Assessment of bone mineral density of the femoral head in dogs with early osteoarthritis

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Objective—To compare the bone mineral density (BMD) of the proximal portion of the femur in dogs with and without early osteoarthritis secondary to hip dysplasia.

Animals—24 dogs (3 Greyhounds, 6 Labrador-Greyhound crossbreeds, and 15 Labrador Retrievers).

Procedure—Computed tomography (CT) of the pelvis, including a bone-density phantom, was performed for each dog. Centrally located transverse CT slices and a computer workstation were used to identify 16 regions of interest (ROIs) in the proximal portion of the femur. For each ROI, the mean Hounsfield unit value was recorded; by use of the bone-density phantom and linear regression analysis, those values were converted to equivalent BMD (eBMD). Mean eBMD values for the subchondral and nonsubchondral ROIs in dogs with and without osteoarthritis (determined at necropsy) were compared. A mixed-model ANOVA and post hoc linear contrasts were used to evaluate the effects of osteoarthritis, breed, and sex on the BMD value.

Results—At necropsy, osteoarthritis was detected in 14 hip joints in 9 dogs; all lesions included early cartilage fibrillation. After adjusting for breed and sex, eBMD in subchondral ROIs 8 and 12 (adjacent to the fovea) were 8% and 6% higher, respectively, in osteoarthritis-affected dogs, compared with unaffected dogs; in the nonsubchondral ROIs, eBMD was 10% higher in osteoarthritis-affected dogs.

Conclusions and Clinical Relevance—Compared with findings in unaffected dogs, increased eBMD in hip joints of dogs with early osteoarthritis supports a strong relationship between the subchondral and epiphyseal regions and articular cartilage in the pathogenesis and progression of osteoarthritis. (Am J Vet Res 2006;67:796–800)

Osteoarthritis is a chronic, progressive disease characterized by the degradation of articular cartilage with accompanying alterations of the joint, including sclerosis and thickening of subchondral bone, osteophyte production, and alterations of the synovium. Findings of early osteoarthritis research suggested that even in the first stages of the disease, changes in the articular cartilage were accompanied by changes in the bone. The role of subchondral bone in the initiation and progression of osteoarthritis has been examined. Specifically, alterations of the subchondral bone have been reported in later stages of osteoarthritis, and increased turnover of the subchondral bone has been shown to predict osteoarthritis progression (worsening) over time. As yet, it is uncertain whether the onset of these bony changes precedes or follows injury to the articular cartilage.

Hip dysplasia results in subluxation of the hip joint and is a common cause of osteoarthritis in dogs; thus, it is a model for naturally occurring disease. The use of this model facilitates the study of articular cartilage lesions and bone alteration in the incipient stages of hip joint osteoarthritis and has the advantage of being nonsurgically induced. Further, hip dysplasia in dogs is an important disease in veterinary medicine, being a common cause of osteoarthritis with associated pain and loss of function in that species. The use of CT for the assessment of BMD has been described. This technique has been applied to the elbow joint of clinically normal dogs. By use of a routine CT scan that includes a density-standard phantom in the field of view, the diopotassium phosphate eBMD of defined ROIs can be derived. Because a CT scan provides cross-sectional images, precise anatomic localization of an ROI is possible; therefore, this modality is ideal for localizing changes in the subchondral region of bone in a noninvasive manner.

The purpose of the study of this report was to compare the BMD of the proximal portion of the femur in dogs with and without early osteoarthritis secondary to hip dysplasia. We hypothesized that the subchondral bone density of the femoral head of dogs with hip joint osteoarthritis would be greater than that of dogs without hip joint osteoarthritis.

