Osteoporosis increases the severity of cartilage damage in an experimental model of osteoarthritis in rabbits

E. Calvo M.D., Orthopaedic Surgeon†‡, S. Castañeda M.D., Rheumatologist, Service of Rheumatology§, R. Largo Ph.D., Research Assistant∥, M. E. Fernández-Valle Ph.D., Research Assistant¶, F. Rodríguez-Salvanes M.D.¶¶ and G. Herrero-Beaumont M.D., Service of Rheumatology, Head, Professor of Rheumatology†

† Department of Orthopaedic Surgery, Fundación Jiménez Díaz, Universidad Autónoma, Madrid, Spain
‡ Bone and Joint Research Unit, Fundación Jiménez Díaz, Universidad Autónoma, Madrid, Spain
§ Department of Rheumatology, Hospital de la Princesa, Universidad Autónoma, Madrid, Spain
∥ MRI Research Center, Universidad Complutense, Madrid, Spain
¶ Unit of Clinical Epidemiology, Hospital de la Princesa, Universidad Autónoma, Madrid, Spain

Summary

Objective: To evaluate the effect of osteoporosis (OP) in cartilage damage developed in an experimental model of osteoarthritis (OA) in mature female rabbits in order to investigate the relationship between OP and OA.

Methods: OA was experimentally induced by anterior cruciate section and partial medial meniscectomy in the left knee of 12 rabbits. OP was experimentally induced prior to OA in six rabbits by bilateral ovariectomy (OVX) and systemic corticosteroid administration during 4 weeks. Knees were evaluated with high resolution magnetic resonance imaging (MRI) before knee surgery to rule out any detrimental effect of corticosteroids on cartilage. Gross and microscopic cartilage changes were assessed 16 weeks after surgery in bilateral knees. Left knees were considered osteoarthritic or osteoarthritic plus osteoporotic. Right knees were used as osteoporotic and healthy controls, respectively. Bone mineral density (BMD) was measured with dual energy X-ray absorptiometry (DXA) at the lumbar spine, global knee and subchondral knee bone, and its variations correlated with cartilage abnormalities.

Results: MRI before knee surgery disclosed no cartilage or bone abnormalities in any of the studied groups. OP increased the severity of cartilage abnormalities in experimental knee OA significantly (P < 0.05). Cartilage damage was inversely correlated with BMD variations measured at the lumbar spine (r = –0.74; P = 0.015). BMD changes in global and subchondral knee bone also showed a trend to correlate inversely with cartilage damage.

Conclusions: Prior induction of OP increases the severity of cartilage damage in experimental OA. Increase in cartilage damage correlates with bone loss. These findings suggest a direct relationship between OP and OA.

Key words: Osteoporosis, Osteoarthritis, Bone mineral density, Cartilage.

Introduction

Osteoarthritis (OA) and osteoporosis (OP) are the most prevalent skeletal diseases related to age and are associated with considerable morbidity. Clinical experience suggests that the conditions occur together uncommonly in the same patient, but the relationship between the two diseases remains unclear. Many studies indicate that bone mass expressed as bone mineral density (BMD) of the lumbar spine and/or hip is higher in patients with OA than in healthy subjects, but long term follow-up studies have failed to demonstrate that patients with OA have a lower rate of fractures despite higher BMD. Furthermore, other investigations have stated that subchondral bone density in OA is lower than normal. To increase the confusion, recent experimental reports have pointed out that estrogen deficiency could result in cartilage degeneration. This controversy is due to the fact that data on the relationship between OA and OP are commonly obtained from clinical cross-sectional studies. Few longitudinal studies have been undertaken looking at the association between these two entities. Although longitudinal studies would ideally be better, they are also more difficult to perform because of the length of time needed. In addition, key tools for studying OP, such as BMD analysis by dual energy X-ray absorptiometry (DXA), can easily be biased by the presence of osteophytes or other abnormalities commonly associated to age, making their results difficult to interpret. These limitations may be overcome with experimental studies. However, experimental investigations addressing the relationship between OP and OA are limited.
The purpose of this study is to analyze the effect of OP in joint degeneration developed in an experimental model of OA in mature female rabbits.

