARANCE

The mean (SD) thickness of the prepared subchondral bone plate specimens in each of the patient groups was 0.69 (0.19) mm in the OA group (n=73), 0.60 (0.11) mm in the normal group (n=32), and 0.56 (0.17) mm in the OP group (n=74). Only the OA specimens were significantly different from normal ($p<0.05$) and there was no significant variation with site or interaction between site and disease group as shown by two way ANOVA. Despite being thicker the OA bone was difficult to prepare as it was coarse in texture, crumbled easily, and the boundary with the underlying cancellous bone was indistinct. OP subchondral bone was thinner but appeared qualitatively stronger. In all groups, removal and preparation of an intact specimen from

![Figure 3](http://ard.bmj.com/)

**Figure 3** Density of the subchondral bone plate from the femoral heads of patients with OA or OP compared with normal controls. Both OA and OP groups are significantly reduced from normal and OP is slightly but significantly less than OA. (* $p<0.05$).

![Figure 4](http://ard.bmj.com/)

**Figure 4** Composition of normal, OA, and OP subchondral bone plate from human femoral heads expressed as a fraction of the wet mass of the sample. Both OP and OA differed from normal but in different ways. (* $p<0.05$).
some of the sites, especially the inferior region, was difficult and not always possible because of their thinness or fragility. In these cases further measurements were not always possible.

STIFFNESS AND DENSITY
Figure 2 shows the median stiffness and figure 3 the density of subchondral bone from all sites in each of the three patient groups. The stiffness (19.8 MPa) and density (mean (SD)) (1.79(0.08) g cm⁻³, n= 29) were both greatest in the normal group, those in the OP group were lowest (14.9 MPa and 1.64 (0.13) g cm⁻³, n=65) and the OA group lay in between though nearer to the OP than to the normals (17.0 MPa and 1.69 (0.13) g cm⁻³, n=67). The number of samples removed intact and tested is denoted by n. Both the stiffness and the density are significantly lower (p<0.05) in the OA and the OP groups than in the normal group and in neither case was there any significant interaction between disease group and site as shown by two way ANOVA. This also showed that across all the disease groups the stiffness of the superior region was significantly greater than that of the inferior (p<0.05) though there was no corresponding variation in density.

COMPOSITION
The compositions of the OA and the OP groups both differed from the normal group but in different ways (fig 4). The OA samples had a reduced mass fraction of mineral and an increased mass fraction of water compared with normal, both of which were significant (p<0.05). The OP samples also had a significantly smaller mass fraction of mineral compared with normal but the organic and water contents were both greater than normal, though only the organic component significantly (p<0.05). Two way ANOVA showed that there was no significant interaction between the disease group and the site for any of the composition variables. Multiplying the mass fraction by the tissue density yields a measure of the composition as mass per unit volume (fig 5). From this it may be seen that OP subchondral bone has organic and water contents that are similar to normal, simply a reduction in the amount of mineral (p<0.05). In contrast the OA bone is not only hypomineralised compared with normal (p<0.05) but there is an increased water content (p<0.05) though no change in organic content.

RELATION BETWEEN STIFFNESS AND DENSITY
Density is one of the main determining factors of bone stiffness and a low but significant correlation was found between the stiffness and density in all three groups (fig 6). The equations of the regression lines and the adjusted correlation coefficients were:
Normal:K = -10.5 + 16.6 \rho (R^2 = 0.24, p = 0.004)
OA: $K = -13.1 + 17.4p(R^2_{adj} = 0.39, p < 0.0001)\)

OP: $K = -21.4 + 22.1p(R^2_{adj} = 0.40, p < 0.0001)\)

A Student’s $t$ test showed that there was no significant difference between these slopes ($p>0.5$ for all comparisons). Only in the OA and OP groups was there a significant correla-

SITE VARIATION

There was very little site variation in any of the parameters measured (fig 7(A)). A two way ANOVA showed that for all disease groups the stiffness of the bone from the inferior region was less than that from the superior ($p < 0.05$). A more detailed analysis using one way ANOVA within each group showed that there was no site variation in any group apart from the OP group in which the inferior was significantly ($p < 0.05$) less than the superior. Expressing these as a fractional change compared with the values obtained from the corresponding site in the normal tissue, using the formula (diseased-normal)/normal, showed that at nearly all sites from both OA and OP groups there was a reduction in the stiffness of the subchondral bone (fig 7(B)) and only in the inferior and posterior regions was this noticeably greater in the OP than in the OA group. These results were reflected in the density measurements where there was not found any significant site variation (fig 8(A)) and the fractional changes were marked only in the anterior and inferior regions, especially in the OP group (fig 8(B)). No significant variation was found in the composition measurements from the various sites.