ABBREVIATIONS

CT Computed tomography

eBMD Equivalent bone mineral density

ROI Region of interest

DLS Dorsolateral subluxation

DI Distraction index

OFA Orthopedic Foundation for Animals

HU Hounsfield unit
**Materials and Methods**

**Animals**—Twenty-four dogs from a colony maintained for the study of hip dysplasia were allocated to 1 of 3 groups on the basis of breed; group 1 consisted of 3 Greyhounds (6 hip joints) obtained as adults, group 2 consisted of 6 Labrador-Greyhound crossbreeds (12 hip joints), and group 3 consisted of 15 Labrador Retrievers (29 hip joints). The right hip joint from 1 Labrador Retriever was excluded from the analysis because of complete luxation that eliminated articular cartilage contact. For dogs in group 2, imaging examinations were performed at 6 months of age, and for dogs in group 3, the mean age was 11.3 months (age range, 8 to 36 months); dogs in group 1 were 4 years old when obtained and underwent similar imaging examinations at that age. Large-breed dogs are skeletally mature at 8 months of age, and femoral capital physeal closure is detected radiographically at approximately 8 months of age.14 All protocols were approved by an institutional animal care and use committee. Some of the dogs were also used for other studies.15,16

**Anesthesia**—For each dog, all imaging examinations were performed during 1 episode of anesthesia. Each dog was premedicated with acepromazine (0.02 mg/kg, IV) and glycopyrrolate (0.01 mg/kg, IM). For dogs in groups 2 and 3, anesthesia was induced with thiopental (10 mg/kg, IV); for dogs in group 1, anesthesia was induced with propofol (6 mg/kg, IV). After intubation, anesthesia was maintained by use of inhalant anesthesia.6

**Radiographic examination**—Hip joints of dogs were evaluated via 3 recognized radiographic examination procedures: assessment of DLS score and evaluation of distraction radiographs and standard ventrodorsal extended hip joint radiographs.1 Distraction radiographs of the hip joints were submitted to a commercial organization for measurement of the DI.18 The DLS score is expected to be lower and the DI is expected to be higher in dogs with hip dysplasia than in unaffected dogs.17 Standard extended hip joint radiographs were evaluated by a board-certified radiologist (NLD), who was unaware of other results. The assessment scheme used was similar to that used by the OFA as follows: grade 1, excellent hip joint conformation; grade 2, good hip joint conformation; grade 3, fair hip joint conformation; grade 4, borderline (indeterminate) hip joint conformation; grade 5, mild hip dysplasia; grade 6, moderate hip dysplasia; and grade 7, severe hip dysplasia.19

**CT scans**—Dogs were positioned in the DLS position, which mimics a weight-bearing position in an anesthetized dog and has been previously described as a valuable method for evaluation of dogs for hip dysplasia.

Computed tomographic exposures were performed at 130 kVp and 125 mA; scan parameters were 2-mm thickness, 1-mm index, and 512 × 512-matrix and were viewed in a bone window (window width, 1,500; window level, 250).4 A density phantom and hydroxyapatite rod were placed in the field of view.

**Image analysis**—All CT images were manipulated on a single workstation by the same examiner (HJC), who was unaware of the group assignment, radiographic scores, and postmortem findings. For each hip joint, the most central transverse slice was selected and the femoral head and neck were manually divided into 16 ROIs (Figure 1). The subchondral region ROIs were defined as ROIs 8 and 12; ROIs 4 and 16 were located just peripheral to the subchondral ROIs and were defined as partially subchondral. The other 12 ROIs were considered nonsubchondral. For each ROI, the mean HU value was recorded. A mean HU value was also recorded for each segment of the density phantom.

**Density phantom**—For each CT scan, mean HU values for each segment of the density phantom and the hydroxyapatite rod were compared with the known density concentrations via linear regression analysis. For the intercepts, P values were > 0.2 for 23 of 24 dogs (significance was set at a value of P < 0.05). Because most dogs had nonsignificant intercepts, a second linear regression analysis was performed for each dog in which the intercept was 0. By use of these slope data, a 1-way ANOVA was performed to compare the slope data among the 3 groups, and the results were F = 0.44 and P = 0.650. As the Barlett test suggested unequal variance (P = 0.079), a Kruskal-Wallis test for nonparametric analysis of variance was performed. The Kruskal-Wallis statistic was 0.804, and P = 0.669. Thus, by use of the mean slope, a regression equation was created and used for all dogs to convert HU values into dipotassium phosphate eBMD values. The eBMD data were used for further analysis.