Material and methods

EXPERIMENTAL ANIMAL MODEL

A total of 12 sexually and skeletally mature white New Zealand female rabbits (8 months old, 3.5–5 kg body weight) were obtained from B&K Universal, Pamplona, Spain. The animals had free access to water and standard rabbit chow (Panlab, Barcelona, Spain). After 2 weeks of acclimatization, rabbits were randomly allocated to two study groups (OP plus OA or OA alone) to assess the effect of OP in cartilage lesions.

OP was experimentally induced in six rabbits by a combination of bilateral ovariectomy (OVX) and systemic corticosteroid administration as previously described20. Bilateral OVX was performed through a sagittal medial laparotomy under general anesthesia with intramuscular injection of 0.5 ml/kg xylazine (Rompun®; Bayer, Leverkusen, Germany) and 1.5 ml/kg of ketamine HCl (Ketolar®; Parke-Davis, Barcelona, Spain) 1:3. Antibiotic prophylaxis with cefonicid (100 mg/kg) (Monocid®; Smith & Beecham, Madrid, Spain) was administered before and during the 5 days following surgery. Figure 1 illustrates the protocol for the experimental induction of OP and OA in the same rabbits. Two weeks postoperatively they began to receive daily i.m. injections of methylprednisolone hemisuccinate (MPH) at a dose of 1 mg/kg/day for 4 weeks. Six weeks after OVX, OA was experimentally induced in the left knee by anterior cruciate ligament section and partial medial meniscectomy21, 22. Rabbits were anesthetized and antibiotic prophylaxis was used according to the protocols previously described. The knee was approached through a median parapatellar incision under sterile conditions. The anterior cruciate ligament was sectioned at its femoral insertion with a nr. 11 blade. Using iris scissors, the meniscotibial ligament was incised, the peripheral attachment of the anterior half of the meniscus was released and finally excised. The knee was closed by layers and a bulky Robert-Jones bandage applied for 4 days. All animals were permitted free cage activity after surgery.

Experimental OA was induced simultaneously in the six remaining rabbits following the same protocol without prior induction of experimental OP. Rabbits were euthanized by intracardiac administration of sodium pentobarbital (50 mg/kg) (Pentotal, Abbott, Madrid, Spain) 16 weeks after the knee operation. Both knee joints were carefully dissected to characterize the progression of their stiffe joint disease and to observe the influence of OP on the cartilage lesions. Left knees from ovariectomized rabbits were osteoporotic and osteoarthritic (OPOA knees), while left knees obtained from rabbits which had not been ovariectomized were OA alone (OA knees). Right knees from ovariectomized rabbits were used as non-osteoaarthritic but osteoporotic controls (OP knees), and right knees from non-ovariectomized animals served as healthy (normal knees) controls. The research complied with national legislation and with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and had local ethical committee approval.

BMD MEASUREMENTS

DXA analyses of BMD were performed in all rabbits at baseline, 6 weeks after OVX and 16 weeks after experimental OA, immediately before sacrifice according to a previously reported protocol20 (see Fig. 1). DXA analyses were carried out using a Hologic QDR-1000/W™ pencil beam densitometer (Hologic Inc., Waltham, MA, USA) with a 1 mm diameter collimator on the X-ray output. The densitometer was daily calibrated and a specific software for small sample analysis which increases the spatial resolution was used (space per scanning line of 0.0254 cm and resolution for each point of 0.0127 cm; version 6.2 (Hologic Inc., Waltham, MA, USA)). BMD was measured in the lumbar spine and in the left stifle joint. Measurements were performed in vivo with animals placed in supine decubitus position under general anesthesia with xylazine and ketamine HCl as previously described. After digital radiological screening, a point located 3 cm below the navel was used as the external guide to focus the DXA pencil beam at about L3–L4. For the knee DXA analysis, rabbits were placed lying on a methacrylate bed specifically designed for the study with 30 degrees of lateral tilt to obtain a true posterior-anterior joint view, and DXA was carried out with the leg in full extension and internal rotation, with the beam focused immediately distal to the joint line20.

Mean absorptiometric values for the third and the fourth vertebrae were calculated for the lumbar spine, and global joint and subchondral bone values were evaluated in the left knee20. A region of interest of 266 lines of width and 22 lines above and under the joint space was defined for global knee BMD values, and the mean values of four differentiated regions corresponding to medial and lateral femoral condyles and tibial plateaux \((17 \times 11\) pixels, 0.06 cm² each), located, respectively, 1 mm above and below the joint line at the areas of maximum contact between the femoral condyles and the tibial plateaux were computed for subchondral bone BMD values (Fig. 2).