Discussion

This study shows that changes occur in the subchondral bone plate immediately adjacent to the articular cartilage in the femoral heads of patients with OA or OP. Of greatest significance is that the stiffness of the bone from patients with either OA or OP is less than that from a non-clinical group but that the causes of this reduction are different in each group. The stiffness of the osteoporotic bone was reduced by about 22% and its density by about 8% while the stiffness of the osteoarthritic bone was 14% less than that of normal and the density was reduced by 6%. Though both patient groups showed similar reductions in the amount of mineral, which being the most dense is the main component that would account for these changes, the relative amounts of organic material and water were different.

There was very little site variation in any of the disease groups though numbers in some of the groups were small, for instance only four in the normal inferior group, which may be why some of the measured changes did not reach significance. Other studies have suggested a correlation between the thickness of the subchondral bone plate and the prevailing mechanical load\(^{18}\) and it may be that the tissue responds to load by regulating the amount of bone rather than the material properties of the bone. We did not measure the thickness of the subchondral bone plate as both surfaces are irregular and the thickness can only be measured properly by cutting vertical sections. This was not compatible with having uniform, circular, parallel faced specimens for ultrasound measurement.

Obtaining normal tissue for postmortem examination is difficult and there will always be
doubt in a study like this as to how ‘normal’ are the normal group. Because of the requirement for matching by age and sex, the tissue was obtained from an elderly population, which could easily have contained people with sub-clinical OA or OP. We excluded any femoral heads with marked degeneration of the articular cartilage but were not able to collect samples in the same number as can be obtained from the operating theatre. Using a clinical definition of disease means that we can define the ‘normal’ group to be clinically normal though we cannot be sure that there is no overlap in the material properties of the groups. However, it is essential to have some group to which the disease groups can be compared otherwise it is not possible to determine whether and in which ways they have deviated from normal. The self consistency of the results from this and our other studies gives us reasonable confidence that what we have measured are real changes in the bone. Clearly both the OA and the OP samples are from the end stages of the disease, as shown by their removal at surgery, and this was deemed sufficient to be able to identify the significant changes that have taken place. Further studies could look for changes during the course of the disease by comparison with an appropriate scoring system but this would require many more samples to have sufficient stages represented. In addition neither the Mankin score for OA cartilage nor the Singh index for OP bone have a scientific basis and provide only a qualitative description of some of the observable changes, indeed the Singh index has been shown to be unreliable as a radiological indicator of bone content in femoral heads.20 The specimens were prepared so that highly polished, parallel faces were produced, and these were checked visually. Scanning electron microscopy shows that there is little or no calcified cartilage in the OA samples and the appearance of the OP and normal specimens strongly suggested that little of the softer calcified cartilage remained after grinding and polishing.

Ultrasound has successfully been used before to measure the elastic stiffness modulus of bone.17,21-25 Ultrasonic measurement offers certain advantages in that it measures the elastic properties of the material; viscoelasticity and its associated time dependency is effectively removed due to the high frequencies involved. It is also non-destructive, although this second factor also means that it cannot provide information on yield properties or ultimate strength. Successive demineralisation of bone pieces has shown that the longitudinal sonic plesio-velocity increases linearly with density.22 However, it is also affected by the anisotropy and porosity of the bone.22 The subchondral bone plate in the femoral head is a compact calcified tissue, with a typical thickness of less than a millimetre, which makes difficult the processing of samples for physical measurements. It is certainly impossible to cut a piece of suitable size and regular shape for testing with a conventional materials testing machine and, perhaps because of this, we are aware of only two other studies investigating the physical properties of subchondral bone plate,23,26 and those both used an indentation technique to measure the hardness. The results of one study showed a reduction in the hardness of the medial tibial plateau in both OA and rheumatoid arthritis26 while the other found no appreciable difference between normal and OA bone in the human patella, though no statistical comparison was presented. In our experiments, obtaining

Figure 8 (A) Site variation of density of the subchondral bone plate from human femoral heads. (B) Density of each site expressed as a fractional change compared with the equivalent site from the normal group.
suitable samples, especially from the inferior region, was not always possible and the sample numbers and the larger errors shown in the site variation of stiffness are a consequence of this. We also found a poor correlation between velocity and density, which suggests that there are changes in the structure of the bone and not in the mineral content alone.