**Necropsy procedures**—Dogs in groups 2 and 3 were euthanized with an overdose of pentobarbital, and the hip joints were examined. The necropsy methods have been described elsewhere, and at least 10 tissues from each dog were used by investigators in other studies.15,16 The hip joints were examined for the presence or absence of osteoarthritis within 2 hours after death. A score of 0 was assigned if the gross appearance of the articular cartilage was normal, and a score of 1 was assigned if cartilage fibrillation was detected in the perifoveal region of the femoral head. Joints with a score of 1 were considered to have secondary osteoarthritis, and none of these had more severe changes than early cartilage fibrillation.

Dogs in group 1 were not euthanized, and gross examinations of the hip joints were not performed. Group 1 dogs were presumed not to have hip dysplasia osteoarthritis on the basis of breed, radiographic measures, and the absence of signs of secondary hip joint osteoarthritis on ventrodorsal extended hip joint radiographs obtained at 2 to 3 years of age.20,21 Thus, these dogs were presumptively assigned a score of 0.

**Statistical analysis**—Independent variables considered to affect CT measures included breed and sex. Variables that were tested in a model that always included breed and sex were as follows: age at CT scan, age at necropsy, hip (left or right), DLS score, DI, hip joint classification (assessed on extended hip joint radiographs), and presence or absence of cartilage lesion (ie, presence or absence of osteoarthritis) at...
Results

At necropsy, 14 hip joints of 9 dogs were assigned an articular cartilage score of 1 and were considered to have secondary osteoarthritis. All lesions identified at necropsy were characterized by focal fibrillation of articular cartilage and were indicative of early osteoarthritis (Figure 2). Within each femoral head (after adjusting for breed and sex), dogs with osteoarthritis had higher eBMD in the subchondral region (at ROIs 8 and 12) than dogs without osteoarthritis (Table 1). The eBMD in these ROIs, which were subjacent to the cartilage lesions seen at necropsy, was 6% to 8% higher in dogs with osteoarthritis than in unaffected dogs. In the partially subchondral region (at ROIs 4 and 16), no difference in eBMD was detected between dogs with osteoarthritis and unaffected dogs. In the 12 nonsubchondral (epiphyseal) ROIs, mean eBMD was 10% higher ($P = 0.013$) in dogs with osteoarthritis, compared with the value in dogs without osteoarthritis. As the DLS score decreased, the eBMD in ROIs 4 and 8 increased significantly, and as DI increased, eBMD in ROI 8 also increased significantly for both the left and right hips, but the coefficients of determination were not high ($all < 0.5$). When the OFA-style hip joint grade was dichotomized at the borderline grade, it had no effect on BMD.

Discussion

Results of the present study have indicated that the BMD of the femoral head in dogs with early osteoarthritis of the hip joint (as a result of hip dysplasia) is greater than that in dogs without osteoarthritis of the hip joint. Because a central slice within the femoral head was selected for density measurements, changes in subchondral BMD at ROIs 8 and 12 coincided with the location of femoral head impingement on the lateral acetabulum in a subluxated hip joint. The occurrence of focal regions of altered density is likely attributable to altered loading of the femoral head in a dysplastic hip joint that initiates bone remodeling and results in increased BMD.

Although the DLS score and DI were significantly correlated with eBMD, the dichotomized OFA-style hip joint grade did not have an effect on eBMD. This was most likely attributable to the early stage of osteoarthritis in hip joints of the dogs, which was not sufficient to result in radiographic signs of osteoarthritis, and to the moderate sensitivity of the OFA grade for identification of early hip dysplasia in young dogs. Additionally, dichotomized data have lower power in statistical analyses.