---

**Fig. 1.** Diagram showing the schedule of experimental interventions.
CARTILAGE EVALUATION WITH MAGNETIC RESONANCE IMAGING

Since the aim of this study was to evaluate the effect of OP in the development of osteoarthritic articular lesions, the left knees in all rabbits were examined with high resolution magnetic resonance imaging (MRI) immediately prior to knee operation. MRI appearance of both groups of animals was compared in order to exclude any detrimental effect upon joint cartilage due to OVX or corticosteroid treatment.

MRI was performed on a Bruker Biospec 47/40 spectrometer (Bruker Medizintechnik GmbH, Ettlingen, Germany) equipped with a 4.7 T superconducting magnet (Oxford Instruments Ltd., Oxford, UK) and high-performance actively shielded gradients with a maximum gradient strength of 50 mT/m, following a modified, previously described protocol. The radiofrequency probe used was a 4 cm home-made surface coil. Articular abnormalities were evaluated in coronal images obtained using a T1 weighted spin-echo sequence [repetition time (TR) = 700 ms, echo time (TE) = 15 ms, 5 cm Field Of View (FOV), 256 × 256 image matrix giving 195 μm² in plane spatial resolution from a 1 mm slice thickness]. In addition, cartilage thickness of the weight bearing area of the medial femoral condyle was automatically measured in sagittal images following a method based on digitized image analysis of variations in signal intensity. The cartilage thickness was evaluated using a specifically developed IDL program (Fig. 3). The sequence acquired for image analysis of cartilage thickness was a 3D-spoiled gradient-echo with TR/TE 100/8 ms, a flip angle of 30°, 1 number of average experiments (NEX). The FOV was 5 × 5 × 1.6 cm³ and the image acquisition size was 256 × 256 × 16. The weight bearing area was selected because it has been demonstrated that it is the zone where the earliest abnormalities can be detected with MRI. The MR images were interpreted by two blinded observers (EC and SC).

GROSS PATHOLOGY

At sacrifice, the infrapatellar synovial pad and the femoral and tibial cartilages were inspected for gross pathologic changes by a blinded observer experienced in cartilage pathology (EC) following a semiquantitative scale. The synovial pad was graded as 0 (normal) or 1 (fibrous and proliferative appearance). For the femoral condyle and tibial plateau cartilage the severity of macroscopic changes was categorized as 0 (normal), 1 (discoloration, mild surface irregularities or pitting), 2 (partial-thickness erosion or fibrillation), and 3 (full-thickness erosion or and osteophytes). A score was allocated to each joint surface (medial and lateral femoral condyles and tibial plateaux) and an overall score was obtained by adding the severity scores for synovial pad and each surface evaluated in order to quantify the joint alterations with regard to both the severity and the extent of tissue damage.

HISTOLOGY

The purpose of the histological evaluation was to define and quantify the overall degree of joint changes, to score abnormalities of cells, matrix, structure and calcified cartilage, and to correlate each histological alteration with the variations of BMD detected by DXA in the ovariectomized rabbits.

After macroscopic examination, isolated femurs and tibias were fixed in buffered formalin for 24 h and then decalcified for 6 weeks in an EDTA solution (2 mM
ethylenediaminetetraacetic acid (EDTA), 0.5 mM tartrate sodium–potassium, pH 1) for further histological evaluation. The decalcified knee joints were cleaved in a sagittal plane along the central portion of the articular surface of each medial femoral condyle corresponding to the weight bearing area before embedding in paraffin wax. Sections (5 µm) were stained with hematoxylin and eosin (to assess cellularity and structural abnormalities) and Alcian blue to evaluate matrix abnormalities. The weight bearing area of the femoral condyle was delimited and histopathologically assessed using the Mankin’s grading system by an experienced cartilage pathologist (EC)\(^25\). The observer was blinded with respect to group, laterality, and macroscopic description, and the samples were presented in random order. A partial score for each category of the Mankin scale (structure abnormalities, cellularity, matrix staining and tidemark integrity) was allocated, and the scores in each of these categories were combined for each section. The evaluation was performed at the weight bearing surface of the medial femoral condyle because it shows the earliest and most severe histological abnormalities\(^24\).