The changes in mass fractions of the major components of the bone clearly show that there are changes in composition with disease. However, these results are not easy to interpret because, when expressed as a fraction, a change in only one component would alter the apparent values of the other components. For instance, it is readily calculated that if there is no change in the absolute amounts of organic and water but the mineral mass fraction is reduced from 48.3% to 43.4% then the mass fractions of organic and water will appear to increase to 33.3% and 23.3% respectively, which are almost identical with the values we measured from the osteoporotic bone. Further insight into what is occurring may be gained by expressing the content of each component as the mass per unit volume. These data (fig 5) further support the result of this calculation, that there is no absolute change in the water and organic contents, and suggests that in the OP group there is a loss of mineralisation of the bone. Whether this is due to hypomineralisation of the matrix or an increase in the amount of unmineralised osteoid needs to be investigated.

In contrast, changes in the composition of the OA bone are more complicated. Not only is there a reduction in mineral content but there are also significant changes in the relative amounts of water and organic material. In a previous study we have found similar changes in the material density and composition of cancellous bone from all sites over the femoral head and neck and, contrary to expectation, the most heavily loaded regions had the lowest material density even though the apparent density (reflecting the amount of bone) was greatest. These results confirm another study, which used a density fractionation technique on finely ground bone and showed that OA subchondral bone is less dense, though in this case ‘subchondral’ was a 1 cm deep core from the surface and must have comprised mainly cancellous bone. This method produced a histogram with binned values of densities that were not related directly to the composition. Our study extends these findings by measuring the stiffness and composition as well as the exact density of the bone thereby enabling the relations between these parameters to be determined. Mineralisation has been shown to have an effect on the packing fraction of collagen fibrils; increased mineralisation leading to closer packing of the collagen molecules and a reduced water content.

Our results from the OA bone are consistent with a defect in the mineralisation of the collagen resulting in a reduced mineralisation and a decreased packing fraction. This would result in an increased water content and could be the source of at least a part of the reduction in mechanical stiffness.

The results of other studies have shown that subchondral bone becomes thickened in OA in both humans and in many animal models. Our results support these findings but with the considerable reservation that we measured the thickness of the samples only after preparation for ultrasonic stiffness measurements. We took care to remove only the minimum amount of bone compatible with obtaining smooth parallel faces, so our results will be a reflection of the thickness but not an accurate measurement of it. This thickening has been related to the reduction in the density of the OA subchondral bone to absorb energy, which is suggested, could in turn lead to breakdown of the cartilage. It has always been assumed that the bone material itself is normal. This study clearly shows, however, that the bone is abnormal; exhibiting a loss of stiffness allied to a change in composition. Taken together, a thickening of more compliant bone ought, in principle, to result in an increased ability to absorb energy—thick, soft cushions are better shock absorbers than thin, hard ones. Changes in the subchondral bone plate alone then would not appear to provide a mechanism for provoking the mechanical breakdown of the articular cartilage. However, it is important not to consider the subchondral bone plate in isolation. We have shown elsewhere that, though similar changes are found in the composition of the underlying cancellous bone, the proliferation of bone leads to a considerable increase in stiffness of the bulk material. Energy absorption will occur not only in the subchondral bone plate but also in the underlying cancellous bone, so it may be the overall increase in stiffness that is a factor in the degeneration of the articular cartilage. In addition, the changes in the cancellous bone were found at all sites over the femoral head and neck suggesting that defective bone is not a phenomenon localised to the joint surface under an osteoarthritic lesion. Proliferation of defective bone, both subchondral and cancellous, throughout the joint could be symptomatic of a more generalised disease of the bone.

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