Remodeling of bone occurs in response to mechanical forces placed on the bone, which was first described by Wolff and is referred to as Wolff's law. The mechanisms of bone response to loading have generated multidisciplinary interest but as yet are not fully understood. Currently, at least 3 general outcomes to mechanical loading of bone are recognized: modeling, wherein there is periosteal and endosteal deposition or resorption of bone; bone turnover involving the coordinated resorption and deposition of bone; and quiescence, in which no response is generated. In bone under stress, it has been shown that BMD increases and subchondral sclerosis is a well-documented manifestation of osteoarthritis. In our study population, the increased BMD in the perifoveal region in dogs with osteoarthritis, compared with dogs without osteoarthritis, may be the result of remodeling of the subchondral bone in response to altered loading of the femoral head in subluxated dysplastic hip joints. However, the mechanism by which this new bone is added has not been elucidated and may, in fact, comprise a response to a combination of both local and systemic factors. Furthermore, these changes may reflect biochemical alterations and not just altered mechanical loading. A proposed mechanism of subchondral sclero-

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Table 1—Least squares mean (± SEM) eBMD (dipotassium phosphatise equivalent density) of selected ROIs from the femoral heads of 24 dogs with and without signs of early osteoarthritis of the hip joints detected at necropsy. The ROIs 8 and 12 are in the perifoveal subchondral region; the value for the nonsubchondral ROIs is the mean eBMD of the 12 nonsubchondral ROIs.

<table>
<thead>
<tr>
<th>Group</th>
<th>ROI 8</th>
<th>ROI 12</th>
<th>Nonsubchondral ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoarthritis of the hip joint (14 femurs in 8 dogs)</td>
<td>$569 \pm 27^*$</td>
<td>$653 \pm 191$</td>
<td>$615 \pm 281$</td>
</tr>
<tr>
<td>No osteoarthritis of the hip joint (33 femurs in 18 dogs)</td>
<td>$521 \pm 24$</td>
<td>$617 \pm 14$</td>
<td>$553 \pm 22$</td>
</tr>
</tbody>
</table>

*Value significantly ($P = 0.05$) different from the ROI 8 value of the other group. Value significantly ($P = 0.007$) different from the ROI 12 value of the other group. Value significantly ($P = 0.013$) different from the value for the nonsubchondral ROIs of the other group.
sclerosis is increased bone deposition on existing trabecular bone with decreased active resorption. However, as bone remodeling is generally regarded as the product of both osteoclastic and osteoblastic activities, a phase or component of decreased BMD may occur in the remodeling process, but this was not detected in our study. Indeed, subchondral plate remodeling is increased below osteoarthritic cartilage, yet the type of remodeling that occurs is unclear. Initially postulated to involve the development and subsequent repair of microfractures, further research has failed to reveal increased numbers of microfractures in osteoarthritic hip joints, compared with control hip joints without discernible signs of disease.

In humans, trabecular thickening of both the subchondral bone and femoral neck has been detected in advanced osteoarthritis of hip joints; it is unclear whether this finding is a footprint of previous microfracture remodeling.

Subchondral bone, which is a mixture of woven and cancellous bone, provides biomechanical support to articular cartilage and has an important role in transferring and distributing loads from the articular surface to the underlying epiphyseal cancellous bone. The subchondral bone has been proposed to be an important impact absorber during joint loading. As such, stiffening of the subchondral plate secondary to sclerosis has been theorized to play a key role in cartilage destruction associated with osteoarthritis. Yet, there is evidence that subchondral bone changes do not induce osteoarthritis in all models. Attempts to induce full-thickness cartilage degeneration with metal implants that increased the stiffness of the subchondral bone in sheep resulted in tidemark advancement at 2 years, but did not result in overt deterioration of the articular cartilage at 5 to 7 years after implantation. Mathematical models have indicated that the biomechanical properties of subchondral bone must become excessively altered before even a modest alteration in the load of overlying cartilage is expected, implying that the required stress to induce such subchondral stiffening exceeds normal physiologic loading. On the basis of such findings, the theory that subchondral bone stiffening induces osteoarthritis has been largely discounted.