**STATISTICAL ANALYSIS**

All statistical analyses were performed using commercially available software (SPSS v 11.0, Windows\(^5\), Chicago, IL, USA). Results are expressed as mean ± standard error measurement (S.E.M.) unless otherwise stated. Data from multiple groups were compared using Kruskal–Wallis and Mann–Whitney non-parametric analyses as appropriate. A Spearman correlation test was calculated to determine the extent to which the cartilage abnormalities evaluated according to the Mankin grading system correlated with the variations in BMD measured with DXA (\(r\) = partial correlation). Differences were considered significant when the \(P\) value was less than 0.05.

**Results**

One rabbit undergoing experimental OA without previous OVX died following meniscectomy. Consequently, data from 11 rabbits were available: five animals with experimental OA, and six undergoing experimental OP and OA (22 knees, six OPOA, six OP, five OA and 5 healthy controls).

**CARTILAGE EVALUATION WITH MRI AND GROSS PATHOLOGY**

No morphologic cartilage or bone changes could be identified in any knee at MRI examination prior to experimental induction of OA. **Figure 3** shows a characteristic high peak representing the cartilage signal intensity. Mean values of cartilage thickness measured with high resolution MRI at the weight bearing area of the left medial femoral condyle were 0.56 ± 0.04 mm and 0.54 ± 0.04 mm in the groups of rabbits with and without previous OVX, respectively. Differences were not statistically significant at comparison.

The scores corresponding to the macroscopic joint assessment after evaluating the 22 knees are shown in Table I. While no abnormalities could be found on gross inspection in the OP and normal control right knees, those knees corresponding to the groups where OA had been previously experimentally induced showed evident alterations consisting of synovial thickening, cartilage pitting and discoloration, ulcers and osteophytes. These abnormalities were more severe in the medial compartment of the knee. Although they were distributed along the whole articular surface, cartilage lesions were more intense in the weight bearing area of both femoral condyle and tibial plateau (**Fig. 4**). Interestingly, total scores individually given to both medial femoral and tibial compartments in the OPOA knees were significantly higher than those obtained by OP and control knees (\(P < 0.05\)). While macroscopic cartilage surface changes showed a trend to be more severe in OA than in OP and control knees, the differences were not statistically significant (\(P = 0.06\)).

**HISTOLOGY**

As illustrated in **Fig. 5**, those knees where OA had been experimentally induced (OA and OPOA left knees) showed significantly higher overall Mankin scores than normal healthy knees (\(P < 0.05\)). Moreover, there were also significant differences when the scores obtained from osteoarthritic knees in ovariectomized rabbits (OPOA knees) were compared with OA knees. Overall Mankin scores in OA knees were higher than that in OP knees, but the differences were not significant. However, OP knees showed significantly higher scores than normal healthy knees (\(P < 0.05\)), suggesting that OP could have an aggravating detrimental effect on the development of OA lesions.

The majority of OA and OPOA knees had abnormalities in structure, cellular density and matrix staining but there were no significant differences between the two groups of knees and the tidemark appeared normal, without any breach by blood vessels, in the majority of samples evaluated. Structural irregularities consisted of fibrillation and clefts or ulcers that reached the radial zone. However, the detrimental effect of OP in cartilage was more pronounced in the matrix staining with Alcian blue (**Fig. 6**). While OA showed mild to moderate reduction in the matrix staining, this reduction was moderate to severe in OPOA knees, and the combined histological scores used for assessment of matrix staining were significantly higher in all knees where OA had been experimentally induced.

---

**Table I**  
**Macroscopic evaluation of the cartilage**

<table>
<thead>
<tr>
<th>Group(^a)</th>
<th>Synovial pad</th>
<th>Medial femur</th>
<th>Lateral femur</th>
<th>Medial tibia</th>
<th>Lateral tibia</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>OP</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>OA</td>
<td>0.8 ± 0.2</td>
<td>2.4 ± 0.6</td>
<td>0.6 ± 0.4</td>
<td>1.6 ± 0.7</td>
<td>0.6 ± 0.6</td>
<td>6 ± 1.7</td>
</tr>
<tr>
<td>OPOA</td>
<td>0.5 ± 0.2</td>
<td>2.7 ± 0.3(\frac{1}{2})</td>
<td>1 ± 0.6</td>
<td>3 ± 0(\frac{1}{2})</td>
<td>0.8 ± 0.5</td>
<td>8 ± 1.4(\frac{1}{2})</td>
</tr>
</tbody>
</table>

\(\text{\(a\)}\)Normal: healthy control right knees of rabbits in which experimental OA was induced in the left knee. OP: osteoporotic control right knees of rabbits in which OP was induced. Data are expressed as mean ± S.E.M. \(N = 5\) (normal and OA)–6 (OPOA and OP). \(\ast P < 0.05\) vs normal; \(\| P < 0.05\) vs OP.
OPOA and OP knees than in controls. With regard to cellular changes, OA knees showed mild to moderate alterations in chondrocytes and hypercellularity, but the cartilage was usually hypocellular, and clones could be appreciated more frequently in OPOA knees. The clones were localized in the superficial and deep zones of the tissue (Fig. 6). Although differences did not reach statistical significance, partial scores from matrix staining and cellular density showed a trend to be more severe in OPOA than in OA knees ($P = 0.067$).

![Gross pathological macrographs from OA (A, B, C) and OPOA (D, E, F) knees. (A) OA knee where the distal joint surface of the femoral condyles appears normal, but the rim of the medial condyle shows a whitish osteophyte. Although no ulcers or fissuring can be detected, the weight bearing area of femoral condyles appears eburnated. (B) OA knee: the load bearing area shows partial-thickness ulcer in the medial femoral condyle and irregularities and pitting in the lateral condyle. (C) Tibia from an OA rabbit where changes of OA, including erosion of the medial plateau and an osteophyte at the edge of the lateral plateau can be seen. (D) OPOA knee: normal whitish bright appearance of the femoral cartilage in the femoral condyles has disappeared and an extensive osteophyte can be observed along the medial rim of the femoral condyle. Note partial-thickness ulcers located at the anterior part of the lateral femoral condyle and weight bearing area of the medial femoral condyle. (E) Full-thickness ulcer in the load bearing area of the medial femoral condyle. (F) Extensive inflamed ulceration can be observed in the medial joint surface and osteophytes border the medial and lateral margins of the tibial plateaux.](image)

![Bar graphs show the histological microscopic evaluation at the weight bearing area of the medial femoral condyle, with the total Mankin score and the partial scores obtained for each category of the scale. HEALTHY = healthy knees, OP = osteoporotic knees, OA = osteoarthritic knees, OPOA = osteoporotic and osteoarthritic knees. *$P < 0.05$ vs healthy; **$P < 0.05$ vs OP; †$P < 0.05$ vs OA.](image)
BMD MEASUREMENTS AND CORRELATION WITH HISTOPATHOLOGY

BMD measurements for the different anatomical locations analyzed with DXA in the groups of knees studied are shown in Table II. Six weeks after OVX, OP rabbits showed a significant decrease in BMD when compared to baseline ($P < 0.05$). BMD values in this group, measured immediately before knee surgery, were also significantly lower than those from animals that did not undergo experimental OP. The BMD values, in this group, were also within the normal range, consistent with previously published data\textsuperscript{20}. These data demonstrate that OA was surgically induced in both OP and healthy rabbits. Sixteen weeks after experimental induction of OA, BMD values showed no significant change, and the differences between the two groups of animals (i.e., rabbits with and without previous OP) were maintained in the three anatomical sites evaluated ($P < 0.05$).

With regard to the relation between BMD variations and cartilage injury, it is notable that microscopic cartilage abnormalities evaluated by the Mankin grading system correlated with the decrease of lumbar BMD [Fig. 7(A), $r = 0.743$; $P = 0.014$]. This tendency was also observed in the global and subchondral bone of the knee [Fig. 7(B and C), $r = -0.55$ and $-0.56$; $P = 0.10$ and 0.09, respectively].

Discussion

The connection between OA and OP has attracted considerable attention. However, reports concerning the relationship between OA and OP are contradictory. This controversy may be the result of difficulties in undertaking adequate sequential studies. Most data pertaining to this relationship have been obtained from human cross-sectional studies. As accurate methods to assess the initial stages of cartilage degeneration in humans are not available, it is almost impossible to establish the potential role of OP in the etiopathogenesis of OA. OP and OA have a heterogeneous pathogenesis where bone integrity plays a key role. There is, therefore, a need for experimental studies where both pathologies can be induced independently without interference from other confounding factors which could influence their development. In this investigation we developed an experimental model of OP and OA in the rabbit where the interplay between both conditions can be studied in a longitudinal manner.