A histomorphometric study of the proximal portion of the femur of dogs has revealed increased trabecular alignment in the medial aspect of the femoral head in dogs with advanced osteoarthrosis of the hip joints that is not evident in dogs with moderate osteoarthrosis of the hip joints. Those investigators concluded that increased trabecular alignment accompanies the progression of osteoarthritis over time. Previously, changes in trabecular alignment have been attributed to changes in mechanical stress. In the present study, the increase in subchondral BMD in regions subjacent to areas of early cartilage fibrillation provides further evidence that altered biomechanics of the subchondral plate exist in the earliest stages of osteoarthritis. In addition, the overall increase in BMD in nonsubchondral portions of the femoral head in dogs supports the concept that osteoarthritis is a so-called global disease, in that it affects the entire synovial joint and associated structures.

Computed tomographic densitometry uses the Hounsfield scale or CT number to express the x-ray attenuation of a given volume of tissue, called a voxel. There is a direct relationship between the CT number and the mean linear attenuation coefficient of the tissue in that voxel. The use of a known density phantom is critical in this technique. The density phantom is placed in the field of view for each scan and can be used to assess consistency of the CT scanner during the study period. By use of simple linear regression analysis, the conversion of HU values via a calibration equation into eBMD values is also possible.

In our ROI design, the subchondral region included the true subchondral bone and subjacent epiphyseal bone. Selective determination of BMD of the subchondral region of the femoral head was facilitated by the use of CT. Transverse CT scans of the hip joint provided a cross-sectional image for analysis. This is an advantage over the use of dual-energy x-ray absorptiometry, which incorporates all tissue nonselectively into a 2-dimensional image, on which BMD is measured by drawing ROIs. The importance of precise location of the sampling region when describing BMD has been acknowledged. By use of a biomechanical model, it has been predicted that changes in BMD or stiffness must be within 2 to 3 mm of the osteochondral junction to affect stresses borne by the overlying cartilage. In our study in dogs, differentiation of changes in the subchondral region from overall changes in the bone of the femoral head and neck and identification of precise sites of these changes were possible.

In a previous investigation by our group, it was determined that during progression of osteoarthritis in dogs, synovial membrane alterations occur simultaneously with development of articular cartilage lesions. Consistent with findings of another study, the BMD measurements determined in the present study indicated that subchondral bone alterations occur concurrently with pathologic changes in the synovium and articular cartilage in hip joints during incipient stages of osteoarthritis. These tissue changes are consistent with a mechanical etiology of osteoarthritis in dysplastic hip joints. Excessive local load on the peril-articular cartilage at the lateral acetabular rim in subluxated hip joints is associated with remodeling of the subchondral bone plate and development of articular cartilage fibration; in addition to involvement of the subchondral region, higher mean BMD also developed in the remainder of the femoral head. Identification of these changes in dogs with early osteoarthritis provides further evidence of a strong relationship between the subchondral region and articular cartilage in the development of osteoarthritis.

a. Veterinary pentothal, Abbott Laboratories, North Chicago, Ill.

b. Diprivan, Zeneca Pharmaceuticals, Wilmington, Del.

c. Halothane, Halocarbon Labs, River Edge, N.J.


e. Picker PQS, Picker International, Cleveland, Ohio.

f. Cam-Genant K2HPO4 phantom (0, 50, 100, and 200 mg/mL), Boston, Mass.

g. Hydroxyapatite rod (800 mg/mL), CIRS, Norfolk, Va.

h. Voxell Q, Picker International, Cleveland, Ohio.
References