Contrary to conventional wisdom, which suggests that OP and OA are inversely related, we have demonstrated a direct association between these two entities in this study.
Experimental OA and the decrease in matrix staining, followed by changes in cellularity are the earliest histological abnormalities detected in OA, while breaches of the tidemark and injury to cartilage structure are only prominent at advanced stages. Our findings suggest that OP accelerates cartilage damage without altering the sequence of changes or the histopathological characteristics of OA.

Since a few reports have indicated that systemic corticosteroids may result in cartilage damage, it could be argued that cartilage degeneration in this experimental model could be secondary to transient methylprednisolone administration. For this reason we performed a study with high resolution MRI before the experimental induction of OA. We have previously demonstrated in an experimental study that at the very early stages of OA high resolution MRI is sensitive enough to detect cartilage swelling, and that this finding correlates histologically to changes in matrix staining, which represents proteoglycan depletion. As no joint lesions could be detected on MRI in the current study, we considered it likely that the cartilage lesions are due to OP and OA.

The observation that cartilage damage is increased by OP is consistent with data from previous studies indicating that the incidence and prevalence of OA are increased in postmenopausal women. Recent publications in monkeys and rats have provided strong evidence that OVX induces OA-like changes in articular cartilage. These studies suggested an apparent chondroprotective effect of endogenous estrogens on cartilage turnover. The suggestion that cartilage metabolism may be influenced by estrogens is made credible by several reports which clearly demonstrated that chondrocytes in articular cartilage possess functional estrogen receptors. Furthermore, this putative chondroprotective effect of estrogens could have critical therapeutic implications for OA. Experimental and epidemiological and case-control studies have demonstrated that the administration of exogenous estrogens and estrogen-like drugs, such as selective estrogen receptor modulators, might prevent cartilage destruction and decrease the incidence of OA. However, whether established cartilage lesions can be effectively reversed using such medications remains to be investigated.

It is interesting that not only did OP increase cartilage lesions in experimental OA, but also the severity of the cartilage lesions correlated with the amount of BMD lost at the lumbar spine and knee, verifying a direct relationship between both these pathologies. With regard to subchondral bone, BMD was significantly lower in OPOA knees than in OA knees in this investigation. Lower than normal BMD values in subchondral regions of both femoral head and knee joints of patients with hip and knee OA have been reported. Subchondral bone rendering articular cartilage more susceptible to mechanical stresses has been incriminated in the pathogenesis of OA. However, this finding suggests that changes in articular cartilage precede structural alterations in subchondral bone in OA, as we have previously demonstrated.

In summary, OP increases the severity of cartilage damage in experimental OA. Cartilage damage is inversely correlated with BMD measured at the lumbar spine and knee. Since chondrocytes possess estrogenic receptors, the effect of OP on OA lesions might be explained by the potential protective effect of estrogens on cartilage. Abnormalities in the mechanical properties of osteopenic subchondral bone in OP could also be incriminated in the etiopathogenesis of OA. The current experiments do not allow us to ascertain if the acceleration of OA in OP is the result of estrogen insufficiency, abnormal subchondral
bone biomechanics, or both. Further studies on the relationship between OP and OA are needed as better understanding of the pathogenesis of these two disorders could have important therapeutic implications for both of these common diseases.

Acknowledgments

The authors wish to thank Dr O. Sánchez-Pernaute, from the Bone and Joint Research Unit (Fundación Jiménez Díaz, Universidad Autónoma, Madrid), and Dr A. Herrera and Dr E. Sáez Barajas, from the CAI of RMN (Universidad Complutense, Madrid) for their valuable advice and technical assistance in this study. This work was partially supported by research grants from the Spanish Ministry of Education (SAF 2003/08379) Comunidad Autónoma de Madrid (GR/SAL/0798/2004), and the Fondo de Investigaciones Sanitarias (CP03/00011; G03/152; PI04/0259).

References

34. Li B, Aspden RM. Material properties of bone from the femoral neck and calcar femorale of patients with osteoporosis or osteoarthritis. Osteoporos Int 1997;7:450–6.